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LORENA SILVA PINHO

# Obtaining, encapsulation, and application of carotenoid-rich extract from guaraná peel (*Paullinia cupana*)

Pirassununga
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**I dedicate this thesis to my Family.**

**I could not go through my doctorate without them.**

## BIOGRAPHY

Lorena Silva Pinho, daughter of Grácia Pinho and Antônio Cezar Pinho, was born in Feira de Santana, Bahia - Brazil, in June, 1989. Lorena attended the Universidade Estadual de Feira de Santana (UEFS), Bahia - Brazil, where she graduated in Food Engineering (2008 - 2014). She collaborated with the Association of Food Engineering Students during her undergraduate program, planning events and helping undergraduates. Besides, she carried out scientific initiation for two years, in which one year was voluntary and one year with a scholarship. For six months, Lorena also worked as a physics teacher in the Universidade Para Todos program of the Federal Government of Brazil. In 2012, she went on a student exchange for one year in the Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisbon – Portugal, with a scholarship from Brazil Government - Ciência sem Fronteiras Program. She also did an internship at Ferreira Lapa Laboratory, in Lisbon, as a wine analyst.

After returning to Brazil, Lorena had a professional experience as a Professor at Centro Territorial de Educação Profissional do Portal do Sertão, Bahia – Brazil, for a semester. At the end of her undergraduate program, she did an internship at Consultali Treinamentos e Consultoria em Alimentos Ltda, Bahia - Brazil, a consulting company for the food industry.

Lorena joined the Master's Program in Food Science at the Universidade Federal Bahia, Bahia-Brazil (2014-2016), with FAPESB scholarship. During this period, she worked in collaboration with different projects of the research group. After master's degree she had an opportunity to work as a Professor at Serviço Nacional de Aprendizagem Industrial, SENAI, Bahia – Brazil (2016).

In 2017, Lorena enrolled her PhD in the Doctoral Program in Food Engineering Sciences at Faculdade de Zootecnia e Engenharia de Alimentos – Universidade de São Paulo (FZEA- USP), Brazil, with FAPESP scholarship. Within this period, she went on a student exchange in the Ohio State University, Ohio - USA, with BEPE/FAPESP scholarship (2019-2020). She worked on her project and also collaborated with other projects of the research group.

Nowadays, she is working at The Good Food Institute as an Analyst of Food Science and Technology.

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**“Let food be the medicine be the food.”**

**Hippocrates**

## RESUMO

PINHO, L. S. **Obtenção, encapsulação e aplicação de extrato rico em carotenoides da casca de guaraná (*Paullinia cupana*).** 2021. 186 f. PhD. Tese - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

A tendência de reaproveitamento de subprodutos para minimizar a tensão global, relacionada ao crescimento populacional e à expansão da produção de alimentos, continua aumentando. Embora muitas estratégias de gestão de resíduos tenham sido desenvolvidas, é necessário valorizar esses subprodutos com baixo custo e menores ameaças ambientais. No Brasil, com a biodiversidade da Amazônia e o próspero agronegócio, é fundamental buscar novas fontes de compostos bioativos que possuam méritos econômicos e ambientais. Como a maioria destes compostos são sensíveis ao calor e ao oxigênio, a encapsulação destes componentes em micropartículas é um pré-requisito para aplicações em alimentos. Assim, o presente estudo teve como objetivo extrair compostos bioativos da casca de guaraná, subproduto da agroindústria, e utilizá-los como matéria-prima para o desenvolvimento de micropartículas que possam ser incorporadas a produtos funcionais. Na primeira seção deste trabalho, foi investigada a caracterização da casca de guaraná, incluindo sua composição, como teor de alcalóides, compostos fenólicos e carotenoides. As condições de extração também foram avaliadas para estabelecer os parâmetros ideais para a recuperação dos pigmentos (β-caroteno e luteína). Nas condições ideais, a capacidade antioxidante dos extratos foi avaliada por meio de ensaios *in vitro*. Os resultados indicam que os subprodutos do guaraná são excelentes fontes de carotenoides, os quais possuem forte capacidade antioxidante e, possivelmente, ocasionam efeitos promotores da saúde. No entanto, como pigmentos naturais instáveis, os carotenoides são altamente suscetíveis à oxidação e isomerização após a extração. Para aumentar sua estabilidade, na segunda parte deste trabalho, o extrato rico em carotenoides foi encapsulado por *spray drying* (SD), *chilling* (SC) e pela combinação das duas técnicas (SDC) para formar micropartículas. As propriedades físico-químicas das micropartículas foram investigadas, e sua estabilidade foi avaliada durante 90 dias de armazenamento. Micrografias eletrônicas de varredura revelaram forma esférica e heterogeneidade de tamanho das amostras. Além disso, a análise dos termogramas indicou que o material carreador e o processo de encapsulamento afetam o comportamento térmico das micropartículas. Da mesma forma, os dados de sorção de água por sorção de vapor dinâmico (DVS) das amostras foram principalmente relacionados aos

agentes carreadores usados para a encapsulação. Apenas alguns estudos abordaram se a encapsulação poderia proteger os carotenoides sob estresse termo-mecânico. Essas informações estão relacionadas à sua aplicação, uma vez que a temperatura e o cisalhamento são usados principalmente durante a produção de alimentos. Assim, a última parte deste estudo conduziu um processamento para simular as condições de extrusão e produzir uma pasta de aveia enriquecida com micropartículas carregadas com carotenoides. Após o processamento, avaliou-se a influência das micropartículas nas propriedades da pasta de aveia. Observou-se que a retenção do β-caroteno foi maior do que a da luteína após o tratamento. Condições menos severas (temperatura e cisalhamento) levaram a maior estabilidade dos carotenoides. Este estudo propôs que a encapsulação e aplicação de carotenoides em alimentos à base de amido de aveia, que foi processado em altas temperaturas e cisalhamento, têm potencial para desenvolver produtos funcionais. No entanto, a incorporação de novos ingredientes pode afetar as propriedades sensoriais dos produtos finais e a bioatividade dos compostos. Por este motivo, trabalhos futuros especificamente no desenvolvimento de produtos alimentícios com atributos de qualidade desejáveis e benefícios nutricionais são essenciais.

**Palavras-chave**: *Paullinia cupana*; carotenoides; encapsulação; extrusão; subprodutos.

## ABSTRACT

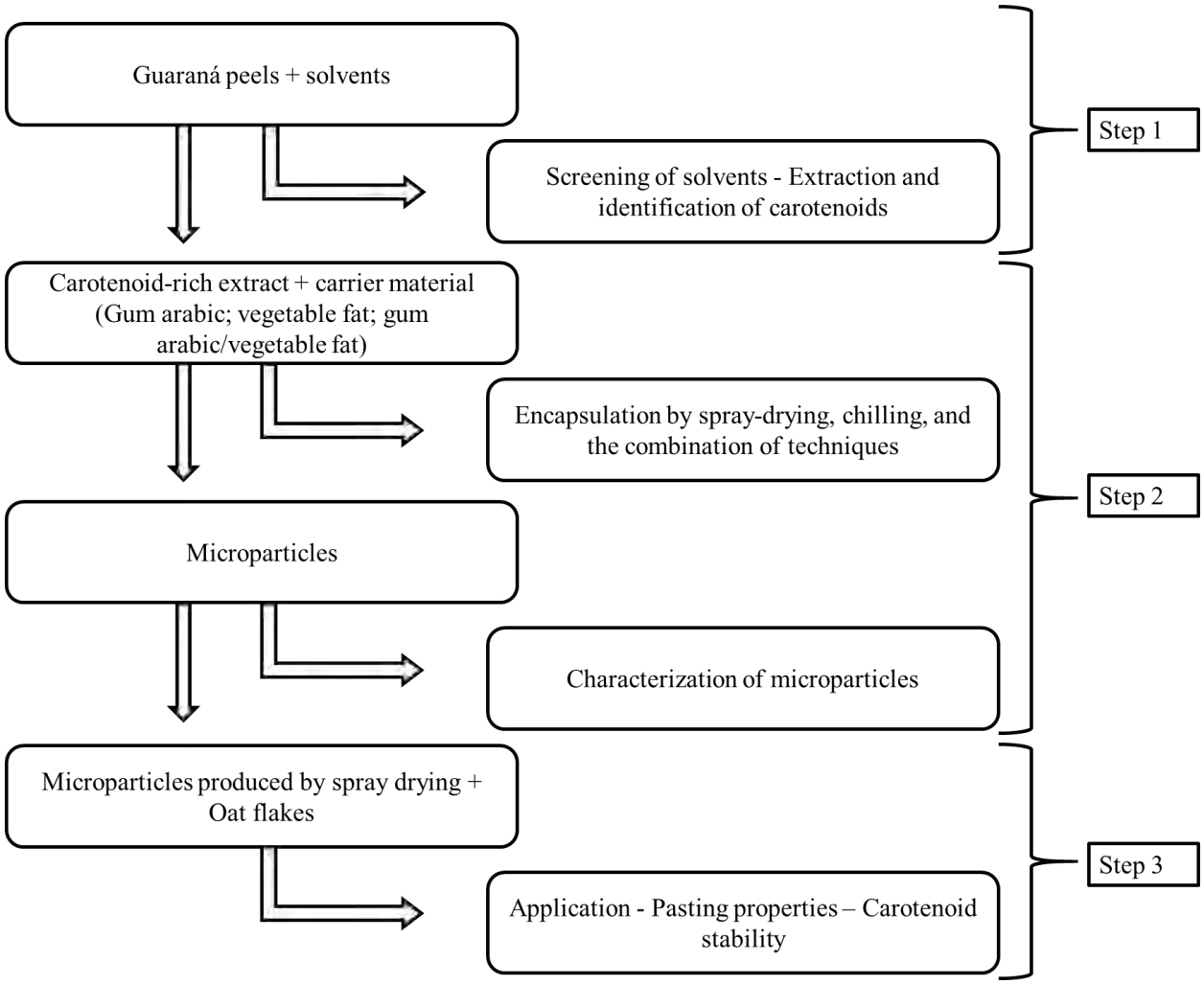
PINHO, L. S. **Obtaining, encapsulation, and application of carotenoid-rich extract from guaraná peel (*Paullinia cupana*).** 2021. 186 f. PhD. Thesis - Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The trends to upcycle by-products to alleviate the global concern related to population growth and food production expansion keep increasing. Although many strategies in waste management have been developed, it is worthwhile to valorize these by-products with low cost and minor environmental threats. In Brazil, with the biodiversity of the Amazon Region and the prosperous agribusiness, seeking new sources of bioactive compounds that possess economic and environmental merits is crucial. As most bioactive compounds are vulnerable to heat and oxygen, entrapping them into microparticles is a pre-requisite for food applications. Thus, the present study aimed to extract bioactive compounds from guaraná peel, a by-product from the agroindustry, and use them as core materials to develop microparticles that can be incorporated into functional products. In the first section of this work, characterization of the guaraná peels was investigated, including their compositions, such as the content of alkaloids, phenolic compounds, and carotenoids. The extraction conditions were also assessed to establish the optimal parameters for the recovery of pigments (β-carotene and lutein). Under the optimal conditions, the antioxidant capacity of the extracts was measured using *in vitro* assays. The results indicate guaraná by-products are excellent sources of carotenoids that possess a strong antioxidant capacity and likely bring health-promoting effects. However, as unstable natural pigments, carotenoids are highly susceptible to oxidation and isomerization after extraction. To increase their stability, in the second part of this work, the carotenoid-rich extract was encapsulated by spray drying (SD), chilling (SC), and their combination (SDC) to form microparticles. The physicochemical properties of the microparticles were explored, and their stability was evaluated during 90 days of storage. Scanning electron micrographs revealed spherical shape and size heterogeneity of samples. Moreover, the analysis of thermograms indicated that the carrier material and encapsulation process affect the thermal behavior of the microparticles. Similarly, water sorption data from the samples' dynamic vapor sorption (DVS) mainly were related to the carrier agents used during encapsulation. Just a few studies approached whether encapsulation could protect the carotenoids under thermo-mechanical stress. Such information correlates with their application, as temperature and shear are mostly

used during food production. Thus, the last part of this study conducted pasting processing to simulate the extrusion conditions and produce oatmeal paste enriched with microparticles loaded with carotenoids. After the processing, the influence of microparticles on the pasting properties of the oatmeal paste was evaluated. We found β-carotene retention was higher than those of lutein after treatment. Less severe conditions (temperature and shear) led to higher stability of the carotenoid. This study proposed that the encapsulation and application of carotenoids into food based on oat starch, which was processed at higher temperatures and shear, has the potential to develop functional products. However, incorporating new ingredients can affect the final products' sensory properties and the bioactivity of mixed compounds. For this reason, future works specifically on the development of food products with desirable quality attributes and nutritional benefits are essential.

**Keywords:** *Paullinia cupana*; carotenoids; encapsulation; extrusion; by-products.

## SCHEMATIC REPRESENTATION OF EACH STEP OF THIS STUDY



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## ACRONYMS AND ABBREVIATIONS

*a** – *Redness*
*b** – *Yellowness*
ABTS – 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid
°C – Degree Celsius
DPPH – 2,2-diphenyl-1-picrylhydrazyl
DSC – Differential scanning calorimetry
DVS – Dynamic vapor sorption
FRAP – Ferric reducing ability of plasma
g – Gram
*g – Gravitational force equivalent*
GPE – Guaraná peel extract
GRAS – Generally Recognized as Safe
h – Hour
HPLC – High performance liquid chromatography
HSP – Hansen solubility parameters
L – Liter
L* – Lightness
Ln – Natural logarithm
mg – Milligram
min – Minute
mL – Milliliter
MPa – Mega Pascal
ORAC – Oxygen radical absorbance capacity
QTOF – Quadrupole time of flight mass spectrometer
rpm – Revolutions per minute
s – Second
SME – Specific mechanical energy
µg – Microgram
µm – Micrometer
v/v – Volume/volume
w/v – Weight/volume
w/w – Weight/weight

**SUMMARY**

# 1 CHAPTER 1

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## INTRODUCTION

One-third of the food produced in the world is either wasted or lost (FAO, 2021). The waste generation in food industries contributes to this fact, and it is an urgent matter considering all-embracing sectors of food processing, including animal- and vegetable-derived. In the animal-derived wastes, most items consist of carcasses, hides, hoofs, heads, feathers, offal, viscera, bones, fat, and meat trimmings (Waldron, 2009). Whereas in vegetable-derived food manufacturing, such as peels, stems, seeds, pomaces, and trimmings residues are dominant after extraction of oil, starch, juice, and sugars ( Helkar, Sahoo, & Patil, 2016; Nasrin & Matin, 2017; Ben-Othman, Jõudu, & Bhat, 2020).

Agribusiness in Brazil is considered one of the drivers of the economy. Therefore, the agroindustries have expanded significantly, and, as a result, the increase in production creates a concurrently large amount of by-products and residues, which could be used more efficiently. In this scenario, food processing is the primary sector responsible for the generation of the massive amount of by-products, which encompass the fruit, vegetable, wine, and beer industries (Bordiga, Travaglia, & Locatelli, 2019; Puligundla, Mok, & Park, 2020; Villacís-Chiriboga, Elst, Van Camp, Vera, & Ruales, 2020, Comunian et al., 2021).

The different types of wastes require appropriate management, and in light of this, by-products and residues have distinct conceptions according to the market and applications. The former has some economic value, rarely recycled or used as animal feed (da Silva et al., 2020). On the other hand, the residue corresponds to the low-value products, which are eventually disposed of in landfills, composted, or incinerated (Pavlenko & Searle, 2020). In general, these under-utilized materials generate a negative response by releasing into the environment and causing pollution. This occurs due to the characteristics of the organic wastes that affect the ecosystem (Kuryntseva, Galitskaya, & Selivanovskaya, 2016).

The characteristics of by-products are the primary concern regarding the perishability of those materials, including poor biological and oxidative stability, the concentration of organic compounds, high water activity, and enzymatic activity. The high lipid content leads to rapid oxidation, besides the presence of fatty acids can enhance the issues to handle with food waste, mostly related to the rancid-smelling compounds produced over time (Arancon et al., 2013).

Despite the environmental issue, not many efforts have been devoted to food waste. From an economic point of view, the surplus cost of waste processing and time-consuming may be some investments that the manufacturer is not willing to consider. Generally, due to the material specificity, an additional food-processing step is not attractive to the industries

(Waldron, 2007). However, the difficulties of waste usage are rather unreasonable, given that those materials are cheap and available. Besides, they are initially food-grade and potentially rich in bioactive compounds (Panusa et al., 2013).

Considering the current landscape, it is also important to mention the global food crisis. With the increase of population, the demand and price of food surely will also increase. Taking all together, the concept of circular bioeconomy through the recycle would emerge as a sustainable alternative; meanwhile, the effectiveness of those materials from food processing would complement the global food supply to alleviate this problem (Martillanes et al., 2020). The upcycling would succeed in an expansion, which would demand the development of new products for recent or new markets.

Developing strategies to use all of these materials competently can minimize a substantial loss of great resources, even though it is challenging. There are certain approaches to upcycling, but they consist of modest uses and do not explore the proper value of the material. Thus, research on new pathways to deal with waste management is crucial to reducing costs and promoting waste valorization. In addition, the trial and scale-up for the functional food development using by-products are fields to be explored, as necessary to endorse its feasibility by food industries.

Developing sustainable processes based on the valorization of food waste is important to ensure the stability of those materials and consider the refinement of the original resource (Morone et al., 2019). In this respect, refinement initially involves adequate transportation and refrigerated storage of by-products. In addition, pre-treatment using drying (Stramarkou, Papadaki, Kyriakopoulou, & Krokida, 2017) or irradiation processes (Casarotti, Borgonovi, Batista, & Penna, 2018) may reduce the degradative reactions and facilitate the extraction of the bioactive components contained in the material. Ultimately, the isolation of bioactive components can be performed through the methodologies and processes, which make the possibility of their recovery, besides their application in food formulations (Galanakis, 2012).

The bioactive components generally coexist with other compounds; therefore, their recovery process from the food matrix must be efficient to extract the highest content of the desired component. Moreover, a comprehensive characterization of these materials is crucial to attribute better the functional activities of the by-products or their extracts to the responsible compounds. Furthermore, extraction techniques must also be suitable to perform with the sustainability standpoint of revalorizing by-products. For that, different methods have been

used, consuming eco-friendly solvents, minimizing time extraction, and improving the process conditions to enhance the extraction yield (de la Luz Cádiz-Gurrea et al., 2020).

## 1.1 By-products as a source of bioactive compounds

The continuously growing market attentive to the enormous variety of fruits from the Amazon region, such as açaí, buriti, camu-camu, cupuaçu, and guaraná, has been stimulating the intensive exploration of them by Brazilian agroindustries. The sensory features and their nutritional value related to health profits are the keys to the prosperous interest of the Amazonian exotic fruits. Considering that by-products fraction comprises almost up to 60% of the entire vegetable (Ayala-Zavala et al., 2011), the quantities of food wastes have been increased, and concomitantly the necessity to design practical projects to replace underutilized by-products into the supply chain. The bioactive compounds recovered from by-products of some native fruits from Amazon are summarized in Table 1.

Tropical fruits from Amazon region are important sources of polysaccharides, minerals and vitamins. Furthermore, bioactive compounds are the main drivers responsible for elevating the demand by countless companies (Xiong et al., 2020). Among the diversity, açaí berry (*Euterpe oleracea*) is one of the notable North species, creating new markets and processes. The consumption of the edible part requires industrial treatment that involves pulp extraction (16%) from the highly fibrous fruit, generating by-products composed of some pulp, seeds, and peels (Buratto, 2019). In addition to açaí, babaçu (*Orbignya speciosa)* coconut is a wide species used to extract its oil, rich in natural antioxidants. However, the oil represents 7% of the fruit weight, and the by-products from the processing can be used to recover bioactive components or produce ethanol (Cinelli et al., 2014; da Silva et al., 2020). Similarly, the oil extraction from buriti (*Mauritia flexuosa*) fruit generates massive by-products, including peels, endocarp, and pulp. This oil contains high contents of fatty acids and carotenoids, which come from the pulp and are mainly applied in pharmaceutical and cosmetics products (Resende, Franca, & Oliveira, 2019). Moreover, the by-products have great potential for underutilization as an additive in new functional foods.

