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ENCAPSULATION OF PHENOLIC COMPOUNDS FROM POMEGRANATE FOR APPLICATION IN GUMMY CANDIES

ENCAPSULAÇÃO DE COMPOSTOS FENÓLICOS DE ROMÃ PARA APLICAÇÃO EM BALAS DE GOMA

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Thesis presented to the Faculty of Food Engineering at the Universidade Estadual de Campinas as part fulfillment of the requirements for the degree of Doctor in Food Engineering.

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RESUMO

As balas de goma são produtos de confeitaria que podem ser produzidos com ingredientes naturais, visando um produto mais saudável. Os polifenóis do extrato de romã (PE) têm funções benéficas para os consumidores quando adicionados aos alimentos. Além disso, o encapsulamento desses componentes pode proporcionar proteção e controle de liberação, além de melhorar aspectos sensoriais. Formulações de pectina e amido foram avaliadas quanto à produção de partículas pela técnica de gelificação iônica, utilizando métodos de *dripping* e *jet cutting*. Partículas com 2,85% de sólidos, dos quais 70% representam pectina e 30% amido de milho ceroso, mostraram-se tecnologicamente viáveis e foram secos satisfatoriamente em leito fluidizado, enquanto a secagem em tambor rotativo não foi eficiente. O encapsulamento convencional de PE adicionado a soluções biopoliméricas apresentou baixa eficiência (4,7% a 43,7%) e a taxa de liberação foi maior para partículas produzidas por gotejamento (~70%). Para reduzir a transferência de massa e consequentemente aumentar a retenção de PE, algumas estratégias foram utilizadas. A eficiência de encapsulamento (EE) foi melhorada (42% para 101%) com a alteração da concentração do meio externo, mas a maior retenção de PE foi observada nas partículas de amido-pectina obtidas por adsorção (2960,26±26,92 mg de ácido gálico equivalente/100g amostra). O revestimento foi capaz de reduzir a taxa de liberação na maioria dos testes, mas durante a execução da técnica houve perda de cerca de 32% dos compostos fenólicos na solução de quitosana, reduzindo o EE. Por fim, foi proposto um estudo de aplicação em balas de goma, com foco na comparação do desempenho de PE encapsulado e não encapsulado. A adição de PE na forma de partículas maiores (*dripping*) apresentou maior aceitação pelo mapa preferências interno e maior intenção de compra. Além disso, o maior teor fenólico e capacidade antioxidante das amostras pode ser um indício de maior potencial para esta forma de aplicação. Porém, mais pesquisas são necessárias para garantir maior aceitação sensorial pelos consumidores.

Palavas-chave: gelificação iônica; pectina; liberação controlada; adsorção.

ABSTRACT

Gummies are confectionery products that can be produced with natural ingredients aiming at a healthier product. Pomegranate extract (PE) polyphenols have beneficial functions for consumers when added to food. Furthermore, the encapsulation of these components allows for protection and release control, in addition to improving sensory aspects. Pectin and starch formulations were evaluated for particle production by the ionic gelation technique, using dripping and jet cutting methods. Granules with 2.85% solids, 70% pectin and 30% waxy corn starch, proved to be technologically viable and were satisfactorily dried in a fluidized bed, while drying in a rotating drum was not efficient. Conventional encapsulation of PE added to biopolymeric solutions showed low efficiency (4.7% to 43.7%) and the release rate was higher for particles produced by dripping (~70%). In order to reduce mass transfer and consequently increase PE retention, some strategies were used. The encapsulation efficiency (EE) was improved (42% to 101%) with changing the concentration of the external medium, but the highest PE retention was observed in pectin-starch particles obtained by adsorption (2960.26±26.92 mg of gallic acid equivalent /100g sample). The coating was able to reduce the release rate in most tests, but during the execution of the technique there was a loss of about 32% of the phenolic compounds in the chitosan solution, reducing the EE. Finally, an application study on gummy candies was proposed, focusing on the comparison of the performance of PE encapsulated and non-encapsulated. The addition of PE in the form of larger beads (*dripping*) showed greater acceptance by the internal preference map and greater purchase intention. Furthermore, the higher phenolic content and antioxidant capacity of the samples may be an indication of greater potential for this form of application. However, more research is needed to ensure greater sensorial acceptance by consumers.

Keywords: ionic gelation; pectin; controlled release; adsorption.

SUMMARY

CHAPTER 1

General introduction, Goals and Thesis structure

CHAPTER 1 - General introduction, Goals and Thesis structure

1.1 General Introduction

Gummy candies represent the most common confectionery consumed in the world and they are also among the fastest-growing product groups in the current market (Altınok et al., 2020; Periche et al., 2014). The presence of high amounts of sweetening agents in these confectioneries is strongly dependent on the final textures obtained (Burey et al., 2010) and sugar replacers such as mannitol, xylitol, and stevia (Cai et al., 2017) or formulations with lower sugar content (Gok et al., 2020) have been extensively studied in last years to manufacture healthier gummies. Moreover, the interest in the addition of natural components in gummy candies has also increased. Coloring and functional ingredients like antimicrobials, anthocyanins, soluble fibers, and probiotics into gummies can be shifting them towards healthier and non-artificial products (Karimi et al., 2020; Moghaddas Kia et al., 2020; Otálora et al., 2019).

Functional ingredients such as polyphenols inserted directly into the candy matrix can be degraded during processing or storage due to their thermolability (Patras et al., 2010). Pomegranate extract, for example, is a rich source of polyphenols that are ability to counteract free radicals, prevention of degenerative diseases (Anbinder et al., 2011), antimutagenic, antifungal, and antimicrobial properties (Aqil et al., 2008). Nevertheless, certain naturally-occurring polyphenolic compounds undergo deterioration when subjected to elevated pH levels (Friedman & Ju, 2000) and the antioxidant capacity decreases as the processing temperature rises (Réblová, 2012).

Microencapsulation avoids the interaction among the compounds from formulation and enables the antioxidant activity in food products by favoring protection against adverse environmental conditions. Moreover, it can improve bioactivity by controlling the release of specific places or at defined rates (Etchepare et al., 2015; Goula & Adamopoulos, 2012).

Encapsulation by ionic gelation is probably the technique more appropriate for the incorporation of polyphenols due to the compatibility of the gelled beads with the gummy candies matrix, providing an additional barrier against rapid release. Moreover, it presents a relatively low cost and does not require specialized equipment, high temperature, or organic solvents (Kurozawa & Hubinger, 2017; Rodrigues et al., 2014). This technique was previously employed for the incorporation of anthocyanins from hibiscus resulting in enteric

protection (Moura et al., 2019), but improved protection under simulated gastrointestinal conditions and homogeneous gummies can be obtained by reducing the particle size and using other drying methods. Another work using the same strategy for betalains, another class of pigment (Otálora et al., 2019) did not evaluate the encapsulation effect of beads in the product, since beads were disintegrated before manufacturing the gummy candies.

Here, we are proposing a study of combined pectin and starch formulations to identify the best biopolymer ratio based on the characteristics of the solutions and the physical properties of the beads. Next, we propose to evaluate the diffusive behavior in the drying and rehydration and proposed techniques to increase the retention of pomegranate extract and decrease release in water. Finally, the study of formulation and characterization of gummy candies containing encapsulated and non-encapsulated extract was done.

1.2 Goals

1.2.1 Main goal

To produce gummy candies added of pomegranate peel extract encapsulated by ionic gelation.

1.2.2 Specific goals

1) Understand the effect of composition on the behavior of solutions and physical properties of pectin and starch beads.
2) Adjust the operational parameters of the jet cutter to reduce the particle size obtaining reduced mass losses in the process.
3) Set the ideal drying parameters for pectin/starch beads (time, load, air flow, temperature).
4) Produce pomegranate beads by different techniques aiming for higher encapsulation efficiency.
5) Produce PE gummy candies with pomegranate extract with high antioxidant activity and good sensory acceptance.

1.3 Thesis Structure

The research project development stages are presented in 8 chapters. In this Chapter 1 – General Introduction, Goals and Thesis Structure, the main theme of this study, the intended objectives and the steps involved in achieving them are presented. Figure 1.1 illustrates the activities developed in each of the practical stages, subdivided into 4 articles.

Figure 1.1 – Schematic representation of the research project development stages

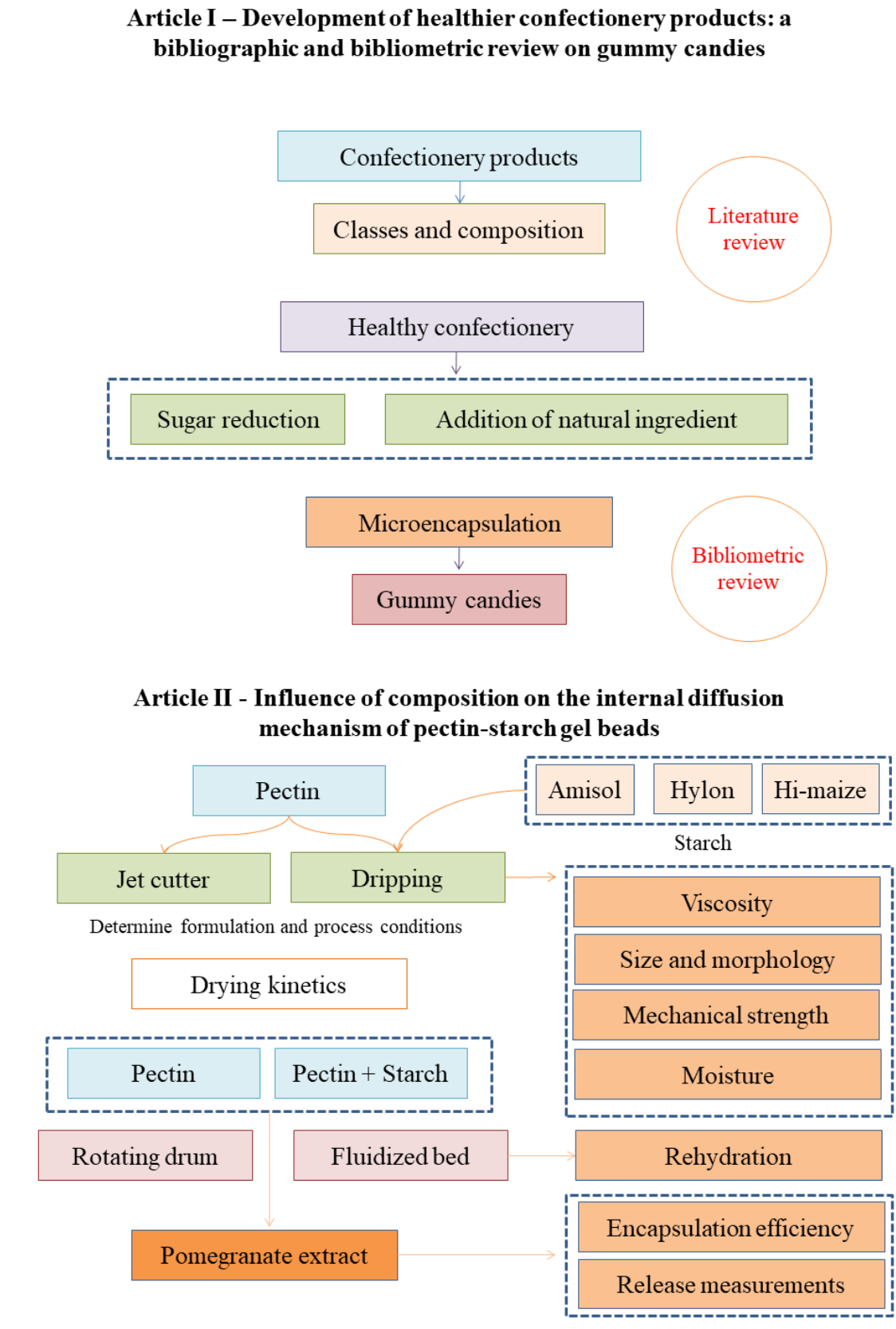
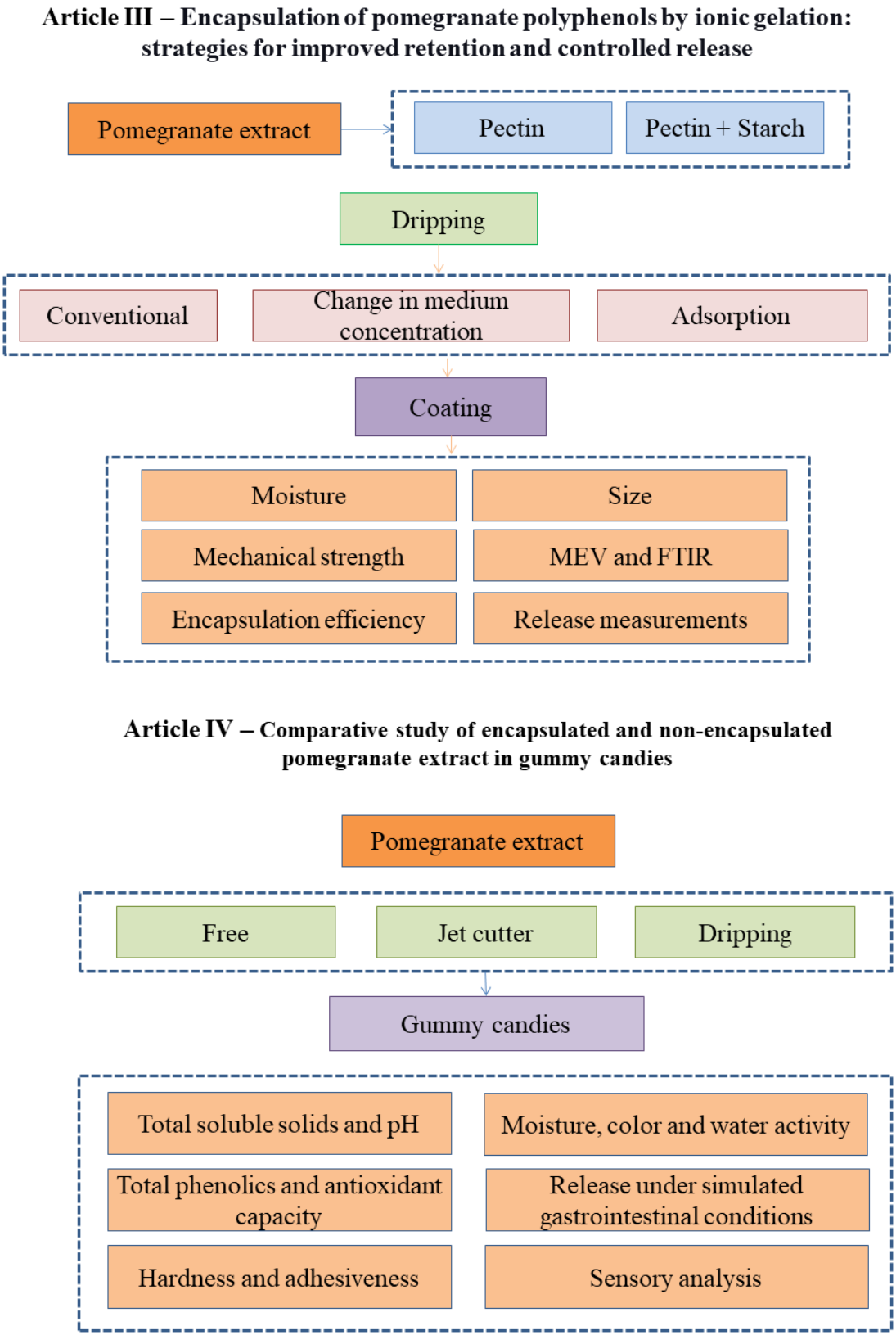


Figure 1.1 – Schematic representation of the research project development stages (continuation)



In **Chapter 2 – Theoretical Foundation**, information is presented that reinforces the importance of the research, the problems that generated the study and the strategies that are being developed to solve these problems. In **Chapter 3 - Development of healthier confectionery products: a bibliographic and bibliometric review on gummy candies**, the main trends in the healthy confectionery market are shown, with an emphasis on the use of encapsulation techniques for application in gummy candies. **Chapter 4 - Influence of composition on the internal diffusion mechanism of pectin-starch gel beads** provides experimental data based on formulation studies that seek to define the best operational conditions for different encapsulation mechanisms, using pectin and starch. These wall materials were further evaluated for the retention and release of hydrophilic compounds.

In **Chapter 5 - Encapsulation of pomegranate polyphenols by ionic gelation: strategies for improved retention and controlled release**, the results for different bead production techniques used as a strategy to improve the encapsulation efficiency of pomegranate extract are presented. In **Chapter 6 - Comparative study of encapsulated and non-encapsulated pomegranate extract in gummy candies**, the results of the production of gummy candies with pomegranate extract are presented and discussed.

In **Chapter 7 – General Discussion**, a discussion addressing the main results obtained in this work is presented. Finally, **Chapter 8 – General conclusion and suggestions for future work**, summarizes the main results obtained in this study and presents suggestions so that the techniques can be improved.

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CHAPTER 2

Theoretical Foundation

CHAPTER 2 – Theoretical Foundation

2.1 Healthier and functional gummies

The modern lifestyle resignificates the role of food, which beyond satisfying basic nutritional needs are rather used to the prevention of nutrition-related diseases and improvement of the physical and mental well-being of consumers (Comunian et al., 2020). Natural bioactive compounds include a broad diversity of structures and functionalities that provide many types of molecules for the production of nutraceuticals, functional foods, and food additives (Joana Gil-Chávez et al., 2013).

Following this trend, confectionery products such as candies have been reformulated for greater healthiness. The demand primarily arises from health concerns, including issues like obesity, tooth decay, and hyperglycemia, all of which are linked to excessive sugar intake (Periche et al., 2014). Substitutes for sugar can influence the product's processability parameters due to variations in water solubility and crystallization characteristics of the sweetener. The introduction of new ingredients additionally impacts viscosity, interactions with components in the formulation, and sensory attributes (Gok et al., 2020).

Altinok et al. (2020) evaluated the addition of skin and seed powders of grapes in soft candies aiming to include nutrients, bioactive compounds, and fibers. The authors found that particle sizes strongly influence the texture, and rheological properties of soft candies and concluded that grape-derived components could be successfully used in the formulation if the particle size is below 200 μm.

Considering the numerous physic-chemical properties and their influence on the texture and sensory properties of gummy candies, an optimization study should rigorously evaluate the confectionery composition, where the interaction of sugar alternatives with the quality features is used.

2.2 Encapsulation of phenolic compounds

Encapsulation of polyphenols is widely studied in the literature focusing on spray drying techniques (Cihat & Erdo, 2014; Fang & Bhandari, 2011; Roopchand et al., 2013; Tsali & Goula, 2018). The advantages associated with this drying method are that the particles have great storage stability due to the low water content. However, such particles can be soluble in products with high humidity, which compromises protection during processing.

In pomegranate juice, there is a high content of polyphenols and anthocyanins but these compounds are unstable molecules and the fresh juice has a short shelf life. In this context, the stabilization of this compound for use in industrial purposes could be aided using microencapsulation technologies (Robert et al., 2010). Pomegranate peels and seeds contain high amounts of phenolics and could be a good source for producing high-value antioxidants (Kaderides & Goula, 2019).

Ionic gelation is an encapsulation method wherein polymers like alginate and pectin create insoluble gels when exposed to oppositely charged ions, such as calcium. This technique is conducted under gentle conditions, specifically at room temperature, without the need for organic solvents or high shear forces, rendering it advantageous (McClements, 2017). Due to the hydrophilicity of the matrix, for the entrapment of polyphenols the use of substances such as fillers and electrostatic deposition coating can increase retention and modify release rates. Especially due to the compatibility of the gelled beads with the jelly candies matrix, this application can provide an additional barrier against rapid release. Moreover, the beads produced by ionic gelation can be stabilized and water removal through the drying process at relatively low temperatures is a viable alternative.

The particle size is extremely important for the sensory characteristic of food products, influencing mainly the appearance and texture. Moura et al. (2019) concluded that due to the large size of particles produced by dripping-extrusion, the hibiscus extract dispersion through the jelly candy was not uniform, while smaller particles produced by atomization gave rise to homogeneous with better color distribution jelly candies. Thus, ionic gelation with a mechanism that reduces beads size, as jet cutting, can be interesting for this application. A jet cutter is a cutting tool comprised of multiple wires that, as it rotates, divides the jet into uniform segments. The spheronization process takes place during the droplet's descent into the bath, akin to the simple dripping process, that involves the application of tension forces as it falls under gravity and interacts with the cross-linking solution (Paulo et al., 2017).

The application of gelled beads can be limited by high moisture content, for incompatibility with the food matrix. Thus, the use of drying can be important to complement the process of obtaining particles, promoting greater stability. Aiming to reduce the water content of gelled beads some authors performed drying mainly at convective oven (Otálora et al., 2019) or freeze-dryer (Dehkordi et al., 2020). For such dryers, the drying rate is very low which can be associated with the preservation of beads structure. However, the increase in the

water removal rate is essential for industrial viability. Thus, techniques using relative movement between air-particle phases, for example, with the suspension of beads in an airstream (fluidized bed) or with the mechanical movement of the particles (rotating drum) must be explored with this aim.

2.2.1 Encapsulation of pomegranate

The encapsulation of pomegranate peel and seed juice, along with pomegranate seed oil, has been a subject of study in various research works, as indicated in Table 2.1. For example, Karaaslan et al. (2014) delved into the impact of temperature on drying kinetics and the thermal degradation of phytonutrients found in pomegranate arils. Their findings revealed that the quickest drying occurred at 75°C, but the highest levels of anthocyanin-phenolic compounds and antioxidant capacity were observed in the arils dried at 55°C. Thus, the use of relatively lower temperatures is intriguing to prevent the degradation of bioactive compounds during the drying process.

Table 2.1. Encapsulation of pomegranate

Active	Encapsulating agente	Drying method	Operating conditions	Results	Reference
Extract of pomegranate peels and seeds	Modified starch (capsul)	Spray-drying	IAT(°C):167 AF (L/h): 600 AP (MPa): 0.5 FR (mL/min): 2.5	Peel extracts showed a punicalagin content of 0.4–9.5% and seed extracts showed a punicic acid content of 65.1–78.4%. The encapsulation efficiency ranged from 35.1% to 72.4% for peel extracts and from 68.2% to 92.7% for seed extracts.	Bustamante et al., 2017
Pomegranate seed oil	Skimmed milk poder	Spray-drying	IAT(°C):150-190 AF (m^3/h): 17.5-22.8 AP (bar): 5 FR (g/min): 1.75	Encapsulation efficiency was about 95.6%.	Goula & Adapaloumos, 2012
Pomegranate seed oil	Sodium alginate or trehalose	Freeze-dried	-20°C and 13.3 Pa	Sodium alginate microcapsules are more heat resistant than trehalose microcapsules	Gupta et al., 2011
Pomegranate peel	Maltodextrin/whey protein isolate (50:50)	Spray-drying	IAT(°C):150-190 AF (m^3/h): 17.5-22.8 AP (bar): 5 FR (g/min): 1.75	The encapsulated phenolic extract was found efficient in improving the shelf life of hazelnut paste, despite the limited solubility of the crude extract in such a high lipid content matrix.	Kaderides et al., 2015

Table 2.1. Encapsulation of pomegranate (continuation)

Active	Encapsulating agente	Drying method	Operating conditions	Results	Reference
Pomegranate peel extract	Orange juice by-products	Spray-drying	IAT(°C):150-190 AF (m^3/h): 17.5-23.1 AP (bar): 5 FR (g/min): 1.75	The achieved efficiency value (99.77%) was notably high, whereas the yield value (12.99%) proved to be significantly lower than those obtained using common wall materials, a problem related to glass transition temperature of the powder.	Kaderides et al., 2019
Juice and ethanolic extracts	Maltodextrin (MD) or soybean protein isolates (SPI)	Spray-drying	IAT(°C): MD (140-160) SPI (100-140) AF (L/h): 600 AP (psi): 20 FR (mL/min): 10	The encapsulation efficiency of polyphenols was notably improved in the SPI matrix, while for anthocyanins, it was superior in the MD matrix. In the yogurt, the stability of the bioactive compounds exhibited a comparable pattern to those without encapsulation, except for PE-MD.	Robert et al., 2010

IAT: Inlet air temperature; AF: air flow; AP: atomisation pressure; FR: feeding rate.

The spray-drying process involves atomizing the wet solution at high velocity and generating dry granulated powders by introducing hot air with temperatures of up to 200°C (Arepally & Goswami, 2019). This technique is widely used for encapsulating polyphenols (Kaderides & Goula, 2019; Rodrigues et al., 2020). However, depending on the application, for example, for the addition of these compounds in gummy candies, ionic gelation can be more viable. Therefore, applications in food products can be improved using techniques less explored as ionic gelation.

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CHAPTER 3

Development of healthier confectionery products: a bibliographic and bibliometric review on gummy candies

CHAPTER 3 - Development of healthier confectionery products: a bibliographic and bibliometric review on gummy candies

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ABSTRACT

Due to the high sugar content and normally low nutritional value, the consumption of confectionery has been associated with obesity, diabetes, cardiovascular diseases, and hypertension. Many studies have been conducted aiming to reduce the sugar content and increase the natural ingredients in candies. Therefore, the development of such products using bioactive compounds and beneficial substances may offer valuable solutions to the confectionery industry. In this work, an overview of the classes and composition of traditional confectionery products was addressed. Studies about confectionery products with health appeal were compiled and discussed, which led to the final part of this review: the use of encapsulation as a technique to enable the production of more healthy confectionery products. A bibliometric analysis associated with a review of the literature was conducted for exploring and analyzing scientific data, to understand the global scenario and trends regarding specifically gummy candies. Encapsulation has been used in gummy food matrices with an emphasis on bioactive compounds such as polyphenols, natural pigments, probiotics, and vitamins. However, it is an area that still can be explored to increase the variety and load of beneficial compounds, producing candies with nutritional quality, naturalness, and convenience, with sensorial acceptance and attractive costs.

Keywords: encapsulation, enriched food, sugar reduction, natural ingredients

3.1 Introduction

Dynamic changes in the global food industry are affected by an increased health consciousness of the consumers, impacting new demands such as the reduction of sugar and calories and the search for organic, vegan, and “clean-labeled” food products (Seremet et al., 2022). In the current context, confectionery is the class of food products that moves around $198,335.3 million annually and is the one that most needs to adapt since its use is usually associated with pleasure and not a nutritional need (Global Data, 2021).

The reduction of sugar in confectioneries is a central point to meet public health needs (WHO, 2015) and government reformulation programs. However, added sugars contribute to some physical properties in solid food products so significantly reducing the sugar content remains a challenge (Erickson and Carr, 2020). On the other hand, the addition of natural compounds in candies has been highlighted for offering sensory benefits and health appeal, but they also present some difficulties for direct application in free form (Otálora et al., 2019).

The encapsulation technique enables a protective layer to form that prevents the active ingredients from coming into direct contact with the other components of candy, such as sugar, gelling agent and flavoring agents, which could impair its quality, stability or flavor. In the context of gummy candies, encapsulation can be used to improve stability, modify the release of flavors and aromas, add nutrients or functional ingredients (Kumar et al., 2020).

In parallel with the sugar reduction policies, new formulations and processing may make this group of products to be considered a convenient carrier of vital nutrients. This review is focused on exploring the confectionary market adaptation to health appeals. The first part makes an analysis of the groups of confectionaries and traditional formulations in terms of carbohydrates and sugar. After, an analysis of the current studies about technological strategies adopted to transform confectionery into more healthy products. And finally, a section is dedicated to the use of encapsulation emphasizing gummy candies. A mixed methodology of bibliometric analysis and literature review was adopted to understand research trends and future perspectives for the food industry.

3.2 Literature research methodology and bibliometric network

The literature review was a integrative theoretical study, a method that permits summarizing knowledge through a systematic and rigorous process (Mendes et al., 2019),

being carried out by tracking scientific articles that focus on the enrichment of sugar confectionery products by sugar replacement and addition of functional, natural and active ingredients. The scientific databases ScienceDirect (https://www.sciencedirect.com), Scopus (http://www.scopus.com), Scielo – Scientific Electronic Library Online (https://www.scielo.org/), PubMed (MEDLINE) (http://www.ncbi.nlm.nih.gov/pubmed), and CAPES/MEC Journal Portal (http://www.periodicos.capes.gov.br) were used to find articles. Market data were obtained from books, company databases and specialized sites, public domain research reports, technical and scientific publications. The exclusion of those that did not fit the objective of the research or repeated articles was carried out.

For the bibliometric analysis data collection, a search was carried out in the database of the Web of Science © (WOS) Main Collection in August 2022. The data were filtered using the terms “gummy OR candy” and “encapsulation OR encapsulated” in the topic item that includes the title, summary, keywords, and keywords plus; no year filter was used to ensure that all the studies present in the database were obtained. The search was performed and it found 48 works comprising 45 research articles (93.7%) and 3 review articles (6.3%). From the data, we extracted and analyzed the 10 most relevant papers. Additionally, the VOSviewer software (Java Version 1.6.14) was used to gather information concerning the most frequent keywords to evaluate the trends in encapsulation for application in confectionery.

3.3 Sugar confectionery products

3.3.1 Classification, composition and quality attributes

Confectionery products, classified as energy-dense food items, are derived from the processing of carbohydrate raw materials and are primarily characterized by their sweet taste due to the inclusion of sucrose and other sugars as key ingredients (Žuljević and Akagi, 2020).

Confectionery sugar products can be broadly categorized into two main groups: hard confectionery (i) and soft confectionery (ii). Another way to classify them is based on the physical state of the sucrose or its substitute bulking agent or sweetener. This classification includes fluid, amorphous, glassy, partially crystalline, and crystalline (Gunes et al., 2022).

The most used sweeteners for sugar-based confections comprise cane or beet sugar (sucrose), liquid sugar, glucose syrup, high fructose syrup and invert sugar (obtained by the sucrose hydrolysis). Glucose syrup is used for controlling sucrose crystallization and invert sugar as a humectant and also to enhance flavor release (Hartel et al., 2018; Bender, 2009).

From the hardening of marshmallows to the graining of hard candies, moisture plays a crucial role in defining the quality and longevity of sugar-based confections. Water is a vital factor in confectionery production, influencing texture, and often acts as the key factor during storage that regulates shelf life (Ergun et al., 2010). Table 3.1 presents the moisture and water activity (Aw) of some confectionery products, as well as the sugar content (glucose syrup and sucrose).

Table 3.1 - Moisture, water activity (Aw) and sugar composition of sugar confectionery products

Sugar confectionery product	**Moisture (%)**	**Aw**	**Glucose syrup (%)**	**Sucrose (%)**
Hard candy	2-5	0.25-0.40	20-30	50-60
Caramel, fudge and toffee	6-18	0.45-0.60	5-20	30-65
Chewy candies	6-10	0.45-0.55	46-60	30-45
Nougat	5-10	0.40-0.65	32-45	40-55
Marshmallow	12-20	0.60-0.75	35-45	30-40
Gummies and jellies	8-22	36-42	36-42	30-36
Fondants and creams	10-18	0.65-0.80	9-16	64-71

(Ergun et al., 2010; Hartel et al., 2018; Ranken et al. 1997)

Fruit preparations, including jams, marmalades, and preserves, consist of fruits, vegetables, and sugar, and are specifically produced for commercial distribution to end users. These products have an extended shelf life and are manufactured using techniques such as thermal processing, water activity control, and pH regulation to ensure long-term storage stability. Fruit preparations serve as versatile intermediate ingredients utilized in a variety of dairy products like yogurt, ice cream, and cheese, as well as in biscuits, confectionery, and other similar food products (Fugel et al., 2005).

The main mass in soft candies is sugar syrup and the character of the final product is determined by processing factors such as heating and cooling temperature, foam formation,

and the use of different hydrocolloids as a stabilizer. In this category are gummy candies with a soft and chewy texture, usually made with gelatin, starch or pectin, sugar, flavorings and colorings, resulting in a smooth and elastic consistency (Gok et al., 2020). Each hydrocolloid provides unique organoleptic properties, gelatin, for example, provides a springy and transparent gel, while starch increases hardness and opacity (turbidity) in jelly-type soft confectionery (Gunes et al., 2022)**.**

The color, flavor and texture of confectionery products are critical factors in consumer acceptance. The texture of soft candies is the main quality characteristic and depends on factors such as moisture and water activity, pH, temperature and processing time, type and content of sugar and gelling agents (Ranken et al., 1997).

Fortunately, water activity in sugar confectionery generally falls below the critical values for microbial growth, with few exceptions (some molds and yeasts in Aw above 0.6). Therefore, the end of shelf life due to microbial growth in confections is generally not an issue. However, the shelf life of hydrocolloid-based candies (gummies and jellies) is primarily related to moisture migration causing the candy to either get too hard (moisture loss) or become sticky (moisture uptake). Thus, changes in textural properties are often the main problem in confections and can be avoided using packaging with good barrier properties (Ergun et al., 2010).

3.3.2 Global market and healthy trends

In recent years, the food consumption habits have undergone changes, with a focus on adopting a healthier lifestyle. For instance, the percentage of Americans who reported following a specific diet or eating pattern increased by 13% in 2022 compared to the previous year (rising from 39% in 2021 to 52% in 2022). The primary motivations for this change remained consistent with those in 2021, with 35% of individuals aiming to protect their long-term health and 34% seeking to lose weight. (IFIC, 2022).

In 2025, the global confectionery market is forecast to have a volume of 15,248.4 million kg, an increase of 5.8% since 2020. The global confectionery market is projected to grow from $194.37 billion in 2021 to $242.53 billion in 2028 (3.8%) (FBI, 2021).

As per the findings of Konar et al. (2022), there is an anticipation that, despite concerns about their high calorie content, the consumption of confectionery products will not experience significant declines. These products are primarily enjoyed for the pleasure they

bring, and, therefore, it remains crucial for them to maintain quality characteristics that meet consumers' expectations, particularly in terms of sensory and textural attributes. Nonetheless, the momentum driven by the growing consumer preference for healthier food options is likely to play a pivotal role in shaping the future landscape of sugar confectionery products.

Trends in the confectionery market in Brazil in 2020 were summarized by Queiroz et al., (2014). The authors emphasize the 5 major: 1) Control and adequacy: reducing the consumption of fats, sugars and sodium, increasing the consumption of fiber and polyunsaturated fatty acids; 2) Nutrition and functionality: increasing consumption of functional foods added probiotics, antioxidants, vitamins, minerals and proteins; 3) Naturalness and authenticity: high demand for products of natural origin and restriction of ingredients with potential risk. Increasing preservatives, colorings and natural sweeteners; 4) Premiumization and experience: use of rare, exotic, artisanal and natural ingredients; 5) Sustainability and transparency: products with an organic seal and environmental self-declaration.

It can be seen that all these aspects are associated with global trends and involve a series of industry adaptations to meet the academic and market research that has been carried out in this segment.

3.3.2.1 Reducing sugar in foods

In 2015, the World Health Organization (WHO) published guidelines suggesting that sugars should not exceed 10% of the population's daily energy intake, and preferably not exceed 5%, both in adults and children. These recommendations were adopted by the US Department of Agriculture (USDA, 2020) who also recommended to avoid food and drinks with added sugars for children under 2 years old.

Sugar-sweetened beverage (SSB) taxation has been recommended as a tool in the package of policy actions to tackle obesity and the non-communicable disease (NCD) crisis (WHO, 2017). As a result of these changes, taxes on SSBs have gained popularity as a fiscal policy implemented by governments. According to real-world assessments, taxes that have been implemented in various jurisdictions across the globe seem to have successfully decreased the purchase and consumption of SSBs (Teng et al., 2019).

In 2018, an agreement was signed between the Brazilian government and the food and beverage industries to gradually reduce sugar in the production of processed products by

144.6 thousand tons over a period of 4 years. The products included in the resolution were separated into 23 food categories, divided into five groups: filled biscuits (reduction of 62%), sweetened beverages (reduction of 33%), dairy products (reduction of 53%), ready-made cakes and cake mixes (reduction of 34% and 46%, respectively) and powdered chocolate powder (reduction of 10%) (ABIA, 2023).

One of the alternatives to raise awareness of the population and encourage the reduction of sugar in industries is the "front-of-package" (FOP), an approach to provide consumers with quick and easy information about the nutritional attributes, ingredients or characteristics of the product, generally through icons, seals, slogans or other graphic representations. Some examples of FOP labeling systems include the "Traffic Light System" in the UK, the "Nutri-Score" in France and other European countries (Zhang et al., 2023).

The substitution of sugar with sweeteners is increasingly becoming the conventional approach to reduce its consumption. Up to now, sweeteners like stevia and xylitol have been widely employed for this purpose. Several studies have indicated that technological advancements can, to some extent, make up for the decrease in sweetness resulting from sugar reduction. These innovations include cross-modal interactions that enhance sweetness with aroma, nanofiltration for filtering disaccharides and higher, enzyme-catalyzed sugar hydrolysis, and microbial fermentation that converts sugar into sugar alcohol (Zhang et al., 2022).

3.4. Healthier and functional sugar confectionery products

Many recent studies propose formulations of sugar-reduced and sugar-substitute blends for being used in sweet confectioneries. Sugar-replacing agents may affect the processability parameters of the product as a consequence of differences in water solubility, and crystallization properties of the sweetener (Gok et al., 2020) but also interaction with the gelling agent, which impacts the structure, hygroscopicity, and flavor release.

Besides this approach, the new trends in the food industry are focused on natural ingredients with specific functionalities and health benefits. Even though candies are typically consumed as treats, they can serve as an effective medium for incorporating functional substances and aiding in the consumption of health-promoting compounds (Moura et al., 2019).

Enrichment of the products to be developed with bioactive components such as probiotics, vitamins, herbal extracts, and polyunsaturated fatty acids that especially support the immune and gastrointestinal systems may increase the satisfaction level of health-conscious consumers. However, in products consumed for pleasure, such as sugar confectionery products, quality characteristics (physico-chemical, texture and sensory) are also of great importance (Konar et al., 2022).

Besides evaluating the substitution of sugar, over the past few years, many studies approached the addition of natural and active ingredients in confectionery products. Table 3.2 presents studies covering foods such as jellies, candies, and gummies which will be discussed below.

Table 3.2 - Use of natural and active ingredients to enrich sugar confectionery products

Food Product	Active ingredient	Application mode	Reference
Jellies	Pomegranate	Juice	Cano-Lamadrid et al., 2020
Soft gummies	Vitamin D	Nanoemulsions	Ahmed et al., 2022
Gummies and bars	Resveratrol	Spray-dried powder	Koga et al., 2016
Commercial Confectionary Hydrogels	Blueberry	Cryoconcentrated juice	Casas-Forero et al., 2020
Hard panned gummies	Uvaia	Dried and milled peels	Avelar et al., 2019

3.4.1 Reduction in calories and sugars

Blending natural bulk sweeteners with high-intensity sweeteners presents a promising industrial solution for maintaining the sensory qualities of chocolates, jams, jellies, or marmalades. Depending on the specific sweetener or combination used, up to 50% of the sucrose content can be replaced without negatively impacting sensory approval. In this context, xylitol and stevia are recognized as effective alternatives to sucrose (Belscak-Cvitanovic et al., 2015; Souza et al., 2022).

Consumer evaluation using the just-about-right technique exhibited satisfactory acceptance of sugar-free jellies produced by Riedel et al (2015). The application of a combination of polydextrose, oligofructose, sucralose and erythritol resulted in a sensory sweetness profile, texture and sensory properties that were comparable to that of a sugar-containing standard product.

Another challenge to this product type is the incorporation of fiber or to development of new fiber-rich products. Aiming that requirement, good stability and texture results has been obtained for a healthy product similar to a fruit confectionery jam. In general, the fiber combination (wheat>psyllium>bamboo>apple) decreased the viscoelastic properties of the gels. However, mixing psyllium with other fibers produced a desirable effect on the mechanical properties of the gels, keeping the syneresis at 0 g/100 g (Figueroa and Genovese, 2018)**.**

Andreone et al. (2022) produced sweet confectioneries with different non-caloric and reduced-calorie sugar blends using erythritol, rebaudioside A and polydextrose (dietary fiber). The confectionery was perceived as very healthy and two systems were selected due to similar physicochemical, rheological and sensory like the full-sucrose options.

3.4.2 Fruit extracts: colorants and antioxidants compounds

Fruit extracts and their byproducts have been added to confectionery products, aiming to replace synthetic dyes, increase antioxidant activity and improve sensory and nutritional attributes. During the assessment of a Brazilian fruit byproduct, "uvaia" (*Eugenia pyriformis*), as a coloring agent in sugar hard-panning confections, a sensory preference was noted for naturally colored confections compared to synthetic caramel color and a natural fruit/plant-based alternative. Additionally, the inclusion of the uvaia byproduct led to a notable increase in the hardness and glass transition temperature of the confection. This change may contribute to improved stability in terms of maintaining the confection's crunchiness (Avelar et al., 2019).

In the context of pomegranate jelly production, fruit juice was utilized to enhance both functional and sensory qualities. The key factors influencing preference were the vivid red color and pronounced brightness. An optimal formulation included 20% gelatin, pure "Mollar de Elche" pomegranate juice, 1% citric acid, and sucrose as the sweetener, resulting in the

highest quality jellies in terms of color, texture, antioxidant capacity, and sensory characteristics (Cano-Lamadrid et al., 2020).

Altinok et al. (2020) evaluated the addition of skin and seed powders of grape in soft candies aiming to include nutrients, bioactive compounds, and fibers. The authors found that particle sizes were the main sensory attribute and strongly influenced the texture, and rheological properties of soft candies. Thus, grape-derived components could be successfully used in the formulation if the particle size is below 200 μm.

In a comparison of the physicochemical properties and stability of betanin in pitaya juice, spray-dried with maltodextrin (MDp) and resistant maltodextrin (RMDp) for use in sugar confections, it was observed that RMDp exhibited superior betanin retention post-processing, measuring at 78.13%, compared to MDp, which registered 69.06%. However, over a storage period of 3 months at 25 °C and 40 °C, the stability of betanin in candies containing RMDp declined below that of candies containing MDp. This indicates that the latter had greater stability, as reported by Shaaruddin et al. in 2017.

3.4.3 Addition of essential oils and natural preservative substances

Essential oils (EO) are complex mixtures of volatile fractions resulting from the secondary metabolism of the plants that present antimicrobial, antiviral, antioxidant and insecticidal properties. The use of EOs in confectionery products has shown a positive effect on the conservation and sensory characteristics (Burt, 2004).

Multicomponent essential oils and their isolated major compounds have been used as ingredients in candies. Kokina et al, 2019, conducted the encapsulation of *Origanum majorana, Achillea millefolium, Foeniculum vulgare, Juniperus communis* and *Anethum graveolens* essential oils (EOs) in alginate capsules as a means of controlling the fast release of volatile constituents. The results of sensorial analysis of candies added with encapsulated OEs showed changes in the taste and the strong herbal odor was found as “uncommon in confectionery but pleasant”. The authors also concluded that encapsulated EOs have to be added as a final step of a recipe to save their antimicrobial and antioxidant potential.

3.5. Encapsulation in gummy candy

Gummy candies (GCs) are confections primarily composed of gelling agents (such as gelatin, starch, gums, and pectin), sweeteners (such as sucrose, glucose, and corn syrups), acids, flavors, and food colorants (Gok et al., 2020). This sugar confectionery product have been used as a novel drug delivery system in the pharmaceutical and food industry, known for their confectionary appearance and taste which make them more appealing to children and some adults. For this reason, gummies came up as an appropriate option to improve the palatability and stability of substances such as pigments and polyphenols (Otálora et al., 2019).

The industry and researchers have put forth the concept of microencapsulating natural bioactive compounds, with the aim of enhancing the stability of these compounds throughout processing and storage. This approach also facilitates the control and sustained release of these natural compounds within food product matrices, thereby extending their bioactivity over an extended period. Recent years have seen the exploration of various advanced techniques for the microencapsulation of bioactive substances, including essential oils, phenolic compounds, flavonoids, flavoring compounds, enzymes, and vitamins (Mehta et al., 2022). In this regard, through bibliographic and bibliometric analysis, the most recent study themes were identified and trends were pointed out.

3.5.1 Bibliometric analysis of the application of encapsulated particles in gummy candies

Bibliometric analysis was applied to better understand the dynamics of scientific developments and outcomes. Many keywords were used only once, indicating that there is not an extremely specific or much-studied line of research in recent years (Figure 3.1).

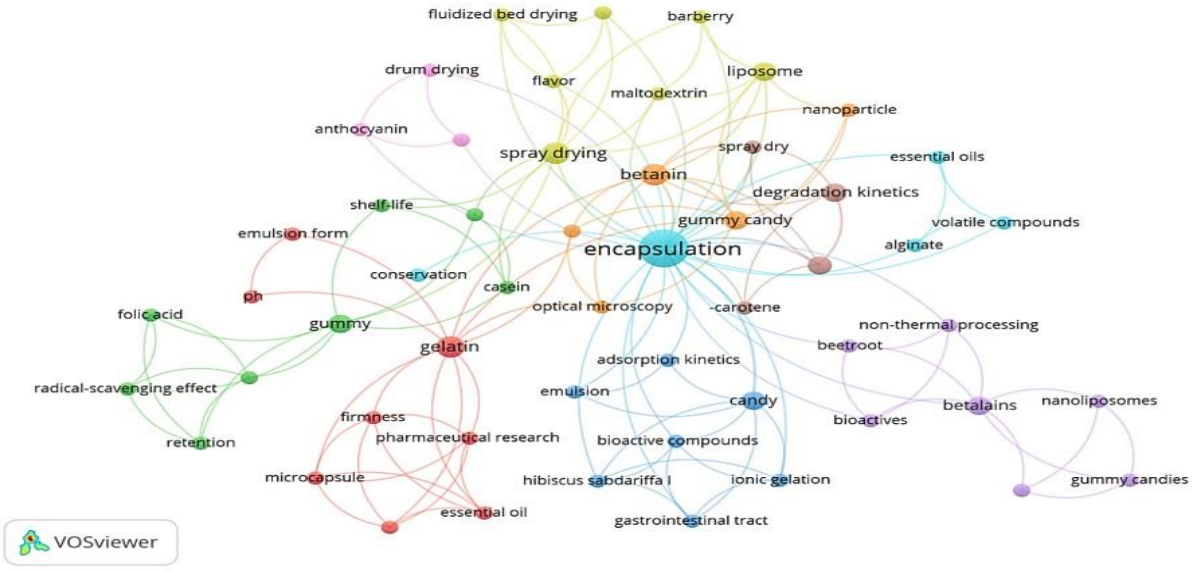

Figure 3.1 - Keyword trend analysis in the application of encapsulated compounds in gummy candy according to data from the Web of Science Core Collection database.

From 48 works found, the 10 most relevant studies about encapsulation for application in gummy were selected and evaluated. It can be seen from Table 3.3 that in most of the papers the encapsulated compounds are mainly polyphenols and natural pigments. Concerning the encapsulation techniques, it was observed that spray-drying, ionic gelation and entrainment in liposomes were prioritized.

Table 3.3 - 10 most relevant publications about the application of encapsulated compounds in gummy candy

Ranking	NC	Active ingredient/function	Encapsulation technique	Reference
1	1	Betacyanins from *Bougainvillea glaba* bracts extract/coloring	Ionic gelation using sodium alginate and inulin	Silva de Azevedo et al., 2021
2	76	Betanin from red beet extract/ coloring	Liposomal nanocarriers	Amjadi et al., 2018
3	30	Betalains of *Opuntia-ficus-indica*/coloring	Ionic gelation with calcium alginate	Otálora et al., 2019
4	-	Phenolic extract of peppermint/ antioxidant and flavoring	Co-crystallized powder	Sarabandi et al., 2022
5	13	Betalains from *Basella rubra L.* fruits/coloring	Lecithin nanoliposomes	Kumar et al., 2020
6	21	Ascorbic acid/ antioxidant	Spray-drying using casein gel	Yan et al., 2021
7	31	Anthocyanins from the hibiscus extract/ antioxidant	Ionic gelation	Moura et al., 2019
8	1	Ground ivy (rosmarinic acid)/ antioxidant	Liposomes	Seremet et al., 2022
9	43	Eggplant peel extract/ coloring and antioxidant	Spray-drying	Sarabandi et al., 2019
10	7	Peppermint/ flavoring	Spray drying, melt extrusion and fluidized bed drying	Kim et al., 2019

NC: number of citations

3.5.1.1 Main encapsulated active ingredients

a) Natural coloring and flavoring

The replacement of artificial colorants with natural pigments that exhibit health benefits is a trend in the food industry for advancing fortified food formulations (Song et al., 2022). Thus, betalains, water-soluble natural pigments, have been used in many studies aiming for a stable product, with improved nutritional value and good sensory acceptance. Within the class of betalains, the betacyanins, are capable to confer red-purple and betaxanthins yellow-orange colors to different foods (Otálora et al., 2019). The stability of betalains limits its use in industrial food processing, for this reason, encapsulation techniques such as spray-drying, ionic gelation and liposomes have been used (Sravan et al., 2020).

Betanin (E162) is the commercial extract from beetroot widely used as a natural colorant in model food systems. This natural pigment was loaded in liposomal nanocarriers (LN) with an encapsulation efficiency of 80.35±1 %. The betanin content and antioxidant activity of gummy candy containing betanin loaded in LN were at least twice those of samples containing free betanin and there was no difference in the sensory parameters of panelists (Amjadi et al., 2018). This pigment was encapsulated in lecithin nanoliposomes (NLs) and incorporated in gummy candies (GuCa) to improve its color stability. The betalain retention, color, texture, antioxidant activity, and shelf-life of the GuCa during storage (5 °C, 28 days) demonstrated its efficacy (Kumar, 2020).

The degradation kinetics of betacyanins were examined by incorporating a capsule formulation containing 20% (w/w) inulin into gummy candies, serving as a food model for a storage period of 35 days. During this time, the betacyanins content in the gummy candies decreased from 3.28 to 0.12 mg/g, with only 3.66% retention. This reduction in betacyanins content was likely influenced by the higher water activity of the gummy candies, which stood at 0.954 (Silva de Azevedo and Noreña, 2021).

The co-crystallized powder containing 10% peppermint extract showed the highest antioxidant capacity in DPPH inhibitory (64.07%), ABTS+ (52.9%), TEAC (1.38 mM) and OH radical scavenging activity (77.65). The gummy candy formulated using 75% powders had the highest score in terms of color, texture, and taste (Sarabandi et al., 2022). In a study conducted by Kim and collaborators (2019), peppermint flavor was employed as a model, and various encapsulation methods (such as spray drying, melt extrusion, and fluidized bed drying) were used to encapsulate this flavor. These encapsulated peppermint flavors were then integrated into a soft, chewable candy. The findings of the study revealed that the overall and dynamic flavor experiences were significantly influenced by the chosen encapsulation technologies and particle sizes. Moreover, it was demonstrated that it is possible to fine-tune these flavor perceptions by combining flavor particles created through different encapsulation techniques, depending on the specific application and desired flavor profile.

b) Probiotics and vitamins

A polymeric encapsulation system for the formulation and storage of *Bifidobacterium adolescentis*, a model anaerobe that loses viability in aerobic incubation at 37°C within 1 day was developed by Qiu et al., 2021. The strain remained viable under aerobic conditions for 14

days at 4°C, enabling formulation development such as solution casting and air drying in an aerobic environment. Next, encapsulation with poly-vinyl alcohol (PVA) was proposed and shown to act as an oxygen barrier and facilitate long-term storage of *B. adolescentis*. Lastly, PVA-based formulations can produce oral capsule-loaded films and edible gummy bears, demonstrating its compatibility with both pharmaceutical and food dosage forms.

The results of storage studies revealed that the microcapsules integrated into the gummy confection retained 92% of vitamin C during accelerated tests conducted over a span of ten weeks. In contrast, the free vitamin C present in the gummy exhibited a lower retention rate of only 79%. Additionally, the microcapsules displayed a slower release profile and provided superior protection for vitamin C within the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). This development of vitamin C microencapsulation holds promise for enhancing the stability of water-soluble vitamins in gummy candies both during processing and storage (Yan et al., 2021).

In a study conducted by Ahmed and colleagues in 2022, oil-in-water (O/W) nanoemulsions were formulated and integrated into edible gummies with the aim of improving bioavailability and stability. The nanoemulsion effectively shielded the active vitamin D from degradation, preserving over 97% of its potency for a span of 15 days, as opposed to 94.5% retention in the oil solution. Furthermore, these soft matrices offered ease of chewing and swallowing, promoting enhanced patient compliance.

c) Phenolic compounds

The inclusion of pure phenolic compounds in foods is constrained by several factors, including their rapid release, low water solubility, limited bioavailability, and susceptibility to degradation under various environmental conditions. These challenges restrict their effectiveness and the potential health benefits they can offer when added to food products (Kaderides and Goula, 2019). Also, polyphenolic compounds form complexes with salivary proteins, playing a role in the sensation of astringency or bitterness (Condelli et al., 2006).

A powdered formulation, encapsulated with eggplant peel extract to serve as a natural source of color and antioxidants, was produced using maltodextrin at a temperature of 170°C. This encapsulated powder demonstrated a high Total Phenolic Content (TPC) of 5.2 mg/g, significant DPPH radical scavenging activity at 73.4%, substantial ABTS radical scavenging at 90.5%, a Trolox Equivalent Antioxidant Capacity (TEAC) of 2.5 mM, substantial hydroxyl

radicals scavenging activity at 79.1%, and reducing power in comparison to other samples. Moreover, the results of sensory evaluation indicated that the addition of this powder in gummy candy showed improved their color and overall acceptability (Sarabandi et al, 2019).

Anthocyanins are a type of polyphenol, non-toxic water-soluble that contribute to food color and present a wide range of biological activities, including antibacterial, anti-inflammatory, anti-diabetic, anti-obesity, and anticancer effects. However, the low stability and non-targeted release of anthocyanins have become the main obstacles in realizing their biological benefits in food systems (Braga et al., 2019). To overcome this challenge, Moura et al. (2019) proposed the microencapsulation of hibiscus anthocyanin by dripping-extrusion and it was observed to improve the enteric protection of bioactive compounds. Application in jelly candy has shown to be technically feasible, with retention of up to 73% of bioactive compounds and mean sensorial acceptance of 70% tasters.

Lamduan (*Melodorum fruticosum Lour*.), a native Thai fruit rich in anthocyanins, was encapsulated and introduced into gummy jellies at concentrations of 10 g/kg, 20 g/kg, or 30 g/kg, replacing artificial colorants. During shelf-life storage of the jellies (conducted at 25°C and 35°C for 8 weeks), there was a gradual decline in anthocyanin levels and antioxidant capacities across all treatments. The rate of degradation of anthocyanins was inversely related to their initial content, and the incorporation of encapsulated lamduan extract into the jellies not only resulted in an appealing red color but also boosted their antioxidant capacity (Sakulnarmrat and Konczak, 2022).

Resveratrol, a polyphenol found in red wine and peanuts, is known for its bitterness and susceptibility to light-induced degradation, which can pose challenges when incorporating it into food products. To address these issues, microencapsulation within a sodium caseinate matrix was employed as a strategy for enhancing stability. This approach was used in the production of gummy candies and bars with encapsulated resveratrol. The encapsulation matrix proved effective in safeguarding the compound, preserving its bioactivity, and ensuring that the overall taste of the bars remained similar to the control sample. However, in the case of gummies, products containing resveratrol microcapsules received a significantly lower overall liking score compared to the control samples with the same protein and/or resveratrol content (Koga et al., 2016).

Liposomes derived from ground ivy, a medicinal plant rich in rosmarinic acid (RA), were explored as encapsulation systems in a recent study. The ground ivy extract exhibited notable levels of total phenolics (1186.20 mg GAE/L) and RA (46.04 mg/L). The researchers

achieved a liposome formulation with high encapsulation efficiency for RA (97.64%), characterized by a double bilayer structure and a polydisperse particle size distribution. In another approach, alginate microparticles fortified with rice proteins demonstrated the highest encapsulation efficiency for RA (78.16%). When liposomes were coated with an alginate-rice protein gel, they facilitated a prolonged and controlled release of RA during simulated gastrointestinal digestion. Similar controlled release was observed when these coated liposomes were integrated into agar-agar candies. (Seremet et al., 2022).

3.6. Conclusions and perspectives

Many studies have been conducted using encapsulation as a delivery system in the gummy food matrix with emphasis on bioactive compounds as polyphenols, natural pigments, essential oils and vitamins. However, it is an area that still can be much explored in order to increase the variety and the load of beneficial compounds, producing candies with nutritional quality, naturalness and convenience, with sensorial acceptance and attractive costs.

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Declaration of interests

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.

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CHAPTER 4

Influence of composition on the internal diffusion mechanism of pectin-starch gel beads

CHAPTER 4 - Influence of composition on the internal diffusion mechanism of pectin-starch gel beads

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ABSTRACT

Pectin is a hydrocolloid commonly used as a wall material for encapsulation, meantime using a filler such as starch can improve the retention in the matrix and control the release in specific places. Formulations of pectin and starch were evaluated for bead production by ionic gelation technique using dripping and jet cutter methods. In addition, beads were dried in a fluidized bed and rotating drum at 50° C. The mean diameter, mechanical strength, yield, moisture content, the morphology of wet and dry beads, and rehydration behavior were evaluated. Furthermore, the pomegranate extract (PE) encapsulation efficiency and release in water were measured. The beads with 2.85% solids content of which 70% are pectin and 30%, waxy corn starch proved to be technologically viable and were satisfactorily dried in a fluidized bed, while on a rotating drum, drying was not efficient. Compared with pure pectin, the pectin-starch beads showed a spherical shape, smoother and more homogeneous with greater mechanical strength and greater water absorption capacity after drying in a fluidized bed. The conventional encapsulation of PE added to biopolymer solutions showed low efficiency (4.7% to 43.7%) and the release velocity was higher for drip beads (~70%).

Keywords: ionic gelation, jet cutter, drying, fluidized bed, rotating drum.

4.1 Introduction

The incorporation of pure phenolics in foods is restricted by many reasons: fast release, low water solubility, low bioavailability, and easy destruction under environmental stresses which limits their activity and their potential health benefits [1]. Pomegranate extract, for example, is a rich source of polyphenols that are associated with the antiradical activity, prevention of degenerative diseases [2], antimutagenic, antifungal, and antimicrobial properties [3]. Among the environmental conditions, the exposure to high pH [4] and increased processing temperature are reported as the main source of damages that decrease the antioxidant capacity of naturally occurring polyphenolics compounds [5,6]. These degrading processes can be avoided through encapsulation techniques such as spray-drying, freeze-drying and ionotropic gelation [7].

Over the past few decades, there has been increasing interest in the use of natural polysaccharides to form structures for controlled delivery applications [8,9] since if properly designed, they afford the protection and release of challenging active compounds such as small molecules, hydrophobic substances and conditions-sensitive compounds like probiotics, vitamins, and polyphenols [10,11]

Sodium alginate, a biomass polysaccharide extracted from brown algae, is the most widely used biomaterial for beads formation by ionotropic gelation. However, Ca-alginate beads are not chemically stable and may disarrange or eventually dissolve after several repeatable usages or certain periods of usage [12–14].