**Table 1.** Bioactive compounds present in fruit by-products from the Amazon region

| Fruit | By-product (Fruit part) | Bioactive compounds | References |
|---|---|---|---|
| Açaí (*Euterpe oleracea*) | Seeds | Procyanidin B1 and B2, catechin, and epicatechin | Melo et al., 2021 |
| | Pulp and seeds | Phenolic, proanthocyanin, and anthocyanin | Xiong et al., 2020 |
| Babaçu (*Orbignya speciosa*) | Peels | Phenolic compounds | Maniglia & Tapia-Blacido, 2016 |
| Buriti (*Mauritia flexuosa*) | Peels, pulp, endocarp | Carotenoids, proanthocyanidins, and phenolic compounds | Resende, Franca, & Oliveira, 2019). |
| Camu-camu (*Myrciaria dubia*) | Seeds, peels, and residual pulp | Phenolic compounds | Das Chagas et al., 2021 |
| Cupuaçu (*Theobroma grandiflorum*) | Seeds | Phenolic compounds | Da Costa et al., 2020 |
| | Seeds | Phenolic compounds | Silva da Costa et al., 2019 |
| Inajá (*Attalea maripa*) | Pulp | Catechins and procyanidins | De Souza Silva et al., 2021 |
| Peach palm (*Bactris gasipaes*) | Pulp and seeds | Carotenoids | Matos et al., 2019 |
| | Stem portion, leaf sheaths, and peels | Phenolic compounds | Giombelli, Iwassa, da Silva, & Barros, 2020 |
| Piquiá (*Caryocar villosum*) | Peels | Phenolic compounds | Roxo et al., 2020 |
| Tucumã (*Astrocaryum vulgare*) | Pulp and seeds | Carotenoids | Matos et al., 2019 |
| Guaraná (*Paullinia cupana*) | Peels | Carotenoids, phenolic compounds, and alkaloids | Pinho et al., 2021 |
| | Peels and depleted seeds | Ascorbic acid, tannins, and phenolic compounds | Santana, Zanini, Macedo, 2020 |

Reference: Own source.

The advance of new markets focus on functional activities is favored by the presence of bioactive ingredients in various other species. Foods derived from camu-camu (*Myrciaria dúbia)* and cupuaçu (*Theobroma grandiflorum)* are progressively consumed because of their nutritional benefits and sensorial characteristics (Pugliese et al., 2013). Industrial processing involves major pulp part, but exploitation of their by-products, such as peel and seeds, have been growing. Furthermore, the commercial interest of inajá (*Attalea maripa)* related to edible oil extracted from the fruit nut emerges as a source of organic acids, carotenoids and phenolic compounds; however, the pulp also has high potential, which lies in the content of natural antioxidants (Barbi et al., 2019).

Peach palm (*Bactris gasipaes*) is extensively cultured in Brazil, and the edible portion of the stem consists of the heart-of-palm, which is commercialized as a minimally-processed product or as a canned vegetable (Neri-Numa et al., 2018). The agroindustrial by-products include pulp, seeds, stem portion, leaf sheaths, and peels. Such materials contain high-value molecules, for example, carotenoids and phenolic compounds. In addition, piquiá (*Caryocar villosum)* and tucumã (*Astrocaryum vulgare*) are other fruits from the Amazon region, used for oil extraction with nutritional effects considering the bioactive composition. Hence, previous studies have demonstrated the upcycling of their by-products through the recovery of carotenoids and phenolic compounds (Matos et al., 2019; Roxo et al., 2020).

Guaraná (*Paullinia cupana*) is another Brazilian fruit native to the Amazon region known for its therapeutic effects, including its stimulant and healing properties (Schimpl, da Silva, Gonçalves, & Mazzafera, 2013). The fruit of guaraná comprises a red peel, containing an inner with brown/black seed partially covered by a white aril (Figure 1).

**Figure 1**. Guaraná fruit

Reference: Own source.

Globally, Brazil is the largest producer of guaraná, with minor areas planted in Venezuela and Peru. Brazilian guaraná production is predominant in seven states, with Bahia, Amazonas, and Mato Grosso representing 95.1% of national production in 2019. Smaller-scale production occurs in the states of Rondônia, Pará, Acre, and Santa Catarina. The national productivity in 2019 was 2761 t, showing an increase of 3.7% compared to the previous year. Guaraná produced in Brazil, in addition to being processed and used by the national industries, is commercialized to the international market (CONAB, 2021).

The industrial handling of guaraná refers only to the seeds that are processed and either directly consumed or used as ingredients in cosmetics, pharmaceuticals, energy drinks, and soda industries. The application of guaraná seed attributed to the stimulant property and health benefits is associated with its methylxanthines (caffeine, theophylline, and theobromine) and polyphenols (catechin, epicatechin, and epicatechin gallate) contents. Taking together, the composition of guaraná seed is a well-known source of different powerful antioxidant compounds.

Given that only seeds are used, the actual processing entails the generation of valuable by-products from guaraná fruits. Indeed, as mentioned earlier, the project of waste management combined with the revalorization of agroindustrial by-products arises as an important tactic to explore the bioactive potential of these materials. Few studies are reported in the literature regarding the upcycling of guaraná by-products, in which the prevalent compounds include carotenoids, polyphenols, alkaloids, and organic acids (Santana, Zanini, & Macedo, 2020; Pinho et al., 2021). Moreover, they also make evident the high potential of these materials on the topic of their antioxidant capacity.

## 1.2 Bioactive compounds

The active compounds in fruit by-products can be explored, focusing on a sustainable destination as food additives, since the interest in functional ingredients from natural sources is increasing. Among the main ways of using by-products as nutraceuticals, some of them reside in the usage of materials directly incorporated into new food products or the extraction of active components for later application. Thus, to better understand the origin of the phytochemicals, it is important to explore the biological activities of plants that result in a wide group of bioactive components.

Plants produce a broad assortment of organic compounds that can be divided into two domains. The primary metabolites are the chemical substances responsible for growth and development. Some examples of primary metabolites include carbohydrates, amino acids, proteins, and lipids (Wu & Chappell, 2008). In contrast, the secondary metabolites have no function in growth, although they may influence the survival ability of plants (Lattanzio, 2013; Rosa, Moreno-Escamilla, Rodrigo-García, & Alvarez-Parrilla, 2019). Such metabolites are formed by different taxonomic groups, according to the specific need of the species, and have particular chemical structures.

In the secondary metabolites group, the substances can act in biological systems, playing as bioactive compounds. They are classified into three categories: (a) alkaloids, (b) phenolic compounds, and (c) terpenes and terpenoids. All these categories cover the well-known active substances obtained from plants, which may have pharmacological or toxicological effects in humans and animals (Bernhoft, 2010).

In human metabolism, some reactive species are produced, including reactive oxygen species (ROS) and nitrogen-oxygen species (NOS). They can play an important role in physiological functions, as in phagocytosis mechanisms, with biological properties (da Silva et al., 2020). However, the oxidative stress caused by the excessive concentration of those reactive systems can overwhelm the organism and lead to serious harm. Thus, components with antioxidant potential can act minimizing/avoiding oxidative processes that may favor some chronic diseases, including diabetes, neurological deficits, cardiovascular risk and cancer (Correia, Borges, Medeiros, & Genovese, 2012). These reactions involve the scavenging of free radicals, chelation of heavy metals, and deactivation of oxygen by reducing hydroperoxides (da Silva et al., 2020).

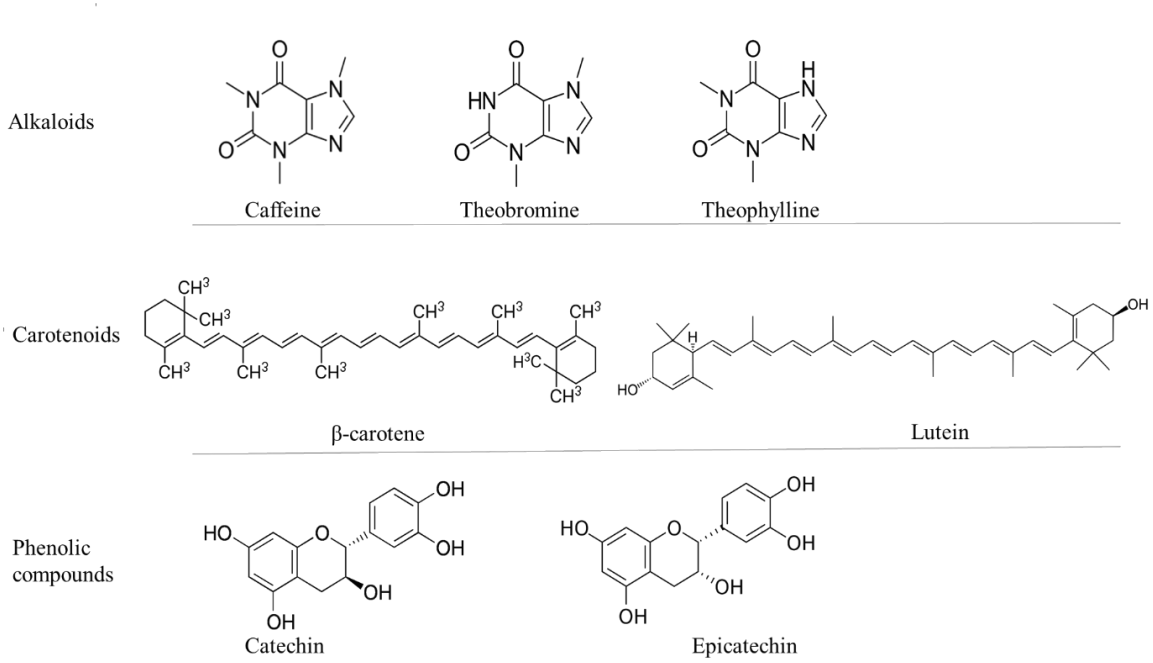
Some of the most common bioactive compounds, with claimed nutritional value related to antioxidant capacity and health-promoting activities, are alkaloids, phenolic compounds and carotenoids (Figure 2). Among them, alkaloids consist of an expansive group of secondary metabolites found mostly in plant-kingdom but also produced by micro-and marine organisms. This group comprises approximately 12000 natural N-containing components and includes compounds with diverse structures.

Overall, alkaloids can be defined as crystalline substances found as free bases, salts with organic and inorganic acids, glycosides, among others. The presence of nitrogen in the molecule is the main character that represents its structure. Generally, this nitrogen has an origin in amino acids and is associated with a heterocyclic ring (Bribi, 2018). Besides nitrogen, carbon, and hydrogen, most alkaloids have oxygen, and these elements can confer to them the potential to act as antioxidant agents.

According to the evidence indicating the antioxidant capacity of alkaloids, such as the xanthine derivatives (caffeine, theobromine, and theophylline), it is important to mention the physiological activity of these compounds. In light of this, Azam, Hadi, Khan, & Hadi (2003) reported that the mentioned alkaloids suppressed the oxidative DNA breakage and the production of hydroxyl radicals. However, the same compounds could act as prooxidant agents if the concentration of the alkaloids and copper ions were high in the system (Azam, Hadi,

Khan, & Hadi, 2003). Moreover, Petrucci, Zollo, Curulli, & Marrosu (2018) claimed the hydroxyl free radical scavenging capacity and the potential antioxidant power towards ROS via electron-transfer for caffeine. The benefits to human health regarding these methylxanthines include psychoactive and cardioprotection activities, preventing neurodegenerative diseases, diabetes, and acting as anti-cancer and enhancement of fertility agents (Beaudoin & Graham, 2011; Oñatibia-Astibia, Franco, & Martínez-Pinilla, 2017; Monteiro, Alves, Oliveira, & Silva, 2019)

**Figure 2.** Chemical structure of alkaloids, carotenoids, and phenolic compounds




Reference: Own source.

Another extensive group of secondary plants metabolites is the phenolic compounds or polyphenols, which currently cover more than 8000 structures. Several classification methods can be used, from general to more specific approaches (Santana-Galvez & Jacobo-Velázquez, 2018): (1) Flavonoids and non-flavonoids; (2) number of aromatic rings (Kabera, Semana, Mussa, & He, 2014); (3) number of hydroxyl groups (Kabera, Semana, Mussa, & He, 2014); (4) organization of carbon atoms in the molecule (basic carbon skeleton) (Evans & Harborne, 1991); (5) chemical structure, including functional groups, saturations, and type of bonds, among other methods. Although the combination of methods (4) and (5) is commonly used for

reasonable description (Pereira, Valentão, Pereira, & Andrade, 2009; Cheynier et al., 2013), the classification will be selected according to the purpose of the study. For this reason, the classification of phenolic compounds remains confusing and complex (Kabera, Semana, Mussa, & He, 2014).

The structure of phenolic compounds consists of a chain with an aromatic ring containing one or more hydroxyl substituents (Tsao, 2010). The antioxidant potential of these compounds is related to the degree of hydroxylation and position of these hydroxyl groups considering the carboxyl functional group (Balasundram, Sundram, & Samman, 2006). Their structure is an important factor associated with the radical-scavenging and metal-chelating capacity. Therefore, the antioxidant activity of phenolics can occur by redox reactivity, regarding their properties as hydrogen- or electron-donating agents. Consequently, the interaction between these compounds and reactive species can lead to a chemically stable form of the free-radical, interrupting the cycle of the generation of new radicals. Besides, the ability of phenolics as metal chelators, including iron and copper, can also eliminate metal-catalyzed free-radical generation (Pereira, Valentão, Pereira, & Andrade, 2009). Overall, the balance among these pathways is based on the reaction environment.

In addition to the antioxidant ability, polyphenol-rich diets have been related to various health benefits, including reduced risk of cardiovascular disease, obesity, diabetes and neurocognitive benefits. Phenolic acids, flavonoids, stilbenes, and tannins might prevent oxidative damage to biomolecules, which play crucial roles in the metabolism of the human body (Hollman, 2001; Ho, Ferruzzi, & Wightman, 2020)

Ultimately, carotenoids are the bioactive secondary metabolites known for their biological functions. These phytochemicals belong to the isoprenoids and generally have a 40-carbon skeleton. The structure of most carotenoids comprises a polyene chain with nine conjugated double bonds and a terminal group at both borders (da Silveira Vasconcelos et al., 2020). Photosynthetic organisms, including plants, cyanobacteria and algae, besides some non-photosynthetic prokaryotes and fungi, synthesize them (Maoka, 2020). Thus, since human beings and most animals cannot synthesize carotenoids, including these components in the diet is recommended to improve physiological activities (Engelmann, Clinton, Erdman, 2011; Rodriguez-Concepcion et al., 2018).

Carotenoids are classified majorly into two categories: carotenes, which are hydrocarbons chains (β-carotene and lycopene, for example), and xanthophylls that are derivatives whose contain one or more oxygen atoms in their molecule (lutein, astaxanthin, and

cryptoxanthin). Conjugated double bonds of carotenoids can absorb the energy from other molecules, and that is the reason why these compounds present antioxidant properties *in vivo* (Namitha & Negi, 2010). Moreover, the carotenoid end groups may have some effect on their polarity, favoring eventual interaction with biological membranes (Britton, 1995). Among the vast health benefits related to carotenoids, some of them comprise the anti-inflammatory properties, provitamin A activity, reduction of the symptoms of many diseases, such as cancer, Alzheimer, cerebral ischemia, macular degeneration, diabetes associated with obesity and hypertension (Bhatt & Patel, 2020; Nabi et al., 2020).

## 1.3 Extraction of bioactive compounds

Considering the health-promoting bioactive compounds, obtaining the extracts rich in them is an important step for further applications. The extraction process of these substances from by-products usually depends on the nature of the bioactive component, the matrix properties, and extraction parameters.

Taking into account that solvent influences the extraction efficiency (Cowan, 1999), the polarity of the component of interest is the main factor to consider for choosing the proper solvent. The extraction can be performed using different solvent systems formed from only one solvent or a mixture. However, polar solvents such as methanol, ethanol, or ethyl-acetate are more appropriate for hydrophilic components. While for more lipophilic components, dichloromethane or hexane consist a better choice. Given that plants are complex matrices and the extraction of specific components is challenging, the strategy of performing screening of solvents can lead to the solvents potentially suitable to obtain bioactive-rich extracts (Pinho et al., 2021).

Besides the molecular affinity, economic feasibility, public health, and environmental issues are other relevant topics to contemplate (Azmir et al., 2013). In light of this, processes have been re-thought to incite the use of ‘‘bio-solvents’’ and, in the meantime, assure the safety and quality of this extract. Furthermore, management of technology and the environment are some of the main concerns nowadays and is a matter of interest by different industries. For this reason, the use of solvents Generally Recognized as Safe (GRAS) for human consumption for extraction of biocomponents is not only an eco-friendly approach but also politically influential (de Oliveira, de Barros, & Gimenes, 2013).

In addition to defining a proper solvent system, some precautions must be conducted to guarantee that targeted compounds are not lost or destroyed over the extraction process. Regarding the extraction steps, they primarily embrace a pre-processing phase, which is also a crucial factor in determining the amount of bioactive compounds recovered from plant matrices. The operations involve pre-treatment through pre-washing, drying or freeze-drying, and milling of the sample. As a consequence, it can facilitate material homogenization, as well as improve the contact of the sample surface with the solvent and enhance the kinetics of extraction (Sunday, 2017).

The phytochemicals can be extracted from vegetable by-products using different methods that are classified into two main groups: novel and conventional techniques. Promising novel techniques include supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, pulsed electric field assisted extraction, pressurized liquid extraction, or a combination of them (Ghafoor, Park, & Choi, 2010; Liew, Ngoh, Yusoff, & Teoh, 2016; Luo et al., 2019; Pashazadeh et al., 2020). Some advantages are the extraction efficiency, selectivity, and kinetics of extraction. Additionally, the facility of automation and reduction in organic solvent favors the employment of these techniques for bioactive compounds extraction. However, shortcomings are associated with upscale extraction, which needs to consider the technique's safety, sustainability, and cost-effectiveness. In this way, conventional techniques are still usually used instead, since they correspond to inexpensive and feasible methods for bioactive compounds extraction at the industrial or pilot level (Sagar et al., 2018; Wen et al., 2020).

The conventional techniques consist of classical methods, which have been employed for a long time. They are based on the solvent system and the heat/mixing treatment. Namely, the most conventional techniques are Soxhlet, hydro-distillation, and maceration (Khoddami, Wilkes, & Roberts, 2013). Maceration is well-known as a low-cost, common and the simplest technique used for solid-liquid extraction of bioactive components and essential oils (Vieitez, Maceiras, Jachmanián, & Alborés, 2018). Although it is time-consuming and requires more solvent than some novel techniques, maceration does not require complicated equipment and skilled workers; besides, it is an energy-saving process (Rasul, 2018).

The maceration extraction process is composed of some operations, including: (1) the grinding of samples into small particles; (2) mixing samples with the appropriate quantity of the solvent; (3) diffusion of a solvent through the particles of sample to solubilize the bioactive present in the matrix; (4) recovering of the extract by pressing the solid residue; and (5) filtration

to remove impurities. Occasionally, agitation during maceration is applied to increase the recovery of the compounds. This supplementary operation can promote the maximum extraction yield and increase the diffusion of the solvent into the sample (Sasidharan et al., 2011; Azmir et al., 2013). Beyond this, solvent type, process temperature and extraction time are the most significant operating conditions to be configured either to improve the bioactive compound extraction technique or the quality of extracts for a potential application.

Indeed, the upcycling of by-products to extract bioactive components is a relevant initiative toward sustainable development since these materials are rich sources of phytochemicals (Galanakis, 2012). However, to successfully apply the bioactive-rich extract in food products, it is necessary to protect these compounds against conditions that may degrade them during processing. Phenolic and carotenoids, for example, can be degraded mainly by exposure to light, heat, oxygen and enzymes. Considering these aspects, microencapsulation emerges as a tool able to ensure the stability of those substances, facilitate their storage, transport, and, eventually, improve the efficacy of their application.

## 1.4 Microencapsulation of bioactive compounds from by-products

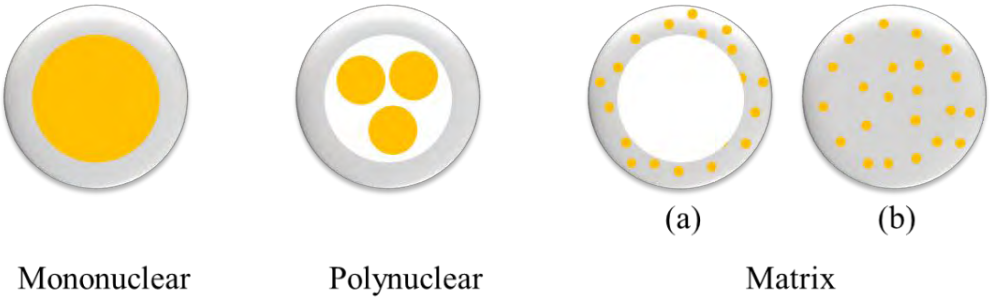
Microencapsulation comprises the entrapment of a component of interest in a secondary material. The compound to be protected, known as the core, nucleus, or active ingredient, can be in solid, liquid or gas form, composed of one or more substances. The carrier material, also called wall, coating, shell, or encapsulate agent, can be applied alone, as a combination, or with some additives (e.g., emulsifiers, surfactants) in a single or double layer.