In turn, pectin, a by-product of the citrus industry, similar to alginate, forms gelled beads in the presence of calcium cations and previous studies have shown that Ca-pectinate beads are less sensitive to ions and chemical agents that destabilize Ca-alginate beads [15]. Moreover, pectin-based polymers have tolerance to a wide range of conditions, including pH and temperature [16]. Despite the application of pectin for bioencapsulation purposes to be attractive, the high water content and porosity of these hydrogels beads impair the retention of hydrophilic compounds, lost by the quick diffusion rate driven by the difference of concentration and the high diffusivity [17].

From several strategies that have been proposed in the literature to overcome the low retention of such beads, the insertion of fillers such as starch, inulin, or other polysaccharides proved to improve the retention and additionally mechanical and, morphological and physical properties of beads ranging 2 to 4 mm [12, 18]. However, the size reduction of beads

produced by extrusion dripping is of utmost importance for food applications due to sensory attributes [19]. Moreover, the size reduction facilitates drying processes, which is important to complement the process of obtaining particles, since the high moisture content sometimes leads to an incompatibility with the food matrix and causes limited stability of the beads [20]. In this sense, the Jet Cutter can be interesting because it is a tool able to produce gelled beads about 80.8 % smaller than extrusion dripping and improve the productivity (mass/time) by 87 % [21]. However, when reducing the size of the particle, the effects of adding a filler may not compensate for the reduced mass transfer resistance created by reducing the diameter.

Thus, our objective is to identify the mass transfer ability of pectin-based beads by regarding the retention behavior of a hydrophilic compound as well as the effect of diameter on drying rates, and to investigate strategies that may contribute to improve the retention of the compound. Starch is being used as filler to be a highly convenient feedstock since they are readily available, renewable, inexpensive, and provide great stereochemical diversity [22]. The concentration of filler was varied, and gel characteristics, flow rate and viscosity, as well as bead size, shape and mechanical strength were evaluated. The effect of the presence of filler and size on retention of pomegranate extract was evaluated. Moreover, drying conditions in a fluidized bed and a rotating drum were tested regarding the maintenance of the bead structure and functionality for future food applications.

4.2 Material and methods

4.2.1 Material

Waxy corn starch - WCS (Amisol 4000, amylose 2 %), high amylose corn starch - HACS (Hylon 260, amylose 70 %), resistant starch - RS (Hi-maize VII, amylose 70 %) was kindly donated by Ingredion (Mogi Guaçu, SP, Brazil). Low-methoxy (LM) pectin Genu® 104-AS-Z was obligingly donated by CP Kelco (Limeira, SP, Brasil). Dihydrate calcium chloride ($CaCl_2$ - 147.01 g/mol) was purchased from Dynamics (Indaiatuba, SP, Brazil), and the pomegranate extract was purchased from Heide Vegetable Extracts (Pinhais, PR, Brazil).

4.2.2 Preparation of the biopolymeric solutions and bead production

Different formulations identified by letters were prepared by altering the total solids (2%, 2.85% and 4% w/w) and the pectin:starch ratio, according to Table 4.1.

Table 4.1 – Formulations for bead production

Formulation	Total solids*	Starch proportion (%)	Pectin*	Starch*
P	2.00	0	2.00	0.00
A	2.00	30	1.40	0.60
B	2.00	50	1.00	1.00
C	2.85	30	2.00	0.85
D	4.00	50	2.00	2.00

*gram/100 grams of solution

The solutions of pectin were stirred at room temperature for 12 h for complete dissolution. The starch solutions were pre-heated at 95 °C for 15 minutes for gelatinization and cooled to room temperature. A solution of $CaCl_2$ was prepared in the concentration of 0.015 g/mL. For the production of Ca-pectin beads, the pH of the pectin solution and of $CaCl_2$ solution were pre-adjusted to 3.0.

Apparent viscosity (η) of all solutions was measured at a shear rate of 60 rpm in triplicate using a Brookfield rotational rheometer (Brookfield RVDV-IIIþ, Stoughton, USA) using a cylinder spindle (S15) with an external to internal radius ratio of 0.75. The sample temperature was maintained at 25.0 ±0.5 °C using a water bath. This temperature was the same as that used in the ionic gelation process.

The formulations from Table 4.1 were dropwised by a peristaltic pump (Reglo Analog MS-2/6, Ismatec, Germany) and silicone tube (I.D. 3 mm; wall thickness = 1 mm) to the cross-linking solution ($CaCl_2$) using through to a jacketed double fluid atomizer nozzle (I.D. 1 mm). The distance between the nozzle and the cutting tool was 1 cm, and the distance between the tool and bath was 4 cm [21].

The addition of the pomegranate extract (20 g/100 g of solution) was carried out under gentle stirring at room temperature and kept for 15 minutes.

For the dripping method, the solution was pumped at 6.5 mL/min directly over the cross-linking solution. For the jet cutting method, the solution was pumped at 51.4 mL/min and the rotary cutting tool was placed at 4 cm from the gelling bath. The hardening time of

the beads was fixed at 15 minutes. The crosslinking solution was placed in a rectangular container 96 cm long, 65 cm wide and 33 cm height.

4.2.3 Beads characterization

4.2.3.2 Morphology

The morphology of the beads produced was observed by an optical microscope (Axioscope 5, Carl Zeiss, China).

4.2.3.1 Bead size and size distribution

Due to the size restriction, large and small beads were differently characterized. Larger beads: the morphology of beads produced by dripping were determined using a stereomicroscope (Citoval 2; Carl Zeiss Jena, Oberkochen, Germany) and the mean bead diameter were based on the measurement of at least 100 beads per sample, using the ImageJ software. Smaller beads: the mean diameter was determined by laser diffraction using LV 950-V2 equipment (Horiba, Kyoto, Japan). Wet and dry beads were evaluated by wet way, using water and ethanol as dispersant, respectively. All mean bead sizes were expressed as the volume mean diameter $D_{4,3}$. The polydispersity of smaller beads was given by the span index, which was calculated according to Equation (4.1).

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \quad (4.1)$$

Where: D_{10}, D_{50}, and D_{90} correspond to the diameters relative to 10%, 50%, and 90% of the accumulated size distribution, respectively.

4.2.3.3 Uniaxial compression to fracture

Compression measurements were performed using a universal texturometer TA-XT Plus Texture Analyzer (Stable Micro Systems, UK) at a controlled temperature of 25 °C. The beads were analyzed exerting a compression force on the sample using an aluminum cylindrical probe (diameter 36 mm). Eight larger beads (± 2 mm) were uniformly distributed

in the external perimeter over a flat plate and the mechanical strength was obtained by compression of the beads at 50% of the original height. The measurements were conducted at least in triplicate.

4.2.3.4 Moisture content

The moisture content of the dried beads was determined gravimetrically at 105 ± 5 °C until constant weight following the official AOAC method (No 925.09, 2005).

4.2.4 Analysis of the water removal

4.2.4.1 Drying kinetic curves

Isothermal drying curves (140 °C) for the beads of P and C formulations produced by jet cutting and dripping were constructed. One gram of each bead was weighed into an aluminum pan liner (diameter: 12 cm), in triplicate, and dried until constant weight in a halogen moisture analyzer (MOC63, SHIMADZU, Japan). Data was collected every 10 s from a computer linked to the analyzer. For comparison purposes between samples, the curves were plotted as a function of dimensionless moisture content (X*) calculated following Equation (4.2):

$$X^* = \frac{X}{X_0} \quad (4.2)$$

Where: X is the average water content of the sample (dry basis) at any time, X_0 is the initial sample water content (dry basis).

4.2.4.2 Fluidized bed and rotating drum drying

The drying process was conducted in a fluidized bed (FBD 1.0, LabMaq, Brasil) which has a stainless-steel AISI 304 conical base (spouted base dimensions: d_1 = 0.185 m, d_2 = 0.051 m and height = 0.20 m; fluidized base dimensions: d_1 = 0.186 m, d_2 = 0.095 m and height=0.134 m), with a cylindrical glass column above it (diameter = 0.184 m, height = 0.393 m). A perforated plate was used to ensure adequate air distribution. The operating

conditions were: sample mass of 200 g; air flow rate of 0.8 ± 0.1 m^3/min; inlet air temperature of 60 °C; product temperature of 50 ± 2 °C; and pressure drop of 3 ± 0.22 cmH_20.

The drying process in the rotating drum was studied by adapting equipment (110E, Incal, Brasil) used in the development of confectionery products as chocolate and hard sugar panned candies with the capacity to process up to 5 L of particles per batch. The drum rotation was maintained at 55 rpm with operating air temperature of 50 °C.

4.2.5 Analysis of water absorption: swelling behavior

The beads were immersed in a beaker with 25 mL of deionized water for 24 h. At predetermined times (20, 40, 60, 120, 240, 480 and 1440 min), the beads were removed from the medium and the swelling degree (SD) was measured by the gravimetric method based on the weight of the dry and swollen beads, according to the Equation (4.3):

$$SD\ (\%) = \frac{W_s - W_i}{W_i\ x\ 100} (4.3)$$

Where: W_s is the weight of swollen beads and W_i is the initial weight of dry beads.

4.2.6. Analysis of the release behavior of pomegranate extract

4.2.6.1 Encapsulation efficiency (EE)

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method after that beads were placed in sodium citrate solution (2% w/v). 900 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added to 100 µL aliquot of diluted sample. After 5 minutes, 1000 µL of sodium carbonate solution (7% w/v) and 400 µl of distilled water were added. The mixture was thoroughly mixed and incubated at 25 ± 2 °C in the dark for 90 min and then its absorbance was read at 750 nm. The TPC was obtained from a gallic acid standard curve (r^2 = 0.9984) in the range of 40 to 200 µL/mL concentrations. Absorbance readings (in triplicate) were made in a UV/Visible spectrophotometer (T60, PG Instruments, United Kingdom). Beads without the bioactive compound were considered as a control sample to disregard the effect of interfering substances on quantifications. The EE was calculated as demonstrated in Equation (4.4):

$$EE\ (\%) = \frac{TPC\ in\ beads}{TPC\ in\ polymeric\ solution} x100 \quad (4.4)$$

4.2.6.2 Release measurements

The release of the pomegranate extract (PE) in water was determined by the addition of 5.0 g of drip beads or 30.0 g of jet cutter beads in 100 mL. The samples were placed in a cylindrical support with stainless steel walls and an exchange area of 220 cm^2 porous size 25 m, according to the system developed by Prata et al. (2008). The support was adjusted in a 250 mL beaker and fixed in a Dubnoff bath (Tecnal, Piracicaba, Brazil). The beakers were covered and maintained at slow and constant stirring rate and temperature (25 °C) throughout the experiment. After 5, 10, 15, 20, 40, 60, 120, 180 and 240 min, 1 mL aliquots were removed from the external part of the container, with solvent replacement. The TPC of the samples was determined and release curves were expressed as the percentage of PE released (PEr) in relation to the (PEi) PE initial mass in beads.

4.2.7 Statistical analysis

Analysis of variance (ANOVA) were used to evaluate the data, and differences between the mean values obtained were compared through the Tukey's test with 5 % of significance, using Statistica 7.0 software.

4.3 Results and discussion

4.3.1 Evaluation of the degree of starch substitution in pectin formulations

Beads produced by dripping with different formulations were characterized, as well as, their original solution. The results for the flow rate and apparent viscosity of pectin and starch solutions, and the mean diameter of the beads produced are shown in Table 4.2.

Table 4.2 – Flow rate and apparent viscosity of solutions, and diameter ($D_{4.3}$) of beads obtained by dripping.

Assay	Flow rate (g/min)	Apparent viscosity (cP)	$D_{4.3}$ (µm)
P	3.59 ± 0.07^{bcd}	215.33 ± 2.07^{b}	3473.3 ± 46.5^{ab}
A -WCS	3.79 ± 0.01^{a}	83.27 ± 0.05^{ef}	3360.7 ± 27.4^{abc}
A-HACS	3.64 ± 0.07^{abc}	41.70 ± 0.00^{g}	3338.3 ± 65.2^{abc}
A-RS	3.45 ± 0.02^{d}	41.70 ± 0.00^{g}	3372.2 ± 101.1^{abc}
B-WCS	3.69 ± 0.08^{b}	72.23 ± 3.91^{f}	3332.1 ± 118.6^{abc}
B-HACS	3.67 ± 0.07^{b}	25.00 ± 0.00^{h}	3339.7 ± 96.4^{abc}
B-RS	3.48 ± 0.02^{cd}	22.23 ± 3.91^{h}	3172.7 ± 91.6^{c}
C-WCS	$3.56\pm0.03b^{bcd}$	219.43 ± 3.94^{b}	3313.2 ± 65.8^{bc}
C-HACS	3.67 ± 0.07^{b}	88.90 ± 3.96^{e}	3330.6 ± 56.3^{abc}
C-RS	3.65 ± 0.03^{b}	88.90 ± 3.96^{e}	3424.3 ± 26.4^{abc}
D-WCS	3.67 ± 0.02^{b}	408.37 ± 11.79^{a}	3434.9 ± 114.7^{abc}
D-HACS	3.79 ± 0.02^{a}	147.23 ± 3.91^{c}	3550.0 ± 103.8^{ab}
D-RS	3.78 ± 0.03^{a}	119.47 ± 3.91^{d}	3601.5 ± 27.1^{a}

WCS - Waxy corn starch; HACS - high amylose corn starch; RS – resistant starch

The type of starch influenced the viscosity of the solution and higher values of apparent viscosity were observed for beads with WCS in comparison with the other starches. But in general, the flow rate was independent of the apparent viscosity of the solution and did not have significant variation between samples. Despite the variation of the size of beads, there was not a direct correlation with the flow rate. The standard deviation obtained indicates the polydispersity of the beads. The mean sizes of the beads were approximately 3.4 mm.

Previous work demonstrated a dependence of mechanical strength on the diameter of the beads [21]. The strength force of such beads was plotted as a function of solid content and apparent viscosity (Figure 4.1).

The mechanical strength has shown dependence on the apparent viscosity. With the same proportion of starch and pectin, as the total solids content increases (from 2% to 2.85% of Formulations A and C), the mechanical strength also increases (Figure 4.1 - orange rectangle). This increase was proportional with the increment observed for apparent viscosity for all samples.

When keeping the amount of pectin constant in 2 g in the formulation (Figure 4.1 - green rectangle), the same trend was observed when increasing the total solids from 2.85 to 4% (Formulations C and D), indicating that the viscosity is dependent on the presence of starch in the formulation.

It was noted that the value of apparent viscosity is not directly related to the amount of starch. The increase of 0.85 g (Formulation C) to 2 g (Formulation D) represents an increase of 135%, which is not observed in the final value of apparent viscosity. Instead, the apparent viscosity is related to the type of starch.

Comparing the type of starch in the formulations HACS and RS starches were very similar in terms of mechanical strength. The viscosity of HACS, however, was greater than RS formulations. Also, for WCS formulations, a greater mechanical strength is observed in relation to the other types. The waxy starch forms a more viscous solution due to the occurrence of pre-gelatinization. This phenomenon occurs when starch is heated in an aqueous solution and consists of the irreversible swelling of the granules, in which there is a loss of structural organization and melting of the crystals [22]. Probably, the force developed by WCS formulations is related to the higher viscosity of the gel.

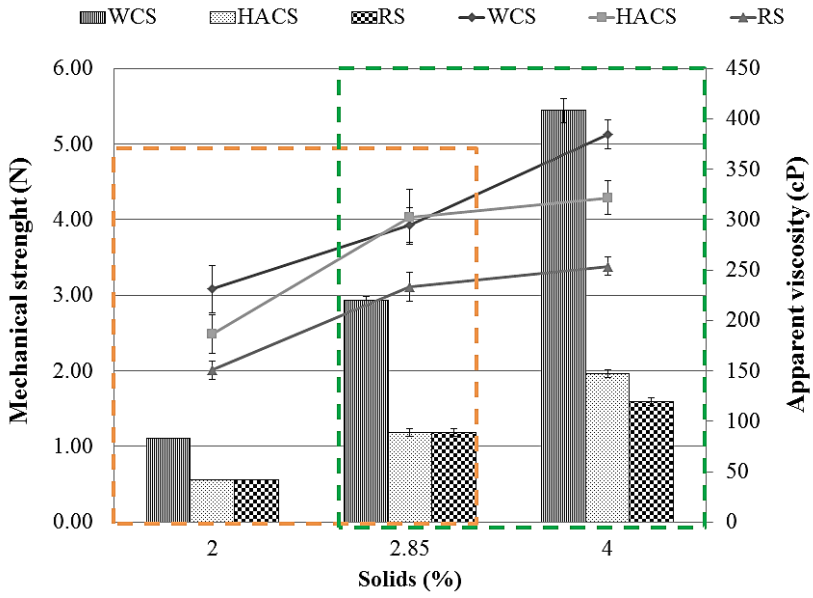

Figure 4.1 - Mechanical strength and apparent viscosity of the pectin/ starch beads with different solids content (A, C and D). WCS - Waxy corn starch; HACS - high amylose corn starch; RS – resistant starch. Lines represent the apparent viscosity and bars the mechanical strength. Orange rectangle indicates formulations with the same pectin:starch ratio and different total solids. Green rectangle, indicates formulations with the same pectin content (2 g).

In the Figure 4.2 formulations containing 2% solids and different amounts of starch (P: 0%, A: 0.3% and B: 0.5%), the increase of starch content resulted in a reduction in apparent viscosity and mechanical strength for all starches studied.

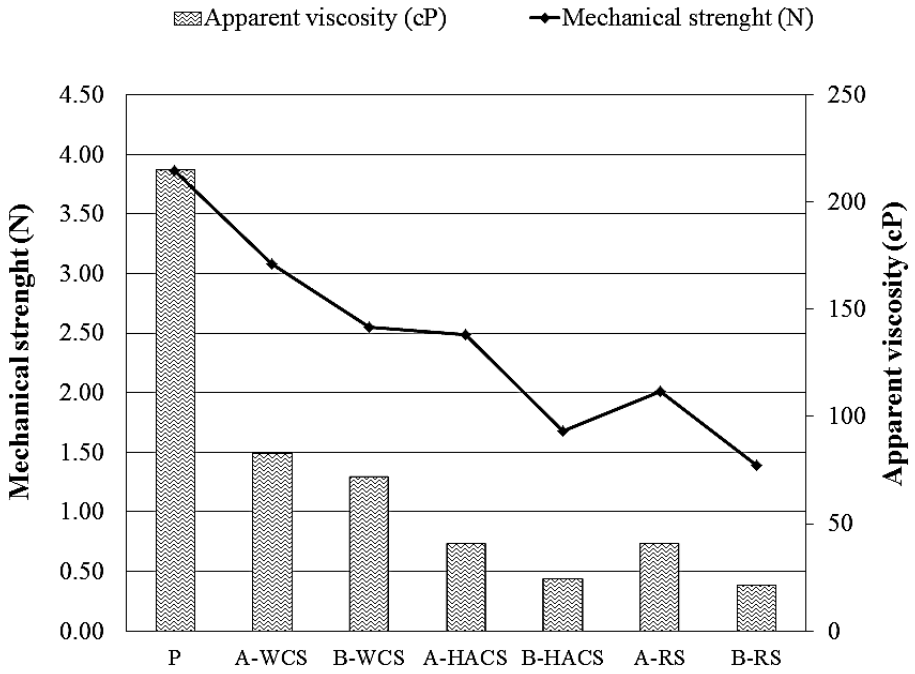

Figure 4.2 - Mechanical strength and apparent viscosity to formulations with 2% solids content (P, A and B). WCS - Waxy corn starch; HACS - high amylose corn starch; RS – resistant starch. Lines represent the apparent viscosity and bars the mechanical strength.

Starch and pectin in a 50/50 ratio (Formulations B and D) did not present technologically satisfactory results. For B formulation, low viscous solutions were obtained and the drop formation was impaired, while for D formulation the high solids content resulted in extremely viscous solutions that were difficult to process due to the flow instability. Thus, to produce particles by jet cutting, formulations A and C were selected, which present the same proportion of pectin and starch (70/30).

4.3.2 Definition of operating conditions and production of beads for the jet cutting technique

The definition of the rotation speed for the jet cutting method was conducted with a formulation containing only pectin (P). The flow rate used for the jet regime was defined in

preliminary tests, considering the force necessary to avoid dragging the disc and passing through the cutting tool.

The jet cutter, adapted to a continuous jet regime, allowed reduction from 5.2 mm to less than 1 mm in Ca-alginate bead size with the increase of the rotation speed from 200 to 900 rpm. However, this increase resulted in higher tangential velocity of the beads, requiring larger collect recipients [21]. In the present study, a larger container was employed, allowing to use speeds up to 2400 rpm.

In general, maintaining the jet regime at a flow rate of 54 mL/min, the bead diameter reduces when increasing the rotation speed. However, above 1500 rpm this reduction is not significant (Table 4.3). All samples showed a high polydispersity index, and the lowest polydispersity was obtained for the speed of 1500 rpm. The mass of collected particles decreased quantitatively due to the increase in the projection angle at which the particles are thrown with increased speed. Therefore, in the subsequent tests, a speed of 1500 rpm was used.

Table 4.3 - Mass yield, mean diameter D_{50}, $D_{4.3}$ and polydispersity index (Span) for beads obtained by jet cutting method

Rotation speed (rpm)	**$D_{4.3}$ (µm)**	**D_{50} (µm)**	**Span**	**Mass yield***
900	1282.1±40.6^{a}	1190.1±37.8^{a}	1.35±0.07^{bc}	120.7±1.1^{a}
1200	1047.8±160.3^{a}	991.4+128.4^{a}	1.42±0.16^{b}	114.9±2.7^{a}
1500	662.1±16.2^{b}	682.8±17.2^{b}	1.12±0.04^{c}	89.2±5.1^{b}
1800	621.8±37.8^{b}	598.6±48.7^{b}	1.47±0.10^{b}	68.2±1.3^{c}
2100	608.3±55.4^{b}	531.0±55.6^{b}	1.94±0.18^{a}	52.3±4.2^{d}
2400	666.2±28.8^{b}	581.3±31.2^{b}	1.95±0.17^{a}	46.1±4.3^{d}

*g of drained beads/100 g of pectin solution

The moisture of the beads in formulation A did not show a significant difference ($P < 0.05$) in relation to the treatment containing pure pectin (P) (Table 4.4). However, as expected, with higher solids content (C) the moisture was lower. In formulation A, the mean diameter was greater than that of the particles containing pure pectin (P) for all types of starch (Table 4.4). The bead sizes were similar for HACS and RS (Formulation C) while for WCS

the beads were significantly larger. As the purpose of using the jet cutter is to reduce size, it is observed that in the Formulation C, this reduction was more effective.

Table 4.4 - Moisture, mean diameter D_{50}, $D_{4.3}$ and polydispersity index (Span) for beads obtained by jet cutting method

Assay	Moisture (%)	$D_{4.3}$ (µm)	D_{50}(µm)	Span
P	96.26±0.05^{bc}	705.62±31.58^{bc}	715.38±29.09^{bc}	1.20±0.04^{ab}
A-WCS	96.31±0.07^{a}	1004.54±36.29^{a}	957.97±28.66^{a}	1.41±0.06^{ab}
A-HACS	96.11±0.08^{abc}	847.90±71.03^{abc}	812.00±49.83^{ab}	1.14±0.15^{b}
A-RS	96.25±0.05^{ab}	885.14±48.18^{ab}	840.90±34.68^{ab}	1.21±0.10^{ab}
C-WCS	95.80±0.05^{c}	692.04±64.13^{c}	632.01±60.85^{c}	1.73±0.30^{a}
C-HACS	95.74±0.06^{bc}	729.33±35.91^{bc}	721.39±61.60^{bc}	1.03±0.12^{b}
C-RS	95.52±0.05^{a}	973.57±61.38^{a}	910.01±50.28^{a}	1.13±0.10^{b}

WCS - Waxy corn starch; HACS - high amylose corn starch; RS – resistant starch

The Figure 4.3 shows that all samples had a spherical shape with irregularities in the surface structure. The micrographs of the beads containing HACS and RS show the presence of starch granules. This result was expected, since during processing it was possible to observe that these starches do not gelatinize at temperatures up to 95 °C. Because of their linearity, amylose molecules align more easily and have more extensive hydrogen bonds. Consequently, more energy is needed to break these bonds and gelatinize the starch (>T gelatinization) [23].

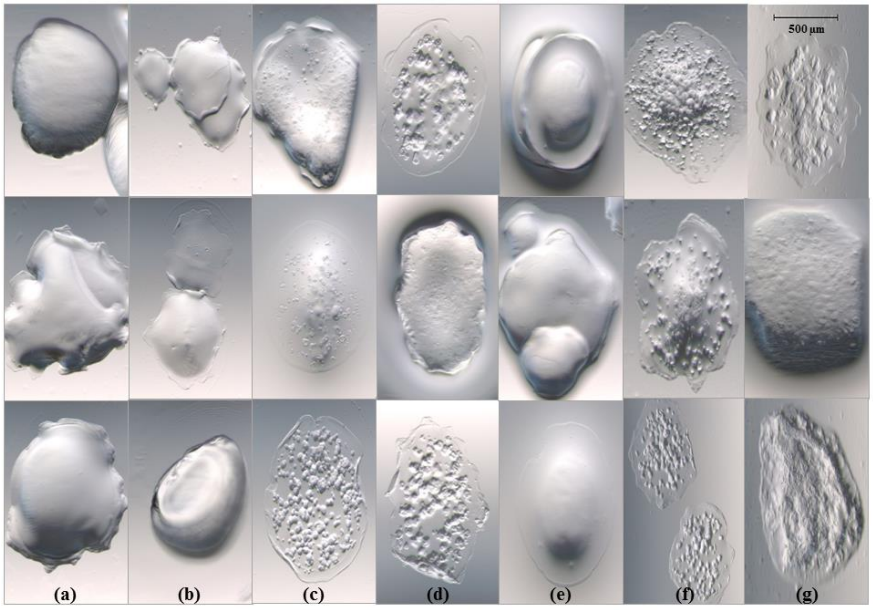

Figure 4.3 – Morphology of beads (10 x magnification). (a) Formulation P, (b) Formulation A with WCS, (c) Formulation A with HACS, (d) Formulation A with RS, (e) Formulation C with WCS, (f) Formulation C with HACS, (g) Formulation C with RS.

4.3.3 Analysis of the release behavior

The concentration of the pomegranate extract in the polymeric matrix was defined taking into consideration the maximum proportion that could carry a significant amount of active and enable the dripping. Above 20% of extract, there was an increase in the viscosity of the solution due to the interaction of pectin with the alcohol present in the pomegranate extract, so technologically it was the most viable concentration. The flow rate was similar to both formulations, however the apparent viscosity of solutions containing gelatinized starch was higher (Table 4.5).

Table 4.5 - Flow rate and apparent viscosity of solutions with 20% of extract, bead diameter ($D_{4.3}$) and mechanical strength of beads obtained by dripping

Formulation	Flow rate (g/min)	Apparent viscosity (cP)	$D_{4.3}$ (µm)	Mechanical strength (N)
P	3.77 ± 0.02^{a}	149.97 ± 13.59^{b}	2819.8 ± 31.9^{a}	4.11 ± 0.17^{a}
C	3.75 ± 0.04^{a}	450.03 ± 31.18^{a}	2921.2 ± 53.1^{a}	4.33 ± 0.01^{a}

P: pectin 2% and C: pectin 2.0% and WCS 0.85%

There was a loss of phenolic compounds in the calcium chloride solution, probably due to its hydrophilicity and matrix porosity. The EE was higher for dripping beads because the bead size reduction favored mass transfer, preventing the compound from remaining retained in the matrix (Table 4.6). WCS as filler in association with pectin, favored mechanical strength and morphology, however no significant difference was observed in the retention of phenolic compounds.

Table 4.6 - Total phenolic content (TPC) and encapsulation efficiency (EE) of the beads with pomegranate extract

Method	Formulation	TPC*	EE (%)
Dripping	C	408.08±12.54[a]	43.44±0.94[a]
	P	376.40± 23.80[a]	41.25±1.95[a]
Jet cutting	C	42.42± 0.79[b]	4.90± 0.39[b]
	P	48.73± 1.64[b]	4.68± 0.12[b]

*mg of gallic acid equivalent/100g sample. P: pectin 2% and C: pectin 2.0% and WCS 0.85%

The release measurements showed that there was no significant difference between the formulations. However, the bead size influenced the diffusion path and the release velocity (Figure 4.4). For drip beads, there was an increase from 40% to 65%-70% of release from 5 to 40 minutes and after this period the release rate remained constant. In the case of jet cutter beads, the proportion of extract released was lower and remained constant since the beginning of the test, with values between 20% and 30%. Similar values were found for polylactic-co-glycolic particles added of pectin and alginate. EE of tilmicosin ranged from 22%–57% and release rate showed agreement with the increased matrix porosity and swelling behavior of biopolymers [24].

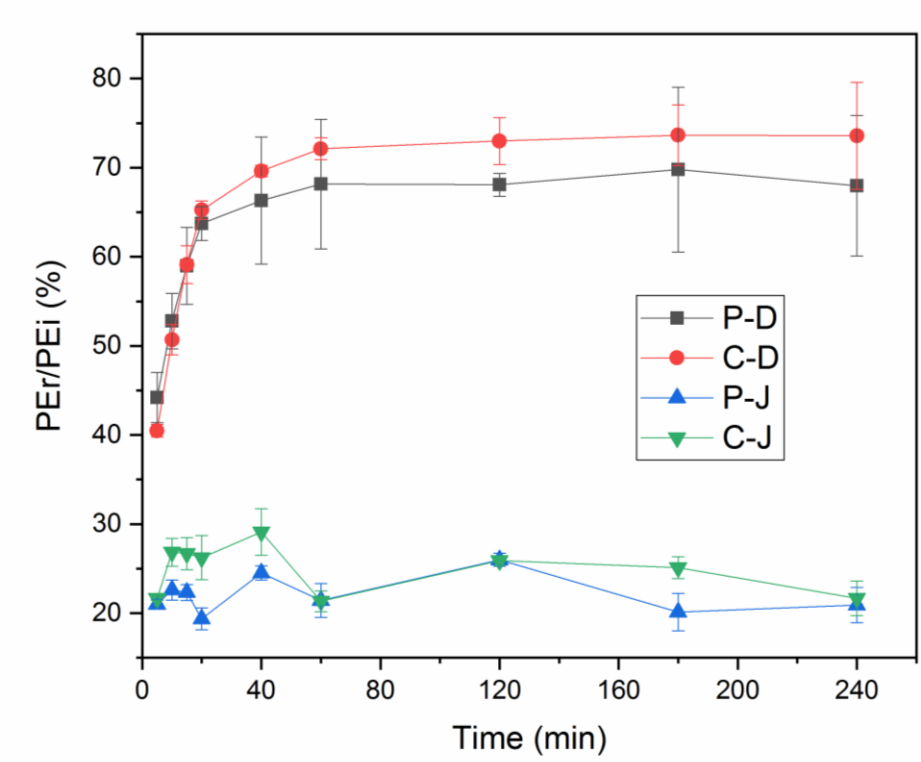

Figure 4.4 - Release behavior of the pomegranate extract pectin–starch gel beads. The legends present the letter that represents formulation (P: pectin 2% and C: pectin 2.0% and WCS 0.85%), followed by the method of production of the beads (J: jet cutter and D: dripping).

When effects of changes in the geometry of wet beads can be neglected, such as those caused by erosion or swelling, the release of TPC from the beads may be described using the Fick's second law (Equation 4.5), that, in spherical polar coordinates renders as:

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\left[\frac{\partial}{\partial r}\left(r^2 D_k \frac{\partial C}{\partial r}\right) + \frac{1}{sin\theta}\frac{\partial}{\partial \theta}\left(sin\theta D_k \frac{\partial C}{\partial \theta}\right) + \frac{D_k}{sin^2\theta}\frac{\partial^2 C}{\partial \phi^2}\right] \quad (4.5)$$

Where: D_k is the apparent diffusion coefficient [m²/s], C is the concentration [kg/m^3], r, θ and ϕ are spherical polar coordinates, and t represents time [s].

The release driven by Fickian diffusion allows the calculation of diffusivity, a parameter dependent on the affinity between matrix and diffusing species. The molecular diffusion occurring inside the pores must consider the comparative resistance of the pores diameter and the diameter of the diffusing molecule (apparent diffusion coefficient). Equation

(4.5) represents the changing of the phenolic concentration over time and position, and, since it normally occurs radially it is a function of the bead diameter.

During the gel network formation, diffusion is facilitated by the opening of the gel matrix not completely gelled (higher D_k). But after to be formed, the shortest diffusive path in the particles produced by the jet cutter determines the lower retention (Table 4.6) inside these beads.

With higher retention due to the larger diameter, and since the difference in concentration is the driving force of mass transfer, the amount released over time from the particle produced by dripping is greater despite the diffusion of the pomegranate extract happens more slowly (Figure 4.4).

To improve the retention of the compounds, it might be suggested to reduce the gradient concentration through increasing the extract concentration in the external environment, to reduce the diameter of external pores by adding a coating material, or to reduce losses during production, by using the adsorption technique (inverse gradient of concentration) to entrap the active compounds into the beads.

4.3.4 Water diffusion in beads

Depending on the food product, the use of dry gelled beads may be more viable than wet beads. In addition, the drying of beads containing phenolic compounds at mild temperatures can increase the load of substances of interest by reducing the moisture content. Considering the low retention of phenolic compounds in wet beads, a study was carried out on the behavior of the matrix during drying, as well as its rehydration behavior.

4.3.4.1 Analyses of drying curves

The isothermal drying rate reflects the rate of water diffusion during drying, thus providing a parameter for evaluating the matrix. Materials which quickly form a thin and dense matrix during drying create a barrier against oxygen transfer, thus assisting in the retention and protection of volatile substances. Therefore, the ideal curve would show a rapid decrease in drying rate with a decrease in moisture content [25]. Considering the particles produced by jet cutting, it was observed that 60% of the initial water content was lost in approximately 3.4 and 4.2 minutes for the particles with (C) and without (P) starch,

respectively (Figure 4.5). For the larger beads, produced by dripping, this time was higher, 5.9 (C) and 5.1 minutes (P). These results suggest that particle size reduction facilitates water diffusion along the particle. It can be observed that moisture ratio (X*) decreases continuously with drying time and no constant drying rate period exists, indicating that beads drying can be considered to occur in the falling rate period, during which internal molecular diffusion is the predominant mass transfer mechanism [26].

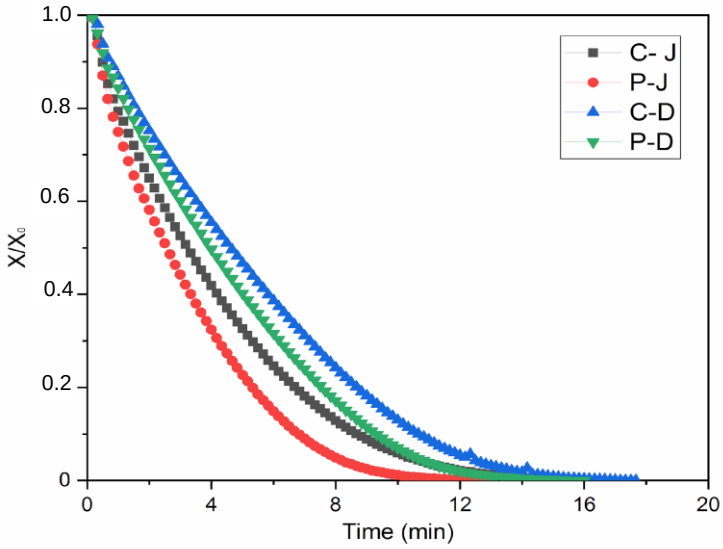

Figure 4.5 - Experimental drying curves of beads. The legends present the letter that represents formulation (P: pectin 2 % and C: pectin 2.0 % and WCS 0.85 %), followed by the method of production of the beads (J: jet cutter and D: dripping).

Although the smaller size beads containing only pectin (P) have shown higher initial moisture, the drying time was shorter due to the higher water removal flow. However, after removing water from the outermost layers, the rate was similar for both formulations (Figure 4.6-a). Higher drying rates for the jet cutting beads can be associated with the increase in the surface area that contributes to the smaller diffusion path of the water.

The beads produced by dripping showed a lower drying rate and there was an influence of the composition with the highest removal flux in the beads without starch (P) throughout the entire drying period (Figure 4.6-b). This difference may be related to the properties of the dry beads, resulting in a more rigid external layer and with deformations that make the rehydration process difficult for the application of pectin beads in food.

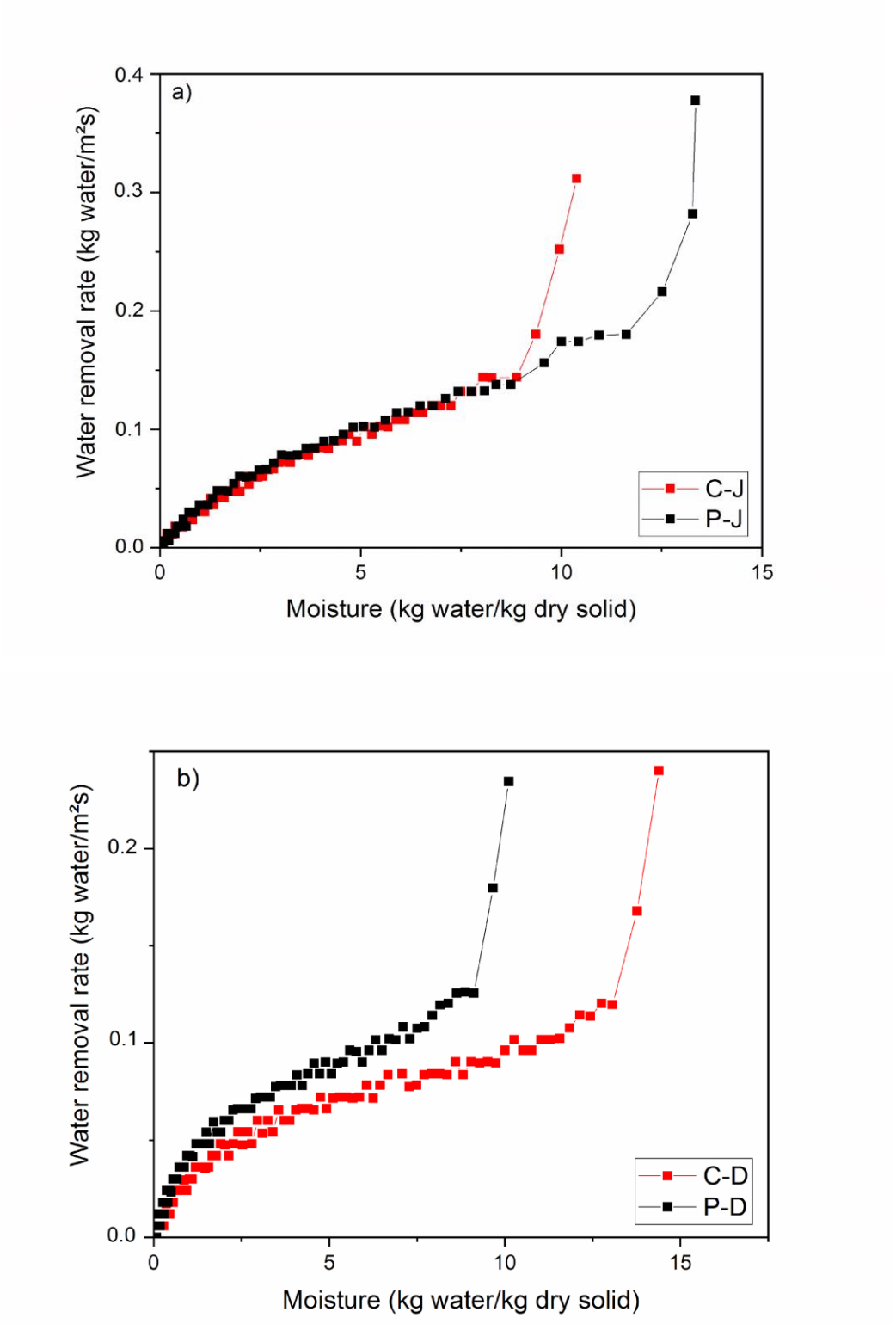

Figure 4.6 – Water removal rate versus moisture for the jet cutting (a) and dripping beads (b). The legends present the letter that represents formulation (P: pectin 2% and C: pectin 2.0% and WCS 0.85%), followed by the method of production of the beads (J: jet cutter and D: dripping).

The application of gelled beads can be limited by high moisture content, for incompatibility with the food matrix. Thus, the use of drying can be important to complement the process of obtaining particles, promoting greater stability. Aiming to reduce the water content of gelled beads, some authors performed drying with a convection oven [27,28] or in a freeze-dryer [29]. For such dryers, the drying rate is very low which can be associated with the preservation of beads structure. However, the increase in the water removal rate is essential for industrial viability. Thus, techniques using relative movement between air-particle phases, for example, with the suspension of beads in an airstream (fluidized bed) or with the mechanical movement of the particles (rotating drum) were explored with this aim.

4.3.4.2 Influence of convection in the drying behavior

a) Fluidized bed drying (upward movement)

In general, the fluidized bed drying shows high thermal inertia of solids, a good degree of mixing, a short time and high drying rate, due to the high heat and mass transfer coefficients and the large exchange area between the particles and gas [30]. Therefore, it is possible to use gentle drying conditions as well as reduced processing times in comparison to ovens. Moreover, fluidized bed dryers are very efficient in terms of energy and maintaining the quality of dried products, without damaging heat sensitive materials [31].

The drying time varied with the particle mass and bed height. For the movement of particles in the bed, it was necessary to reduce the initial moisture. Therefore, the particles were exposed to room temperature for 8 days. During this period, there was a reduction in humidity from 95.9% to 75.33% for the beads produced by jet cutting and from 95.7 to 91.39% for the produced by dripping.

Bead size decreased after drying, due to shrinkage promoted by water evaporation. For samples produced by dripping there was a reduction of about 55% in the size of the beads, while for those produced by jet cutting the reduction was 85%. The samples showed high polydispersity and the jet cutting beads were the most polydisperse. Theremore, the final moisture of the smaller particles was also lower compared to those produced by dripping (Table 4.7).

Table 4.7 - Moisture, mean diameter D_{50}, $D_{4.3}$ and polydispersity index (Span) for beads obtained by dripping and jet cutting method and dried by fluidized bed

Method	Assay	Moisture (%)	$D_{4.3}$ (µm)	D_{50} (µm)	Span
Dripping	P	19.38±0.17	1459.92±21.85	1360.42±9.96	0.78±0.05
	C	16.32±0.17	1581.30±23.65	1509.11±18.02	0.67±0.03
Jet cutting	P	14.02±0.16	608.49±6.81	571.43±4.70	1.01±0.01
	C	17.13±0.12	376.00±7.56	335.52±5.45	1.38±0.04

P: pectin 2.0 % and C: pectin 2.0 % and WCS 0.85 %

In general, the dried beads showed rough surface, with some cracks observed especially in the pectin (P) ones (Figure 4.7). Similar results were obtained by Sampaio et al. (2019) [32] that used alginate and pectin for encapsulation of a lycopene-rich watermelon concentrate by ionic gelation with drying in vacuum oven drying [32]. Belščak-Cvitanovic et al. (2016) reported that the addition of whey proteins or hydroxypropyl methylcellulose (HPMC) in the formulation of alginate and pectin hydrogels improved the surface appearance and particles morphology of freeze-dried beads. According to the authors, these polymers, when added, are embedded in the gel matrix, acting as “structural supports” and thus controlling the beads shrinking and fractures after drying. As seen in Figure 4.7, the surface of the particles containing starch (C) appears smoother, more uniform and spherical, which corroborates with the findings of the authors [6].

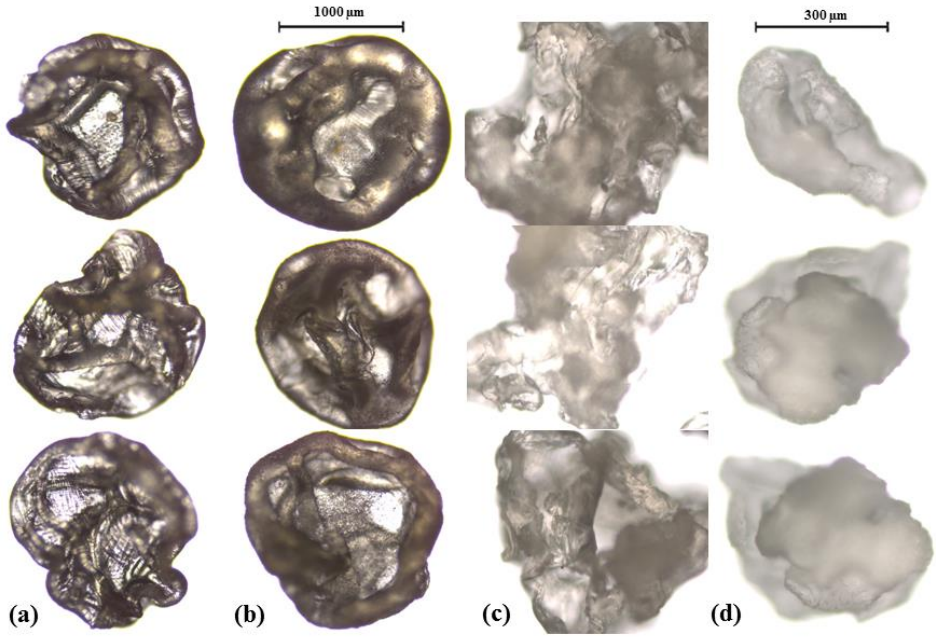

Figure 4.7 – Morphology of beads dried in a fluidized bed. Formulation P (a) and C (b) produced by dripping (4 x magnification). Formulation P (c) and C (d) produced by a jet cutter (10 x magnification).

a.1) Water absorption behavior of dried particles

Swelling degree (SD) of dry pectin gel beads (P) occurred faster than beads containing starch, however after 8 and 24 h the SD was approximately 2 times smaller (Figure 4.8).

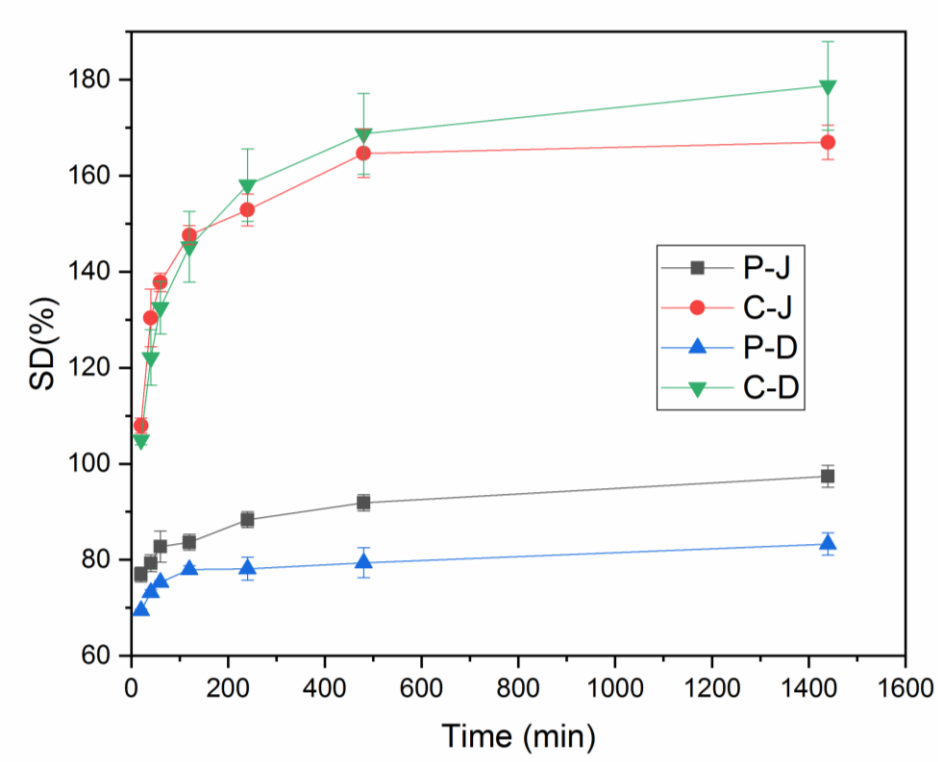

Figure 4.8 - Swelling behavior of the pectin–starch gel beads. The legends present the letter that represents formulation (P: pectin 2% and C: pectin 2.0% and WCS 0.85%), followed by the method of production of the beads (J: jet cutter and D: dripping).

Swelling is a significant factor in the stability and adsorption properties of hydrogels. The water absorption is attributed to the presence of a large number of hydrophilic COOH groups on the polymer chains of pectin, while the resistance to dissolution in the surrounding medium owes to cross-linking between the polymer chains [36]. To low-methyl esterified pectins (degree of methyl esterification < 50%), the gels are formed in the presence of divalent cations in a two-step process, and the dimers from "egg box" structure occurs in a molar ratio of 0.25 Ca2+/free galacturonic acid [37]. Then, in an excess of calcium, the crosslinking density of the matrix will be the same and cannot influence the release of the active compound. However, the properties of pectin gel crosslinked with Ca2+ ions can be controlled by pH, other monovalent counterions, and temperature [35].

There was no visible change in the morphology and the physical integrity of the rehydrated beads was maintained compared to dry beads. The images confirm that the starch-containing particles were crack-free and more spherical (Figure 4.9). Similarly, carboxymethyl sago pulp (CMSP)(15%)/pectin (5%) hydrogel beads synthesized by

calcium crosslinking was able to stay intact for 24 h in swelling medium at pH 7.4 [33], and apple pectin beads swelled more at pH 7.4 and 8.0 than at pH 5.0 [35].

Popov et al. (2022) verified that pectin beads collapsed in phosphate-buffered saline (PBS) at pH 3.0, which may be associated with the protonation of carboxyl groups or leaching of calcium ions by displacement of sodium, potassium, and phosphate ions from PBS. The crosslinking of ionized carboxyl groups of pectin chains with calcium ions at pH values higher than pKa values increases gel stability, whereas electrostatic repulsion of pectin chains promotes swelling [35].

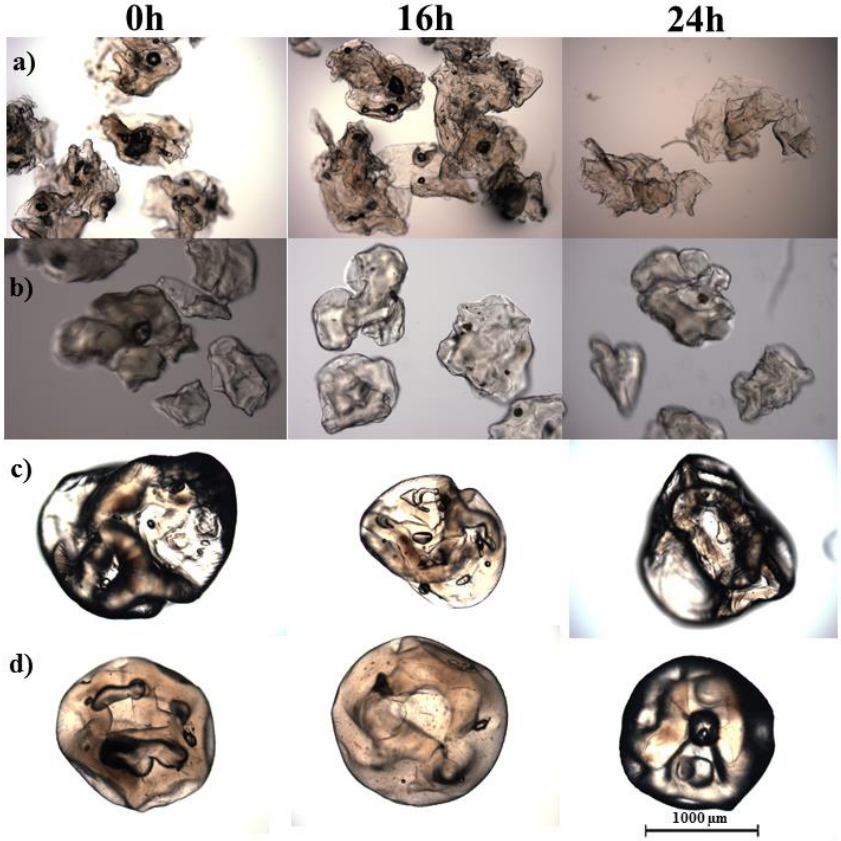

Figure 4.9 – Morphology of dried (0h) and rehydrated beads (16 and 24 h). Formulation P (a) and C (b) produced by a jet cutter (4 x magnification). Formulation P (c) and C (d) produced by dripping (4 x magnification).

The mechanical strength of dry starch-containing beads was 11.7 times greater than that of wet beads, while pure pectin beads showed an increase of 4.7 times (Table 4.8). As expected, during the rehydration process there was a reduction in mechanical strength due to the increase in water content.

Table 4.8 - Mechanical strength (N) of beads obtained by dripping during the rehydration

Formulation	Time (h)		
	0	**16**	**24**
P	18.25 ± 0.88^{Ab}	3.97 ± 0.23^{Bb}	3.24 ± 0.30^{Bb}
C	47.36 ± 1.63^{Aa}	7.54 ± 0.54^{Ba}	7.42 ± 0.75^{Ba}

Capital letters indicates comparison between lines and lowercase letters between columns. P: pectin 2% and C: pectin 2.0% and WCS 0.85%.

Despite the higher mechanical strength for the beads containing starch, the higher water absorption resulted in a decrease of 84% from the initial value. Considering all the rehydration parameters, the use of starch seems interesting because it favors the beads resistance, maintains the morphology and has a greater water absorption capacity.

b) Rotating drum drying (rotative movement)

Rotating drum is an alternative often available in the food industry for the production of dredging. One advantage of this equipment is their ability to handle varied feedstock, i.e. granular material having a wide distribution of size, density, shape, roughness or else [34].

The selected formulation from the previous tests (C-WCS) was dried by the rotating drum technique. There were operational difficulties and it was not effective in reducing particle moisture (Table 4.9). As well as in fluidized bed drying, the size reduction in particles produced by dripping was lower (45.5%) than those produced by jet cutting (84.0%). The polydispersity index was higher for particles produced by jet cutter as the size distribution was not uniform.

Table 4.9 - Drying time, moisture, mean diameter D_{50}, $D_{4.3}$ and polydispersity index (Span) for beads dried in rotating drum

Method	Drying time (min)	Moisture (%)	$D_{4.3}$ (µm)	D_{50} (µm)	Span
Dripping	120	71.42±0.11	1806.09±13.50	1884.08±14.76	0.64±0.01
Jet cutting	80	33.65±0.26	529.99±6.77	562.81±8.46	1.18±0.01

Beads produced by dripping showed spherical shape with some hollows commonly seen in the dry bead structure. There was a loss of the spherical structure of beads produced by the jet cutter. It is believed that the friction caused by the rotation of the equipment and the contact of the particles with the wall promoted their fracture and agglomeration, according to Figure 4.10.

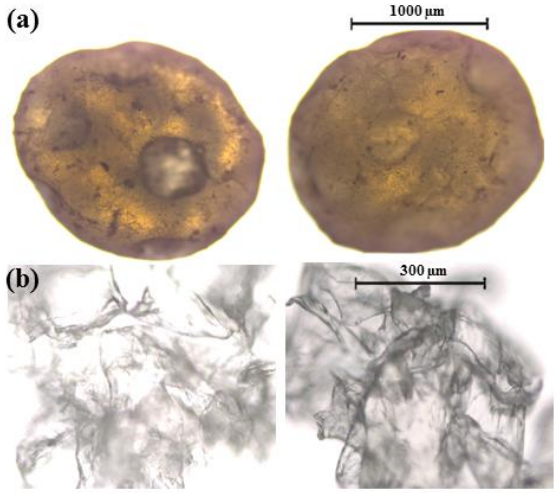

Figure 4.10 – Microscopy of beads dried in rotating drum. (a) - C beads produced by dripping (4 x magnification); (b) – C beads produced by jet cutter (10 x magnification)

The drying of gel particles containing bioactive compounds can increase the load of substances of interest by reducing the moisture content and seems to be interesting for the encapsulation by ionic gelation proposed in this work. Among the techniques evaluated for this purpose, the fluidized bed drying proved to be efficient from an operational point of view and resulted in structurally better particles than rotating drum drying.

4.4. Conclusion

Pectin and starch were evaluated as wall materials for bead production by ionic gelation technique using dripping and jet cutting method. The mechanical strength of beads was influenced by the type and concentration of starch, being the highest values obtained for the formulation D with WCS (5.13±0.19 N) and the lowest values for the formulation B with RS (1,39±0.09 N). These results are strongly associated with the apparent viscosity of the biopolymeric solutions. The use of a jet cutter could reduce the pectin bead sizes from 3.6 mm

(dripping method) to about 0.67 mm at the chosen rotational speed (1500 rpm). The particles with 2.85% solids content containing pectin (70%) and waxy corn starch (30%) (Formulation C-WCS) proved to be technologically viable and were satisfactorily dried in a fluidized bed. For this formulation the spherical structure was maintained and EE was higher than pure pectin. Even so EE values were low for both formulations produced by jet cutting, which makes it necessary to use techniques to improve retention and fast release, such as adsorption and coating. This study allowed a solid understanding of how the composition affects the bead formation, providing guidance for establishing appropriate operational conditions and combinations of the wall material for the insertion of active compounds and subsequent application in food.

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CHAPTER 5

Encapsulation of pomegranate polyphenols by ionic gelation: strategies for improved retention and controlled release

CHAPTER 5 - Encapsulation of pomegranate polyphenols by ionic gelation: strategies for improved retention and controlled release

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ABSTRACT

This study aimed at producing pectin hydrogel beads by ionic gelation process to carry pomegranate extract (PE) evaluating approaches to increase its retention and protect the polyphenols from environmental conditions that interfere in the stability and color of these compounds, such as the pH of the medium. Several strategies were tested to reduce the mass transfer and consequently increase its retention. The insertion of a filler (gelatinized starch), the employment of different concentrations from the external environment, the adsorption using blank pectin-starch beads, and the electrostatic coating using chitosan were performed. The release of entrapped compounds over time was employed to evaluate the release pattern of PE in water media. Diffusion coefficients calculated from these experiments were then used to estimate the PE release behavior. The encapsulation efficiency (EE) was significantly improved (42 % to 101 %) when equalizing the concentration of the external medium with that from the beads formulation. Furthermore, the increase in the PE concentration was proportional to the rise in the mechanical strength (MS) of the beads which indicates a modification of internal structure due to the presence of polyphenols. The adsorption was efficient in entrapping the active compound, and despite the high PE content observed for all beads (average value of 2960.26 mg of gallic acid equivalent/100 g sample), they had the lowest diffusion coefficient from the release in water media. Finally, the coating was able to reduce the release rate in most of the tests (D_{AB} uncoated = 0.5 D_{AB} coated), however, during

the electrostatic deposition a loss of about 32 % of the phenolic compounds in the chitosan solution was observed which led to a reduced EE. Despite the obtention of retarded release, coating studies need to be improved. Some adjustments in the execution of this technique are necessary so that the losses are reduced and the process becomes viable for the use of beads in food.

Keywords: ionic gelation; release behavior; diffusion coefficient; chitosan.