Some factors are crucial to indicate the suitable carrier material, which includes the purpose of microencapsulation, sensory characteristics, properties of the active ingredient, core: carrier material compatibility, and potential application. Moreover, these materials may be natural or synthetic; however, they must be GRAS for human health (Nazzaro, Orlando, Fratianni, & Coppola, 2012). Among the specific requirements, the encapsulate agent has to be chemically inert and non-toxic (Tolve et al., 2016). In this way, the most used carrier material are polysaccharides (starch, maltodextrin, chitosan, gum arabic, alginate, and carrageenan), proteins (whey protein, gelatin and albumin), or lipids (wax, oils and hydrogenated vegetable fats) (Silva et al. 2014).

Microparticles formed by core:carrier material usually present functional properties covering structure and stability under process or storage. Their structure can be categorized into

microcapsules, which are related to the reservoir system, including mononuclear and polynuclear types, and microspheres that correspond to the matrix system (Figure 3). In the mononuclear type, the carrier material completely covers the core, while many cores are within the shell in the polynuclear. Nevertheless, in the matrix type, the core is homogenized and entrapped into the carrier material. The microsphere can exhibit hollowness inside (Figure 3a) or massive particle (Figure 3b). All these microspheres act as a physical barrier between the core and the external conditions and mask off-flavors, tastes, or colors, improving the dispersibility of some substances and providing a controlled release of them (Tolve et al., 2016).

**Figure 3.** Cross-sectional illustration of the different types of microspheres



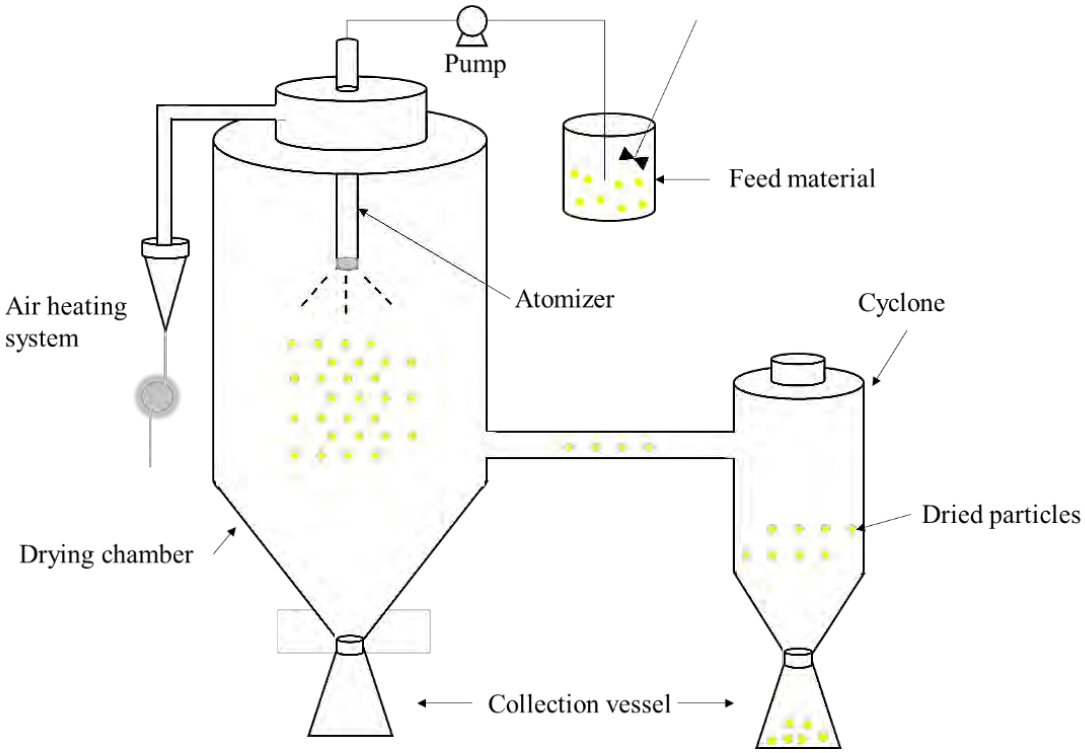

Reference: Own source.

For the encapsulation of phytochemical-rich extracts from vegetable sources that consist of complex matrices, not only the encapsulating agent but also the technique must be carefully selected. This is surely relevant to ensure adequate component protection. Furthermore, it is necessary to optimize the operating conditions to warrant obtaining microparticles with the desired properties.

Overall, microencapsulation techniques can be categorized into three groups: chemical (e.g. *in situ* polymerization), physicochemical (e.g. coacervation), and physic-mechanical (e.g. spray drying and chilling) (Giro-Paloma, Martínez, Cabeza, & Fernández, 2016; Shao, Xuan, Wu, & Qu, 2019). Although the existence of a large number of encapsulation techniques, some of the widely applied to food ingredients comprise spray drying and spray chilling, which are illustrated in Figure 4 and described in the following. This introduction will focus on these two techniques typically used for microencapsulation and applied in the current study. Moreover,

recent reviews about the microencapsulation of bioactive compounds were published (de Freitas Santos et al., 2021; Huang, Yuan, & Baojun, 2021).

**Figure 4.** Schematic illustration of spray drying and chilling processes




Reference: Own source.

## 1.5 Spray drying

Spray drying is the most traditional technique applied in the food industry to microencapsulate bioactive components. The process is defined by the transformation of liquid feed material into a dried powder (Gallo & Corbo, 2010). The procedure initially comprehends a mixture and homogenization of the active ingredient in the solution of a water-soluble polymer, which can generate a suspension, dispersion, or emulsion. Following this, the feed material is sprayed inside the hot chamber. The solvent evaporation from the droplet causes the solidification of the encapsulating agent, and matrix-type microparticles are formed. Lately, the dried particles are transported from the hot chamber by air to the cyclone or filter and, finally, collected.

After the atomization of bioactive-rich extract, some authors have reported the characteristics of microparticles, including process yield, encapsulation efficiency, hygroscopicity, moisture content, water activity, retention and stability of bioactive component after process or over storage (Daza et al., 2016; Ramakrishnan, Adzahan, Yusof, & Muhammad, 2018; Barrientos et al., 2021). All of the structural, morphological and functional features of particles produced by spray drying are associated with the processing conditions.

Among the diverse process parameters, namely, temperature, drying air flow rate, feed flow rate, atomizer pressure, carrier material, and concentration of the feed solution, the inlet air temperature present a prevalent effect on the final characteristics of the bioactive-rich particles (Schoubben et al., 2010). Furthermore, specifically, this operational condition requires attention, considering that carotenoids and phenolics are thermosensitive components, as aforementioned, and the heat-treatment can impact their retention during and after the process.

Chuyen et al. (2019) reported that during the encapsulation of gac peel oil at higher temperatures (180°C), the total carotenoid content of microparticles had the lowest retention compared to the minimum condition tested (temperature of 160°C). Interestingly, Moser, Ferreira, & Nicoletti (2019) indicated that the combination of higher temperature and feed flow rate (190°C and 6 mL/min) or lower temperature and feed flow rate (160°C and 2 mL/min) during atomization of buriti oil enhanced the carotenoid retention concerning the samples produced through milder settings. In the first case, the high temperature may induce rapid film formation, which mitigates the material migration to the particle surface. In the second case, the main strategy was to reduce exposure to heat and preserve the particle structure.

The formation of a crusted surface in the particles at higher temperatures during a short time favors rapid evaporation of water, and this can improve the chemical stability of the bioactive encapsulated. However, temperatures around 190 °C and 200 °C may contribute to either oxidation and/or degradation reactions or the creation of fissures, pores and rupture of the particles, diminishing the encapsulation efficiency (Corrêa-Filho et al., 2019). Additionally, the temperature also influences the moisture content and water activity of the resultant particles directly. Taken all together, these responses are relevant aspects extracted from spray-dried samples, given that they are associated with the quality, safety and capacity of preservation of active ingredients.

From a structural point of view, active compounds encapsulated can be found dispersed all over the volume of the particles, as the air-drying promotes the evaporation of the water present in the droplets. These particles are usually spherical, hollow and shrunken. Some studies

demonstrated the appearance of concavities and wrinkles in the particle surface and, this is also a result of the water elimination from the droplets.

One of the critical points of the process is the atomization into the vessel, as the size of the resultant particle depends on the droplets' magnitude, distribution, and velocity. Additionally, the nozzle atomizer and the physical properties of the feed material will affect the final product (Fujita et al., 2017; Sablania & Bosco, 2018). All these aspects can influence the occurrence of aggregates and non-uniform sizes, which can vary between 10-100 μm (Ribeiro, Shahgol, Estevinho, & Rocha, 2020). However, these phenomena may interfere with the sensory characteristics, considering the application of the particles in food products.

As external process parameters, the type of encapsulating agent and its concentration in the feed material consist of crucial factors that can impact the particles' features and stability. In view of the hydrophobicity of carotenoids, for example, the suitable carrier material must have the emulsifying capacity because the stability of the emulsion (feed material) may reflect on the particles properties. Although numerous carriers are used to atomize carotenoid-rich extracts, such as maltodextrin, whey protein and gum arabic, the latter is the most promising, as demonstrated, for instance, by Álvarez-Henao et al. (2018) and Tupuna et al. (2018).

The predominance of the carrier agent over the characteristics of particles loaded with bioactive compounds is attributed to the chemical structure of these materials. Particularly, gum arabic has a complex molecular structure composed of: (1) a highly branched polysaccharide chain; (2) a fraction formed by arabinogalactan–protein complex; and (3) a fraction with the highest protein content, which comprises a glycoprotein with amino acids chains of different sizes (Dror, Cohen, & Yerushalmi-Rozen, 2006). This natural composite is mainly derived from exudates of Acacia senegal and Acacia seyal trees. Due to its chemical domains and ramified chain, gum arabic possess good structural support and film-forming capacity, serves as an excellent emulsifier, and can act as a long-term stabilizer in products with oil-water interfaces (Montenegro, Boiero, Valle, & Borsarelli, 2012). Moreover, it has low viscosity in aqueous solution, satisfactory retention properties and may provide protection against higher temperatures (Chranioti & Tzia, 2014).

In fact, spray drying is a simple technique, in which some advantages include the speediness of the process, availability of equipment, the low-cost operation, and the ease of scale-up. Moreover, the decisive advantage is that it is a continuous process, whereas most processes used for encapsulation are batch. However, there are a few disadvantages related to the process, and one of the most significant harms is the high temperature of the system.

Considering that bioactive compounds, like phenolic and carotenoids, are heat-sensitive, this factor becomes an important issue to be ameliorated.

## 1.6 Spray chilling

Spray chilling is a technique in which an active ingredient is dispersed or emulsified in a lipid carrier material melted and atomized through a heated nozzle into a cooling chamber by injection of cold air or liquid nitrogen. The contact of the droplets with the cold air leads to their solidification and production of matrix-type microparticles. The resultant solid lipid particles are collected in the cyclone vessel (Champagne & Fustier, 2007; Saifullah et al., 2019). This process can be executed by using spray drying equipment.

The stabilization of the feed material (emulsion or suspension) may be necessary throughout the method, and this depends on the time in between sample preparation and atomization. For this reason, it is highly recommended the maintenance of feed material in agitation until it is pumped into an atomizer to ensure an efficient entrapment of the bioactive compounds in the matrix.

Besides, it is utter of importance to know which the main factors of process parameters can influence particle characteristics. The spray chilling process is dependent upon the material properties and operational parameters, such as (1) melting point of the carrier agent, (2) the temperature of the feed material melted during atomization, (3) chamber temperature, (4) atomizing air temperature and pressure, and (5) feeding flow of the molten mixture (Okuro, Matos Junior, & Favaro-Trindade, 2013; Đorđević et al., 2015). Predominantly, the ideal chamber condition consists of holding the system at a temperature below the melting point of the material over the complete atomization. About the carrier material, it is indispensable the selection of lipophilic materials with a melting point higher than room temperature. The droplet size and its surface area contribute to the cooling capacity and consequently its solidification.

During the process, the material already atomized undergoes different phases of cooling, droplet cooling, solidification and particle cooling. Conducting the temperature of the lipid material during solidification is essential since it influences the polymorphism of fats and consequently affects the release of the encapsulated bioactive compound. The crystallization process is associated with lipid chilling, in which rapid cooling leads to small and unstable crystals and a slow cooling favor the formation of the β-form, as the crystal packing of the stable form (Eldem, Speiser, & Altorfer, 1991).

Regarding the particles features, the production of particles with uniform size is attributed to high-speed atomization and low viscosity. Heat treatment applied to the feed material can lead to low viscosity, which produces small particles, while the addition of solids can result in larger particle sizes (Albertini, Passerini, Pattarino, & Rodriguez, 2008; Okuro et al., 2013). Spray chilling usually generates micrometric particles, with sizes ranging from 20 to 200 μm and impermeable to water. In addition, the particles are generally dense, do not exhibit porous on the surface, and tend to be intact under agitation.

Nevertheless, the encapsulation efficiency of spray chilling is associated with the type of atomizer, and this response can vary according to the equipment available. In fact, atomizers have resembled settings, and none of them is proper to process high viscosity molten mixture. Thus, to avoid occasional difficulty during the processing, either the melting point of the material or core: carrier ratio must be taken into account. In this way, it is recommended that the proportion of the active component in the feed material not exceed 30 and 50%, in the case of solid and liquid cores, respectively (Albertin, Passerini, Pattarino, & Rodriguez, 2008).

Spray chilling is an easy technique to upscale, and it does not involve organic solvents. Usually, lipophilic materials, such as fatty acids, triacylglycerols, waxes and hydrogenated vegetable fat, with a high melting point, are the most appropriate carrier agents selected to encapsulate bioactive ingredients by this process. For this reason, lipid particles obtained by spray chilling are insoluble in water, which may limit or not their applications in food (Đorđević et al., 2015). For example, chilled particles can be incorporated in bakery products, dry soup mixes, and food containing high levels of fat (Barbosa-Cánovas, Ortega-Rivas, Juliano, & Yan, 2005). Moreover, this technique is suitable for the encapsulation of thermosensitive ingredients, such as carotenoids and phenolics.

However, some drawbacks related to this process include low encapsulation efficiency of active components and the eventual expulsion of active ingredients during storage (Okuro et al., 2013). Besides that, the matrix-type particles produced by spray chilling often have some core material at the surface, which may reduce the bioactivity of the ingredient. In view of overcoming this feasible issue, one of some alternatives is to combine both techniques, spray drying and chilling. This strategy may minimize the loss of the potential activity of this component and open new possibilities for applications. In this way, the combination of spray drying and chilling has been demonstrated as an efficient tool to preserve the stability of prebiotics (Arslan-Tontul & Erbas, 2017) and functional oils (Fadini et al., 2018).

Since bioactive components, such as carotenoids, phenolics, and alkaloids, have nutritional properties, including provitamin A activity and antioxidant capacity, the main purpose of incorporating microparticles into conventional food is to add value to products. The application of microparticles obtained through different encapsulating methods in a pilot-scale model promotes the understanding of the behavior of microparticles in food, such as the storage stability after incorporation into the product. Furthermore, information collected in relation to the physicochemical, nutritional and sensory properties of fortified foods is also highly relevant in order to assess their potential for applications at an industrial level.

## 1.7 Application of microparticles loaded with bioactive compounds

The incorporation of bioactive compounds in food products allows the transformation of conventional products into functional foods. Thus, functional foods contain a variety of nutrients that can provide health benefits to consumers. In general, functional foods are related to the concepts of (i) products with the ability to offer health benefits in addition to basic nutrition, (ii) foods rich in natural ingredients included in the daily diet that exhibit effects on specific biological functions, and (iii) foods that act to regulate physiological processes and reduce disease risks (Onwulata, 2013). Although functional foods play an important role in the state of well-being and health, they are not medicines, therefore, have no therapeutic effects.

Currently, the food industry has developed functional foods in response to the demand of consumers who are increasingly interested in health benefits (Jacobs & Tapsell, 2007). In fact, research in different areas of science is providing and highlighting the role of the bioactive components, which also can attribute attractive color and technological properties to the product.

Polyphenolic extract of pomegranate peel, when encapsulated using maltodextrin, showed preferable characteristics and increased the shelf life of the cupcake (Sharayei, Azarpazhooh, & Ramaswamy, 2020). Besides, Bernardes et al. (2019) reported that the incorporation of bioactive-rich microparticles into the gelatin was favorable as long as it ensured protection to them and provided the best gelatin color over storage.

Coronel-Aguilera & Martin-Gonzalez (2015) investigated the addition of β-carotene powder obtained by spray drying, followed by fluidized bed coater into yogurt as a model system. The authors compared the enriched product with commercial peach yogurt. The results showed that the total color parameters were compared with the standard values, and the new

product was stable in an acidic medium over four weeks of storage at 4 °C. Another study reported the development of functional foods, in which yogurt, pasteurized milk, and cake were enriched with encapsulated gac oil using whey protein concentrate and gum arabic. After the incorporation of microparticles, the products presented minimum color change, as well as a slight degradation of β-carotene and lycopene during storage. According to the authors, the degradative reactions were mostly driven by auto-oxidation, photo-oxidation, and photo-isomerization through the storage period (Tuyen, Nguyen, Roach, & Stathopoulos, 2015). Interestingly, a ready-to-serve beverage was produced incorporating microencapsulated extract of marigold flowers into the water. Physicochemical and phytochemical characteristics of the lutein-rich product suggested its potential as an alternative to stimulate the daily intake of this bioactive compound (Pal & Bhattacharjee, 2018).

Moreover, encapsulated annatto seeds extract was applied in an isotonic tangerine soft drink. The study demonstrated that the addition of microparticles contributed to the color and increased carotenoid stability of the new beverage during storage under accelerated conditions (heat and light) (Tupuna-Yerovi et al., 2020). According to the examples above presented, most of the researches upon the application of encapsulated bioactive compounds is promising. It is demonstrated by the effective protection reached after encapsulating techniques and the feasibility of the process.

In order to produce bioactive ingredients for the food industry, microencapsulation also has been successfully employed for extrusion processes focused on the development of functional products. Since the extrusion cooking process might induce the degradation/oxidation of compounds with antioxidant capacities such as carotenoids, mainly attributed to the heat-mechanical stress, the microencapsulation can improve the stability of those compounds over processing and storage. Regarding this topic, the influence of the extrusion parameters on carotenoid stability will be further discussed in the next chapter.

## References

Albertini, B., Passerini, N., Pattarino, F., & Rodriguez, L. (2008). New spray congealing atomizer for the microencapsulation of highly concentrated solid and liquid substances. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*(1), 348-357. https://doi.org/10.1016/j.ejpb.2007.09.011

Álvarez-Henao, M. V., Saavedra, N., Medina, S., Cartagena, C. J., Alzate, L. M., & Londoño-Londoño, J. (2018). Microencapsulation of lutein by spray-drying: Characterization and stability analyses to promote its use as a functional ingredient. *Food Chemistry*, *256*, 181-187. https://doi.org/10.1016/j.foodchem.2018.02.059

Arancon, R. A. D., Lin, C. S. K., Chan, K. M., Kwan, T. H., & Luque, R. (2013). Advances on waste valorization: new horizons for a more sustainable society. *Energy Science & Engineering*, *1*(2), 53-71. https://doi.org/10.1002/ese3.9

Arslan-Tontul, S., & Erbas, M. (2017). Single and double layered microencapsulation of probiotics by spray drying and spray chilling. *LWT*, *81*, 160-169. https://doi.org/10.1016/j.lwt.2017.03.060

Ayala-Zavala, J., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W., ... & González-Aguilar, G. A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, *44*(7), 1866-1874. https://doi.org/10.1016/j.foodres.2011.02.021

Azam, S., Hadi, N., Khan, N. U., & Hadi, S. M. (2003). Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. *Medical Science Monitor*, *9*(9), BR325-BR330.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99, 191–203. https://doi.org/10.1016/j. foodchem.2005.07.042.

Barbi, R. C. T., de Souza, A. R. C., Hamerski, F., Teixeira, G. L., Corazza, M. L., & Ribani, R. H. (2019). Subcritical propane extraction of high-quality inajá (Maximiliana maripa) pulp oil. *The Journal of Supercritical Fluids*, *153*, 104576. https://doi.org/10.1016/j.supflu.2019.104576

Barbosa-Cánovas, G. V., Ortega-Rivas, E., Juliano, P., & Yan, H. (2005). *Food powders*.

Barrientos, M. A. E., Maeda, J. M. K., Chaves, I. E., Tulini, F. L., de Souza, V. B., Thomazini, M., ... & Favaro-Trindade, C. S. (2021). Production of vitex (Vitex agnus-castus L.) extract in powder form using spray-drying: Potential for the production of functional foods. *Journal of Food Processing and Preservation*, *45*(4), e15333. https://doi.org/10.1111/jfpp.15333

Beaudoin, M. S., & Graham, T. E. (2011). Methylxanthines and human health: epidemiological and experimental evidence. *Methylxanthines*, 509-548. https://doi.org/10.1007/978-3-642-13443-2_21

Ben-Othman, S., Jõudu, I., & Bhat, R. (2020). Bioactives from agri-food wastes: Present insights and future challenges. *Molecules*, 25(3), 510. https://doi.org/10.3390/molecules25030510

Bernardes, A. L., Moreira, J. A., Tostes, M. D. G. V., Costa, N. M. B., Silva, P. I., & Costa, A. G. V. (2019). In vitro bioaccessibility of microencapsulated phenolic compounds of jussara (Euterpe edulis Martius) fruit and application in gelatine model-system. *LWT*, *102*, 173-180. https://doi.org/10.1016/j.lwt.2018.12.009

Bernhoft, A. (2010). A brief review on bioactive compounds in plants. *Bioactive compounds in plants-benefits and risks for man and animals*, 50, 11-17.