5.1 Introduction

Pomegranate extract (PE) is a rich source of polyphenols, including tannins (gallic acid, ellagic acid, punicalagin A&B, and punicalin A&B) and flavonoids (anthocyanins, catechins, and other complexed flavonoids) (Saparbekova et al., 2023; Smaoui et al., 2019) that are associated with antiradical activity, prevention of degenerative diseases, antimutagenic, antifungal, and antimicrobial properties (Kandylis & Kokkinomagoulos, 2020). Therefore, the application of PE as a food ingredient has a nutraceutical potential, but its effectiveness depends on preserving its integrity and appropriate concentration.

Since some naturally occurring polyphenolic compounds are damaged when exposed to high pH and the antioxidant capacity decreases with increasing processing temperature (Réblová, 2012; Rahman et al., 2021), the utilization of encapsulated polyphenols instead of free compounds can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound in vivo and in vitro (Fang & Bhandari, 2011).

Encapsulation by ionic gelation produces hydrogel beads, which is advantageous in terms of biocompatibility and structural similarity to the highly hydrated structure of the human body and because of their tunable properties for controlling the release profile of the entrapped molecules (Zhao et al., 2014b; Skicki, 2021). On the other hand, this hydrophilic property impairs the encapsulation of compounds that tend to leak into an aqueous media. Its network formation by ionic crosslinking results in an open lattice structure with an average pore diameter that provides low transport resistance for solutes with molecular weight (MW) lower than 44 kDa (Li, Altreuter & Gentile, 1996). Then, molecules that have high water affinity and low MW tend to freely diffuse to the surrounding media. Several works in the literature have confirmed this behavior. The PE-loaded beads produced by dripping had

41.25% retention (Silveira et al., 2023), comparable to alginate beads containing other hydrophilic compounds, whose encapsulation efficiency was around 20-40% (Essifi et al., 2021; Sohail et al., 2012).

Recently, our research group evaluated the effect of bead size on the retention of PE in pectin beads. Considering Fickian diffusion, the smaller the particle the faster the rate of release, due to the dependence of diffusion time on the diffusion length. The reduction from 2.8 mm to 0.7 mm led to a reduction of 10 times in PE retention (Silveira et al., 2023). However, relatively fast releases are observed even if adopting larger particle sizes (2.8 mm) with a total release in about 30 min (Chan, Lee & Heng, 2006). Thus, the current challenge for hydrophilic compounds is to increase encapsulation efficiency and enhance controlled release properties (Kurozawa & Hubinger, 2017). For large molecules such as proteins, their diffusion time through the hydrogel (radius 1-3 mm) may be sustained up to 30 hours (Skicki, 2021).

To favor the effect of steric hindrance on molecules diffusion, the porosity may be modified by physical approaches such as using polymer filler in bead matrix, coating the particle surface via electrostatic charge interactions, or making internal ionic gelation (Barbut & Foegeding, 1993; Belščak-Cvitanovic et al., 2016; Chan, Lee & Heng, 2006; Vallejo-Castillo et al., 2020). Even though, the high concentration gradient between beads and media should be reduced to increase the retention with time (Basha et al., 2014; Belščak-Cvitanovic et al., 2016; Li et al., 2021) or to use the gradient in the opposite direction to perform the adsorption of unloaded beads (Carvalho et al., 2019).

All these approaches were tested separately in the literature for larger beads. In this work, we are proposing the evaluation of the effect of each one of these alternatives to improve the retention of polyphenols from PE in beads produced by ionic gelation. The presence of a filler, change of concentration of the external environment, adsorption, and electrostatic coating approaches are proposed considering their effect on mass transfer. The release of entrapped compounds over time is also employed to evaluate the release pattern of PE in water media.

5.2 Material and methods

5.2.1 Material

Waxy corn starch - WCS (Amisol 4000) was kindly donated by Ingredion and Low-methoxyl (LM) pectin Genu® 104-AS-Z by CP Kelco. Dihydrate calcium chloride (CaCl2 - 147.01 g/mol) was purchased from Dinâmica, and the ethanolic extract (70% alcohol) from pomegranate peel was purchased from Heide Vegetable Extracts.

5.2.2 Characterization of pomegranate extract

The analysis to determine the main components of PE was carried out using equipment for high-resolution analysis (mass accuracy) by direct infusion (Thermo Q-Exactive, Orbitrap). The mass range of 50 to 2000 m/z and the polarity of the positive and negative source were used.

5.2.2.1 Antioxidant capacity – DPPH and ABTS

The diphenyl-1-picrylhydrazyl (DPPH) radical scavenging analysis was measured according to Arend et al. (2017). The sample solution was prepared by adding different concentrations of PE (0.1, 0.5, 1, 2.5, and 5 mg/mL) into 70% ethanol solution and kept shaking at room temperature overnight. Then, 160 μL of sample solutions were mixed with 40 μL of methanolic DPPH solution (0.15 mM) and kept in the dark for 30 min before measuring the absorbance at 517 nm in UV-visible spectrophotometer (path length = 1 cm, quartz, T60 PG Instruments).

The 2.2-azino-bis-3-ethylbenzothiazoline-6-sulphonic (ABTS) radical scavenging capacity was measured according to Michalaki et al. (2023). The ABTS was dissolved in water (7.4 mM), mixed with the same volume of potassium persulfate (2.6 mM), and incubated at 25 ◦C in the dark for 16 h. The stock solution of ABTS+ was diluted with 5 mmol/L PBS to absorbance up to 0.7 at 734 nm. Then, 10 μL (0.1 g/mL) of the sample was added to 1 mL of diluted ABTS+ solution, stirred vigorously for 30 s, then placed in the dark for 6 min, and its absorbance read at 734 nm. The standard curve (R^2 = 0.9965) was prepared by reacting 40 μL of Trolox (in the range of 0–100 ppm) with 4 mL of diluted ABTS + solution. The result was expressed as mg of Trolox equivalent (TE) per g of extract.

5.2.2.2 Anthocyanin content

Total anthocyanins were determined in 5 ± 0.01 g of PE by using the pH differential method AOAC (2005). Dilution was performed in a solution at pH 1 and 4.5, and the quantification of the absorbance was performed on a wavelength of 520 and 700 nm in a UV/Visible spectrophotometer (Varian, model Cary 50).

5.2.2.3 pH stability

The stability of PE against pH was conducted by visual inspection of the chroma difference and formation of precipitates, at different pH values. The pH was modified by using NaOH (1M) or HCl (1M) to reach pH 2.0 to 9.0. The color was also measured by a Chromameter (CR-400, Konica-Minolta Sensing), programmed in the CIE L*c*h system. Chroma, hue, and ΔE* values were calculated by Equations (1), (2), and (3,), respectively.

$$Chroma = C^* = \sqrt{a*^2 + b*^2} \quad (1)$$

$$hue = h^* = arctan\left(\frac{b*}{a*}\right) \quad (2)$$

$$\Delta E^* = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \quad (3)$$

5.2.2.4 Total phenolic content (TPC)

TPC was determined according to the Folin Ciocalteu spectrophotometric method according to Moura et al., (2019). PE (0.400 ± 0.001 g) was diluted by completing the volumetric flask of 100 mL with ethanol. Then, 900 µL of distilled water and 100 µL of Folin Ciocalteu reagent were added to 100 µL aliquot of the diluted PE. After 5 minutes, 1000 µL of sodium carbonate solution (7% w/v) and 400 µl of distilled water were added. The mixture was thoroughly mixed and incubated at 25 ± 2 ◦C in the dark for 90 min and then the absorbance was read at 750 nm (path length = 1 cm, quartz, T60 UV-visible Spectrophotometer PG Instruments). The TPC was obtained from a gallic acid standard curve (R^2 = 0.9917) ranging from 40 to 200 µg /mL and the values are expressed as mg gallic acid equivalent (GAE)/ 100 mL of sample.

5.2.3 Beads production

Three methodologies were tested to entrap PE, as schematically represented in Fig. 5.1, and details of production are subsequently described.

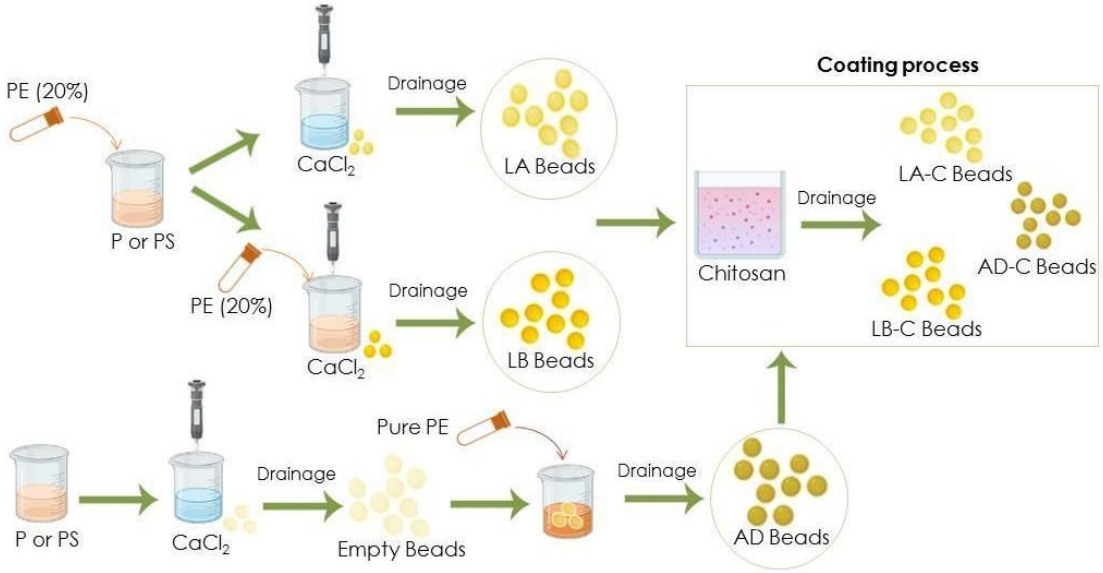

Fig. 5.1. Schematic representation of the encapsulation methods. PE: pomegranate extract; P: pectin; PS: pectin-starch; LA beads - produced in ionic solution; LB beads - produced in ionic solution with PE and AD beads loaded by adsorption.

The initial load (20%) was preliminarily determined with experimental tests. The PE affected the viscosity and gelation of the pectin polymers. Concentrations higher than 20% increased the viscosity of polymeric solution resulting in pumping problems. The interference with the viscosity may also indicate the influence of the ionic crosslinking for allowing the gel formation and consequently, a tendency to have a more porous internal structure. Theoretical loads of 20% were then employed for producing LA and LB beads (Table 5.1).

Table 5.1

Codes and extract concentration per sample

Sample	PE concentration in biopolymeric solution (%)	PE concentration in external medium (%)
LA (Loaded)	20	0
LB (Loaded)	20	20
AD (Adsorbed)	0	100

PE: pomegranate extract

Pectin and pectin-starch beads were prepared by external gelation. Pectin solution (2% w/w in water for 12h) and Pectin-pregelatinized starch (2:0.85 %w/w) were extruded by using a pump (Reglo Analog MS-2/6, Ismatec) at a flow rate of 3.9 mL/min through a jacketed double fluid atomizer nozzle (I.D. 1 mm) in CaCl2 (0.015 g/mL). The pH = 3.0 of calcium chloride solution was adjusted using a 50% citric acid solution. The beads were kept in solution to harden for 15 min and collected by sieving.

Loaded beads were produced by mixing PE (20% w/w) with the solutions before extrusion. concentration of PE was inserted in the crosslinking bath to evaluate the effect of reduced concentration gradient on the retention (LB beads – Table 5.1). The effect of the opposite concentration gradient was evaluated by adding empty hydrogel beads in a bath containing pure PE. These unloaded beads (AD) were inserted separately into a beaker (50 mL) in a ratio of 1:1 (w/w) with pomegranate extract (PE). The adsorption of PE was allowed to occur for 120 min under constant stirring at room temperature. After the adsorption, the PE was drained and the beads partially superficially dried using a paper towel.

The beads produced above (LA, LB, and AD) were coated by electrostatic deposition. Chitosan solution (1% w/w in 0.1M acetic acid, adjusted to pH = 3.7) has high positive charge density at this pH (Gonçalves et al., 2018). Beads were placed into the solution immediately after preparation in a mass ratio of 1:2 (beads: chitosan solution) and kept stirred for 10 min (Carvalho et al., 2019). Then, the chitosan solution was drained and the beads were superficially dried using a paper towel.

5.2.4 Beads characterization

5.2.4.1 Scanning electronic microscopy (SEM)

The beads were frozen at -18 °C for further freeze drying (24 h, at -48 °C and 1.33 Pa) in lyophilizer (LS 3000, Terroni). Microstructure analysis of freeze-dried beads without PE was performed by using a scanning electronic microscope (JSM-5800LV, Jeol). Freeze-dried beads were carefully attached to stubs using two-sided adhesive tape. The microscope was operated with a high vacuum and an accelerating voltage of 10 kV.

5.2.4.2 Fourier transform infrared (FTIR) spectroscopic analysis

The Fourier transform infrared spectrophotometer (IRPrestige-21, Shimadzu) and the IRSolution acquisition software (version 1.60) were used to evaluate the difference between P and PS beads containing PE (LA samples). Freeze-dried beads were crushed in a mortar and pestle. The crushed material was mixed with KBr (1:100), dried, and compressed to pastilles (D = 13 mm, hydraulic press, 80 KN, 10 minutes, SSP-10A, Shimadzu). Spectra were obtained in the range of 400 to 4000 (cm-1), with a resolution of 4 (cm^{-1}), 16 scans, and Happ-Genzel apodization. As a baseline (background), a pure dry potassium bromide (KBr) tablet of spectroscopic grade was used.

5.2.4.3 Bead mean diameter

Bead mean diameter was determined based on the measurement of at least 50 beads per sample, using the ImageJ software. The images of beads were obtained using a stereomicroscope (Citoval 2; Carl Zeiss Jena).

5.2.4.4 Uniaxial Compression to Fracture

Compression measurements were performed using a universal texturometer TA-XT Plus Texture Analyzer (Stable Micro Systems) at a controlled temperature of 25 °C. A compression force at 50% of the original height of the beads was exercised by using an aluminum cylindrical probe (D = 36 mm). The mechanical strength (N) was recorded as a response to this applied force. The measurements were conducted at least 6 times.

5.2.4.5 Encapsulation efficiency (EE)

A known mass of beads was placed into 100 mL of sodium citrate solution (2% w/w) for 180 minutes under agitation. The TPC in the solution was determined according to what was described previously. The analysis was also performed in blank beads. The efficiency of encapsulation (EE) was calculated according to Moura et al., 2019 by comparing the TPC determined in the beads concerning the amount of TPC detected in the solution before producing the beads (Equation 5.4).

$$EE\ (\%) = \frac{TPC\ in\ beads}{TPC\ in\ polymeric\ solution\ x\ 100} \quad (5.4)$$

5.2.4.6 Moisture content

The moisture content of the beads was determined gravimetrically at 105 °C ± 5 °C until constant weight following the official AOAC method (2005).

5.2.5 Release measurements

The release of the PE in water was determined by the addition of a known mass of beads into 100 mL of deionized water. The samples were placed in cylindrical support with stainless steel walls and an exchange area of 220 cm^2 porous size 25 µm, according to the system developed by Prata et al. (2008). This support was fixed in a Dubnoff bath (Tecnal). The beakers were covered and maintained at a slow and constant stirring rate and temperature (25° C) throughout the experiment. After 5, 10, 15, 20, 40, 60, and 120 min, 0.1 mL aliquots were removed from the external part of the container, with solvent replacement. The TPC of the samples was determined and release curves were expressed as the percentage of PE released concerning the PE initial in the mass of beads.

5.2.5.1 Determination of diffusion coefficients

From the release experiments, diffusion coefficients were determined to effectively quantify the differences between the strategies adopted in this study. The release of TPC from the beads can be described with Fick's second law (Equation 5.5), which, in spherical polar coordinates renders as

$$\frac{\partial C}{\partial T} = \frac{1}{r^2}\left[\frac{\partial}{\partial r}\left(r^2 D_k \frac{\partial C}{\partial r}\right) + \frac{1}{sin\theta}\frac{\partial}{\partial \theta}\left(sin\theta D_k \frac{\partial C}{\partial \theta}\right) + \frac{D_k}{sin^2\theta}\frac{\partial^2 C}{\partial \emptyset^2}\right] \quad (5.5)$$

Where: D_k is the apparent diffusion coefficient [m^2/s], C is the concentration [kg/m^3], r, θ, and f are spherical polar coordinates, and t represents time [s].

Several assumptions were adopted to simplify this model:

I. The system was constantly and efficiently stirred which mitigated the mass transfer resistance in the frontier of the bead.

II. The release media was considered to act as sink media. The gallic acid solubility in water is very high (58.95g/mL). The amount of beads used for release assays varied as a function of encapsulation efficiency. The maximum amount employed corresponded to 0.015 g/mL.
III. The shape of the particles may be approximated by a sphere.
IV. The extract is homogeneously distributed in the matrix, in the form of small droplets.
V. Diffusion is considered only in the radial direction, with no angular dependence.
VII. In general, the dimensions of particles remain unchanged with water imbibition in the matrix.
VIII. The diffusion coefficient of gallic acid was considered constant, independent of the water content.
Based on these assumptions, the integrated solution of Eq. 5.5 in terms of released mass is given by Equation 5.6 (Crank, 1975).

$$\frac{M_t}{M_\infty} = \left[1 - \frac{6}{\pi^2}\sum_{n=0}^{\infty} \quad \frac{1}{n^2} exp\left(\frac{-D_{AB}\pi^2 n^2 t}{r^2}\right)\right] \quad (5.6)$$

Comparing the general results from a mechanistic model and the power law equation, the fitting using Eq. 5.6 could better describe the general behavior of release. The R^2 value for the fitting was mostly >0.90 for all curves examined with this function.

2.6 Statistical Analysis

The statistical significance was determined using 2-way ANOVA, for samples (LA, LB, and LC), and for formulations (P, PC, PS, and PS-C). The differences between the mean values obtained were compared through Tukey's test with 5 % significance, using Statistica 7.0 software. The analysis was carried out in triplicate.

5.3. Results and discussion

5.3.1 Characterization of pomegranate extract

Mass spectrometry analysis indicated the presence of punicalagin, punicalin, ellagic acid and epigallocatechin, phenolic compounds present mainly in pomegranate peel. Spectra are shown in Appendix A. The PE was characterized by moisture content and indicators of

phenolic activity. The data showed high values in total phenolic content (TPC), which has a direct relation with the antioxidant capacity (Table 5.2). The TPC of the PE was 4285.66 mg GAE/ 100 g of ethanolic PE. Saleh et al. 2017 performed an extraction from pomegranate peels by using methanol and found a TPC of 5062.5 mg GAE/100 g of methanolic extract. Gözlekçi et al. (2011) evaluated the distribution of TPC in extracts (5 g/25 mL of ethanolic solution 50%) of hand press juice, peel, and seed of Turkish pomegranates, and found that the concentration in the peel is, on average, 125% higher than in the juice. Pomegranate seed extracts had the lowest total phenolic content (135.2 mg GAE/L) concerning the peel (2746.8mg GAE/L) and juice (1218.2 mg GAE/L) extracts.

Table 5.2

Results of the characterization of pomegranate extract

Moisture (%)	**TPC***	**Anthocyanins****	**AC - DPPH*****	**AC - ABTS*****
93.1 5 ± 0.03	4285.66 ± 21.07	0.71 ± 0.10	331.45 ± 11.83	316.97 ± 6.62

*TPC: total phenolic content (mg of GAE/100g sample); **mg of cyanidin-3-glucoside equivalent/100g); ***AC: antioxidant capacity (µmol of Trolox equivalent/g).

The pH of the PE was 4.66 ± 0.02. The results of pH stability agree with the study of Friedman & Ju (2000) which showed that phenolic compounds have lower stability at high pH, and this instability increases rapidly from pH 5-6. In this pH range, there was a noticeable chroma difference, for parameters L, C*, h*, and **(**ΔE***)** (Table 5.3). The color is significantly different amongst the pHs ($p < 0.05$), varying from orange-red in pHs under 5.16 to yellow at higher pHs.

Table 5.3

Evaluation of the color of pomegranate extract with pH variation

pH	L	C*	h*	ΔE*
4.66	22.01±0.18	6.23±0.22	0.08±0.00	-
2.01	22.78±0.13	7.43±0.11	0.13±0.00	1.53±0.20
3.08	22.01±0.11	5.89±0.24	0.10±0.00	0.44±0.10
4.17	21.95±0.05	5.27±0.09	0.10±0.00	1.00±0.21
5.16	21.85±0.11	4.44±0.07	0.09±0.01	1.81±0.21
6.13	29.61±0.14	12.58±0.10	1.42±0.00	12.62±0.20
7.24	41.25±0.34	24.65±0.25	1.52±0.00	29.46±0.40
8.31	44.96±0.17	25.25±0.09	1.53±0.00	32.53±0.19
9.06	42.48±0.15	22.23±0.12	1.51±0.00	28.50±0.27

Above pH 5, there was a formation of precipitate which led to an increase in the turbidity of the medium (Fig. 5.2). The increase in luminosity (L) in the positive direction reveals a tendency towards white color, due to the characteristic coloring of the formed precipitate. The hue angle is a color parameter in which values vary between 0 and 90 to represent red and yellow, respectively. There was a reduction in the red hue of the extract (increase in h*) with increasing pH. Similarly, the saturation, indicated by the chroma parameter, and the total color change (ΔE*) also increased, which may be associated with increased instability.

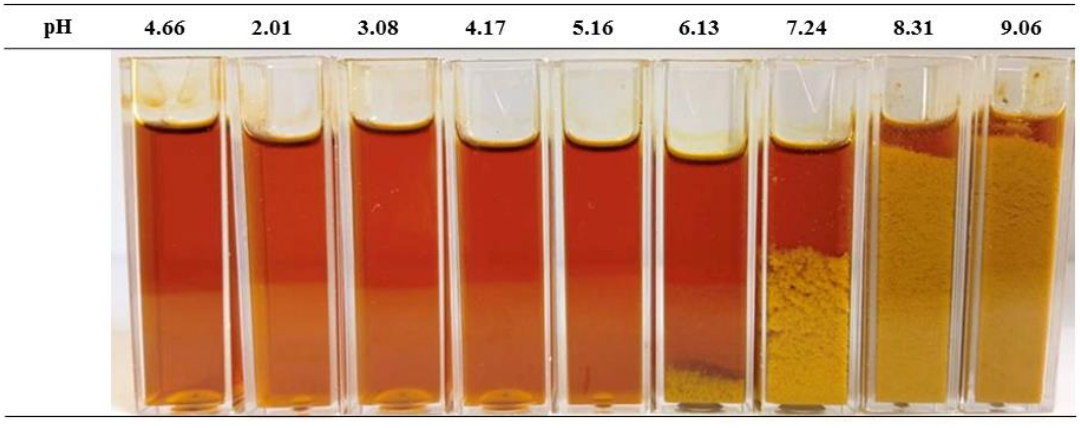

Fig. 5.2. Chroma difference of pomegranate extract at different pH values.

Polyphenol instability and precipitate formation at moderately basic pH have been reported by several authors (Chethan & Malleshi, 2007; Friedman & Ju, 2000; Xiao, 2022;

Zeng et al., 2017). Resonance, tautomers, hydrogen bonding, and hydrated structures as well as colors of polyphenolic anthocyanins are strongly influenced by pH. The instability of compounds such as acid gallic, caffeic, and chlorogenic is related to the impossibility of resonance stabilization of phenoxide ions and quinone oxidation intermediates, mechanisms responsible for the stability of compounds such as ferulic acid for example. In general, multi-ring aromatic structures are more complex than mono-ring phenolic compounds, which are susceptible to the effects of high pH. Therefore, the presence of other mono-ring acids such as ellagic, cinnamic, and p-coumaric in PE may have been responsible for the instability at pH ≥ 6 (Giri et al., 2023; Friedman & Ju, 2000).

Pomegranate ethanolic extract contains considerable amounts of phenolic compounds such as anthocyanins, gallic and ellagic acids, catechins, punicalagin, and punicalin (Pan et al., 2011; Smaoui et al., 2019). Despite being excellent antioxidants, are susceptible to oxidation at high pH, and the browning rate has been related to the presence of groups with higher antioxidant activity. Barbehenn and collaborators (2006) observed at pH 10, that the group of hydrolyzable tannins (ellagitannins) contained the most active tannins examined, forming high concentrations of semiquinone radicals and browning at the highest rates.

5.3.2 Physical properties of beads

The microstructure analysis of the beads produced is shown in Fig. 5.3. The upper panel shows the complete beads (33 x magnification) while the microstructural features of their surface are highlighted in the bottom panel (150 x magnification).

The micrographs of pectin (P) and pectin-starch (PS) gel beads complement the optical microscopy results of our previous study (Silveira et al., 2023), in which it was detected that the beads produced with starch appear more spherical-like geometries. The starch acts as structural support for modulating bead shrinking and surface fracture after drying (Lozano-Vazquez et al., 2015). The presence of coating in P-C and PS-C beads was not distinguished by SEM analysis.

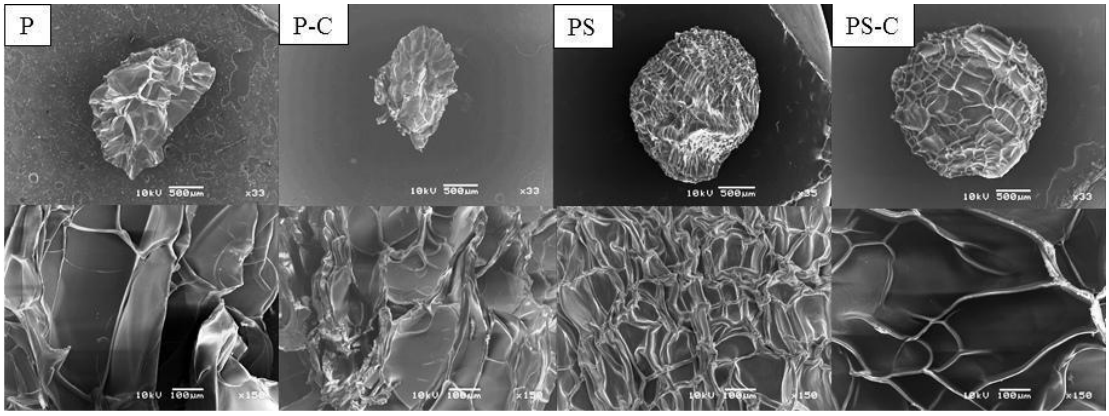

Fig. 5.3. Microstructure of uncoated and coated (C) pectin (P) and pectin-starch (PS) beads.

The FTIR results of both beads (P and P-S) don't show new group formation (Fig. 5.4) which indicates the absence of interaction among the polysaccharide chains (starch and pectin) or polysaccharide-PE that could increase the molecular size of the biomolecule. The emergence of a peak around 1520 cm^{-1} can indicate functional groups of phenolic compounds related to C=C vibrations of aromatic rings (Nisar et al., 2019).

From the FTIR spectra, it can be observed that there has been a spreading of the absorption line in the wave number range of 3000–3600 cm^{-1} with a peak in 3417.861 cm^{-1} due to the stretching and flexion of OH groups (Wani & Uppaluri, 2023). Pectin and starch have absorption bands at 800–1200 cm^{-1}, which are characteristic of polysaccharides. The characteristic bands at 1020.33 and 1103.282 cm^{-1} are associated with the coupled C–O and C–C stretching vibrations (Manrique & Lajolo, 2002), while the absorption peak at 835.177 and 960.550 cm^{-1} was attributed to the symmetrical stretching vibrations of α-pyran (Zhao et al., 2014a; Chen et al., 2022; Ren et al., 2017).

The absorption peak at 1334.74 cm^{-1} corresponded to the stretching vibrations of the glycosidic C-O bonds between saccharide units, while the absorption peak at 1234.42 cm^{-1} could be assigned to the out-of-plane bending vibrations of the C-H bonds of–CH3 (Chen et al., 2022). The carboxylic acid group in the uronic acid molecules of pectins exhibits peaks at 1732.08 cm^{-1} due to deprotonated carboxylate (COO− groups) (Manrique & Lajolo, 2002). The absorption peaks at 1631.78 cm−1 and 1442.75 cm^{-1} could be assigned to the antisymmetric and symmetric COO stretches (Zhao et al., 2014a; Wu et al., 2013).

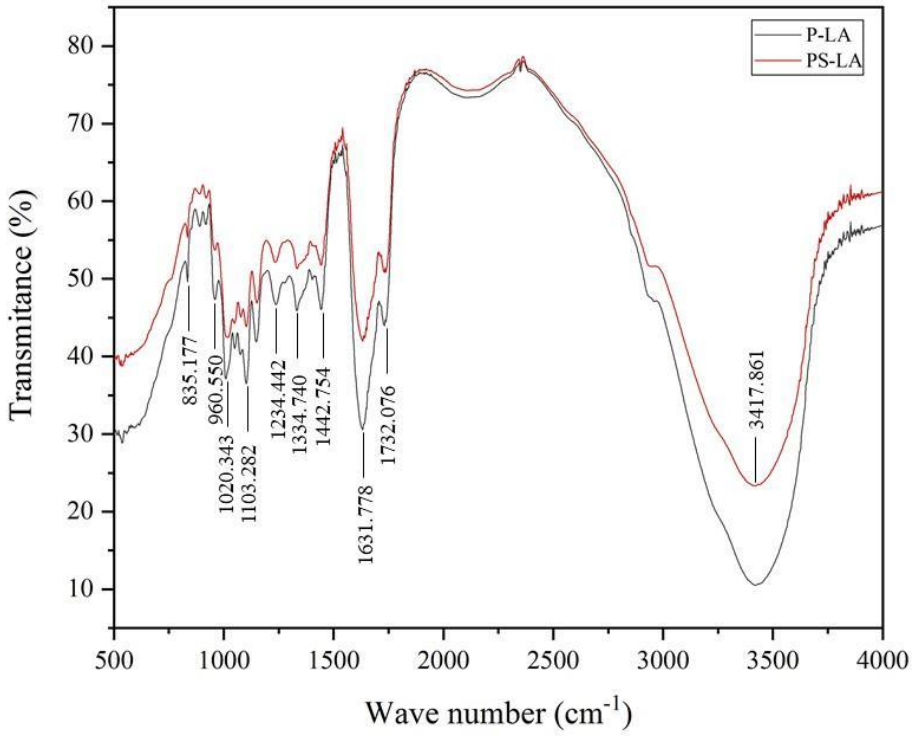

Fig. 5.4. FTIR spectra of pectin (P) and pectin-starch (PS) beads containing PE (LA samples).