Bhatt, T., & Patel, K. (2020). Carotenoids: potent to prevent diseases review. *Natural Products and Bioprospecting*, *10*(3), 109-117. https://doi.org/10.1007/s13659-020-00244-2

Bordiga, M., Travaglia, F., & Locatelli, M. (2019). Valorisation of grape pomace: an approach that is increasingly reaching its maturity–a review. *International Journal of Food Science & Technology*, *54*(4), 933-942. https://doi.org/10.1111/ijfs.14118

Bribi, N. (2018). Pharmacological activity of alkaloids: a review. *Asian Journal of Botany*, *1*(1), 6. doi:10.63019/ajb.v1i2.467

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, *9*(15), 1551-1558.

Buratto, R. T. (2019). Valorization of Euterpe oleracea Mart.(açaí) by-product: characterization, extraction and formulation. Available in: https://core.ac.uk/download/pdf/286337792.pdf

Casarotti, S. N., Borgonovi, T. F., Batista, C. L., & Penna, A. L. B. (2018). Guava, orange and passion fruit by-products: Characterization and its impacts on kinetics of acidification and properties of probiotic fermented products. *LWT*, *98*, 69-76. https://doi.org/10.1016/j.lwt.2018.08.010

Champagne, C. P., & Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology*, *18*(2), 184-190. https://doi.org/10.1016/j.copbio.2007.03.001

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. *Plant physiology and biochemistry*, *72*, 1-20. https://doi.org/10.1016/j.plaphy.2013.05.009

Chranioti, C., & Tzia, C. (2014). Arabic gum mixtures as encapsulating agents of freeze-dried fennel oleoresin products. *Food and Bioprocess Technology*, *7*(4), 1057-1065. https://doi.org/10.1007/s11947-013-1074-z

Chuyen, H. V., Roach, P. D., Golding, J. B., Parks, S. E., & Nguyen, M. H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, *344*, 373-379. https://doi.org/10.1016/j.powtec.2018.12.012

Cinelli, B. A., Lopez, J. A., Castilho, L. R., Freire, D. M., & Castro, A. M. (2014). Granular starch hydrolysis of babassu agroindustrial residue: A bioprocess within the context of biorefinery. *Fuel*, *124*, 41-48. https://doi.org/10.1016/j.fuel.2014.01.076

Comunian, T. A., Silva, M. P., & Souza, C. J. (2021). The use of food by-products as a novel for functional foods: Their use as ingredients and for the encapsulation process. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2021.01.003

CONAB – Companhia Nacional de Abastecimento. Relatório de Análise Mensal do Guaraná, Mês Abril de 2021. Available in: https://www.conab.gov.br/info-agro/analises-do-mercado-agropecuario-e-extrativista/analises-do-mercado/historico-mensal-de-guarana/item/15719-guarana-analise-mensal-abril-2021. (Acessed on: 7 January 2022).

Coronel-Aguilera, C. P., & San Martín-González, M. F. (2015). Encapsulation of spray dried β-carotene emulsion by fluidized bed coating technology. *LWT-Food Science and Technology*, *62*(1), 187-193. https://doi.org/10.1016/j.lwt.2014.12.036

Corrêa-Filho, L. C., Lourenço, S. C., Duarte, D. F., Moldão-Martins, M., & Alves, V. D. (2019). Microencapsulation of tomato (Solanum lycopersicum L.) pomace ethanolic extract by spray drying: Optimization of process conditions. *Applied Sciences*, *9*(3), 612. https://doi.org/10.3390/app9030612

Correia, R. T., Borges, K. C., Medeiros, M. F., & Genovese, M. I. (2012). Bioactive compounds and phenolic-linked functionality of powdered tropical fruit residues. *Food Science and Technology International*, *18*(6), 539-547. https://doi.org/10.1177/1082013211433077

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582. https://doi.org/10.1128/CMR.12.4.564

das Chagas, E. G. L., Vanin, F. M., dos Santos Garcia, V. A., Yoshida, C. M. P., & de Carvalho, R. A. (2021). Enrichment of antioxidants compounds in cookies produced with camu-camu (Myrciaria dubia) coproducts powders. *LWT*, *137*, 110472. https://doi.org/10.1016/j.lwt.2020.110472

da Costa, R. S., de Souza Pinheiro, W. B., Arruda, M. S. P., Costa, C. E. F., Converti, A., Costa, R. M. R., & Júnior, J. O. C. S. (2020). Thermoanalytical and phytochemical study of the cupuassu (Theobroma grandiflorum Schum.) seed by-product in different processing stages. *Journal of Thermal Analysis and Calorimetry*, 1-10. 10.1007/s10973-020-10347-0

da Silva, D. C., Neto, A. D. O. W., Peres, A. E. C., Neto, A. A. D., & Dantas, T. N. C. (2020). Removal of oil from produced water by ionic flocculation using saponified babassu coconut oil. *Journal of Materials Research and Technology*, *9*(3), 4476-4484. https://doi.org/10.1016/j.jmrt.2020.02.075

da Silva, L. M. R., de Sousa, P. H. M., de Sousa Sabino, L. B., do Prado, G. M., Torres, L. B. V., Maia, G. A., ... & Ricardo, N. M. P. S. (2020). Brazilian (North and Northeast) fruit by-products. *Food Wastes and By-products: Nutraceutical and Health Potential*, 127-158. https://doi.org/10.1002/9781119534167.ch5

da Silveira Vasconcelos, M., de Oliveira, L. M. N., Nunes-Pinheiro, D. C. S., da Silva Mendes, F. R., de Sousa, F. D., de Siqueira Oliveira, L., ... & de Melo, D. F. (2020). Analysis of tetraterpenes and tetraterpenoids (carotenoids). In *Recent Advances in Natural Products Analysis* (pp. 427-456). Elsevier. https://doi.org/10.1016/B978-0-12-816455-6.00012-3

Daza, L. D., Fujita, A., Fávaro-Trindade, C. S., Rodrigues-Ract, J. N., Granato, D., & Genovese, M. I. (2016). Effect of spray drying conditions on the physical properties of Cagaita (Eugenia dysenterica DC.) fruit extracts. *Food and Bioproducts Processing*, *97*, 20-29. https://doi.org/10.1016/j.fbp.2015.10.001

de Freitas Santos, P. D., Rubio, F. T. V., da Silva, M. P., Pinho, L. S., & Favaro-Trindade, C. S. (2021). Microencapsulation of carotenoid-rich materials: A review. *Food Research International*, 110571. https://doi.org/10.1016/j.foodres.2021.110571

de la Luz Cádiz-Gurrea, M., del Carmen Villegas-Aguilar, M., Leyva-Jiménez, F. J., Pimentel-Moral, S., Fernández-Ochoa, Á., Alañón, M. E., & Segura-Carretero, A. (2020). Revalorization of bioactive compounds from tropical fruit by-products and industrial applications by means of sustainable approaches. *Food Research International*, *138*, 109786. https://doi.org/10.1016/j.foodres.2020.109786

de Oliveira, R. C., De Barros, S. T. D., & Gimenes, M. L. (2013). The extraction of passion fruit oil with green solvents. *Journal of Food Engineering*, *117*(4), 458-463. https://doi.org/10.1016/j.jfoodeng.2012.12.004

de Souza Silva, A. P., Rosalen, P. L., de Camargo, A. C., Lazarini, J. G., Rocha, G., Shahidi, F., ... & de Alencar, S. M. (2021). Inajá oil processing by-product: A novel source of bioactive catechins and procyanidins from a Brazilian native fruit. *Food Research International*, *144*, 110353. https://doi.org/10.1016/j.foodres.2021.110353

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., ... & Nedović, V. (2015). Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews*, *7*(4), 452-490.

Dror, Y., Cohen, Y., & Yerushalmi-Rozen, R. (2006). Structure of gum arabic in aqueous solution. *Journal of Polymer Science Part B: Polymer Physics*, *44*(22), 3265-3271.

Eldem, T., Speiser, P., & Altorfer, H. (1991). Polymorphic behavior of sprayed lipid micropellets and its evaluation by differential scanning calorimetry and scanning electron microscopy. *Pharmaceutical research*, *8*(2), 178-184. https://doi.org/10.1023/A:1015831801813

Engelmann, N.J., Clinton, S.K., Erdman Jr., J.W., 2011. Nutritional aspects of phytoene and phytofluene, carotenoid precursors to lycopene 1, 2. *Advances in Nutrition*, 2, 51–61. https://doi.org/10.3945/an.110.000075.1.

Evans, C. S. (1991). JB Harborne (ED.) Methods in plant biochemistry: 1. plant phenolics. Academic Press, New York and London, 1990.£ 65.

Fadini, A. L., Alvim, I. D., Ribeiro, I. P., Ruzene, L. G., da Silva, L. B., Queiroz, M. B., ... & Rodrigues, R. A. F. (2018). Innovative strategy based on combined microencapsulation technologies for food application and the influence of wall material composition. *LWT*, *91*, 345-352. https://doi.org/10.1016/j.lwt.2018.01.071

FAO (Food and Agriculture Organization of the United Nations). Food Wastage Footprint Impacts on Natural Resources. Available in: http://www.fao.org/3/i3347e/i3347e.pdf. (Accessed on 6 October 2021).

Fujita, A., Souza, V. B., Daza, L. D., Fávaro-Trindade, C. S., Granato, D., & Genovese, M. I. (2017). Effects of spray-drying parameters on in vitro functional properties of camu-camu (Myrciaria dubia Mc. Vaugh): a typical Amazonian fruit. *Journal of Food Science*, *82*(5), 1083-1091. https://doi.org/10.1111/1750-3841.13668

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*(2), 68-87.

Gallo, M., & Corbo, M. R. (2010). Mincroencapsulation as a New Approach to Protect Active Compounds in Food. *Application of Alternative Food-Preservation Technologies to Enhance Food Safety and Stability*, 188.

Ghafoor, K., Park, J., & Choi, Y. H. (2010). Optimization of supercritical fluid extraction of bioactive compounds from grape (Vitis labrusca B.) peel by using response surface methodology. *Innovative Food Science & Emerging Technologies*, 11(3), 485-490. https://doi.org/10.1016/j.ifset.2010.01.013

Giombelli, C., Iwassa, I. J., da Silva, C., & Barros, B. C. B. (2020). Valorization of peach palm by-product through subcritical water extraction of soluble sugars and phenolic compounds. *The Journal of Supercritical Fluids*, *165*, 104985. https://doi.org/10.1016/j.supflu.2020.104985

Giro-Paloma, J., Martínez, M., Cabeza, L. F., & Fernández, A. I. (2016). Types, methods, techniques, and applications for microencapsulated phase change materials (MPCM): a review. *Renewable and Sustainable Energy Reviews*, *53*, 1059-1075. https://doi.org/10.1016/j.rser.2015.09.040

Helkar, P. B., Sahoo, A. K., & Patil, N. J. (2016). Review: Food industry by-products used as a functional food ingredients. *International Journal of Waste Resources*, *6*(3), 1-6. https://doi.org/10.4172/2252-5211.1000248

Ho, K. K., Ferruzzi, M. G., & Wightman, J. D. (2020). Potential health benefits of (poly) phenols derived from fruit and 100% fruit juice. *Nutrition Reviews*, *78*(2), 145-174. https://doi.org/10.1093/nutrit/nuz041

Hollman, P. C. H. (2001). Evidence for health benefits of plant phenols: local or systemic effects?. *Journal of the Science of Food and Agriculture*, *81*(9), 842-852. https://doi.org/10.1002/jsfa.900

Jacobs, D. R., & Tapsell, L. C. (2007). Food, not nutrients, is the fundamental unit in nutrition. *Nutrition Reviews*, 65(10), 439-450.

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J Pharm Pharmacol*, *2*(7), 377-392.

Huang, K., Yuan, Y., & Baojun, X. (2021). A Critical Review on the Microencapsulation of Bioactive Compounds and Their Application. *Food Reviews International*, 1-41. https://doi.org 10.1080/87559129.2021.1963978

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328-2375.https://doi.org/10.3390/molecules18022328

Kuryntseva, P., Galitskaya, P., & Selivanovskaya, S. (2016). Changes in the ecological properties of organic wastes during their biological treatment. *Waste Management*, *58*, 90-97. https://doi.org/10.1016/j.wasman.2016.09.031

Lattanzio, V. (2013). Phenolic Compounds: Introduction 50. *Natural Products*, 1543-1580. Springer-Verlag Berlin Heidelberg 2013. https://doi.org/10.1007/978-3-642-22144-6_57.

Liew, S. Q., Ngoh, G. C., Yusoff, R., & Teoh, W. H. (2016). Sequential ultrasound-microwave assisted acid extraction (UMAE) of pectin from pomelo peels. *International Journal of Biological Macromolecule*s, 93, 426-435. https://doi.org/10.1016/j.ijbiomac.2016.08.065

Luo, X., Bai, R., Zhen, D., Yang, Z., Huang, D., Mao, H., ... & Fu, C. (2019). Response surface optimization of the enzyme-based ultrasound-assisted extraction of acorn tannins and their corrosion inhibition properties. *Industrial Crops and Products*, *129*, 405-413. https://doi.org/10.1016/j.indcrop.2018.12.029

Maniglia, B. C., & Tapia-Blacido, D. R. (2016). Isolation and characterization of starch from babassu mesocarp. *Food Hydrocolloids*, *55*, 47-55.

Maoka, T. (2020). Carotenoids as natural functional pigments. *Journal of natural medicines*, *74*(1), 1-16. https://doi.org/10.1007/s11418-019-01364-x

Martillanes, S., Rocha-Pimienta, J., Gil, M. V., Ayuso-Yuste, M. C., & Delgado-Adámez, J. (2020). Antioxidant and antimicrobial evaluation of rice bran (Oryza sativa L.) extracts in a mayonnaise-type emulsion. *Food Chemistry*, *308*, 125633.https://doi.org/10.1016/j.foodchem.2019.125633

Matos, K. A. N., Lima, D. P., Barbosa, A. P. P., Mercadante, A. Z., & Chiste, R. C. (2019). Peels of tucumã (Astrocaryum vulgare) and peach palm (Bactris gasipaes) are by-products classified as very high carotenoid sources. *Food Chemistry*, *272*, 216-221.https://doi.org/10.1016/j.foodchem.2018.08.053

Melo, P. S., Selani, M. M., Gonçalves, R. H., de Oliveira Paulino, J., Massarioli, A. P., & de Alencar, S. M. (2021). Açaí seeds: An unexplored agro-industrial residue as a potential source

of lipids, fibers, and antioxidant phenolic compounds. *Industrial Crops and Products*, *161*, 113204. https://doi.org/10.1016/j.indcrop.2020.113204

Monteiro, J., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2019). Pharmacological potential of methylxanthines: Retrospective analysis and future expectations. *Critical Reviews in Food Science and Nutrition*, *59*(16), 2597-2625. https://doi.org/10.1080/10408398.2018.1461607

Montenegro, M. A., Boiero, M. L., Valle, L., & Borsarelli, C. D. (2012). Gum Arabic: more than an edible emulsifier. *Products and applications of biopolymers*, *51*, 953-978.

Morone, P., Koutinas, A., Gathergood, N., Arshadi, M., & Matharu, A. (2019). Food waste: Challenges and opportunities for enhancing the emerging bio-economy. *Journal of cleaner production*, *221*, 10-16. https://doi.org/10.1016/j.jclepro.2019.02.258

Moser, P., Ferreira, S., & Nicoletti, V. R. (2019). Buriti oil microencapsulation in chickpea protein-pectin matrix as affected by spray drying parameters. *Food and Bioproducts Processing*, *117*, 183-193. https://doi.org/10.1016/j.fbp.2019.07.009

Nabi, F., Arain, M. A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., ... & Liu, J. (2020). Health benefits of carotenoids and potential application in poultry industry: A review. *Journal of animal physiology and animal nutrition*, *104*(6), 1809-1818. https://doi.org/10.1111/jpn.13375

Namitha, K.K., Negi, P.S., 2010. Chemistry and biotechnology of carotenoids. *Critical Reviews in Food Science and Nutrition*, 50, 1040–8398. https://doi.org/10.1080/10408398.2010.499811.

Nasrin, T. A. A., & Matin, M. A. (2017). Valorization of vegetable wastes. *Food Processing By-Products and Their Utilization; Anal, AK, Ed*, 53-88.

Nazzaro F, Orlando P, Fratianni F, Coppola R. Microencapsulation in food science and biotechnology. *Current opinion in biotechnology*. 2012 Apr 1;23(2):182-6. https://doi.org/10.1016/j.copbio.2011.10.001

Neri-Numa, I. A., Sancho, R. A. S., Pereira, A. P. A., & Pastore, G. M. (2018). Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. *Food Research International*, *103*, 345-360. https://doi.org/10.1016/j.foodres.2017.10.053

Okuro, P. K., de Matos Junior, F. E., & Favaro-Trindade, C. S. (2013). Technological challenges for spray chilling encapsulation of functional food ingredients. *Food Technology and Biotechnology*, *51*(2), 171.

Oñatibia-Astibia, A., Franco, R., & Martínez-Pinilla, E. (2017). Health benefits of methylxanthines in neurodegenerative diseases. *Molecular nutrition & food research*, *61*(6), 1600670. https://doi.org/10.1002/mnfr.201600670

Onwulata, C. I. (2013). Microencapsulation and functional bioactive foods. *Journal of Food Processing and Preservation*, *37*(5), 510-532. https://doi.org/10.1111/j.1745-4549.2012.00680.x

Pal, S., & Bhattacharjee, P. (2018). Spray dried powder of lutein-rich supercritical carbon dioxide extract of gamma-irradiated marigold flowers: process optimization, characterization and food application. *Powder Technology*, 327, 512-523. https://doi.org/10.1016/j.powtec.2017.12.085

Panusa, A., Zuorro, A., Lavecchia, R., Marrosu, G., & Petrucci, R. (2013). Recovery of natural antioxidants from spent coffee grounds. *Journal of Agricultural and Food Chemistry*, *61*(17), 4162-4168.

Pashazadeh, B., Elhamirad, A. H., Hajnajari, H., Sharayei, P., & Armin, M. (2020). Optimization of the pulsed electric field-assisted extraction of functional compounds from cinnamon. *Biocatalysis and Agricultural Biotechnology*, 23, 101461. https://doi.org/10.1016/j.bcab.2019.101461

Pavlenko, N., & Searle, S. (2020). A comparison of methodologies for estimating displacement emissions from waste, residue, and by-product biofuel feedstocks. *Working Paper*, (2020-22).

Pereira, D. M., Valentão, P., Pereira, J. A., & Andrade, P. B. (2009). *Phenolics*: From chemistry to biology. https://doi.org/10.3390/molecules14062202

Petrucci, R., Zollo, G., Curulli, A., & Marrosu, G. (2018). A new insight into the oxidative mechanism of caffeine and related methylxanthines in aprotic medium: May caffeine be really considered as an antioxidant?. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1862*(8), 1781-1789. https://doi.org/10.1016/j.bbagen.2018.05.011

Pinho, L. S., da Silva, M. P., Thomazini, M., Cooperstone, J. L., Campanella, O. H., da Costa Rodrigues, C. E., & Favaro-Trindade, C. S. (2021). Guaraná (Paullinia cupana) by-product as a source of bioactive compounds and as a natural antioxidant for food applications. *Journal of Food Processing and Preservation*, e15854. https://doi.org/10.1111/jfpp.15854

Pugliese AG, Tomas-Barberan FA, Truchado P, Genovese MI. 2013. Flavonoids, proanthocyanidins, vitamin C, and antioxidant activity of *Theobroma grandiflorum* (Cupuassu) pulp and seeds. J Agric Food Chem. 61:2720–2728.10.1021/jf304349u

Puligundla, P., Mok, C., & Park, S. (2020). Advances in the valorization of spent brewer's yeast. *Innovative Food Science & Emerging Technologies*, *62*, 102350.https://doi.org/10.1016/j.ifset.2020.102350

Ramakrishnan, Y., Adzahan, N. M., Yusof, Y. A., & Muhammad, K. (2018). Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation. *Powder technology*, *328*, 406-414. https://doi.org/10.1016/j.powtec.2017.12.018

Rasul, M. G. (2018). Conventional Extraction Methods Use in Medicinal Plants, their Advantages and Disadvantages. *Int J Basic Sciences App Computing*, *2*(6), 10-14. ISSN: 2394-367X, Volume-2 Issue-6, December 2018

Resende, L. M., Franca, A. S., & Oliveira, L. S. (2019). Buriti (Mauritia flexuosa L. f.) fruit by-products flours: Evaluation as source of dietary fibers and natural antioxidants. *Food Chemistry*, *270*, 53-60. https://doi.org/10.1016/j.foodchem.2018.07.079

Ribeiro, A. M., Shahgol, M., Estevinho, B. N., & Rocha, F. (2020). Microencapsulation of vitamin A by spray-drying, using binary and ternary blends of gum Arabic, starch and maltodextrin. *Food Hydrocolloids*, *108*, 106029. https://doi.org/10.1016/j.foodhyd.2020.106029

Rodriguez-Concepcion, M., Avalos, J., Bonet, M.L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D., Limon, M.C., Melendez-Martı´nez, A.J., Olmedilla-Alonso, B., Palou, A., Ribot, J., Rodrigo, M.J., Zacarias, L., Zhu, C., 2018. A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. *Prog. Lipid Res*. 70, 62–93. https://doi.org/10.1016/j.plipres.2018.04.004.