Ionotropic gelation results from the association of sequences of galacturonate residues from pectin with ions calcium. The general characterization of the beads produced shows some differences among the entrapment procedures. Even if by SEM the starch provided some structure to the beads, the presence of starch was not able to change the internal structure of pectin gels, and similar mechanical strength ($p < 0.05$) was observed for P and PS, concerning LA and LB beads.

The formation of the "egg box" structure requires specific ion binding characteristics, but the stabilization of cooperative egg-box structures will take place by van der Waals interactions and hydrogen bonds in addition to electrostatic interactions (Fraeye et al., 2010). We verified that increased PE concentrations have an influence on viscosity and gelling properties. Considering the same PE concentration in the pectin solutions, their viscosity was similar, which reflected no difference in sizes ($p < 0.05$) of LA and LB beads (Table 5.4). No difference in bead morphology was observed compared to our previous work (Silveira et al., 2023).

Table 5.4

Moisture content, bead diameter, and mechanical strength of the beads

Sample	Formulation	Moisture (%)	Diameter (mm)	Mechanical strength (N)
LA	P	96.51 ± 0.06^{aA}	2.623 ± 0.089^{cC}	3.95 ± 0.16^{bC}
	P-C	96.79 ± 0.02^{aA}	2.773 ± 0.131^{bC}	3.75 ± 0.29^{cC}
	PS	95.28 ± 0.74^{bB}	2.795 ± 0.080^{bB}	4.65 ± 0.23^{aC}
	PS-C	95.72 ± 0.08^{bA}	2.808 ± 0.136^{aB}	4.56 ± 0.23^{aC}
LB	P	95.11 ± 0.03^{cB}	2.762 ± 0.161^{bB}	5.72 ± 0.27^{cB}
	P-C	94.22 ± 0.03^{dB}	2.873 ± 0.096^{aB}	5.51 ± 0.30^{dB}
	PS	96.70 ± 0.02^{aA}	2.677 ± 0.126^{cC}	6.24 ± 0.28^{aB}
	PS-C	95.89 ± 0.32^{bA}	2.714 ± 0.122^{cC}	5.92 ± 0.45^{bB}
AD	P	92.03 ± 0.03^{cC}	2.946 ± 0.097^{cA}	8.97 ± 0.50^{cA}
	P-C	90.88 ± 0.08^{dC}	3.171 ± 0.134^{bA}	7.84 ± 0.35^{dA}
	PS	95.22 ± 0.02^{aB}	2.901 ± 0.123^{bcA}	13.78 ± 1.01^{aA}
	PS-C	93.92 ± 0.25^{bB}	3.325 ± 0.131^{aA}	11.18 ± 0.20^{bA}

The letter C in the formulations indicates that beads were coated with chitosan. LA: loaded, LB: loaded with PE also in the $CaCl_2$ solution, and AD: adsorbed. Uppercase letters represent statistical differences between treatments (LA, LB, and AD), and lowercase letters between formulations (P, P-C, PS, and PS-C).

However, the mechanical strength of the beads increased as the PE concentration in the bath changed, indicating the effect of PE on the network structure. LA beads had PE mixed in the polymeric solution before extrusion and average MS of 4.22 N; LB beads had additional PE in the hardening bath (average MS = 5.85 N) and, finally, AD beads, despite not having PE dispersed in the internal matrix, were kept in contact with concentrated PE for 2 hours and presenting average MS of 10.44 N. This increased hardness may be associated with an expected crosslinking of the matrix. Some polyphenols are used with this aim for biopolymer stabilization (Alavarse et al., 2022; Kaczmarek & Mazur, 2020; Rivero, García & Pinnotti, 2010). Both polysaccharides employed to produce the beads carry many hydroxyl groups along the chains that may potentially be employed for crosslinking with phenolic compounds, either by Michael addition or Schiff-base reactions with more than one chain (Alavarse et al., 2022; Rivero, García & Pinnotti, 2010).

In the literature, some polysaccharides were crosslinked with polyphenols. The addition of tannic acid in chitosan films, for example, increased by 29% tensile strength whereas decreased 24% water vapor permeability (Rivero, García & Pinnotti, 2010). Lee and collaborators (2023) also noted that the resulting chitosan-tannic acid composite films exhibited improved mechanical strength owing to covalent crosslinking resulting from Schiff base and Michael addition reactions between the amino groups of CS and galloyl groups of TA.

Moreover, the beads produced by the adsorption technique showed the lowest moisture (average of 93 %) ($p < 0.05$) (Table 5.4), probably, due to the dehydrating effect of the alcohol from pure PE. The PE adsorption was visually observed to occupy the whole diameter of the beads with no tendency of surface concentration (data not shown). The moisture content may be used as an indicator of release behavior. Hydrogel beads normally tend to have more than 92% moisture content (Carvalho et al., 2019), which was observed for most beads produced in this work.

Regarding the effect of electrostatic coating, despite being not significant for most beads ($p < 0.05$), there was an increase in diameter and a slight reduction of MS in all formulations. The chitosan has a rigid chain structure and is bigger than the proteins normally used for coating, which results in self-repulsion and a lower degree of packing (Pan et al., 2021).

5.3.3 Evaluation of the PE retention

The effect of the producing technique on PE retention can be evaluated by the values of encapsulation efficiency and TPC content of all beads, which are presented in Table 5.5.

Table 5.5

Total phenolic content (TPC) in beads and efficiency of encapsulation (EE)

Sample	Formulation	TPC*in final beads	EE (%)
LA	P	376.4 ± 23.80^{cC}	41.25 ± 1.91^{aB}
	P-C	243.80 ± 12.60^{aC}	27.24 ± 1.08^{cB}
	PS	411.89 ± 12.54^{dC}	43.44 ± 2.25^{aB}
	PS-C	306.49 ± 18.13^{bC}	34.21 ± 1.24^{bB}
LB	P	840.41 ± 29.90^{bB}	101.26 ± 4.41^{aA}
	P-C	578.76 ± 11.28^{cB}	64.67 ± 1.14^{bA}
	PS	904.30 ± 34.80^{aB}	102.71 ± 4.08^{aA}
	PS-C	588.90 ± 16.30^{cB}	62.34 ± 1.15^{bA}
AD	P	2541.69 ± 74.77^{bA}	-
	P-C	1737.76 ± 44.77^{cA}	-
	PS	2960.26 ± 26.92^{aA}	-
	PS-C	1840.42 ± 105.73^{cA}	-

*mg of gallic acid equivalent/100g sample. The letter C in the formulations indicates that beads were coated with chitosan. LA: loaded, LB: loaded with PE also in the CaCl2 solution, and AD: adsorbed. Uppercase letters represent statistical differences between treatments (LA, LB, and AD), and lowercase letters between formulations (P, P-C, PS, and PS-C).

As expected, the conventional encapsulation (LA) had the lowest retention, which on average for all formulations tested was around ~36% (Table 5.5). The combined hydrophilic characteristic and small size of PE led to a facilitated diffusion through the hydrophilic matrix. These particles have about 96% moisture, and pore sizes are considered too large for encapsulation of many small molecular-size biomolecules (Córdoba, Deladino & Martino, 2013). The major part of the extract is lost during the hydrogel network formation. This result was corroborated by the literature with different hydrophilic compounds such as yerba mate polyphenols (55%), nisin (48%), carqueja extract (49%) (Balanc et al., 2016; Córdoba, Deladino & Martino, 2013; Hosseini et al., 2014; Stojanovic et al., 2012).

Inulin and starch have been proposed as fillers in other works to improve the retention in hydrogels (Li et al., 2021; Paulo et al., 2022). However, the presence of starch

aiming to act as a filler in the current study, did not significantly influence retention of LA and LB beads *($p > 0.05$)*. The entrapment efficiency was marginally greater (43.44% and 102.71%, respectively for LA and LB beads). Lozano-Vazquez et al. (2015) studied the alginate: starch ratio and reported that the starch may promote a more tortuous matrix when used in higher concentrations than ours.

Electrostatic interactions used for layer-by-layer bottom-up building onto surfaces of beads are another option to improve certain properties such as digestibility, protection, and tailored release profiles of gel particles, which could work to reduce the loss of hydrophilic compounds (Flamminii et al., 2021; Pan et al., 2021; Picos-Corrales et al., 2023). However, in this work, the coating of all beads using chitosan resulted in comparatively lower retention than uncoated beads, with an average loss of 32% of phenolic compounds during the coating process. Probably the TPC decrease occurred due to the migration of polyphenols due to the porosity of the polymeric matrix and the tendency to move to a less concentrated medium.

Similarly, Deladino et al. (2008) observed that chitosan-alginate particles entrapped with lower yerba mate polyphenols content than the bead without chitosan. According to the authors, significant active compound losses occurred mainly during the immersion in the chitosan solution. There also is a chance to have occurred the complexation of the chitosan with the released phenolic compounds. Anyway, specifically for the electrostatic coating process, the presence of starch helped to retain the phenolic compounds. Chitosan-coated beads containing starch (PS-C) had a lower loss of phenolics (21.2 %) compared to those without starch (34.0%). It seems that during the coating process, the starch delayed internal transport of PE by obstruction of diffusional transport or steric hindrance.

Aiming to reduce the mass transfer to the media, PE was added to the CaCl2 solution during bead (LB) production. This strategy drastically increased retention, as an effect of the reduction in the concentration gradient between the medium and the solution and also, having a contribution of PE adsorption from the media, which can be seen by values of EE greater than 100% (Table 5.5). For both formulations (P and PS) there was an increase of about 2.2 times in TPC. For starch-containing beads (PS) the TPC increased from 376.4 ± 23.80 to 840.41 ± 29.90, while for P beads the increase was from 408.08 ± 12.54 to 904.30 ± 34.80 mg of gallic acid equivalent/100g sample. In the literature, other authors found higher retention when adding the same internal core material to the bath solution (Carvalho et al., 2019; Córdoba, Deladino & Martino, 2014).

The phenolic retention was the greatest with the adsorption technique (AD) in which the higher values of TPC were obtained (Table 5.5). The high values may be expected since a higher PE concentration was employed to produce these beads. For these beads, the presence of starch significantly ($p < 0.05$) made the difference in the TPC values. Beads with only pectin had 2541.69 ± 74.77 mg of GAE/100g sample while with pectin and starch, 2960.26 ± 26.92 mg of GAE/100g sample was quantified.

A proportionality relation between TPC and MS was observed, which can be confirmed by the linear and positive correlation between these parameters (R^2= 0.87) (Fig. 5.5). The greater MS values were observed for beads with higher content of TPC, which corroborate the hypothesis of a higher degree of complexation matrix-phenolic compounds.

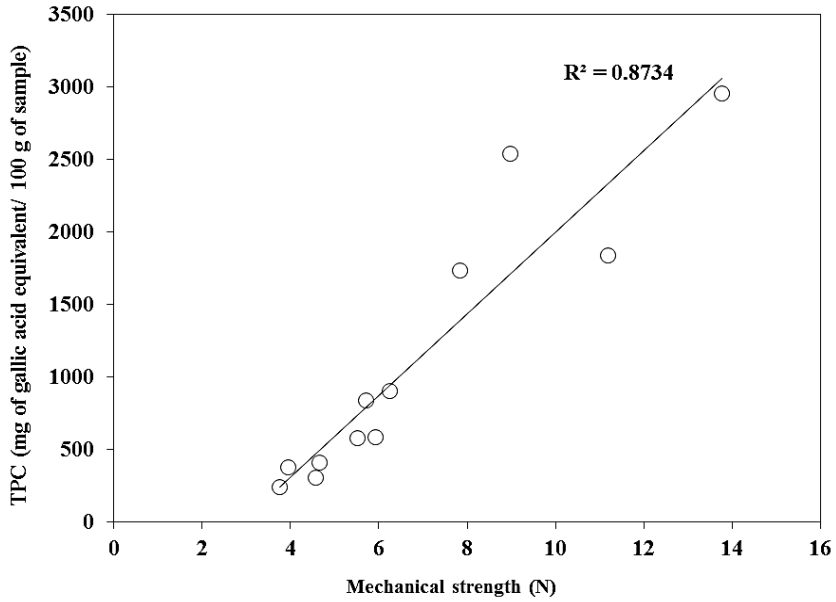

Fig. 5.5. Correlation between mechanical strength and total phenolic content (TPC). The solid line represents the linear regression of the experimental data.

As a preliminary conclusion, the encapsulation efficiency of the beads was deeply related to the fabrication step, considering the hydrophilic characteristic of the production media and the propensity of the PE to be leaked. Aiming to evaluate the effect of formulation in the retention of the entrapped polyphenol, release assays were performed adopting water as release media.

5.3.4 Evaluation of the PE Release

In general, the release curve was composed of a burst release in the first 20 minutes, and a plateau after 60 minutes (Fig. 5.6). In addition, as general behavior, coated beads showed a lower percentage of release, but this effect was more evident for beads-containing starch.

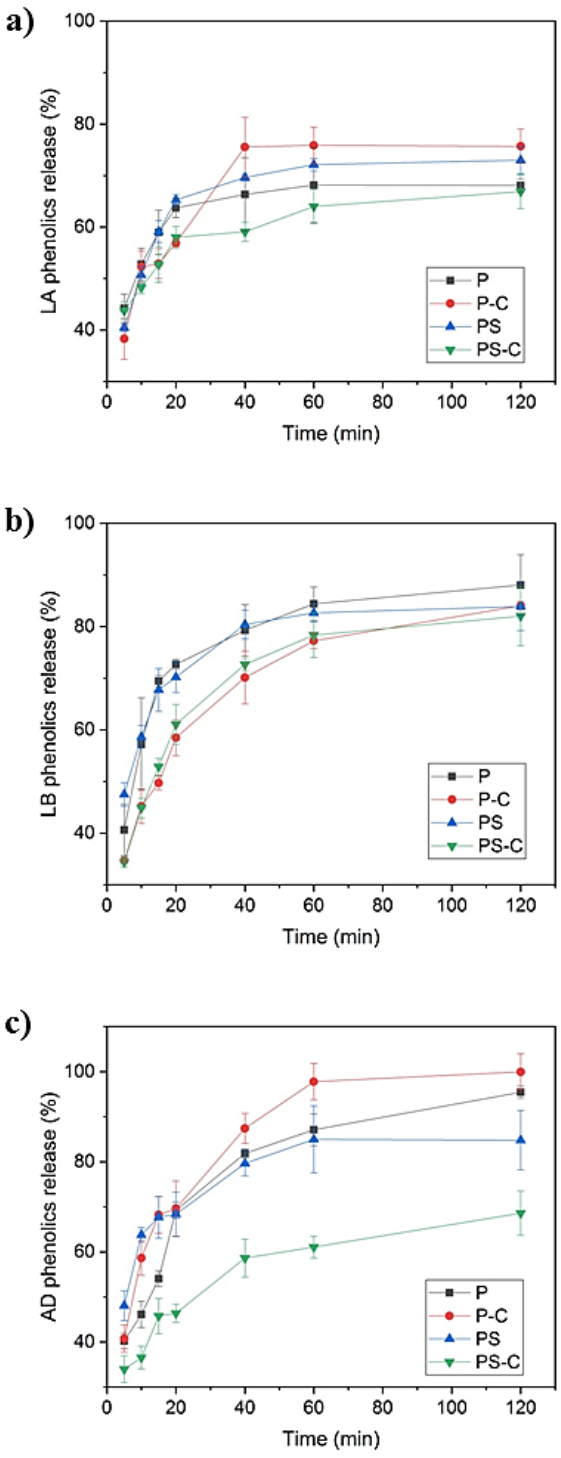

Fig. 5.6. Release behavior to beads produced by the conventional encapsulation method (LA), with PE in external medium (LB) and by adsorption (AD).

According to Fig. 5.6-A, there was a pattern in the release behavior of LA beads, reaching a plateau after 40 minutes. Regarding coated beads, the P-C sample experienced a reduction of approximately 75% in TPC. On the other hand, the PS-C sample exhibited a maximum release of 60%, indicating that the use of starch contributed to the control of the release.

The release for beads produced with PE in the external environment (LB beads) was about 80 % for all samples (Fig. 5.6-B). There is a little difference in reduction in release with the use of coating for both formulations (P and PS). For particles produced by adsorption (AD beads), the coating reduced the release in formulation PS (Fig. 5.6-C). Furthermore, this result reinforced that the use of starch was effective in reducing the release of PE. Then, despite the mass transfer rate being directly associated with the initial amount of PE with the tendency to release more as higher initial PE content, the coating of AD beads with starch was effective in reducing the release from 82% to 60% after 1 hour of release in water.

The burst release is generally associated with the presence of active compounds on the surface or near the surface (Joyce et al., 2020) and, the same release of AD-beads and LB-beads may be observed, confirming that during beads production, there was migration of PE along the radial direction, i.e., for the internal structure of AD-beads.

The retarded release observed for coated particles and with the filler indicated the potential of both strategies to improve the retention by the additional barriers to mass transfer or interaction with the active compound. Similar to the result obtained in this study, alginate beads coated by chitosan gave the most retarded release of tea polyphenols (TP) both in water and in simulated gastrointestinal conditions. Moreover, TP encapsulated in the obtained beads could partly protect them from degradation during storage (Li et al., 2021). The diffusion coefficients present values in the order of 10^{-10}. This magnitude was expected, as it was diffusion in solids. In general, the use of coating reduced the diffusivity values of PE in beads (Table 5.6). Among the 3 tests (LA, LB, and AD), the lowest values of diffusion coefficient were obtained for particles produced by the adsorption technique, which had the greater TPC content and higher MS. Higher moduli might be correlated with higher retention rates. In our conclusion, it can be considered that the combination of adsorption with an electrostatic coating is interesting when considering the potential of crosslinking of polyphenols with pectin egg box structure.

Table 5.6

The diffusion coefficient of the samples

Sample	Formulation	D_{ab} (m^2/s)*10^{10}	R^2
LA	P	4.10 ± 0.29^{cC}	0.925 ± 0.026
	P-C	2.37 ± 0.19^{bB}	0.974 ± 0.006
	PS	4.13 ± 0.31^{cB}	0.979 ± 0.008
	PS-C	1.90 ± 0.08^{aB}	0.931 ± 0.019
LB	P	3.67 ± 0.25^{bB}	0.933 ± 0.064
	P-C	2.03 ± 0.09^{aA}	0.972 ± 0.016
	PS	4.63 ± 0.42^{cC}	0.956 ± 0.027
	PS-C	2.00 ± 0.08^{aB}	0.991 ± 0.005
AD	P	1.67 ± 0.09^{bA}	0.970 ± 0.003
	P-C	2.17 ± 0.19^{cA}	0.984 ± 0.009
	PS	2.33 ± 0.24^{cA}	0.945 ± 0.040
	PS-C	1.07 ± 0.09^{aA}	0.933 ± 0.019

The letter C in the formulations indicates that beads were coated with chitosan. LA: loaded, LB: loaded with PE also in the CaCl2 solution, and AD: adsorbed. Uppercase letters represent statistical differences between treatments (LA, LB, and AD), and lowercase letters between formulations (P, P-C, PS, and PS-C).

Theophylline, vitamin B12, and myoglobin were loaded into alginate polymers to study the release kinetics in water. The diffusion coefficient was similar to those found in this study and varied from $2.65x10^{-10}$ m^2/s to $6.05x10^{-10}$ m^2/s for spherical beads (4.5 at 5.0 mm in diameter) showing lower values with increasing alginate concentration (0.8, 1.5, and 2.5%, w/w) (Pasut et al., 2008). On the other hand, lower values were found in Ca-alginate gel beads determined at conditions suitable for biodegradation studies. Diffusion coefficients for chlorferon (0.34 cm in diameter) and diethyl thiophosphate (DETP) (0.32 cm in diameter) were $2.70x10^{-10}$ m^2/s and $4.28x10^{-11}$ m^2/s, respectively (Ha, Engler & Lee, 2008).

5.4. Conclusion

In conclusion, this study delved into various strategies aimed at enhancing the retention of an ethanolic extract rich in polyphenols. Initially, conventional external ionic

gelation produced pectin beads with a retention rate of 41%. However, by reducing the concentration gradient between the internal and external medium we had significantly improved the retention to approximately 101%. The most noteworthy results were obtained with pectin-starch beads produced through adsorption, boasting 2960.26 ± 26.92 mg of GAE/100g sample, higher microstructure stability, and slower release rates. Although the coating technique effectively reduced the release rate in all beads, it incurred a 32% loss of phenolic compounds in the chitosan solution, consequently diminishing their overall retention. The combined use of adsorption and coating techniques on starch-containing beads emerges as a promising avenue to bolster the retention of hydrophilic compounds. Nonetheless, it's worth noting that some adjustments in the execution of these techniques are essential for the practical application of these polyphenol-loaded beads in food matrices.

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CHAPTER 6

Comparative study of encapsulated and non-encapsulated pomegranate extract in gummy candies

CHAPTER 6 – Comparative study of encapsulated and non-encapsulated pomegranate extract in gummy candies

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ABSTRACT

Health issues are triggering demands for functional gummies (GC) to provide benefits related to vitamin supplementation. This increasing trend is translating into opportunities to broaden the product portfolio. Ionotropic gelation has been used as an efficient method for improving the stability of heat-sensitive compounds such as natural polyphenols. In this work, the performance of encapsulated pomegranate extract (PE) in gummy candies was evaluated. PE-containing beads were produced by two different sizes (DB-dripping beads and JB-jet cutter beads) and the stability of polyphenols-enriched gummies (color, total phenolic content (TPC) and water activity (Aw)) was evaluated after 1, 30, and 60 days. A release study in a simulated digestion system was also proposed. Considering the TPC (1158.98 ± 26.98 mg GAE/100 g of sample) and the antioxidant activity of GCs, the use of DB (3 mm) provided higher values, besides there was greater release control under simulated gastrointestinal conditions. During the storage period, there were few indications of degradation or losses of the active compound. Despite obtaining relatively low sensory acceptability for all samples with PE, in terms of purchase intention and the internal preference map, this sample was also better evaluated. These findings suggest that it was possible to enrich GCs with encapsulated

pomegranate polyphenols, however more research is needed to ensure greater sensorial acceptance by consumers.

Keywords: ionic gelation; sensory analysis; storage stability; phenolic compounds.

6.1 Introduction

Gummy candies (GC) have been enjoyed by people of all ages notably by their outstanding taste, vibrant colors, and chewy texture. However, consumers are seeking beyond enjoyment, healthier confectionery options (Sarabandi et al., 2022). The reduction of sugar, the use of fruit extract, color from natural sources, and the presence of functional ingredients in GC bring opportunities to convert conventional confectionaries into products with enhanced nutritional profiles and potential health benefits (Kim et al., 2019). However, many of these ingredients are unstable and tend to degrade during production and storage. Thus, microencapsulation has been explored to incorporate bioactive compounds into GC due to the possibility of protection and controlled release properties (Moura et al., 2019; Silveira et al., 2023).

Ionotropic gelation is a microencapsulation technique that involves the formation of a hydrogel network by crosslinking polymers in the presence of ions. This process enables the encapsulation of sensitive active compounds due to the absence of high temperature and shear stresses and additionally, the absence of organic solvents and the employment of food-chain polymers (Zhao et al., 2014; Skicki, 2021).

Recently, our research group studied the effect of production technique on the retention and release of pomegranate extract (PE), a rich source of polyphenols, which possess antioxidant, anti-inflammatory, and potential health-promoting properties (Kandylis & Kokkinomagoulos, 2020). Results indicate that the reduction of size impairs PE retention due to the facilitated diffusion related to the small diffusion path. The main route of loss occurred in the beads production with PE leaching to the water media (Silveira et al., 2023). Moreover, different techniques were evaluated to improve the retention and the higher TPC was obtained using the adsorption technique (unpublished data), making possible their application for food enrichment.

By the dripping method, beads of approximately 3 mm in diameter are obtained (Silveira et al., 2023), which are visually comparable to pomegranate arils. On the other hand,

jet cutting is a technique able to produce gelled beads about 80.8% smaller (Paulo et al., 2017). Therefore, it is expected that the different bead sizes result in sensorial and technologically different gummy candies. Thus, this work aims to explore the potential use of pomegranate extract for enriching gummy candies. The work highlights the technological, nutritional and sensorial implications of using the free and encapsulated extract.

6.2 Material and methods

6.2.1 Material

Low-methoxy pectin (LMP) (104-AS-Z, CP Kelco, Limeira, SP, Brazil) and waxy corn starch (WCS) (Amisol 4000, Ingredion, Mogi Guaçu, SP, Brazil) were employed to produce the beads. Genu® 150 pectin (slow set gelling rate, CP Kelco, Limeira, SP, Brazil) was used to produce the gummy candies. Dihydrate calcium chloride ($CaCl_2$ - 147.01 g/mol), sodium citrate ($Na_3C_6H_5O_7.2H_20$ – 294.10 g/mol) and citric acid ($C_6H_8O_7.H_2O$ – 210.14 g/mol) were purchased from Dinâmica (Indaiatuba, SP, Brazil). The ethanolic extract of pomegranate peel (PE) was purchased from Heide Vegetable Extracts (Pinhais, PR, Brazil). Granulated sugar and glucose syrup with equivalent dextrose of 40 were acquired from local supermarkets and bakery ingredients stores, respectively.

6.2.2 Beads production

Pectin-starch beads were prepared by external gelation using dripping and jet cutter techniques. LMP: pregelatinized WCS at a mass ratio of 2:0.85 was used to produce unloaded beads, according to Silveira et al. (2023). The beads were then inserted into a beaker (250 mL) containing the corresponding mass of PE (beads:PE 1:1). The adsorption of PE was allowed to occur for 120 min under constant stirring at room temperature (25 ± 1 °C). After the adsorption, the PE was drained and the beads were superficially dried using a paper towel.

6.2.3 Preparation of gummy candy

The control formulation (C) without PE as well as formulations supplemented with different levels of free PE or PE-containing hydrogel beads are described in Table 6.1. The

beads contain about 50% of the TPC of the free PE, and then, their formulations should contain twice the mass of beads to correspond to the percentage of free PE.

Table 6.1. Water content and active compound added in gummy candy (GC) samples

Sample code	Water (g)	GC-ingredients (g)	Free extract or beads (g)	PE or beads concentration (g/100 g of GC)
C-GC	28	72	0	0
E1-GC	27	72	1	1.3
E2-GC	26	72	2	2.6
D2-GC	26	72	2	2.6
D4-GC	24	72	4	4.9
J2-GC	26	72	2	2.5
J4-GC	24	72	4	5.2

C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

Genu® 150 pectin (1.5% w/w) was hydrated in water at room temperature for 12 h under agitation and after that the solution was heated to temperature of 80 to 90 °C, and stirred using a heating plate (C-MAG HS 7, IKA, Staufen, Germany). Granulated sugar (35% w/w) and glucose syrup equivalent dextrose 40 (34.95% w/w) were then incorporated into the pectin solution, stirred and heated up to approximately 100 °C. The formulations without the PE were boiled and the concentration over time was measured by using a refractometer (Hanna Instruments, HI96800, USA). The final soluble solid content was determined to be 78-80 °Brix.

This solution was allowed to cool to approximately 80 °C with manual stirring, and then citric acid (0.4% of 50% aqueous solution), sodium citrate (0.15 %), and beads (D or J) or free PE were added at two different concentrations. The pH of the syrup was measured in a potentiometer (Scientific Marte, MB-10, Brazil). The final product was transferred to a rectangular mold and after cooling, it was cut into 1 cm cubes. The cubes were transferred to a surface covered with corn starch and allowed to dry (6 to 8% moisture) in chambers at 25 ± 2 °C for 24 h. The schematic experimental procedure can be seen in Figure 6.1.

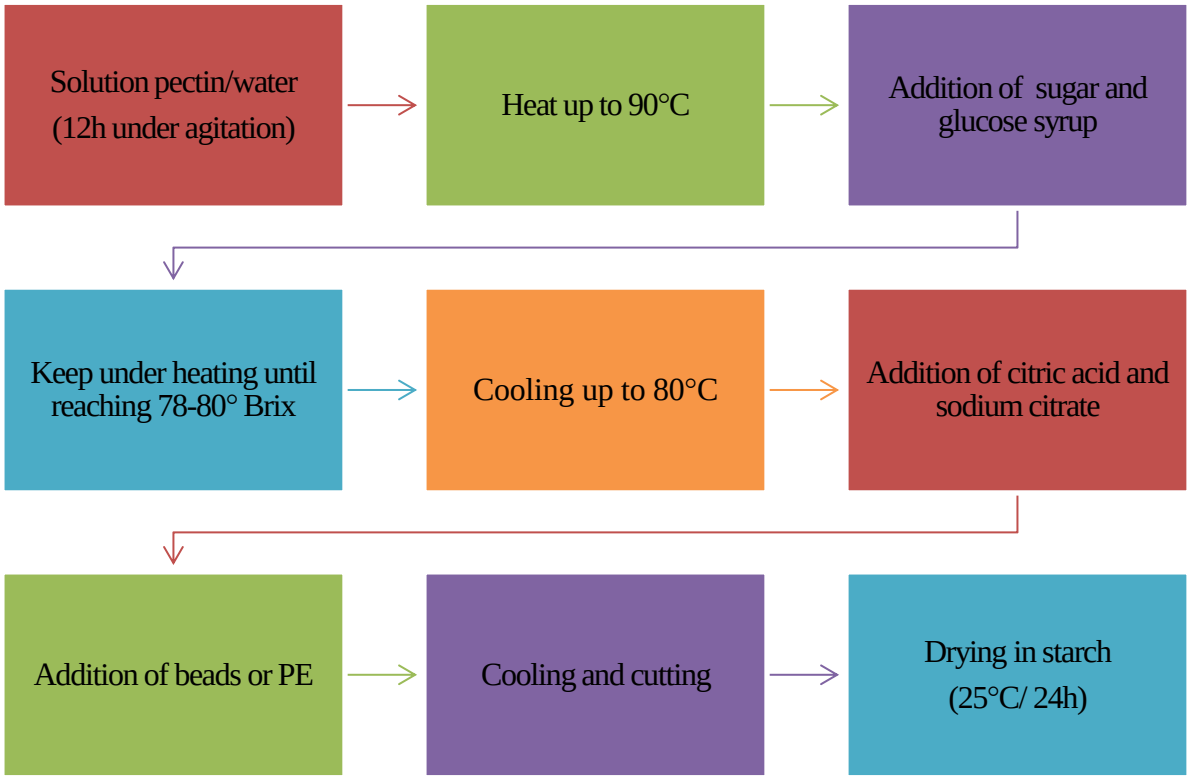

Figure 6.1. Methodology for producing gummy candies (GCs).