Rosa, L. D., Moreno-Escamilla, J. O., Rodrigo-García, J., & Alvarez-Parrilla, E. (2019). Phenolic compounds. *Postharvest physiology and biochemistry of fruits and vegetables*, 253-271. doi:10.1016/B978-0-12-813278-4.00012-9

Roxo, M., Peixoto, H., Wetterauer, P., Lima, E., & Wink, M. (2020). Piquiá Shells (Caryocar villosum): A Fruit by-Product with Antioxidant and Antiaging Properties in Caenorhabditis elegans. *Oxidative medicine and cellular longevity*, *2020*. https://doi.org/10.1155/2020/7590707

Sablania, V., & Bosco, S. J. D. (2018). Optimization of spray drying parameters for Murraya koenigii (Linn) leaves extract using response surface methodology. *Powder Technology*, *335*, 35-41. https://doi.org/10.1016/j.powtec.2018.05.009

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 512-531. https://doi.org/10.1111/1541-4337.12330

Saifullah, M., Shishir, M. R. I., Ferdowsi, R., Rahman, M. R. T., & Van Vuong, Q. (2019). Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: A critical review. *Trends in Food Science & Technology*, *86*, 230-251.

Santana ÁL, Zanini JA, Macedo GA. Dispersion-assisted extraction of guarana processing wastes on the obtaining of polyphenols and alkaloids. Journal of Food Process Engineering. 2020 Apr;43(4):e13381. https://doi.org/10.1111/jfpe.13381

Santana-Gálvez, J., & Jacobo-Velázquez, D. A. (2018). Classification of phenolic compounds. In *Phenolic Compounds in Food* (pp. 3-20). CRC Press.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African journal of*

*traditional, complementary and alternative medicines*, *8*(1). https://doi.org/10.4314/ajtcam.v8i1.60483

Schimpl, F. C., da Silva, J. F., de Carvalho Gonçalves, J. F., & Mazzafera, P. (2013). Guarana: revisiting a highly caffeinated plant from the Amazon. *Journal of ethnopharmacology*, *150*(1), 14-31. https://doi.org/10.1016/j.jep.2013.08.023

Schoubben, A., Blasi, P., Giovagnoli, S., Rossi, C., & Ricci, M. (2010). Development of a scalable procedure for fine calcium alginate particle preparation. *Chemical Engineering Journal*, *160*(1), 363-369. https://doi.org/10.1016/j.cej.2010.02.062

Sharayei, P., Azarpazhooh, E., & Ramaswamy, H. S. (2020). Effect of microencapsulation on antioxidant and antifungal properties of aqueous extract of pomegranate peel. *Journal of food science and technology*, *57*(2), 723-733. https://doi.10.1007/s13197-019-04105-w

Shao, P., Xuan, S., Wu, W., & Qu, L. (2019). Encapsulation efficiency and controlled release of Ganoderma lucidum polysaccharide microcapsules by spray drying using different combinations of wall materials. *International journal of biological macromolecules*, *125*, 962-969. https://doi.org/10.1016/j.ijbiomac.2018.12.153

Silva da Costa, R., de Farias Silva, N., Gabbay Alves, T. V., Fernandes da Silva, M., do Socorro Barros Brasil, D., Ribeiro-Costa, R. M., ... & Carréra Silva, J. O. (2019). Antioxidant Activity of an Industrial Cupuassu Seed By-product: Molecular Modeling of Phenolic Compounds. *Chemical engineering & technology*, *42*(2), 397-406. https://doi.org/10.1002/ceat.201800299

Silva PT, Fries LL, Menezes CR, Holkem AT, Schwan CL, Wigmann ÉF, Bastos JD, & Silva CD. (2014). Microencapsulation: concepts, mechanisms, methods and some applications in food technology. *Ciência Rural,* 44:1304-11. https://doi.org/10.1590/0103-8478cr20130971

Stramarkou, M., Papadaki, S., Kyriakopoulou, K., & Krokida, M. (2017). Effect of drying and extraction conditions on the recovery of bioactive compounds from Chlorella vulgaris. *Journal of Applied Phycology*, *29*(6), 2947-2960.10.1007/s10811-017-1181-8

Sunday, B. O. (2017). *Phytochemical investigation and tissue culture studies on the South African knob trees, Zanthoxylum Capense and Senegalia Nigrescens* (Doctoral dissertation).

Tolve, R., Galgano, F., Caruso, M. C., Tchuenbou-Magaia, F. L., Condelli, N., Favati, F., & Zhang, Z. (2016). Encapsulation of health-promoting ingredients: applications in foodstuffs. *International journal of food sciences and nutrition*, *67*(8), 888-918. https://doi.org/10.1080/09637486.2016.1205552

Tsao, R., 2010. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2, 1231–1246. https://doi.org/10.3390/ nu2121231.

Tupuna-Yerovi, D. S., Paese, K., Flôres, S. H., Guterres, S. S., & Rios, A. (2020). Addition of norbixin microcapsules obtained by spray drying in an isotonic tangerine soft drink as a natural dye. *Journal of food science and technology*, *57*(3), 1021-1031. https://doi.org/ 10.1007/s13197-019-04135-4

Tupuna, D. S., Paese, K., Guterres, S. S., Jablonski, A., Flôres, S. H., & de Oliveira Rios, A. (2018). Encapsulation efficiency and thermal stability of norbixin microencapsulated by spray-drying using different combinations of wall materials. *Industrial crops and products*, *111*, 846-855. https://doi.org/10.1016/j.indcrop.2017.12.001

Tuyen CK, Nguyen MH, Roach PD, Stathopoulos CE. A storage study of encapsulated gac (Momordica cochinchinensis) oil powder and its fortification into foods. *Food and Bioproducts Processing.* 2015 Oct 1;96:113-25. https://doi.org/10.1016/j.fbp.2015.07.009

Vieitez, I., Maceiras, L., Jachmanián, I., & Alborés, S. (2018). Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology. *The Journal of Supercritical Fluids*, *133*, 58-64. https://doi.org/10.1016/j.supflu.2017.09.025

Villacís-Chiriboga, J., Elst, K., Van Camp, J., Vera, E., & Ruales, J. (2020). Valorization of by-products from tropical fruits: Extraction methodologies, applications, environmental, and economic assessment: A review (Part 1: General overview of the by-products, traditional biorefinery practices, and possible applications). *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 405-447 https://doi.org/10.1111/1541-4337.12542

Waldron, K. W. (Ed.). (2009). *Handbook of waste management and co-product recovery in food processing*. Elsevier.

Waldron, K. (2007). Handbook of Waste Management and Co-Product Recovery in Food Processing || Waste minimization, management and co-product recovery in food processing: an introduction. , (), 3–20. https://doi.org/10.1533/9781845692520.1.3

Wen, L., Zhang, Z., Sun, D. W., Sivagnanam, S. P., & Tiwari, B. K. (2020). Combination of emerging technologies for the extraction of bioactive compounds. *Critical reviews in food science and nutrition*, 60(11), 1826-1841. https://doi.org/10.1080/10408398.2019.1602823

Wu, S., & Chappell, J. (2008). Metabolic engineering of natural products in plants; tools of the trade and challenges for the future. *Current Opinion in Biotechnology*, *19*(2), 145-152. https://doi.org/10.1016/j.copbio.2008.02.007

Xiong, J., Matta, F. V., Grace, M., Lila, M. A., Ward, N. I., Felipe-Sotelo, M., & Esposito, D. (2020). Phenolic content, anti-inflammatory properties, and dermal wound repair properties of industrially processed and non-processed acai from the Brazilian Amazon. *Food & Function*, *11*(6), 4903-4914. https://doi.org/10.1039/c9fo03109j

# 2 CHAPTER 2

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## THE INCORPORATION OF CAROTENOIDS ON READY TO EAT FOODS STUDIED THROUGH THEIR STABILITY DURING EXTRUSION PROCESSING*

*This chapter is based on the published review:

Pinho, L. S., Rodrigues, C. E. C., Favaro-Trindade, C. S., & Campanella, O. H. (2021). The Incorporation of Carotenoids on Ready to Eat Foods Studied Through Their Stability During Extrusion Processing. *Food Engineering Reviews*, 1-14. (Copyright is in Attachment A)

**ABSTRACT**

Studies have reported evidence that consumption of carotenoids provides many health benefits. Therefore, assuring the maintenance of their nutritional/nutraceutical properties after food processing is gaining increasing interest. Products such as breakfast cereals and ready-to-eat snacks are commonly consumed foods that potentially could serve as ideal vehicles of bioactive compounds, such as carotenoids. Some of these foods are produced by extrusion. The impact of the extrusion process on bioactive compounds is harsh and may require control of the processing conditions to alleviate the negative effects of the process on the product's nutritional quality. Extrusion process parameters, such as temperature, screw speed, shear rate, and the feed moisture content, can affect the retention of carotenoids in the final product. The influence of those parameters on products is discussed in this review, taking into account aspects of carotenoid stability and the conservation of its nutritional properties. Further, some strategies to increase carotenoid retention during extrusion processes are discussed.

**Keywords:** Extrusion; thermomechanical treatment; carotenoids; retention; foods.

## 2.1 Introduction

The food industry is increasingly focused on meeting consumer demands for nutritious foods that provide health benefits. The development of functional foods including fruits and vegetables offering high contents of bioactive compounds, such as carotenoids, has become an important step to support the requests of these consumers. Carotenoids have been highlighted as important compounds due to their demonstrated evidence for promoting immunomodulatory and anti-inflammatory, antimicrobial, and antiviral activities, in addition to preventing degenerative diseases (Bernal, Mendiola, Ibáñez, Cifuentes, 2011; Christaki, Bonos, Giannenasa, Florou-Paneria, 2013; Buono et al., 2014). Furthermore, some carotenoids (α-carotene, β-carotene, and β-cryptoxanthin) are provitamin A (Ndiaye et al., 2020) and have shown to have antioxidant capacity and to protect against oxidative stress (Guedes, Amaro, Malcata, 2011). The development of products that incorporate bioactive components and can be consumed daily, such as snacks and breakfast cereals, is an important initiative because these products may contribute to a nutritious daily intake of these compounds. The cost-benefit ratio of healthcare and the fact that diet is essential in preventing chronic diseases support the need

for research, development, and investment in the creation of products with nutritional/nutraceutical properties (Singh, Gamlath, Wakeling, 2007; Potter, Stojceska, Plunkett, 2013; Obradović et al., 2014)

The basic problem of adding bioactive compounds in processed foods is the compound's sensitivity to changes in pH, oxygen, temperature, light exposure, and mechanical forces during processing. Extrusion is a processing tool widely employed in the food industry. The interest in the use of extruders stems from the versatility of the extrusion process in terms of the ability to handle different formulations with high solid feed, low space requirements, energy efficiency, and low environmental impact when compared with other food processes (Bouvier & Campanella, 2014). Regarding the quality of extruded foods, the control of process parameters, such as temperature, moisture, screw speed, and raw material, is fundamental to guarantee products with desirable characteristics and properties (Cheftel, 1986; Singh, Gamlath, Wakeling, 2007; Brennan, Derbyshire, Tiwari, Brennan, 2013; Bouvier & Campanella, 2014).

As extrusion cooking involves the application of high thermomechanical stress to the processed material, it is obvious to assume a significant impact on the stability of thermolabile compounds such as carotenoids. The effect of extrusion conditions on carotenoids has been reported (Emin, Mayer-Miebach, Schuchmann, 2012; Waramboi, Gidley, Sopade, 2013). The degree of mixing and homogenization, as well as the extrusion process conditions, can affect the carotenoid stability and active property retention in food systems and promote changes to obtain final products with unique characteristics. In this sense, the present work aims to review the literature in the area to provide important and comprehensive guidelines for evaluation of the stability of carotenoids during extrusion and establish extrusion conditions that optimize their retention and bioavailability.

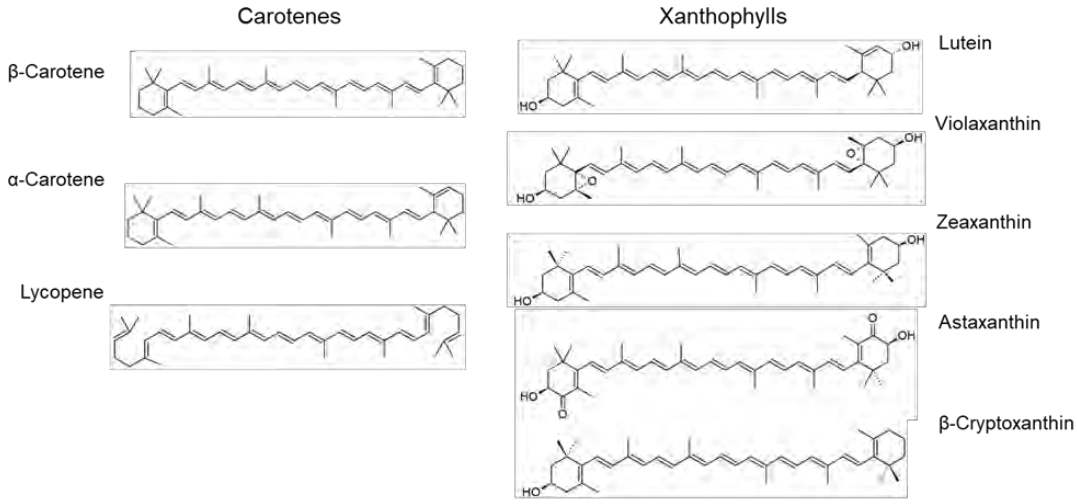
## 2.2 Carotenoids

Carotenoids are natural pigments providing red, orange, and yellow color to leaves, fruits, and flowers of plants, fish, feathers, and shells of crustaceans. They are formed by polymerization of isoprene units to form an aliphatic structure. These compounds are biosynthesized as secondary metabolites by many plants, algae, bacteria, and fungi (Gouveia & Empis, 2003; Robert, Carlsson, Romero, Masson, 2003; Martínez-Delgado, Khandual, Villanueva-Rodríguez, 2017).

Carotenoids belong to the class of tetraterpenes (Stahl & Sies, 1996), which consist of chains that incorporate conjugated double bonds with delocalized π-electrons (Britton, 1995). Their yellow-red color, the well-recognized antioxidant activity, as well as the capacity of these compounds as chelating agents for molecular singlet oxygen are associated with their particular chemical structure. In nature, most carotenoids are present as isomers, with the *trans* conformation more stable than the cis isomeric form.

The term carotenoid comprises a variety of chemicals, which include two main classes of pigments. As illustrated in Figure 5, carotenes (as α-carotene, β-carotene, and lycopene) are exclusively non-oxygenated hydrocarbons, and xanthophylls (such as lutein, violaxanthin, zeaxanthin, astaxanthin, and β-cryptoxanthin) are oxygenated hydrocarbons, which are produced in the plastids of plants and algae (Jaswir, Noviendri, Hasrini, Octavianti, 2011; Oroian & Escriche, 2015).

**Figure 5.** Chemical structures of carotenes and xanthophylls




Reference: Own source.

In general, carotenes are soluble in lipids and organic solvents such as petroleum ether, hexane, chloroform, and present limited solubility in ethanol, whereas xanthophylls have better solubility in more polar solvents. Both classes of pigments can absorb light in the range of the visible spectrum, mainly in the regions of blue (430–470 nm), bluegreen (470–500 nm), and green (500–530 nm) (Britton et al. 1995).

The color of fruits and vegetables can indicate the form and content of the carotenoids present in the product, and the concentration of each pigment can vary among different matrices. Yellow-orange fruits generally consist of β-carotene and α-carotene. In tomatoes, their red color is attributed to lycopene. In green leafy vegetables, lutein (almost 45%), β-carotene (25–30%), followed by neoxanthin (10–15%), and violaxanthin (10–15%) are the types of carotenoids found most (Priyadarshani & Jansz, 2014).

The benefits of different carotenoids in human health are directly associated to their structure, mainly due to their antioxidant potential (Yara-Varón et al., 2016; Kolniak-Ostek et al., 2017). Besides, they are important nutritional constituents, since carotenoids are precursors of vitamin A. Carotenoids have been correlated with a decreased risk of diseases, such as cancer, cardiovascular disorders, cataracts, and eye degeneration because they act as anti-inflammatory compounds and have an immunomodulatory effect (Chávez-Jáuregui et al., 2010; Gammone, Riccioni, D'Orazio, 2015; Khoo et al., 2011; Murphy et al., 2014; Norfezah, Hardacre, Brennan, 2011). Human beings cannot produce these compounds; therefore, they must be obtained from food, with plants and vegetables being the most abundant sources.

There are more than 700 carotenoids found in nature (Saini, Nile, Park, 2015) however, up to 60 carotenoids are found in foods, the main ones being β-carotene, α-carotene, lutein, violaxanthin, zeaxanthin, and β-cryptoxanthin (Van Hal, 2000; Azevedo-Meleiro, Rodriguez-Amaya, 2004; Perera & Yen, 2007). Lutein and zeaxanthin, when introduced into the diet, are selectively absorbed in the macula of the eye and act by absorbing up to 90% of blue light and protecting the eyes against oxidative damage induced by light (Krinsky, Landrum, Bone, 2003; Koushan, Rusovici, Li, Ferguson, Chalam, 2013). The conversion of the biological activity of carotenoids into vitamin A (retinol) activity in the human body suggests the relevance of ingesting these components. Regarding this conversion to retinol, 1 retinol equivalent (RE) corresponds to 6 µg of all-*trans*-β-carotene, and 1 retinol activity equivalent (RAE) corresponds to 12 µg of all-*trans*-β-carotene, 24 µg of α-carotene, or 24 µg β-cryptoxanthin in the diet (Breithaupt, Weller, Wolters, Hahn, 2003; Perera & Yen, 2007).

Furthermore, β-carotene is an antioxidant acting on harmful effects caused by free radicals, which have been associated with various health disorders, such as gastrointestinal and lung cancer (Goralczyk, 2009). According to Obulesu, Dowlathabad, & Bramhachari (2011), β-carotene can also have beneficial effects on Alzheimer's disease. Additionally, violaxanthin has shown antiproliferative activity in human breast cancer, and 9′-cis-neoxanthin has shown

relevant results on preventing human prostate cancer cells (Kotake-Nara, Asai, Nagao, 2005; Pasquet et al., 2011).

The potential biological activity of carotenoids in human health depends, in some aspects, on their absorption, and the properties of these compounds have stimulated interest in their bioaccessibility and bioavailability. Bioaccessibility is related to the fraction of the component that is released from the food matrix to the gastrointestinal system during digestion, promoting absorption in the intestine (Kean, Hamaker, Ferruzzi, 2008), whereas bioavailability consists of the part of ingested carotenoids that reaches the systemic circulation and is metabolized by the organism. In general, bioavailability consists of absorption, metabolism, and bioactivity (Carbonell-Capell et al., 2014). The bioavailability of carotenoids in foods can vary according to endogenous (related to the product) and exogenous (related to the process) factors (Fernández-García et al., 2012). Some studies (Parada & Aguilera, 2007; Courraud, Berger, Cristol, Avallone, 2013) have shown that food processes increase the bioaccessibility of carotenoids by disrupting the food matrix through processing. Nevertheless, high temperatures during some processes or improper storage of products can promote isomerization and formation of by-products, and this will possibly reduce the availability of the bioactive compounds of interest.

To develop functional foods rich in carotenoids, the food industry must take into account the pathway mechanisms that promote the degradation of carotenoids to set the right process conditions to ensure the presence and stability of these components in the product (Boon, McClements, Weiss, Decker, 2010). Carotenoids have a highly conjugated and unsaturated native structure; therefore, they are unstable, mainly when extracted from their biological matrix and susceptible to the harsh conditions imposed by food processes (Britton, 1995; Polyakov & Leshina, 2006).