After drying, excess of starch was removed and gummies were packaged in metallic stand-up pouch bags with a ziplock closure and stored at 25 ± 2 °C for a maximum of 60 days.

Texture profile analysis, antioxidant capacity and sensory properties were evaluated on the first day of storage. The Aw, color and TPC were analyzed on 1, 30, and 60 days of storage**.** All analyses were performed in triplicate.

6.2.4 Gummy candy characterization

6.2.4.1 Texture profile analysis

The mechanical properties were analyzed using a texturometer (Stable Micro Systems, TA.XT.Plus, UK). The evaluation of texture comprised a perforation test with a 2 mm diameter probe P/2. The tests were performed by measuring force on compression, using a 50 kg load cell and a trigger force of 0.05 N. The perforation distance was 3 mm, the pretest speed was 2.0 mm/s, the test speed was 1.0 mm/s, and the posttest speed was also 1.0 mm/s.

The properties evaluated were hardness and adhesiveness. The textural measurements were performed on 12 gummies of each type.

6.2.4.2 Moisture content

The moisture content of the gummy candies was determined gravimetrically at 70 °C with no vacuum for 24 h, followed by vacuum drying for an additional 24 h, according to the official AOAC method (2005).

6.2.4.3 Antioxidant capacity – DPPH and ABTS

For the diphenyl-1-picrylhydrazyl (DPPH) radical scavenging analysis, 100 μL of sample solutions were mixed with 3.9 mL of methanolic DPPH solution (0.06 mM) and kept in the dark for 60 min before measuring the absorbance at 515 nm in UV-Visible Spectrophotometer (path length = 1 cm, quartz, T60 PG Instruments). The standard curve (R^2 = 0.9941) was prepared by reacting 100 μL of Trolox (in the range of 100 to 2000 μM) with 3.9 mL of diluted DPPH solution. The result was expressed as mM Trolox equivalent antioxidant capacity (TEAC)/g sample.

To determine the 2.2-azino-bis-3-ethylbenzothiazoline-6-sulphonic (ABTS) radical scavenging capacity, the ABTS was dissolved in water (7.0 mM), and 5 mL was mixed with 88 μL of potassium persulfate (140 mM) and incubated at 25 ◦C in the dark for 16 h. The stock solution of ABTS+ was diluted with ethyl alcohol to absorbance up to 0.7 at 734 nm. Then, 30 μL of the sample was added to 3 mL of diluted ABTS+ solution, stirred vigorously for 30 s, then placed in the dark for 6 min, and its absorbance read at 734 nm. The standard curve (R^2 = 0.9949) was prepared by reacting 30 μL of Trolox (in the range of 100 to 2000 μM) with 3 mL of diluted ABTS+ solution. The result was expressed as mM Trolox equivalent antioxidant capacity (TEAC)/g sample.

6.2.4.4 Storage stability

The GCs were stored for 60 days at room temperature (25 °C) and then evaluated in terms of total phenolic content, water activity, and color after 1, 30 and 60 days. The color was also measured by a Chromameter (CR-400, Konica-Minolta Sensing), programmed in the

CIE L*c*h system. Chroma, hue, and ΔE* values were calculated by Equations (6.1), (6.2), and (6.3,), respectively.

$$Chroma = C^* = \sqrt{a*^2 + b*^2} \quad (6.1)$$

$$hue = h^* = arctan\left(\frac{b*}{a*}\right) \quad (6.2)$$

$$\Delta E^* = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \quad (6.3)$$

Water activity was quantified in pieces of GC (1 x 1 mm) through LabTouch-Aw equipment (Novasina, Switzerland), at a temperature of 25 °C. The samples were not equilibrated and the measurements were performed in triplicates.

For TPC, a known mass of GC was dissolved into 100 mL of sodium citrate solution (2% w/w) for 180 minutes under agitation. Then, 900 µL of distilled water and 100 µL of Folin Ciocalteu reagent were added to 100 µL aliquot of the diluted PE. After 5 minutes, 1000 µL of sodium carbonate solution (7% w/v) and 400 µL of distilled water were added. The mixture was thoroughly mixed and incubated at 25 ± 2 °C in the dark for 90 min and then the absorbance was read at 750 nm in a UV-Visible Spectrophotometer (path length = 1 cm, quartz, T60 PG Instruments, UK). The TPC was obtained from a gallic acid standard curve (R^2 = 0.9961) ranging from 40 to 200 µg/mL (Concen = 270.86 Abs - 6.6546).

The color of GC was measured using the Hunter Lab system with a colorimeter (CR-400, Konica-Minolta Sensing, Japan). The color reading was performed using 6 squares of gummy candy (1 cm x 1 cm) randomly selected over Petri dishes calibrated with a white tile (Minolta calibration plate, No. 21733001, Y = 92.6, x = 0.3136, y = 0.3196) at 2 observation angle with a C illuminant source. Three readings were taken from each sample. Hunter L (lightness; 100=white, 0=black), a (redness; +, red; −, green), b (yellowness; +, yellow; −, blue) values were recorded.

6.2.4.6 Study of release profile under simulated oral, gastric and intestinal conditions

Simulated salivary fluid (SSF), gastric fluid (SGF), and small intestinal fluid (SIF) were prepared according to Minekus et al. (2014). A mass of sample (0.006 to 0.6 g) was weighed, performing a total of 1 g by adding water. Then, 700 µL of SSF, 5.0 µL of $CaCl_2$ (0.3 M); and 195 µL of milli Q water were added, performing 2 g in the total. The mixture

was heated to 37 °C in a thermoblock and then 100 µL of saline a-amylase (1500 U/mL) was added, and stirred for 2 min. 1000 µL of the supernatant of the oral phase were removed.

For the gastric phase, 750 µL of SGF, 0.5 µL of CaCl2 (0.3 M), 20 µL of HCl (1M) and 69.5 µL of milliQ water were added to the remaining suspension. The suspension was heated to 37 °C in a thermoblock and then 160 µL of pepsin (25000 U/mL) were added, and stirred for 120 min. 1000 µL of the supernatant of the gastric media was collected.

For the intestinal phase, 440 µL of SIF, 100 µL of bile, 1.6 µL of $CaCl_2$ (0.3 M), 6 µL of NaOH (1M) and 52.4 µL of milliQ water were added. The suspension was heated to 37°C in a thermoblock and then 200 µL of pancreatin (8000 U/mL) were added and then stirred for 120 min. 1000 µL of the supernatant of the intestinal media was collected.

These procedures were carried out for the free PE, for beads produced by dripping and jet cutter, as well as for GCs. 100 µL aliquots of fluids were periodically taken (0, 2, 30, 60, 90, 120, 150, 180, 210 and 240 min) for subsequent quantification of TPC (Folin Ciocalteu) of the samples. The results of the release studies were expressed as a percentage, as a ratio of the TPC at a given time and the initial value determined in the sample.

6.2.4.7 Sensory evaluation

Sensory evaluation was carried out at the Laboratory of Sensory Science and Consumer Research (LSCCR), at the Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil.

The research was approved by the ethics and research committee of UNICAMP (number CAAE 70124523.5.0000.5404). Subjects were invited to participate of GC study through specific social media groups created for this purpose and posters on university bulletin boards. They evaluated samples in individual booths with white light in a controlled environment at 21 ± 2 °C, following ISO (2007) guidelines. Before any evaluation, subjects were asked about their willingness to participate in the tests as volunteers and those choosing to collaborate signed an informed consent form. The acceptance analysis was performed with 122 volunteers consumers, employees and students from the Universidade Estadual de Campinas, representing the target public, with no restrictions for sex, age, and social classes (ABEP, 2016).

Four samples (C, FE1, DB2 and JB2) were coded with randomly selected 3-digit numbers and served in disposable 50 mL plastic cups. Samples were evaluated in one

monadic sequential session, per an outline of complete balanced blocks (Meilgaard et al., 2015). Potable water was served for palate cleansing. The acceptance concerning appearance, aroma, flavor, texture, and overall impression was carried out using a nine-centimeter unstructured hedonic scale (Meilgaard et al., 2015), anchored at the ends by the terms “I disliked it very much” on the left, and “I liked it very much” on the right (Lawless & Heymann, 2010).

The purchase intention was assessed using a 5-point scale, ranging from “certainly not would buy” to “certainly would buy” (Meilgaard et al., 2015). The ideal intensities in relation to pomegranate flavor, sweetness, and hardness were also evaluated, using the Just-About-Right (JAR) scale with five (5) categories, being “1=much less than ideal”, “2=less than ideal”, “3=ideal”, “4=more than ideal” and “5=much more than ideal” (Lawless & Heymann, 2010).

Check-all-that-apply (CATA) analysis was applied to the same 122 subjects who participated in the acceptance analysis questionnaire (Pereira et al., 2021). They were asked to evaluate samples and choose the terms that best described them from a list of 18 predefined descriptors, which were: sour taste, sweet taste, bitter taste, astringent, homogeneous mass, bright, aftertaste, gelatinous, good bead size, attractive color, firm, sticky, good consistency, very light color, very dark color, translucent, soft, bad bead size. The sequence of terms in the list was balanced for each subject and kept the same for all samples (Meyners & Castura, 2016).

The results were collected using the FIZZ Network Sensory software (version 2.47b - Biosystemes, Couternon, France).

6.2.5 Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the data, and differences between the mean values obtained were compared through Tukey’s test with 5 % of significance, using Statistica 7.0 software.

Statistical analysis of data from acceptance tests performed by ANOVA and Tukey’s Honestly Significant Difference (HSD) Test of Means ($p \leq 0.05$). Data from purchase intention were plotted in frequency histograms for each sample (Meilgaard et al., 2016). Multivariate statistical analysis based on the principal component analysis was applied to the

representation of individual notes of consumer acceptance concerning overall impression to perform the internal preference map (Bolini et al., 2023).

Data from CATA were analyzed using Cochran's Q test ($P \leq 0.05$) and correspondence analysis (CA) were also performed for data from CATA and correlated to acceptance test data using XLSTAT software version 2023 (Addinsoft, Paris, IF, France).

6.3. Results and discussion

6.3.1 Characterization of gummy candies

The subjective visual analysis of the GCs allows for observing color differences and the presence of beads, mainly using the dripping technique (DB) to produce them (Figure 6.2).

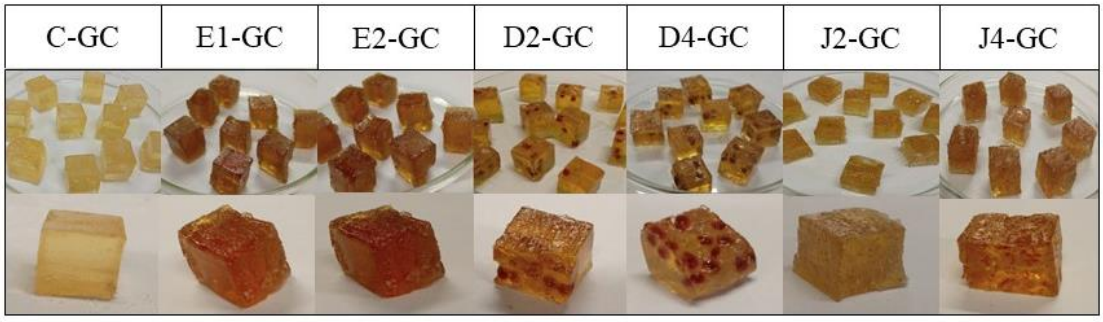

Figure 6.2. Gummy candies (GC). C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

The addition of PE in the formulation (pH = 2.66) reduced the pH values of the GC-syrup. Thus, the control sample (C) differed statistically from the others (Table 6.2). The higher the percentage of PE in the formulation, the lower the pH.

Total soluble solids (TSS) of the formulation after boiling is the parameter used to identify the final point of cooking the GC, so the difference in values was a consequence of the production process. Considering the formulations highlighted in Table 6.2, the resulting Brix indicates an average evaporation of 22%. Typically, GCs have a moisture value from about 9% to about 18% by weight and pH not higher than 4.0 (Yamabe et al., 2017). Therefore, the values found are in the characteristic range of commercial GCs.

Table 6.2. pH, total soluble solids (TSS), moisture, hardness and adhesiveness of gummy candies (GC)

Amostra	pH	TSS (°Brix)	Moisture (%)	Hardness (N)	Adhesiveness (N)
C-GC	3.91±0.03^{a}	78.90±0.24^{a}	12.34±0.09^{e}	1.43±0.06^{b}	-0.69±0.06^{ab}
E1-GC	3.29±0.03^{b}	75.50±0.29^{c}	11.07±0.14^{f}	1.91±0.10^{a}	-0.91±0.06^{a}
E2-GC	3.23±0.02^{b}	76.83±0.29^{b}	14.37±0.09^{d}	1.45±0.07^{b}	-0.78±0.06^{ab}
D2-GC	3.09±0.05^{b}	76.43±0.26^{bc}	15.95±0.18^{ab}	1.11±0.08^{cd}	-0.58±0.05^{b}
D4-GC	3.07±0.04^{b}	79.87±0.26^{a}	16.60±0.07^{a}	1.04±0.07^{d}	-0.61±0.05^{b}
J2-GC	3.45±0.02^{b}	79.83±0.37^{a}	14.85±0.25^{cd}	1.26±0.06^{bc}	-0.74±0.06^{ab}
J4-GC	2.98±0.02^{b}	76.1±0.24^{bc}	15.32±0.37^{bc}	1.28±0.07^{bc}	-0.74±0.06^{ab}

C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

An inverse relationship between moisture and hardness of GC could be observed. Beads without extract (C) and containing free extract (FE) showed lower moisture values and consequent higher hardness. The higher moisture of the samples containing beads is due to their high water content (around 95%). Rivero et al. (2019) evaluated the hardness of different commercial gummy samples, and those of LM pectin produced under conditions similar to this work, presented lower values (0.077 N) than those obtained in this study.

There were small variations in the values of adhesiveness, with the DB samples being more adhesive than the others. The higher the adhesiveness value, the greater the force required to detach the GC from a surface. This can result in a sticky feeling on your teeth or hands when consuming GCs.

The antioxidant capacity of the GC is shown in Figure 6.3. All samples showed dosage dependent reducing power activity and moreover, they were well correlated with the TPC. ABTS scavenging activity of the samples was higher than DPPH radical scavenging. E2-CG and D4-CG exhibited the highest ABTS scavenging activity (about 75 mM TEAC/g, $p<0.05$), while E2-CG and J4-CG exhibited the highest DPPH scavenging activity (about 25 mM TEAC/g, $p<0.05$).

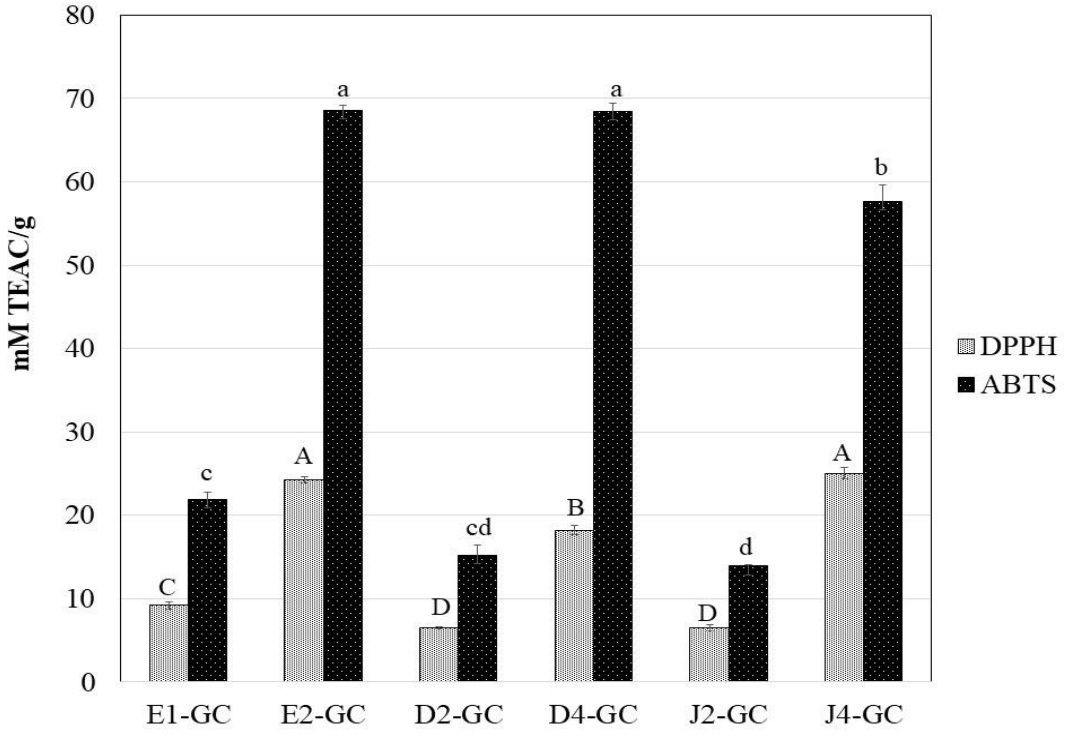

Figure 6.3. Antioxidant capacity. C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

6.3.2 Storage stability

GCs should be stored at room temperature in a cool and dry area (20–25 ◦C, 50–60 % relative humidity), conditions in which the shelf life extends from 12 to 24 months (Appleton, Adams, & Abene, 2018). The shelf life of gummies is primarily related to moisture migration (loss or uptake). Typically, water activity (Aw) for GCs ranges from 0.5 to about 0.7 (Yamabe et al., 2017).

The amounts obtained are in this range, except for the samples produced by dripping in the initial period of storage. Aw of the GCs was reduced during the storage period (Figure 6.4). This may have occurred due to the process of migration of water from GC into the surrounding atmosphere, a process called "moisture loss", leading to a decrease in water activity. This reduction is beneficial for the stability and shelf life of the candies, as the limited availability of water inhibits the growth of microorganisms, such as bacteria and fungi, which can cause product deterioration. The Aw was proportional to the moisture, thus higher values were measured for GC containing DB. Similar values (0.63–0.65) were

obtained by Yan et al. (2021), however gummy containing free vitamin C had higher Aw (0.648) than encapsulated by spray drying (0.636), probably due to the low moisture of the powder.

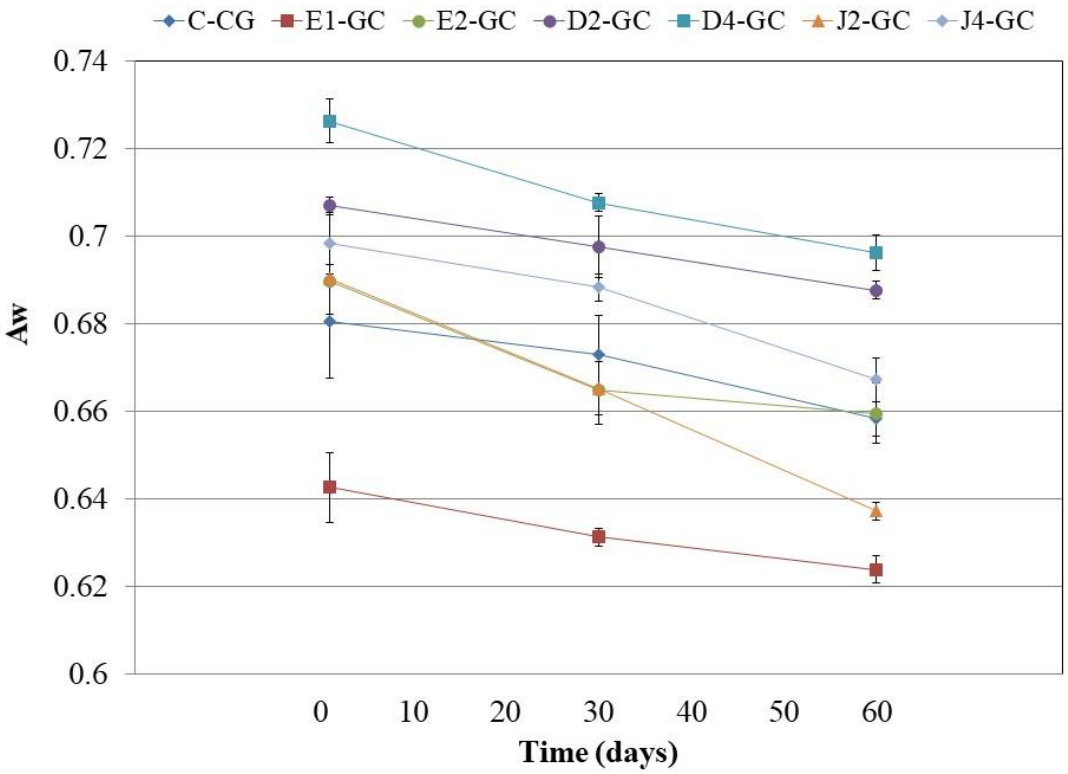

Figure 6.4. Water activity (Aw). C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

The luminosity values (L) showed positive variation over 60 days for all samples, except for the control (C). The PE shows visible coloration in shades of dark orange. The hue angle (h) is a color parameter in which values vary between 0 and 90 to represent red and yellow, respectively. As h variation was very low, it can be considered that there was no degradation of the pigments throughout the storage period. The saturation, indicated by the chroma parameter (C*) presented reduction more pronounced in the period from 1 to 30 days and remained more stable up to 60 days of storage. The variation in the color parameters can be seen in Table 6.3.

Table 6.3. Color parameters. C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

Days	**1**			**30**			**60**			**ΔE***
	L	**C***	**h***	**L**	**C***	**h***	**L**	**C***	**h***	
B-P	41.92±3.45	8.42±0.69	1.54±0.01	35.56±2.19	13.19±0.59	1.56±0.00	30.27±1.23	11.42±0.92	1.56±0.00	12.04±0.43
B-E (1%)	32.03±3.85	15.86±1.29	1.43±0.03	36.97±1.64	8.65±0.58	1.4±0.04	39.13±2.75	8.84±0.19	1.37±0.02	10.01±0.34
B-E (2%)	22.24±1.15	13.53±1.23	0.9±0.08	28.87±2.90	6.29±0.33	0.35±0.05	30.13±0.82	7.77±0.38	0.82±0.09	9.78±0.23
B-D (2%)	24.19±1.56	19.42±1.11	1.52±0.01	37.46±0.89	6.29±0.49	1.37±0.06	36.68±2.48	4.68±0.40	1.2±0.11	19.56±0.15
B-D (4%)	26.60±1.21	14.79±1.53	1.39±0.05	27.11±0.91	10.5±0.57	1.22±0.08	27.63±0.72	12.63±0.25	1.37±0.04	2.93±0.12
B-J (2%)	27.30±0.80	14.63±0.38	1.5±0.01	28.47±0.84	9.25±0.24	1.43±0.02	33.97±0.83	8.39±0.65	1.37±0.06	9.31±0.26
B-J (4%)	25.15±1.53	8.56±0.95	1.27±0.06	25.51±2.21	6.21±0.33	1.4±0.05	37.30±1.60	3.37±0.17	1.45±0.02	13.24±0.35

The TPC of the GC at the day of production and after 30 and 60 days is shown in Figure 6.5. Lower values were observed for GC containing the corresponding to 1% of PE in the formulation. It was not possible to distinguish the effect of incorporation of PE extract in the formulation when using the beads and neither due to size differences among them. Possibly, the pectin matrix of GC is enough to protect the PE, avoiding degradation over time. A slight reduction in the TPC value throughout the storage period could only be observed for formulation with the greater PE concentration (E2-GC, D4-GC and J4-GC) (Figure 6.5). It was also observed that in equivalent proportions of PE, the GCs containing beads obtained by dripping (DB) carried a greater value of polyphenols compared to the GCs containing jet beads (JB), probably due to the losses during production.

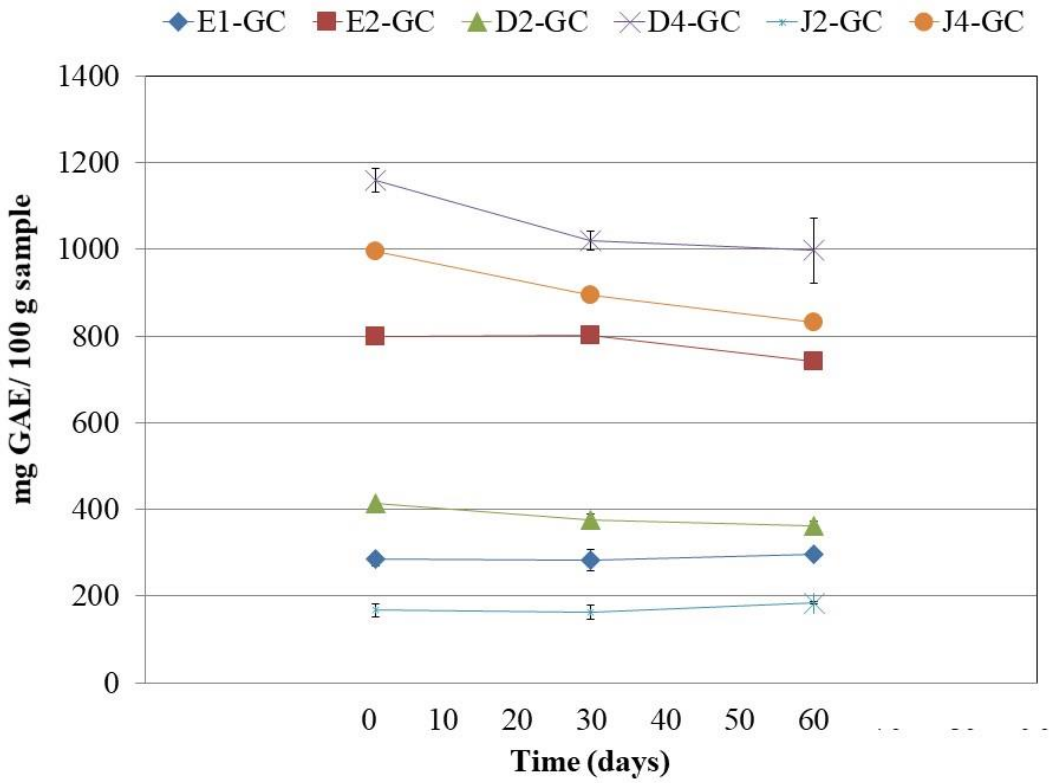

Figure 6.5. Total phenolic content (TPC). C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads.

Considering the absence of significant changes in the degradation or loss of phenolic compounds in the samples, the gummy candy seems to be a promising matrix for carrying compounds of this nature.

6.3.3 Effect of in vitro simulated gastrointestinal digestion on phenolic composition of gummy candies

Simulated digestion conditions were used to verify the degradation of beads and consequent release of PE extract in different phases. Figure 6.6 shows the GCs release profile for phenolic compounds in simulated oral, gastric and intestinal fluids in relation to the initial contents of the samples. It is important to emphasize the control sample had polyphenol content, probably due to the presence of oxidant compounds. This value was discounted off the value obtained from collections in other samples.

It can be seen that the free extract showed the highest percentage of release during digestion (Figure 6.6). Similar behavior was observed by Moura et al., (2019) where the free extract showed total release in 120 min. Particles generated by drip extrusion showed lower release (51%) compared to particles generated by atomization (47%), similar to what was observed in the present study.

The behavior of the GCs (D2 and J2) was similar to that of the corresponding beads (DB and JB) however it can be observed that the percentage released in the GCs was lower, possibly due to the interaction with the other ingredients.