The end groups of the carotenoid chains can degrade under various conditions. It is worth mentioning that the decomposition pathways of carotenoids are induced by the presence of oxidizing agents involved in the beginning of the degradation mechanism. Some of these oxidizing agents have been reported; for instance, Everett et al. (1996) described the interactions of radical species ($NO_2\cdot$, $RS\cdot$, $RSOO\cdot$) with β-carotene, and Hill et al. (1995) explained interactions between $CCI_3O_2\cdot$ and a range of carotenoids. Carotenoid degradation causes the loss of its functional properties, in addition to reducing the nutritional value, color, and organoleptic characteristics they provide to foods. Thus, it is necessary to consider the degrading impacts of factors that may influence the stability of the desirable compound, such

as the presence of oxygen, light, elevated temperature, and changes in pH, among other conditions associated with food processes (Gouveia & Empis, 2003; Herrera et al., 2011).

Preserving carotenoids naturally present in some foods and their incorporating in foods that do not include large quantities of these components can provide benefits to health and favor the novel markets promoting nutritious food products. Considering the important role that carotenoids have in our health and the interest of consumers in consuming healthier foods, as well as the industry's concern in meeting this demand, extrusion can be a good processing alternative to achieve those goals. However, the process can be used provided the effects of the extrusion severe thermo mechanical conditions are alleviated to keep the product textural quality, while maximizing the retention of carotenoids, or alternatively using other means to protect these compounds, for example, encapsulation. Since it is known that carotenoids are highly unstable, some aspects of this process to reduce their degradation must be considered.

## 2.3 Extrusion

Extrusion is a process providing combined unit operations involving mixing, cooking, kneading, shearing, and shaping in just one piece of equipment called an extruder (Fellows, 2000). The extrusion process was invented in 1797 by Joseph Bramah who patented the process. Initially, the extrusion process was used to manufacture lead tubes. In the following years, this technology was applied in the production of plastics, molded metals, and synthetic materials. From the 1930s, the application for the production of foods started in the food industry (Bouvier & Campanella, 2014) with the extrusion of pasta.

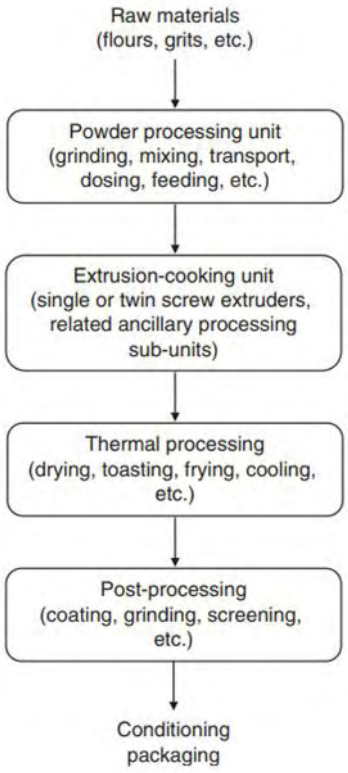
Extrusion cooking has been used to produce ready-to-eat breakfast cereals, bakery products, snacks, texturized vegetable protein, and pet foods (Riaz, Asif, Ali, 2009; Bouvier & Campanella, 2014). It has some advantages over other common thermal processing methods, in terms of its versatility, relatively low cost, energy efficiency, high productivity, automated control, and low or no effluent production and disposal (Alam, Kaur, Khaira, Gupta, 2016). Furthermore, the process can improve the digestibility of starch (Singh, Dartois, Kaur, 2010) the and bioavailability of nutrients, such as catechins (Gu, House, Rooney, Prior, 2008) and carotenoids (Kean, Hamaker, Ferruzzi, 2008).

Depending on the temperature, two forms of extrusion have been used—cold (below 70 °C) and hot (above 70 °C) (Brennan, Derbyshire, Tiwari, Brennan, 2013). Conventional extrusion, or hot extrusion, is operated at temperatures high enough to provide the energy to

transform the material, in addition to the mechanical or shear energy delivered by the rotation of one or two screws inside the extruder barrel. However, if the extrusion process is operated at low temperature (cold extrusion), the thermal effect is reduced compared to the shear action. In this case, the viscosity of the material moving inside the extruder may be higher leading to a high shear energy input on the processed material. This review focuses on the role of hot extrusion (instead of cold extrusion) in the stability of carotenoids.

Although there exist different processing alternatives, typical steps of an industrial conventional extrusion process can be represented by the diagram shown in Figure 6. The process generally starts with raw materials composed of its main macronutrients, starches, and proteins (present in fours or grains). As illustrated in Figure 6, extruded products are produced through the combination of four central operations prior to final conditioning:

**Figure 6.** Core process diagram of industrial extrusion cooking processes



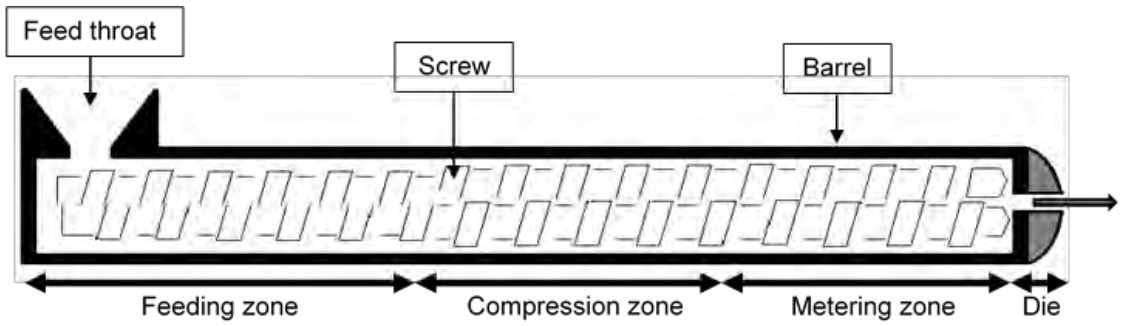

Reference: Bouvier & Campanella, 2014.

In the extrusion process, the raw material is heated in a screw-barrel system where the imposed processing conditions are controlled by the raw material properties, moisture, pressure,

and temperature, to mechanically shear and shape the extruded material, which undergoes many chemical and structural transformations (Rodríguez-Miranda et al., 2011)

During extrusion, one (single screw extruder) or two screws (twin screw extruder) rotate inside a barrel (Figure 7). The rotation of the screws provides the dragging force to move the extruded material from the feeding section to the die. During that transit, the extruded material experiences continuous increases of temperature and pressure, and at the same time, the shear exerted by the rotation screw applies mechanical energy to the product, which translates in what is known as a thermomechanical treatment. At the end of the extruder barrel, the product is pushed at high pressure and temperature through an orifice (the die). The moisture content contained in the product, which due to the high pressure is in liquid form, flashes of from the product at atmospheric pressure at the exit of the die generating the characteristic texture of extruded products (Bouvier & Campanella, 2014).

**Figure 7.** A cross-section of a twin-screw food extruder




Reference: Own source.

The screw profile used in the extruder defines the amount of shear applied in the process. Low-shear screw extruders, characterized by a channel with a large height, defined by the screw geometry (fight), are used to produce pasta and meat products. Medium-shear screw extruders with medium channel heights are used to produce texturized proteins. High-shear screw extruders with very small channel heights are used to produce expanded products and breakfast cereals.

Typical extrusion processing takes place at a relatively low moisture varying of 20–35% that depends on the final product and the properties of the raw materials. The process induces shear-induced and thermal transformation of the material at reasonably high temperatures,

generally above 120 °C, and short residence times, below 1 min. Considering these conditions, the processing of extruded products favors structural changes, producing cooked materials with different properties (Brennan, Monro, Brennan, 2008). The quality of the products resulting from this process depends on the operating conditions of the extruder, for instance, type of equipment, feed moisture, cylinder temperature, screw speed and configuration, and feed rate among other processing parameters that are described by Bouvier & Campanella (2014).

The extrusion processing can generate physicochemical reactions with nutritional implications. These changes might differ according to the raw material composition and the addition of other ingredients and their proportions. In this sense, special importance is given to the presence of components such as starches (Bouvier & Campanella, 2014), proteins (Alonso, Orúe, Marzo, 1998), lipids (Camire & Krumhar, 1990) and vitamins (Brennan, Brennan, Derbyshire, Tiwari, 2011) in foods. Interestingly, the increase in bioaccessibility associated to the thermal stress during extrusion has been related to the disruption of the cell wall structure and protein-carotenoid complexes (Ortak et al., 2017). Some studies revealed that, due to the broken and softened structure of the cell wall after the thermal treatment, there is a greater release of β-carotene from the food matrix (Hornero-Méndez, Mínguez-Mosquera, 2007; Biehler et al., 2010). In addition, protein denaturation induced by the severe thermal treatment causes disruption of protein-carotenoid complexes, which results in the formation of β-carotene molecules that can be simply released (Mulokozi, Hedrén, Svanberg, 2004). It is worth mentioning that mechanical stress can also play an important role in bioaccessibility. The shear promoted by the extruder screws causes a breakdown of the food matrix. Thus, smaller particles with larger surface areas for digestive enzymes are formed (Hedrén, Diaz, Svanberg, 2002; Donhowe & Kong, 2014)

One of the main trends in the food industry is the production of indulgent foods with health benefits. With the development of extrusion technology, the incorporation of ingredients, such as carotenoids, is being investigated to improve the nutritional profile of highly consumed foods. However, although some evidence has indicated the benefits of the extrusion process over the bioavailability/bioaccessibility of carotenoids, other different factors can affect carotenoid stability during the process. As discussed, carotenoids differ in chemical structure and in available forms. The stability during extrusion can vary considering the critical thermomechanical treatments applied, which can promote several complex reactions in the extruded material (Waramboi, Gidley, Sopade, 2013).

## 2.4 Factors that influence the stability of carotenoids during extrusion

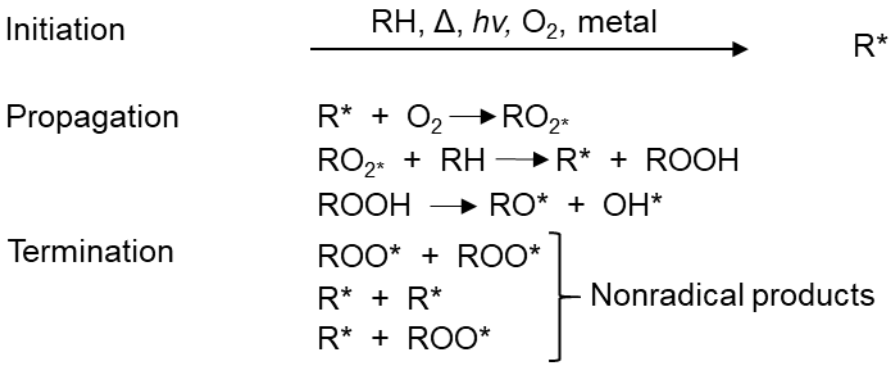
Carotenoids are compounds susceptible to undesirable reactions, such as oxidation and isomerization processes, which lead to their degradation during processing and storage. Oxygen concentration, high temperature processing, available water, and the presence of some specific metals have an important influence on the oxidation of carotenoids (Achir et al., 2010). The mechanisms of carotenoid degradation under high oxygen concentration can follow many paths, including interactions with singlet oxygen and auto-oxidation reactions. Upon heating, and in the presence of oxygen, the structure of carotenoids undergoes cleavage and reactions involving their double bonds, which result in volatile and non-volatile compounds (Boon, McClements, Weiss, Decker, 2010; Oboh, Falade, Ademiluyi, 2014). Thus, the increase of oxygen during extrusion may lead to a loss of carotenoids.

Regarding to available water, the effect of this factor depends on the complexity of the system. The increase of the water content in a solid food matrix can favor more access of dissolved oxygen in the medium and increase the oxidation rate of the carotenoids. However, increasing water content can also dilute heavy metal catalysts or convert them to hydroxides, as well as neutralize the peroxide decomposition, which can slow down the degradative processes (Selim, Tsimidou, Biliaderis, 2000; Lavelli, Zanoni, Zaniboni, 2007). In general, the high moisture extrusion reduces the temperature and viscosity inside of the extrudate, minimizing the degradation of carotenoids.

The basic steps of oxidation of lipids in food components, also including carotenoids, were reported by Karel (1980). This process involves three phases: initiation, followed by propagation, and termination (Figure 8).

In some systems, the medium may already have free radicals to promote oxidative reactions or they may be formed from processing as described above. In the presence of those free radicals, several mechanisms involving the interaction of carotenoids can occur. One of them is the interaction of the free radical with oxygen to form the radical carotenoid peroxyl (ROO·). Due to the presence of the ROO·, interactions between radicals and carotenoids and/or between radicals are possible. In the termination step, final stable or non-reactive products such as alcohols, aldehydes, ketones, esters, and hydrocarbons are generated.

**Figure 8.** Scheme of the carotenoid oxidation process




Reference: Karel, 1980.

In addition to oxidation, other serious damages to carotenoids occur during extrusion and are related to the isomerization and hydroxylation of carotenoids at elevated temperatures (Momoi, Hofmann, Schmid, Urlacher, 2006; Zepka & Mercadante, 2009). These findings have suggested that oxidation is not only limited to the mechanisms indicated above, but it can be favored by the temperatures existing during the extrusion process. Some studies have shown that heat treatments can promote the conversion of trans-carotenoids into cis-carotenoids by isomerization (Dewanto, Xianzhong, Adom, Liu, 2002; Burgos et al., 2012; Basto et al., 2016). Thus, the stability of carotenoids depends on processing parameters used in the extrusion process, such as temperature, moisture, shear, and extrusion time (Riaz, Asif, Ali, 2009; Tiwari & Cummins, 2009).

Many researchers have studied the influence of extrusion conditions on carotenoid retention (Table 2). Marty & Berset (1988) investigated the effects of extrusion parameters on the stability of all-trans-β-carotene, exhibiting 8% of retention in a process conducted at temperature of 180 °C and screw speed of 150 rpm. In this way, different carotenoids and food matrices were evaluated, as well as, processing conditions, and this interest reveals the importance of understanding this phenomenon to improve the retention of these bioactive ingredients during the extrusion.

**Table 2.** Extrusion conditions and carotenoid retention

| Carotenoid | Raw material | Extrusion Parameters | Carotenoid retention (%) | References |
|---|---|---|---|---|
| All-trans- β-carotene | Corn starch<br>All-trans - β-carotene | Twin screw extruder<br>SS: 150 rpm<br>BT: 180 °C<br>MF: not defined | 8 | (Marty & Berset, 1988) |
| All-trans- β-carotene | Wheat flour<br>All-trans p-carotene powder | Twin screw extruder<br>SS: 150 rpm<br>BT: 125-200 °C<br>MF: 18% | 38-73 | (Guzman-Tello & Cheftel, 1990) |
| All-trans- β-carotene | Corn starch<br>All-trans - β-carotene | SS: 150 rpm<br>BT: 180 °C<br>MF: not defined | 8-93 | (Marty & Berset, 1990) |
| β-carotene | Maize starch<br>Synthetic β-carotene | Twin screw extruder<br>SS: 300-800 rpm<br>BT: 135-170 °C<br>MF: 18% | 45-71 | (Emin et al., 2012) |
| β-carotene | Sweet potato cultivars flour | Twin screw extruder<br>SS: 150-300 rpm<br>BT: 120 °C<br>MF: 30-40 % | 48-94 | (Waramboi et al., 2013) |
| β-carotene | Wheat flour<br>Synthetic carotenoid – emulsion | Twin screw extruder<br>SS: 300 rpm<br>BT: 105-155 °C<br>MF: not defined | 50-63 | (Caliskan et al., 2015) |
| β-carotene | Corn starch<br>Carrot pomace powder | Twin screw extruder<br>SS: 100-250 rpm<br>BT: 50–140 °C<br>MF: 15-30 % | 35-75 | (Kaisangsri et al., 2016) |
| Lycopene | Corn grits<br>Tomato pulp | Twin screw extruder<br>SS: 225 rpm<br>BT: 80-160 °C<br>MF:30 % | 24-37 | (Tonyali et al., 2016) |
| Lutein, zeaxanthin | Maize semolina<br>Natural purple corn extract /chia/ quinoa | Twin screw extruder<br>SS: 82 rpm<br>BT: 50-150 °C<br>MF: 14 % | 20-65 | (Cueto et al., 2017) |
| β-carotene, lutein, lycopene | Corn starch<br>Flour from pumpkin tissue/ Jerusalem artichoke/ amaranth seeds | Single-screw extruder<br>SS: 180 rpm<br>BT: 160-180 °C<br>MF: not defined | 5-100 | (Kolniak-Ostek et al., 2017) |
| β-carotene, lutein | Corn grits<br>Carrot pulp | Twin screw extruder<br>SS: 125-225 rpm<br>BT: 80-160 °C<br>MF: 25 % | 45-77 | (Ortak et al., 2017) |
| β-carotene | Orange-fleshed sweet potatoes/Bambara flour | Twin screw extruder<br>SS: 30 rpm<br>BT: 100-130 °C<br>MF: 14% | 60-87 | (Honi et al., 2018) |

| | | | | |
|---|---|---|---|---|
| All-trans-Lutein, all-trans-Zeaxanthin, β-Cryptoxanthin, all-trans-β-Carotene, Xanthophylls, cis-β-Carotene | Biofortified maize genotypes flour | Twin screw extruder<br>SS: 150-300 rpm<br>BT: 120-160 °C<br>MF: 25-35 % | 65-94 | (Ortiz et al., 2018) |
| β-carotene, α-carotene, β-cryptoxanthin | Grain pearl millet<br>Carrot powder/ Baobab/ Moringa powders | SS: 900 rpm<br>BT: 105-121 °C<br>MF: 35 | 60-90 | (Ndiaye et al., 2020) |

SS: Screw speed; BT: Barrel temperature; MF: Moisture Feed.

Reference: Own source.

## 2.5 Temperature

Thermal degradation is the cause that most likely leads to the loss of bioactive compounds during food processing (Cueto et al., 2017). Concerning carotenoids, the decomposition pathways depend on their chemical structure which, as discussed, comprises molecules with conjugated double-bond chains that are highly prone to degradation (Rawson et al., 2011; Oliveira, Pintado, Almeida, 2012). Carotenoid degradation is mainly associated with their low heat susceptibility.

Analyzing data from previous studies, Singh et al. (2007) reported that temperatures of 200 °C impact carotenoid stability during extrusion. However, that value is a little too optimistic because thermal degradation and significant carotenoid reduction (approximately 40%) were observed during extrusion at temperatures ranging from 94 to 140 °C (Delgado-Nieblas et al., 2012). Dar, Sharma, Kumar (2014) reported that a rice-based extrudate enriched with carrot pomace showed high degradation of β-carotene at extrusion temperatures in the range 110–140 °C.

Unfortunately, despite the evidence of carotenoid degradation during extrusion, an exact threshold extrusion temperature has not been determined for different raw material formulations. Different extrusion conditions lead to different local temperatures, which may affect carotenoid retention. The food matrix, the extruder, the process conditions, as well as the method of quantifying the carotenoid content should be standardized to establish consistent retention data. According to Rojas-Garbanzo, Pérez, Bustos-Carmona, Vaillant (2011), carotenoid loss depends on the type of carotenoid in the extruded material. It is noteworthy to mention that Obradović et al. (2015) found that xanthophylls (lutein and zeaxanthin) showed less sensitivity to extrusion than other carotenes (e.g., α-carotene, 9-*cis*-β-carotene, and 13-*cis*-β-carotene). Similarly, Kolniak-Ostek et al. (2017) found that in extruded corn products blended

with pumpkin tissue four, Jerusalem artichoke, and amaranth seeds, the content of carotenoids decreased, in the order of lycopene, followed by β-carotene, and lutein.

Furthermore, the degradation of carotenoids is favored in an oil medium with a high content of unsaturated fatty acids. The higher lipid content may lead to the generation of oxidizing agents that can interact with carotenoids, promoting their damage (Frankel, 1992). Cueto et al. (2017) showed the highest losses of carotenoids during processing of extrudates used in cornflake production in formulations containing chia seeds when compared to control (without chia), indicating that carotenoid degradation probably was due to the presence of chia's highly unsaturated lipid content. Moreover, according to the authors, the high content of lipids may have generated components that favor the degradation of carotenoids, such as free radicals and peroxides.

Other studies found that the extrusion conditions promote isomerization of trans-carotene into *cis*-carotene. Marty & Berset (1988) revealed the production of *cis* isomers and other degradation products after extrusion cooking at 180 °C. Guzman-Tello & Cheftel (1990) described the formation of the 13-*cis* isomer mainly at a barrel temperature of approximately 150 °C, as well as the formation of the 9-*cis* isomer at higher barrel temperatures. According to Ortak et al. (2017), the mutual influence of the thermal and mechanical treatment can explain the loss of β-carotene and lutein content during extrusion to produce snacks made from corn grits mixed with carrot pulp. In this study, the reason for the reduction of β-carotene was supported by the isomerization of β-carotene from its most stable natural *trans* to the *cis* form.

In nature, β-carotene is generally in the form of a *trans* isomer, and the high extrusion temperature promotes isomerization to the *cis* configuration. However, *cis*-β-carotene is more polar than the *trans* isomer, which increases its solubility. Thus, according to Ortak et al. (2017), this phenomenon can compensate for the loss of β-carotene observed in extrusion at high temperatures.

The temperature of carotenoid retention can vary depending on the applied shear, the moisture content, and the feed formulation. Rodríguez et al. (2021) investigated the synergy among these factors to affect carotenoid stability during extrusion. It was found that the combined effect of feed moisture (13.2–13.7%) and barrel temperature (120–132 °C) led to changes in the carotenoid profile of maize grits extruded snacks. Under these extrusion conditions, β-cryptoxanthin and β-carotene increased whereas the content of lutein and zeaxanthin was reduced in the extruded product. Tovar-Jiménez et al. (2015) reported that the optimal processing conditions for carotenoid retention were achieved by setting the feed

moisture around 22–24% and temperatures of 128–130 °C. In addition, other studies suggested that the presence of other components in the formulation, such as proteins (Dehghan-Shoar, Hardacre, Brennan, 2010; Ying et al., 2015) and starch (Paznocht, Burešová, Kotíková, Martinek, 2021), can minimize the degradation of carotenoid by acting as a protective media of these bioactive compounds.

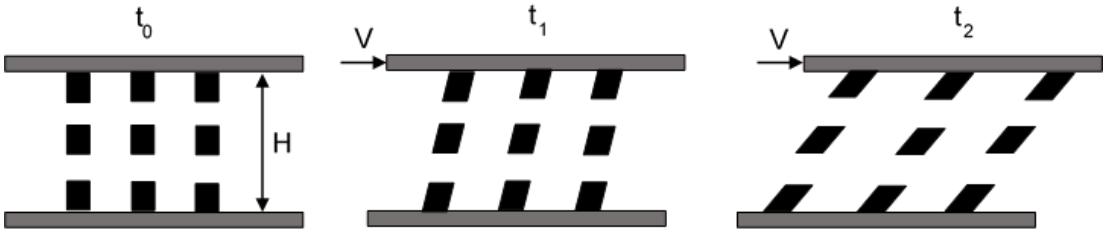
## 2.6 Shear

It is assumed that the degradation of carotenes occurs due to the thermo-mechanical action of the extrusion process (Wyman, 1975). The high rotational speed of the screws produces high friction and increase temperature, which may reduce the local melt viscosity. However, the high shear rate prevailing in the extrusion process can also produce molecular degradation. The conception of shear rate $\dot{y}$ in twin and single screw extruders is based on the velocity (V) of the melt and the depth of the channel (H):

$$\dot{y} = \frac{V}{H} \quad (1)$$

The effects of a simple shear fow are schematically illustrated in Figure 9. At time $t_0$, a local volume of fluid, indicated by a black square, is located between two parallel plates separated by a distance (H). In following times, e.g., times $t_1$ and $t_2$, due to the movement of the top plate with a constant speed (V) due to the shear action, the deformation of the fluid elements is noted.

**Figure 9.** Schematic description of the fluid particle shear deformation under a constant flow




Reference: Bouvier & Campanella, 2014.

According to Bouvier & Campanella (2014), the velocity profile and the shear rate field in the extruder channel are more complex than that illustrated in Figure 9; instead, it varies with

the position of the fluid element in the channel. The prevalent laminar flow owing to the high extrudate viscosity translates in non-uniform different residence times and shear fields in the extruder. Thus, a complete characterization of shear forces applied during the process is important to explain its influence on carotenoid degradation. There is information on the effects of different screw geometries on shear profiles and residence times that should be taken into account to quantitatively assess their effects on molecular degradation of the extrudate main components and quality of the final products (Bouvier & Campanella, 2014).

The degradation of β-carotene during extrusion at 180 °C was more severe than the loss observed after just heating for a long time, 2 h, under the same temperature conditions (Marty & Berset, 1986). This finding confirms the key influence of shear on carotenoid degradation. Furthermore, Kosińska-Cagnazzo, Bocquel, Marmillod, Andlauer (2017) evaluated the carotenoid stability of goji berries after extrusion. Temperatures of 110, 130, and 150 °C were considered to evaluate their effect parameter on carotenoid degradation. They found that approximately 60% of zeaxanthin dipalmitate was lost, regardless of the process temperature which showed no statistically significant differences, suggesting that other process parameters, such as shear, may have caused carotenoid degradation.

The increase of the melt temperature in the range of 135–170°C did not affect β-carotene retention (Emin, Mayer-Miebach, Schuchmann, 2012). However, the change of screw speed from 300 to 500 rpm favored compound retention by approximately 25%. While the screw speed was higher, the viscosity of the system was lower, which probably caused a reduction of shear forces. Furthermore, the residence time of the extrudate has an effect on the retention of carotenoids, because not only the shear rate, but also the shear strain, may affect the retention of carotenoids. In light of this, the results indicate that β-carotene retention is largely related mainly to the mechanical forces generated, especially the shear rate and shear strain in extrusion rather than by thermal effects.

Similarly, Waramboi, Gidley, Sopade (2013) analyzed the effects of extrusion parameters separately and found that a screw speed of 300 rpm retained the carotenoid for more than 80% in extruded sweet potato four cultivars. Conversely, carotenoid retention was lower at a screw speed of 150 rpm. These results show that not only the shear rate applied in the process, but also the amount of shear, or shear strain, affect carotenoid retention. As discussed, shear strain depends on the product residence time. Thus, degrading actions on carotenoids could be minimized using shorter residence time and less degree of filling of the barrel when screw speed is around 300 rpm. Higher screw speeds possibly increase the frequency of

exposure to the maximum shear stresses. However, it is necessary to better understand the flow patterns and the resulting shear stress and strain profiles during extrusion cooking that are described for both single screw and twin-screw technologies by Bouvier & Campanella (2014).

The snack industry uses a parameter associated with the thermomechanical treatment in the extrusion process to associate it with the quality of the product in terms of molecular transformations and texture. The parameter is known as the specific mechanical energy (SME) that can be calculated from the torque applied by the extruder motor, the screw speed, and the mass throughput (Bouvier & Campanella, 2014). According to Dehghan-Shoar, Hardacre, Brennan (2010) the loss of carotenoids during extrusion measured by the lycopene content was related to SME. The study indicated the effects of the mechanical action, assessed by the SME parameter, on lycopene loss during extrusion and showed that increases in the processing temperature (140–180 °C) had no significant effect on lycopene retention in comparison to SME.

Concerning factors associated with processing, Ortiz et al. (2018) reported the influence of moisture content, screw speed, and barrel temperature profile on carotenoid retention. They identified the greatest retention of carotenoids when the moisture was higher and the screw speed was lower, which agrees with what is discussed above considering the effect of the viscosity and frictional heating. Conversely, at low extrusion moisture, the greatest retention of carotenoids was obtained when the screw speed was higher. In other words, the negative effect of the high local shear forces was compensated with a lower residence time associated with the higher screw speed.

As discussed above, depending on the screw configuration, a low moisture condition is a severe condition, and the probability of bioactive compound degradation and molecular transformations are increased (Waramboi, Gidley, Sopade, 2013). Low moisture levels associated with low screw speeds, conditions that favor long residence times, and a larger degree of barrel filling-all degrade carotenoids significantly. It has been reported that depending on the extrusion conditions about 45 to 80% of the bioactivity of carotenoids can be maintained. Under high moisture conditions (above 40%), the retention of carotenoids can be more than 80% (Kosińska-Cagnazzo, Bocquel, Marmillod, Andlauer, 2017).

In the extrusion process, the feed moisture content can be adjusted prior to the extrusion process or through controlled water injection in barrels close to the feeding section. When the material moves along the barrel that is subjected to high temperatures, pressure mixing, and

shear, the water in the product, owing to the high pressures, is in liquid form and acts as a plasticizer.

Optimization of the moisture content used in the extrusion process to maximize carotenoid retention has been extensively studied because it is an important step in the design of the extrusion process considering that high feed moisture contents can offset the negative effects of high temperatures in the retention of the carotenoids. The extrusion conditions to preserve β-carotene in extruded corn starch enriched with passion fruit pulp was reported by Cortés, Guzmán, Martínez-Bustos (2014) as a feed moisture content of 27% and temperatures around 127 °C.

Nevertheless, in addition to optimizing the extrusion processing conditions, there exist other alternatives to preserve carotenoids during extrusion. Therefore, it is worth considering other strategies to retain the activity of these valuable compounds, after identifying the main causes of carotenoid losses in the process

## 2.7 Systems to protect carotenoids during extrusion

As discussed, carotenoids are widely susceptible to degradation both during food processing and storage, due to chemical, thermal, and mechanical effects. Therefore, different approaches have been investigated to develop effective protective and delivery systems to improve the use, bioavailability, and stability of carotenoids in foods.

The increase in β-carotene retention by approximately 40% was possible after dissolving the compound in oil and adding it to the final stages of the extrusion process (Emin, Mayer-Miebach, Schuchmann, 2012). Ying et al. (2015) reported an increase of β-carotene retention during extrusion, about 90%, when mixing the compound in a heated protein-carbohydrate matrix prior to addition to the process. Thus, it was hypothesized that a heated protein-carbohydrate system is able to guarantee the stability of oil-soluble bioactive in extruded foods (Ying et al., 2015). Similarly, Pensamiento-Niño et al. (2018) also postulated the protective mechanism of the protein-carbohydrate matrix.

Since they are very soluble in nonpolar solvents, carotenoids can be incorporated into organic solutions, such as vegetable oils, increasing their bioavailability in the human body. In addition to acting as solvents, organic solutions can help to prevent the degradation of carotenoids. Thus, the incorporation of carotenoids into saturated oils prevents the generation

of radical species that can react with carotenoids, offering greater stability to lipid oxidation (Ambati, Moi, Ravi, Aswathanarayana, 2014).

The presence of polyphenols, flavonoids, tocopherols, and vitamins (C and E) can also ensure the stability of carotenoids. Thus, flours with a high phenolic content added to extrusion formulations (e.g., pumpkin tissue, Jerusalem artichoke, and amaranth seeds) possibly protect carotenoids from processing damage (Kolniak-Ostek et al., 2017). Obradović et al. (2015) added ascorbic acid to enhance the retention of carotenoids present in pumpkin powder included in an extrusion formulation, which affected β-carotene isomer stability as indicated by the preservation of a red color in the extrudates.

Ruiz-Armenta et al. (2018) reported that compounds present in the naranjita fruit bagasse, such as pectins and gums, can alter the rheological characteristics of the mixtures, promoting more fluidity and shorter residence time and, consequently, less damage to carotenoids. Ndiaye et al. (2020) described the increased stability of carotenoids after extrusion of grain pearl millet combined with carrot powder, moringa, and baobab leaf powders, which are additives rich in antioxidants. The extrusion was carried out utilizing a short barrel extruder at 900 rpm of speed, and final temperatures varying between 105 and 121 °C, i.e., a process with a very short residence time.

Protection techniques have been also reported to reduce carotenoid degradation during the extrusion process. Microencapsulation, for instance, consists of covering the bioactive compound by one or more protective materials, generally biopolymers that can form a barrier protecting the bioactive compound from harsh external conditions (Favaro-Trindade & De Pinho, 2008). These techniques include microencapsulation using various polymers, such as chitosan, production of polymeric nanospheres, liposomes, and matrices with β-cyclodextrin or calcium ions (Tachaprutinun, Udomsup, Luadthong, Wanichwecharungruang, 2009). Favaro-Trindade et al. (2020) demonstrated that microencapsulation by coacervation technique is a useful and efficient way to protect cinnamon extract compounds under extrusion conditions. The products obtained at 130 °C, with shear rates of 1000 $s^{-1}$ and 500 $s^{-1}$ and residence time of 0.128 and 0.256 s showed greater protection of the bioactive compound during processing and storage.

As further evidence, Caliskan, Lim, Roos (2015) reported that encapsulation of β-carotene in oil droplets increased its retention for 50 and 63% from the value obtained of compounds protected with a single layer and layer-by-layer emulsions, respectively, suggesting the possibility of using the approach to protect bioactive compounds added to the formulations

of snack-type extrudates. However, the degradation kinetics of β-carotene were considered significant. Carotenoid degradation can be described by a first-order reaction as the one used for β-carotene degradation kinetics expressed by Eq. (2) below:

$$ln\frac{C_{(t)}}{C_0} = -kt \quad (2)$$

where $C_{(t)}$ is the concentration of the bioactive compound at time t, $C_0$ is its initial concentration, and k is the rate constant.

It is important to highlight that in research related to studies on carotenoid degradation, the lack of standards in the chemical analyses can be crucial to establish reasonable associations. For appropriate comparisons, factors such as the extraction method, reagents used, and extraction conditions must be considered, as these factors largely interfere in the evaluation of compounds arisen from the degradation of carotenoids and therefore in the effectiveness of quantifying these compounds in different food matrices.

## 2.8 Conclusions

The incorporation of carotenoids in extruded foods is a vehicle for obtaining functional foods widely consumed so they become available in popular and indulgent foods in addition to the consumption of carotenoids present in the cellular matrix of plants. However, preserving these components during the extrusion process remains a challenge, since these added and processed carotenoids are prone to more degradation than those occurring in plant tissues.

The extrusion processing conditions and their effects on the extruded material are interrelated in a complex manner, thus making it difficult to relate carotenoid retention to one single factor. Critical extrusion parameters, such as temperature above 100 °C combined with moisture below 15% and high screw speed are conditions which combined with inadequate formulations can cause damage of carotenoids. Recent studies have highlighted and elucidated some mechanisms imposed by many processing conditions that are responsible for the damage of these bioactive compounds. This knowledge, with improved extruders and process control, can help to optimize parameters that can produce extruded products with the good texture and enhanced nutritional properties.

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## References

Achir, N., Randrianatoandro V.A., Bohuon P., Lafargue A., & Avallone S. (2010). Kinetic study of β-carotene and lutein degradation in oils during heat treatment. *European Journal of Lipid Science and Technology*, 112(3):349–361. https://doi.org/10.1002/ejlt.200900165

Alam, M.S., Kaur J, Khaira H, & Gupta K. (2016). Extrusion and extruded products: changes in quality attributes as afected by extrusion process parameters: a review. *Critical Reviews in Food Science and Nutrition*, 56(3):445–473. https://doi.org/10.1080/10408398.2013.779568

Alonso, R., Orúe E., & Marzo F. (1998). Efects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chemistry*, 63(4):505–512. https://doi. org/10.1016/S0308-8146(98)00037-5

Ambati, R.R., Moi P.S, Ravi S., & Aswathanarayana R.G. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications - a review. *Marine Drugs*, 12(1):128–152. https://doi.org/10.3390/md12010128

Azevedo-Meleiro, C.H. & Rodriguez-Amaya D.B. (2004). Confrmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. *Journal of Food Composition and Analysis*, 17(3–4):385–396. https://doi.org/ 10.1016/j.jfca.2004.02.004

Basto, G.J., Carvalho, C.W.P., Soares, A.G., Costa, H.T.G.B., Chávez, D.W.H., de Godoy, O., & Pacheco, S. RL. (2016). Physicochemical properties and carotenoid content of extruded and non-extruded corn and peach palm (Bactris gasipaes, Kunth). *LWT - Food Science and Technology*, 69:312–318. https://doi.org/10.1016/j.lwt.2015.12.065

Bernal, J., Mendiola, J.A., Ibáñez, E., & Cifuentes, A. (2011). Advanced analysis of nutraceuticals. *Journal of Pharmaceutical and Biomedical Analysis*, 55(4):758– 774. https://doi.org/10.1016/j.jpba.2010.11.033

Biehler, E., Mayer, F., Hofmann, L., Krause, E., & Bohn, T. (2010). Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. *Journal of Food Science*, 75(1). https:// doi.org/10.1111/j.1750-3841.2009.01417.x

Boon, C.S., McClements, D.J., Weiss, J., & Decker, E.A. (2010). Factors infuencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition*, 50(6):515–532. https://doi.org/10.1080/ 10408390802565889

Bouvier, J. M., & Campanella, O. H. (2014). *Extrusion processing technology: Food and non-food biomaterials*. John Wiley & Sons. https://doi.org/ 10.1002/9781118541685

Breithaupt, D. E., Weller, P., Wolters, M., & Hahn, A. (2003). Plasma response to a single dose of dietary β-cryptoxanthin esters from papaya (Carica papaya L.) or non-esterified β-cryptoxanthin in adult human subjects: a comparative study. *British Journal of Nutrition,* 90(4), 795-801.https://doi.org/10.1079/bjn2003962

Brennan, C., Brennan, M., Derbyshire, E., & Tiwari, B. K. (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science & Technology*, 22(10), 570-575. https://doi.org/10.1016/j.tifs.2011.05.007

Brennan, M. A., Derbyshire, E., Tiwari, B. K., & Brennan, C. S. (2013). Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science & Technology*, 48(5), 893-902. https://doi.org/10.1111/ijfs. 12055

Brennan, M. A., Monro, J. A., & Brennan, C. S. (2008). Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. *International Journal of Food Science & Technology,* 43(12), 2278-2288. https://doi.org/10.1111/j.1365-2621. 2008.01867.x

Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, 9(15), 1551-1558. https://doi.org/10. 1096/fasebj.9.15.8529834

Buono, S., Langellotti, A. L., Martello, A., Rinna, F., & Fogliano, V. (2014). Functional ingredients from microalgae. *Food & Function*, 5(8), 1669-1685. https://doi.org/10.1039/c4fo00125g

Burgos, G., Amoros, W., Salas, E., Muñoa, L., Sosa, P., Díaz, C., & Bonierbale, M. (2012). Carotenoid concentrations of native Andean potatoes as affected by cooking. *Food Chemistry*, 133(4), 1131-1137. https://doi.org/10.1016/j.foodchem.2011.09.002

Caliskan, G., Lim, A. S., & Roos, Y. H. (2015). Beta-carotene stability in extruded snacks produced using interface engineered emulsions. *International Journal of Food Properties*, 18(10), 2256-2267. https://doi.org/10. 1080/10942912.2014.973963

Camire, M. E., Camire, A., & Krumhar, K. (1990). Chemical and nutritional changes in foods during extrusion. *Critical Reviews in Food Science & Nutrition*, 29(1), 35-57. https:// doi.org/10.1080/10408399009527513

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frígola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety*, 13(2), 155-171. https://doi.org/10.1111/1541-4337.12049

Chávez-Jáuregui, R. N., Santos, R. D., Macedo, A., Chacra, A. P. M., Martinez, T. L., & Arêas, J. A. G. (2010). Effects of defatted amaranth (Amaranthus caudatus L.) snacks on lipid metabolism of patients with moderate hypercholesterolemia. *Food Science and Technology*, 30, 1007-1010. https://doi.org/10.1590/S0101- 20612010000400026

Cheftel, J. C. (1986). Nutritional effects of extrusion-cooking. *Food Chemistry*, 20(4), 263-283. https://doi.org/10.1016/0308-8146(86)90096-8

Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2013). Functional properties of carotenoids originating from algae. *Journal of the Science of Food and Agriculture*, 93(1), 5-11. https://doi.org/10.1002/jsfa.5902

Cortés, R. N. F., Guzmán, I. V., & Martínez-Bustos, F. (2014). Effects of some extrusion variables on physicochemical characteristics of extruded corn starch-passion fruit pulp (Passiflora edulis) snacks. *Plant Foods for Human Nutrition*, 69(4), 365-371. https://doi.org/10.1007/ s11130-014-0443-8

Courraud, J., Berger, J., Cristol, J. P., & Avallone, S. (2013). Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. *Food Chemistry*, 136(2), 871-877. https:// doi.org/10.1016/j.foodchem.2012.08.076

Cueto, M., Farroni, A., Schoenlechner, R., Schleining, G., & Buera, P. (2017). Carotenoid and color changes in traditionally flaked and extruded products. *Food Chemistry* 229, 640-645.https://doi.org/10. 1016/j.foodchem.2017.02.138

Dar, A. H., Sharma, H. K., & Kumar, N. (2014). Effect of extrusion temperature on the microstructure, textural and functional attributes of carrot pomace-based extrudates. *Journal of Food Processing and Preservation*, 38(1), 212-222. https://doi.org/10.1111/j.1745-4549.2012.00767.x

Dehghan-Shoar, Z., Hardacre, A. K., & Brennan, C. S. (2010). The physico-chemical characteristics of extruded snacks enriched with tomato lycopene. *Food Chemistry*, 123(4), 1117-1122.https://doi.org/ 10.1016/j.foodchem.2010.05.071

Delgado-Nieblas, C., Aguilar-Palazuelos, E., Gallegos-Infante, A., Rocha-Guzmán, N., Zazueta-Morales, J., & Caro-Corrales, J. (2012). Characterization and optimization of extrusion cooking for the manufacture of third-generation snacks with winter squash (Cucurbita moschata D.) flour. *Cereal Chemistry*, 89(1), 65-72. https://doi.org/10.1094/ CCHEM-02-11-0016

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50(10), 3010-3014. https://doi.org/10.1021/jf0115589

Donhowe, E. G., & Kong, F. (2014). Beta-carotene: digestion, microencapsulation, and in vitro bioavailability. *Food and Bioprocess Technology*, 7(2), 338-354.https://doi.org/10.1007/s11947-013-1244-z

Emin, M. A., Mayer-Miebach, E., & Schuchmann, H. P. (2012). Retention of β-carotene as a model substance for lipophilic phytochemicals during extrusion cooking. *LWT-Food Science and Technology*, 48(2), 302-307.https://doi.org/10.1016/j.lwt.2012.04.004

Everett, S. A., Dennis, M. F., Patel, K. B., Maddix, S., Kundu, S. C., & Willson, R. L. (1996). Scavenging of Nitrogen Dioxide, Thiyl, and Sulfonyl Free Radicals by the Nutritional Antioxidant ²-Carotene (∗). *Journal of Biological Chemistry*, 271(8), 3988-3994.