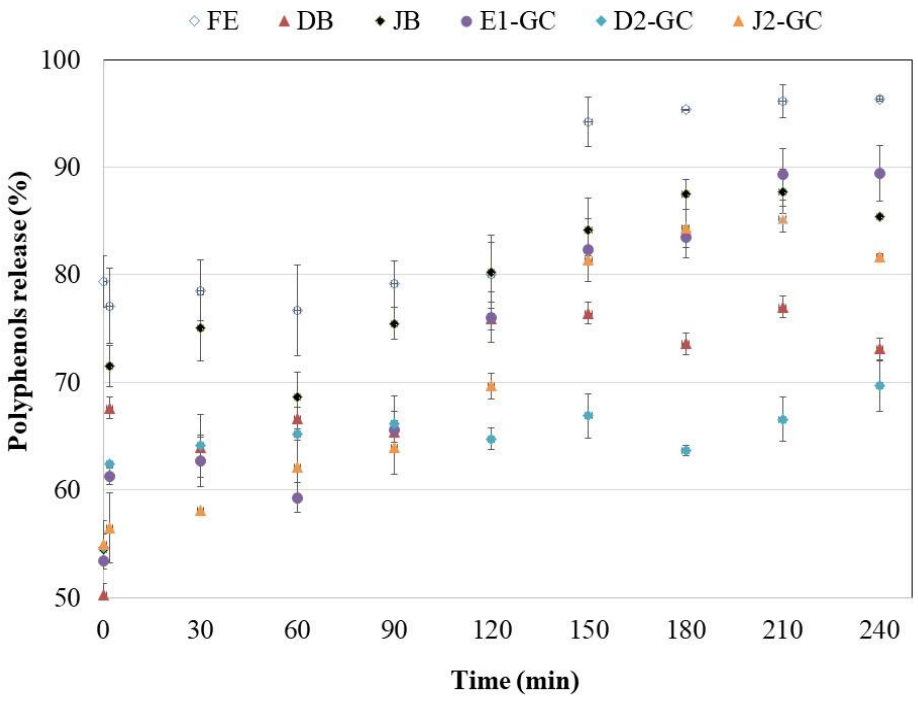

Figure 6.6. TPC in digestion. FE: free PE; DB: dripping beads; JB: jet beads; D2-GC: PE-dripping beads; J2-GC: PE-jet beads; E1-GC: free PE. 0 to 2 minutes: oral phase. 2 to 120 minutes: gastric phase. 120 to 140 minutes: intestinal phase.

3.4 Sensory evaluation

The results of acceptance in relation to appearance, texture and overall impression of the prototypes of the C-GC were slightly better accepted than the others. For acceptance in relation to appearance and aroma, all the samples are in a range that means that the tasters slightly disliked or were indifferent (3.8-5.0), while for flavor and texture some notes demonstrated that tasters liked slightly. Regarding flavor (Table 6.4), there was greater acceptance of C-GC and D2-GC samples ($p<0.05$).

Table 6.4. Means* of acceptance in relation to appearance, aroma, taste, texture and overall impression using hedonic scale.

Sample	Appearance	Aroma	Flavor	Texture	Overall impression
C-GC	5.0^{a}	4.1^{a}	5.6^{a}	5.9^{a}	5.5^{a}
E1-GC	3.9^{b}	4.1^{a}	4.5^{b}	5.3^{b}	4.8^{b}
D2-GC	3.8^{b}	4.2^{a}	5.3^{a}	5.3^{b}	5.0^{b}
J2-GC	4.2^{b}	4.0^{a}	4.8^{b}	5.1^{b}	4.8^{b}

C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads. *Means marked with superscript lowercase letters do not differ significantly according to Tukey's test ($p>0.05$)

For the analysis on the ideal scale, there was a significant difference between the control sample (C) and the others for the three attributes evaluated (Table 6.5). The pomegranate flavor and hardness were considered slightly less than ideal for the C sample, and slightly greater than ideal for the samples containing PE. As for sweetness, the C sample was considered ideal and the others much more than ideal.

Table 6.5. Means* for the ideal pomegranate flavor, sweetness, and hardness (Just-About-Right scale)

Sample	Pomegranate flavor	Sweetness	Hardness
C-GC	2.04[a]	2.62[a]	2.16[a]
E1-CG	3.80[b]	4.79[b]	4.26[b]
D2-CG	4.03[b]	4.75[b]	4.32[b]
J2-CG	4.04[b]	4.69[b]	4.28[b]

C-GC: control without PE; E-GC: free PE; D-GC: PE-dripping beads; J-GC: PE-jet beads. The numbers encoded correspond to the percentage of PE or beads. *Means marked with superscript lowercase letters do not differ significantly according to Tukey's test (p>0.05).

According to the Internal Preference Mapping generated from the results of the overall liking of the consumers, the main components explained, respectively, 39.38% and 34.29%. The DB2 and C samples were positioned close to most consumers (represented by red dots) which indicates greater preference, whereas the other samples were located on the opposite side (lower quadrant), far from most consumers (Figure 6.7).

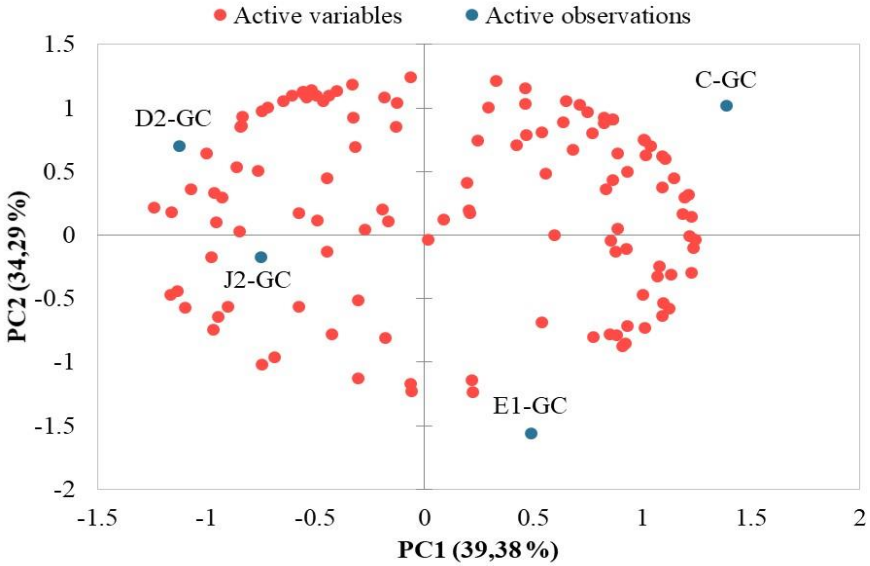

Figure 6.7. Internal preference map of gummy candies samples referring to the consumers´ overall impression (PC1 × PC2: 73.67%).

According to Figure 6.8, about 37% of the tasters stated that they would possibly or certainly buy the larger particle-added candies (D2-CG), while the other PE-added samples had a lower purchase intention (approximately 30%).

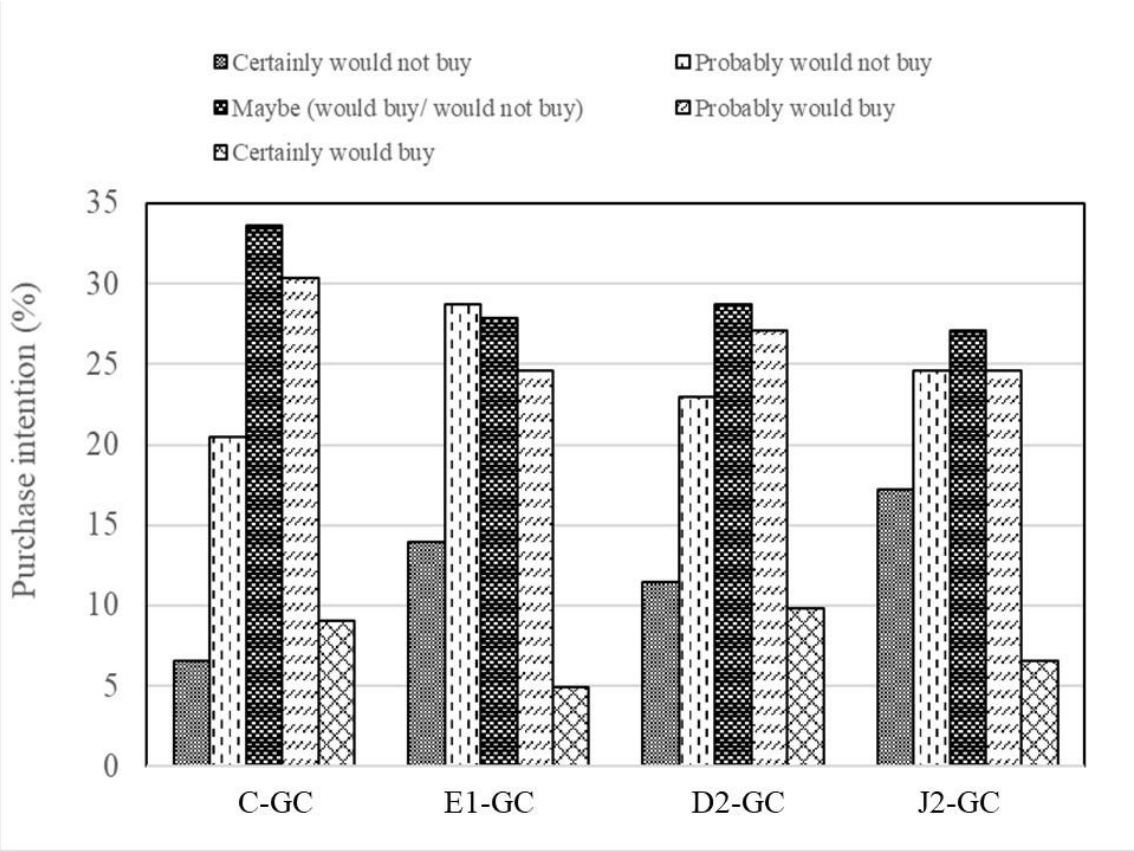

Figure 6.8. Distribution histograms of the consumers' purchase intention of GCs.C-GC: control without PE; E1-GC: free PE; D2-GC: PE-dripping beads; J2-GC: PE-jet beads.

The CATA analysis showed that the attributes sour taste, firm and bad bead size were mainly correlated with the DB2 sample, while very dark color and good bead size were associated with sample JB2 (Figure 6.9). The control sample was characterized by good consistency, bright, sticky and very light color, while the sample containing free extract was described as very dark color, astringent and presented aftertaste. The indication of these descriptors may indicate that characteristics of PE were smoothed by the use of microencapsulation techniques, mainly by dripping.

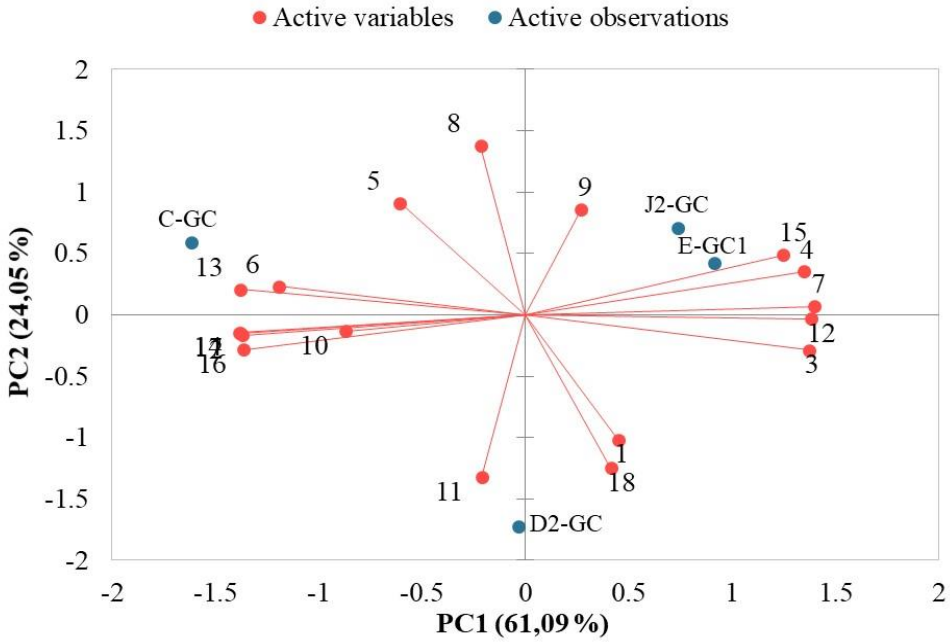

Figure 6.9. Biplot representation of four gummy candy samples (blue) and the sensory attributes (red) used to describe them, in the first two dimensions of Correspondence Analysis (CA) on the frequency presented on the CATA question. C-GC: control without PE; E1-GC: free PE; D2-GC: PE-dripping beads; J2-GC: PE-jet beads. Attributes: 1. sour taste; 2. sweet taste; 3. bitter taste; 4. Astringent; 5. homogeneous mass; 6. bright; 7. aftertaste; 8. gelatinous; 9. good bead size; 10. attractive color; 11. firm; 12. sticky; 13. good consistency; 14. very light color; 15. very dark color; 16. translucent; 17. soft; 18. bad particle size.

In general, the control sample had greater consumer acceptance. The addition of PE in the form of larger particles showed greater acceptance by the internal preference map and greater purchase intention (Figure 6.8). Furthermore, the higher phenolic content and antioxidant capacity of the samples may be an indication of greater potential for this form of application.

6.4 Conclusion

The present study emphasizes the significance of enhancing the sensory attributes of the product to encourage the consumption of a high-antioxidant product. The study of an innovative but not widely known product like pomegranate-enriched GCs may have played a

role in intermediate consumer acceptance. This underscores the importance of conducting such studies and promoting the consumption of healthy products.

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CHAPTER 7

General Discussion

CHAPTER 7 – General Discussion

The food industry is constantly striving to enhance its processes and products to meet the ever-increasing demands of consumers. In recent years, the development of foods with health claims has gained relevance, spanning various sectors such as dairy, bakery, and confectionery. In this sense, this work was developed, mainly aiming to study the ionic gelation process by dripping and jet cutting techniques, using pomegranate peel extract as the active compound. Subsequently, the application of both free and encapsulated PE was evaluated to enhance the nutritional quality into gummy candies (GCs).

In Chapter 3 we present an overview of the confectionery market trends and the usefulness of encapsulation as a technique to enable the production of more healthy confectioneries. From that, a bibliometric analysis associated with a review of the literature was conducted for exploring and analyzing scientific data, to understand the global scenario and identify the trends regarding specifically gummy candies. Encapsulation has been used as a delivery system in gummy food matrices with an emphasis on bioactive compounds such as polyphenols, natural pigments, probiotics, and vitamins. These trends were identified as still little explored, which led to the main objectives of this study: to deeply evaluate the encapsulation matrix by ionic gelation and to apply an extract with a high content of polyphenols to enrich GCs.

One of the great differences of this work was the detailed study of the wall materials. At the beginning of this project, an evaluation of the structure of the gelled beads was made to understand what would be the appropriate proportions of wall material, viscosity, size, and moisture (Chapter 4). Then, the asset was incorporated by different techniques until the selection of the most viable conditions for application (Chapter 5). Finally, the enrichment of the GCs was carried out with the application of beads and the study of the physical-chemical and sensorial characteristics (Chapter 6).

Pectin, a by-product of the citrus industry forms gelled beads in the presence of calcium cations. However, the high water content and porosity of these hydrogels beads impair the retention of hydrophilic compounds. The insertion of fillers such as starch can improve the retention and additionally mechanical, morphological and physical properties of beads. The elaboration of pectin and starch solutions with different concentrations (Chapter 4) allowed the understanding of the rheological behavior and physical-chemical characteristics of the final beads obtained. This led to the selection of beads with 2.85% solid content of

which 70% is pectin and 30% waxy corn starch. With a view to facilitating the application in food matrices and improving conservation, these beads were dried. Fluidized bed drying was technologically satisfactory, while on a rotating drum, drying was not efficient. Compared with pure pectin, the pectin-starch beads showed a spherical shape, smoother and more homogeneous with greater mechanical strength and greater water absorption capacity after drying in a fluidized bed. The conventional encapsulation of PE added to biopolymer solutions showed low efficiency (4.7% to 43.7%) and the release velocity was higher for drip beads (~70%).

Observing the low retention by the conventional encapsulation technique, different ways of incorporating the active were tested to increase the total phenolic content in the particles and decrease the release rate (Chapter 5). The insertion of a filler (gelatinized starch), the employment of different concentrations from the external environment, the adsorption using blank pectin-starch beads, and the complexation process using chitosan coating were performed. The encapsulation efficiency (EE) was improved (42 % to 101 %) with the change of concentration of the external medium, but the highest retention of PE was observed in pectin-starch beads obtained by adsorption (2960.26±26.92 mg of gallic acid equivalent/100 g sample). Furthermore, the increase in the PE concentration was proportional to the rise in the mechanical strength (MS) of the beads. The coating was able to reduce the release rate in most of the tests, however during the performance of the technique there was a loss of about 32 % of the phenolic compounds in the chitosan solution, reducing the EE. The combination of adsorption and coating techniques on particles containing starch proved to be more interesting, considering that higher EE and reduced release rate were achieved.

Considering the greater retention of polyphenols by adsorption, this technique was chosen for the preparation of gummy candies (Chapter 6). Different sizes were tested (by dripping and jet cutting), aiming to evaluate the physical, chemical and sensorial characteristics. The study evaluated the pH, moisture, total solid soluble (TSS), and textural parameters (hardness and adhesiveness) of GCs added from free extract (FE) and encapsulated extract (DB and JB). Furthermore, water activity (Aw), color parameters (L, a*, b*), and total phenolic content (TPC) were measured after 1, 30, and 60 days. In the simulated digestion system, free PE showed the highest percentage of release. The behavior of the candies (D2 and J2) was similar to that of the corresponding beads (DB and JB), and dripping samples presented greater release control. Moreover, the TPC (1158.98 ± 26.98 mg GAE/100 g of sample) and the antioxidant activity of GCs, the use of DB (3 mm) provided higher

values. During the storage period, there were few indications of degradation or losses of the active compound. Despite obtaining relatively low sensory acceptability for all samples with PE, in terms of purchase intention and the internal preference map, this sample was also better evaluated. These findings suggest that it was possible to enrich GCs with encapsulated pomegranate polyphenols, however more research is needed to ensure greater sensorial acceptance by consumers.

CHAPTER 8

General conclusion and suggestions for future work

CHAPTER 8 - General conclusion and suggestions for future work

8.1 General conclusion

Expanding the range and quantity of encapsulated beneficial compounds to create gummy candies with enhanced nutritional quality, naturalness, sensory acceptance, and cost-effectiveness remains a challenging. Nevertheless, in recent years, significant advancements have been made in research within this sector.

Based on the solutions viscosity, mean diameter, mechanical strength, yield, moisture content and, morphology of the beads a formulation with 2.85% solids content of which 70% are pectin and 30% waxy corn starch was chosen to encapsulation PE by ionic gelation. When compared to pure pectin, the pectin-starch beads exhibited a spherical shape, smoother and more homogeneous texture, greater mechanical strength, and enhanced water absorption capacity after drying in a fluidized bed. Conventional encapsulation of polyphenols (PE) in biopolymer solutions showed low efficiency, ranging from 4.7% to 43.7%. By employing techniques aimed at reducing mass transfer and, consequently, enhancing retention, the encapsulation efficiency was improved from 42% to 101% by varying the concentration of the external medium. However, the most significant retention of polyphenols (PE) was observed in pectin-starch beads obtained through adsorption, with a measurement of 2960.26±26.92 mg GAE per 100g sample. The chitosan coating successfully reduced the release rate in most tests, but during the process, there was an approximately 32% loss of phenolic compounds in the chitosan solution, leading to reduced encapsulation efficiency.

Incorporating beads into pectin-based gummies resulted in higher polyphenol content, increased antioxidant activity, and a reduced release rate in gastrointestinal conditions, mainly when were used larger beads produced through the dripping method. Additionally, there was a greater tendency towards positive sensory acceptance for this sample. Nevertheless, overall sensory analysis yielded low scores for all samples. This study underscores the importance of enhancing the sensory attributes of the product and emphasizes the significance of conducting such studies to encourage the consumption of healthy products.

8.2 Suggestions for future work

• Study the use of pomegranate extract in the internal ionic gelation process;

• Study other gelling agents, such as alginate and gums, in addition to polysaccharide mixtures in the polyphenol encapsulation process;

• Evaluate the application of beads in other matrices, such as bakery products, dairy products and beverages;

• Carry out an in vivo digestibility study of food products containing the applied beads;

• Evaluate other gummy candy formulations, aiming for a technologically viable production process.

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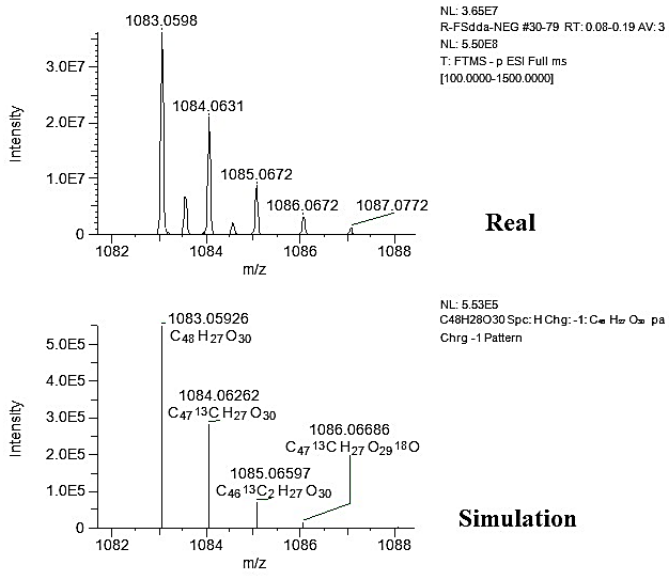
APPENDIX

APPENDIX A

Mass Spectrum (MS) scan with isotopic standard for the main components detected in pomegranate extract

Punicalagin



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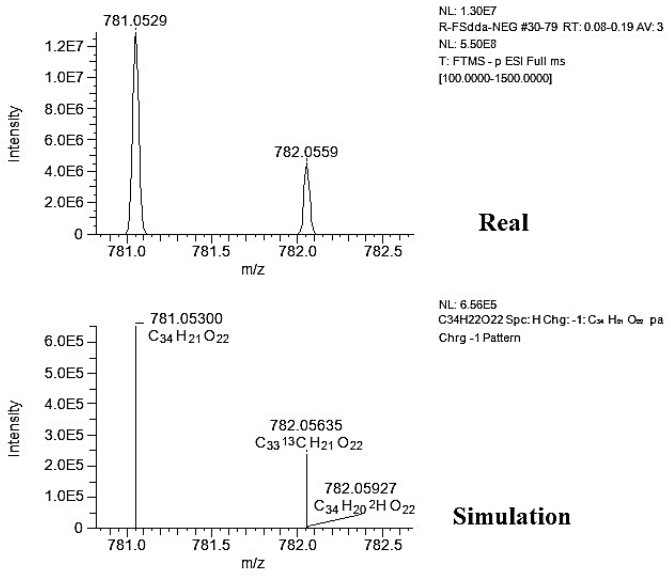
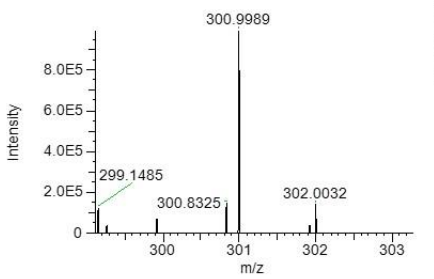
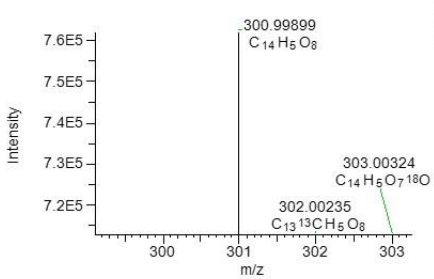


Ellagic acid

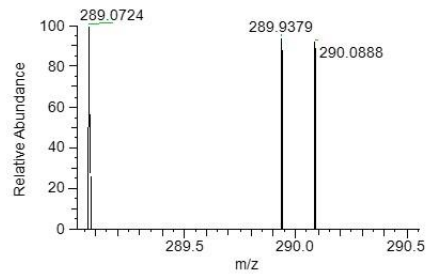


Real



Simulation

Catechin



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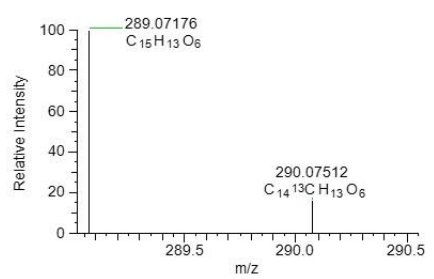
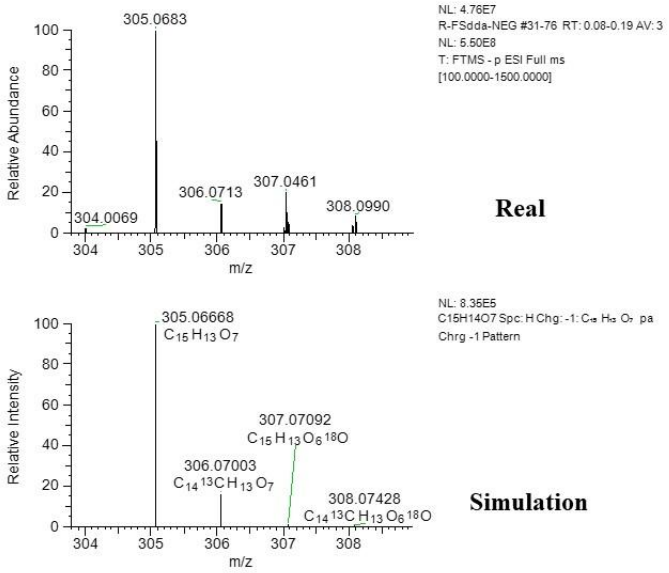


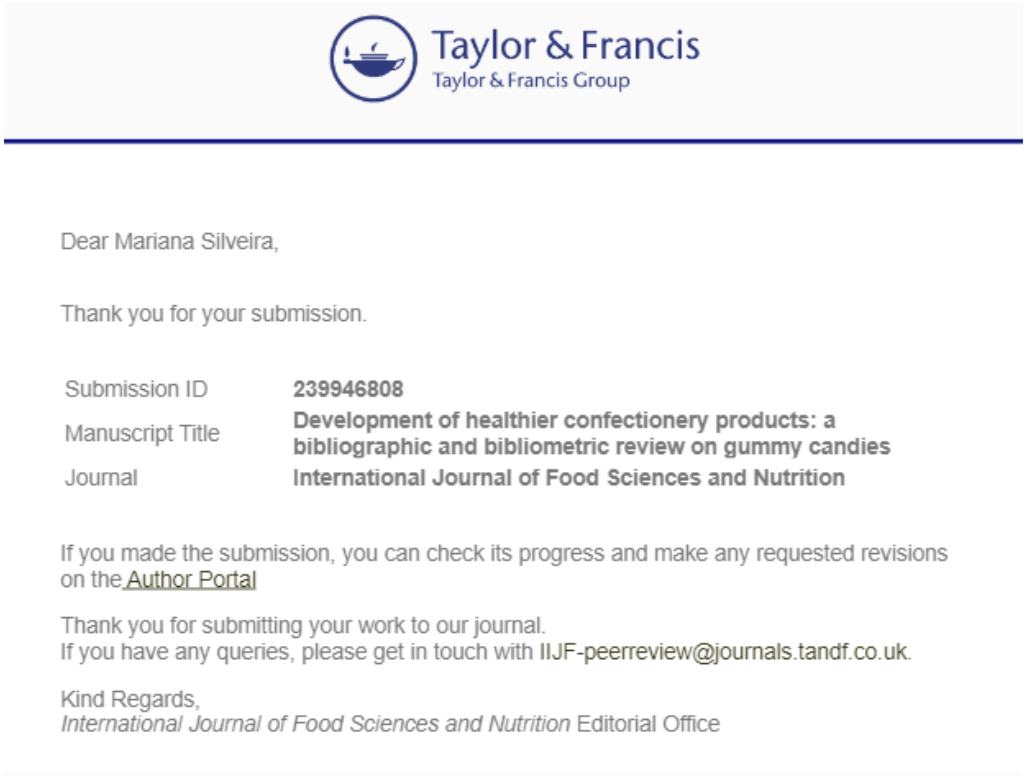
Simulation

Epigallocatechin



APPENDIX B

a) Proof of article submission of Chapter 3 - Development of healthier confectionery products: a bibliographic and bibliometric review on gummy candies



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Thank you for your submission.

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Manuscript Title	**Development of healthier confectionery products: a bibliographic and bibliometric review on gummy candies**
Journal	**International Journal of Food Sciences and Nutrition**

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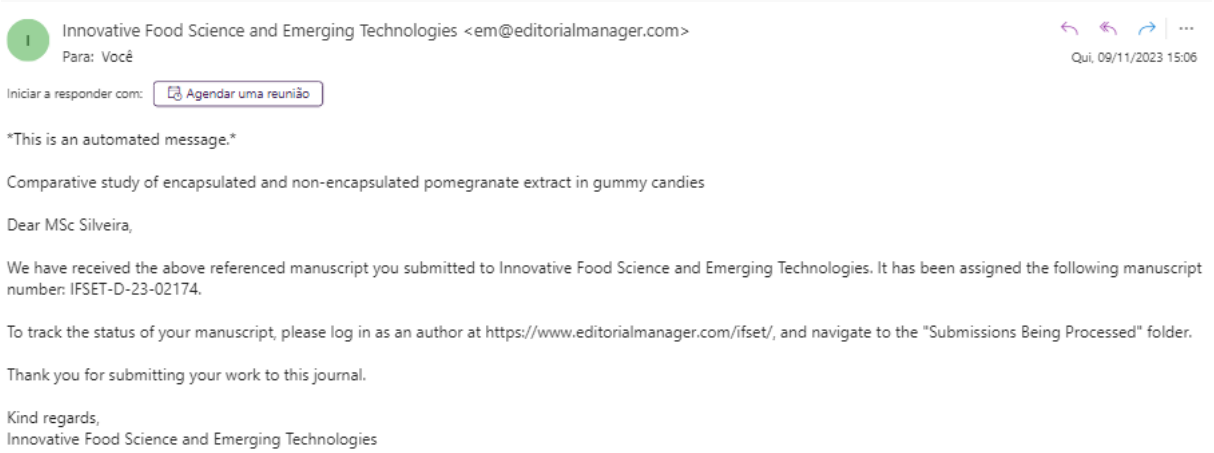

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