Favaro-trindade, C.S. & De Pinho, S.C. (2008). Revisão: Microencapsulação de ingredientes alimentícios. *BR Journal of Food and Technology*, 11:103–112. https://doi.org/10.18406/2316-1817v1n32009223

Favaro-Trindade, C. S., Patel, B., Silva, M. P., Comunian, T. A., Federici, E., Jones, O. G., & Campanella, O. H. (2020). Microencapsulation as a tool to producing an extruded functional food. *LWT*, 128, 109433. https://doi.org/10.1016/j.lwt.2020.109433

Fellows, P. J. (2000). *Food processing technology*: Principles and Practice, 2nd ed. CRC Press, Boca Raton (Chapter 14).

Fernández-García, E., Carvajal-Lérida, I., Jarén-Galán, M., Garrido-Fernández, J., Pérez-Gálvez, A., & Hornero-Méndez, D. (2012). Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International*, 46(2), 438-450.https://doi.org/ 10.1016/j.foodres.2011.06.007

Frankel, E. N. (1991). Recent advances in lipid oxidation. *Journal of the Science of Food and Agriculture*, 54(4), 495-511.https://doi.org/10.1002/jsfa. 2740540402

Gammone, M.A., Riccioni. G., & D'Orazio, N, (2015). Carotenoids: Potential allies of cardiovascular health? *Food & Nutrition Research*, *59*(1), 26762. https://doi.org/10.3402/fnr.v59.26762

Goralczyk, R. (2009). ß-Carotene and lung cancer in smokers: Review of hypotheses and status of research. *Nutrition and Cancer*, 61(6), 767-774. https://doi.org/10.1080/01635580903285155

Gouveia, L., & Empis, J. (2003). Relative stabilities of microalgal carotenoids in microalgal extracts, biomass and fish feed: effect of storage conditions. *Innovative Food Science & Emerging Technologies*, 4(2), 227-233. https://doi.org/10.1016/S1466-8564(03)00002-X

Gu, L., House, S. E., Rooney, L. W., & Prior, R. L. (2008). Sorghum extrusion increases bioavailability of catechins in weanling pigs. *Journal of Agricultural and Food Chemistry*, 56(4), 1283-1288. https://doi.org/10.1021/ jf072742i

Guedes, A. C., Amaro, H. M., & Malcata, F. X. (2011). Microalgae as sources of carotenoids. *Marine Drugs*, 9(4), 625-644. https://doi. org/10.3390/md9040625

Guzman-Tello, R., & Cheftel, J. C. (1990). Colour loss during extrusion cooking of β-carotene-wheat flour mixes as an indicator of the intensity of thermal and oxidative processing. *International Journal of Food Science & Technology*, 25(4), 420-434. https://doi.org/10.1111/j.1365-2621. 1990.tb01099.x

Hedren, E., Diaz, V., & Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *European Journal of Clinical Nutrition*, 56(5), 425-430.https://doi.org/10.1038/ sj.ejcn.1601329

Herrera-Andrade, M. H. (2011). Extracción de la astaxantina y su estabilidad. *Revista Latinoamericana de Recursos Naturales*, 7(1), 21-27.

Hill, T. J., Land, E. J., McGarvey, D. J., Schalch, W., Tinkler, J. H., & Truscott, T. G. (1995). Interactions between carotenoids and the CCl3O2. bul. *Radical. Journal of the American Chemical Society*, 117(32), 8322-8326. https:// doi.org/10.1021/ja00137a004

Honi, B., Mukisa, I. M., & Mongi, R. J. (2018). Proximate composition, provitamin A retention, and shelf life of extruded orange-fleshed sweet potato and bambara groundnut-based snacks. *Journal of Food Processing and Preservation*, 42(1), e13415.. https://doi.org/10.1111/jfpp. 13415

Hornero-Méndez, D., & Mínguez-Mosquera, M. I. (2007). Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. *Innovative Food Science & Emerging Technologies*, 8(3), 407-412.https:// doi.org/10.1016/j.ifset.2007.03.014

Noviendri, D., Hasrini, R. F., & Octavianti, F. (2011). Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. *Journal of Medicinal Plants Research*, 5(33), 7119-7131. https://doi.org/10.5897/JMPRx11.011

Kaisangsri, N., Kowalski, R. J., Wijesekara, I., Kerdchoechuen, O., Laohakunjit, N., & Ganjyal, G. M. (2016). Carrot pomace enhances the expansion and nutritional quality of corn starch extrudates. *LWT-Food Science and Technology*, 68, 391-399.. https://doi.org/10.1016/j.lwt.2015.12.016

Karel, M. (1980). Lipid oxidation, secondary reactions, and water activity of foods. In *Autoxidation in food and biological systems* (pp. 191-206). Springer, Boston, MA.

Kean, E. G., Hamaker, B. R., & Ferruzzi, M. G. (2008). Carotenoid bioaccessibility from whole grain and degermed maize meal products. *Journal of Agricultural and Food Chemistry*, 56(21), 9918-9926.https://doi.org/10.1021/ jf8018613

Khoo, H. E., Prasad, K. N., Kong, K. W., Jiang, Y., & Ismail, A. (2011). Carotenoids and their isomers: color pigments in fruits and vegetables. *Molecules,* 16(2), 1710-1738. https://doi.org/10.3390/molecules16021710

Kolniak-Ostek, J., Kita, A., Pęksa, A., Wawrzyniak, A., Hamułka, J., Jeznach, M., ... & Jariene, E. (2017). Analysis of the content of bioactive compounds in selected flours and enriched extruded corn products. *Journal of Food Composition and Analysis,* 64, 147-155. https://doi. org/10.1016/j.jfca.2017.08.008

Kosińska-Cagnazzo, A., Bocquel, D., Marmillod, I., & Andlauer, W. (2017). Stability of goji bioactives during extrusion cooking process. *Food Chemistry*, 230, 250-256.https://doi.org/10.1016/j.foodchem. 2017.03.035

Kotake-Nara, E., Asai, A., & Nagao, A. (2005). Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells. *Cancer Letters,* 220(1), 75-84. https://doi.org/10.1016/j.canlet.2004.07.048

Koushan, K., Rusovici, R., Li, W., Ferguson, L. R., & Chalam, K. V. (2013). The role of lutein in eye-related disease. *Nutrients*, 5(5), 1823-1839. https:// doi.org/10.3390/nu5051823

Krinsky, N. I., Landrum, J. T., & Bone, R. A. (2003). Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annual Review of Nutrition*, 23(1), 171-201. https://doi.org/10.1146/annurev.nutr.23.011702.073307

Lavelli, V., Zanoni, B., & Zaniboni, A. (2007). Effect of water activity on carotenoid degradation in dehydrated carrots. *Food Chemistry*, 104(4), 1705-1711.https://doi.org/10.1016/j.foodchem.2007.03. 033

Martínez-Delgado, A. A., Khandual, S., & Villanueva–Rodríguez, S. J. (2017). Chemical stability of astaxanthin integrated into a food matrix: Effects of food processing and methods for preservation. *Food Chemistry*, 225, 23-30.https://doi.org/10.1016/j.foodchem.2016.11.092

Marty, C., & Berset, C. (1986). Degradation of trans-β-carotene during heating in sealed glass tubes and extrusion cooking. *Journal of Food Science,* 51(3), 698-702. https://doi.org/10.1111/j.1365-2621.1986.tb13915.x

Marty, C., & Berset, C. (1988). Degradation products of trans-β-carotene produced during extrusion cooking. *Journal of Food Science,* 53(6), 1880-1886. https://doi.org/10.1111/j.1365-2621.1988. tb07866.x

Marty, C., & Berset, C. (1990). Factors affecting the thermal degradation of all-trans-. beta.-carotene. *Journal of Agricultural and Food Chemistry*, 38(4), 1063-1067. https://doi.org/10.1021/jf00094a033

Momoi, K., Hofmann, U., Schmid, R. D., & Urlacher, V. B. (2006). Reconstitution of β-carotene hydroxylase activity of thermostable CYP175A1 monooxygenase. *Biochemical And Biophysical Research Communications*, 339(1), 331-336. https://doi.org/10.1016/j.bbrc.2005.11.023

Mulokozi, G., Hedrén, E., & Svanberg, U. (2004). In vitro accessibility and intake of β-carotene from cooked green leafy vegetables and their estimated contribution to vitamin A requirements. *Plant Foods for Human Nutrition*, 59(1), 1-9. https://doi.org/10. 1007/s11130-004-4305-7

Murphy, M. M., Barraj, L. M., Spungen, J. H., Herman, D. R., & Randolph, R. K. (2014). Global assessment of select phytonutrient intakes by level of fruit and vegetable consumption. *British Journal of Nutrition*, 112(6), 1004-1018. https://doi.org/10.1017/S0007114514001937

Ndiaye, C., Martinez, M. M., Hamaker, B. R., Campanella, O. H., & Ferruzzi, M. G. (2020). Effect of edible plant materials on provitamin A stability and bioaccessibility from extruded whole pearl millet (P. typhoides) composite blends. *LWT*, 123, 109109. https://doi.org/10.1016/j.lwt.2020.109109

Norfezah, M. N., Hardacre, A., & Brennan, C. S. (2011). Comparison of waste pumpkin material and its potential use in extruded snack foods. *Food Science and Technology International*, 17(4), 367-373. https://doi.org/10. 1177/1082013210382484

Oboh, G., Falade, A. O., & Ademiluyi, A. O. (2014). Effect of thermal oxidation on the physico-chemical properties, malondialdehyde and carotenoid contents of palm oil. *Rivista Italiana Delle Sostanze Grasse*, 91(1), 59-65.

Obradović, V., Babić, J., Šubarić, D., Ačkar, Đ., & Jozinović, A. (2014). Improvement of nutritional and functional properties of extruded food products. *Journal of Food and Nutrition Research*, 53(3), 189-206.

Obradović, V., Babić, J., Šubarić, D., Jozinović, A., Ačkar, Đ., & Klarić, I. (2015). Influence of dried Hokkaido pumpkin and ascorbic acid addition on chemical properties and colour of corn extrudates. *Food Chemistry*, 183, 136-143. https://doi.org/10.1016/j.foodchem. 2015.03.045

Obulesu, M., Dowlathabad, M. R., & Bramhachari, P. V. (2011). Carotenoids and Alzheimer's disease: an insight into therapeutic role of retinoids in animal models. *Neurochemistry International*, 59(5), 535-541. https://doi.org/10.1016/j.neuint.2011.04.004

Oliveira, A., Pintado, M., & Almeida, D. P. (2012). Phytochemical composition and antioxidant activity of peach as affected by pasteurization and storage duration. *LWT-Food Science and Technology*, *49*(2), 202-207. https://doi.org/10.1016/j.lwt.2012.07.008

Oroian, M., & Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, *74*, 10-36. https://doi.org/10.1016/j.foodres.2015.04.018

Ortak, M., Caltinoglu, C., Sensoy, I., Karakaya, S., & Mert, B. (2017). Changes in functional properties and in vitro bioaccessibilities of β-carotene and lutein after extrusion processing. *Journal of food science and technology*, *54*(11), 3543-3551.https://doi.org/10.1007/ s13197-017-2812-4

Ortiz, D., Ponrajan, A., Bonnet, J. P., Rocheford, T., & Ferruzzi, M. G. (2018). Carotenoid stability during dry milling, storage, and extrusion processing of biofortified maize genotypes. *Journal of Agricultural and Food Chemistry*, *66*(18), 4683-4691. https://doi.org/ 10.1021/acs.jafc.7b05706

Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*, *72*(2), R21-R32.https://doi.org/10.1111/j.1750-3841.2007.00274.x

Pasquet, V., Morisset, P., Ihammouine, S., Chepied, A., Aumailley, L., Berard, J. B., ... & Picot, L. (2011). Antiproliferative activity of violaxanthin isolated from bioguided fractionation of Dunaliella tertiolecta extracts. *Marine Drugs*, *9*(5), 819-831. https:// doi.org/10.3390/md9050819

Paznocht, L., Burešová, B., Kotíková, Z., & Martinek, P. (2021). Carotenoid content of extruded and puffed products made of colored-grain wheats. *Food Chemistry*, *340*, 127951.https://doi.org/10.1016/j. foodchem.2020.127951

Pensamiento-Niño, C. A., Gómez-Aldapa, C. A., Hernández-Santos, B., Juárez-Barrientos, J. M., Herman-Lara, E., Martínez-Sánchez, C. E., ... & Rodríguez-Miranda, J. (2018). Optimization and characterization of an extruded snack based on taro flour (Colocasia esculenta L.) enriched with mango pulp (Mangifera indica L.). *Journal of Food Science and Technology*, *55*(10), 4244-4255.https://doi.org/10.1007/ s13197-018-3363-z

Perera, C. O., & Yen, G. M. (2007). Functional properties of carotenoids in human health. *International Journal of Food Properties*, *10*(2), 201-230. https://doi.org/10. 1080/10942910601045271

Polyakov, N. E., & Leshina, T. V. (2006). Certain aspects of the reactivity of carotenoids. Redox processes and complexation. *Russian Chemical Reviews*, *75*(12), 1049. https://doi.org/10.1070/ rc2006v075n12abeh003640

Potter, R., Stojceska, V., & Plunkett, A. (2013). The use of fruit powders in extruded snacks suitable for Children's diets. *LWT-Food science and technology*, *51*(2), 537-544. https://doi.org/10.1016/j.lwt.2012. 11.015

Priyadarshani, A.M.B. & Jansz, E.R. (2014). A critical review on carotenoid research in Sri Lankan context and its outcomes. *Critical Reviews in Food Science and Nutrition,* 54(5):561–571. https://doi.org/10.1080/10408398.2011.595019

Rawson, A., Patras, A., Tiwari, B. K., Noci, F., Koutchma, T., & Brunton, N. (2011). Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. *Food Research International*, *44*(7), 1875-1887.https://doi.org/10.1016/j.foodres.2011.02.053

Riaz, M.N., Asif, M., Ali, R. (2009). Stability of vitamins during extrusion. *Critical Reviews in Food Science and Nutrition*, 49(4):361–368. https://doi. org/10.1080/10408390802067290

Robert, P., Carlsson, R. M., Romero, N., & Masson, L. (2003). Stability of spray-dried encapsulated carotenoid pigments from rosa mosqueta (Rosa rubiginosa) oleoresin. *Journal of the American Oil Chemists' Society*, *80*(11), 1115-1120. https://doi.org/10.1007/s11746-003-0828-4

Rodríguez, J. A. R., Ascheri, J. L. R., da Silva Lopes, A. J., Vargas-Solórzano, J. W., Pacheco, S., & de Jesus, M. S. C. (2021). Physical characterization of maize grits expanded snacks and changes in the carotenoid profile. *Plant Foods for Human Nutrition*, *76*(1), 68-75. https://doi.org/10.1007/ s1113-0020-00876-2

Rodríguez-Miranda, J., Ruiz-López, I. I., Herman-Lara, E., Martínez-Sánchez, C. E., Delgado-Licon, E., & Vivar-Vera, M. A. (2011). Development of extruded snacks using taro (Colocasia esculenta) and nixtamalized maize (Zea mays) flour blends. *LWT-Food Science and Technology*, *44*(3), 673-680. https://doi.org/10.1016/j.lwt.2010.06. 036

Rojas-Garbanzo, C., Pérez, A. M., Bustos-Carmona, J., & Vaillant, F. (2011). Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes HBK) flour. *Food Research International*, *44*(7), 2377-2384. https://doi.org/10.1016/j.foodres.2011. 02.045

Ruiz-Armenta, X. A., Zazueta-Morales, J. D. J., Aguilar-Palazuelos, E., Delgado-Nieblas, C. I., López-Diaz, A., Camacho-Hernández, I. L., ... & Martínez-Bustos, F. (2018). Effect of extrusion on the carotenoid content, physical and sensory properties of snacks added with bagasse of naranjita fruit: optimization process. *CyTA-Journal of Food*, *16*(1), 172-180. https://doi.org/10.1080/19476337.2017.1368717

Saini, R.K., Nile, S.H., & Park, S.W. (2015). *Carotenoids from fruits and vegetables*: Chemistry, analysis, occurrence, bioavailability and biological activities. 76:735–750

Selim, K., Tsimidou, M., & Biliaderis, C.G. (2000). Kinetic studies of degradation of safron carotenoids encapsulated in amorphous polymer matrices. *Food Chemistry*, 71(2):199–206. https://doi.org/ 10.1016/S0308-8146(00)00156-4

Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science & Technology*, *21*(4), 168-180. https://doi.org/10.1016/j.tifs.2009.12.001

Singh, S., Gamlath, S., & Wakeling, L. (2007). Nutritional aspects of food extrusion: a review. *International Journal of Food Science & Technology*, *42*(8), 916-929. https://doi.org/10.1111/j.1365-2621.2006.01309.x

Stahl, W., & Sies, H. (1996). Lycopene: a biologically important carotenoid for humans? *Archives of Biochemistry and Biophysics*, 336(1), 1-9.

Tachaprutinun, A., Udomsup, T., Luadthong, C., & Wanichwecharungruang, S. (2009). Preventing the thermal degradation of astaxanthin through nanoencapsulation. *International Journal of Pharmaceutics*, 374(1-2), 119-124.

Tiwari, U., & Cummins, E. (2009). Nutritional importance and effect of processing on tocols in cereals. *Trends in Food Science & Technology*, *20*(11-12), 511-520.https://doi.org/10.1016/j.tifs.2009.06.001

Tonyali, B., Sensoy, I., & Karakaya, S. (2016). The effect of extrusion on the functional components and in vitro lycopene bioaccessibility of tomato pulp added corn extrudates. *Food & Function*, *7*(2), 855-860. https://doi.org/10.1039/c5fo01185j

Tovar-Jiménez, X., Caro-Corrales, J., Gómez-Aldapa, C. A., Zazueta-Morales, J., Limón-Valenzuela, V., Castro-Rosas, J., ... & Aguilar-Palazuelos, E. (2015). Third generation snacks manufactured from orange by-products: physicochemical and nutritional characterization. *Journal of Food Science and Technology*, *52*(10), 6607-6614. https://doi.org/10.1007/s13197-015-1726-2

Van Hal, M. (2000). Quality of sweetpotato flour during processing and storage. *Food Reviews International*, *16*(1), 1-37. https://doi.org/10. 1081/FRI-100100280

Waramboi, J.G., Gidley, M.J., & Sopade, P.A. (2013). Carotenoid contents of extruded and non-extruded sweet potato fours from Papua New Guinea and Australia. *Food Chemistry*, 141(3):1740–1746. https://doi.org/10.1016/j.foodchem.2013.04.070

Wyman CE (1975). Theoretical model for intermeshing twin screw extruders: axial velocity profile for shallow channels. *Polymer Engineering & Science* 1.5 (8), 606.

Yara-Varon, E., Fabiano-Tixier, A. S., Balcells, M., Canela-Garayoa, R., Bily, A., & Chemat, F. (2016). Is it possible to substitute hexane with green solvents for extraction of carotenoids? A theoretical versus experimental solubility study. *RSC Advances*, *6*(33), 27750-27759. https://doi.org/10.1039/c6ra03016e

Ying, D., Cheng, L.J., Chibracq, G., Sanguansri, L., Oiseth, S.K., & Augustin, M.A. (2015). The format of β-carotene delivery afects its stability during extrusion. *LWT-Food Science and Technology* 60(1):1–7. https://doi.org/10.1016/j.lwt.2014.09.034

Zepka, L.Q. & Mercadante, A.Z. (2009). Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. *Food Chemistry*, 117(1):28–34. https://doi.org/10.1016/j.foodchem.2009. 03.071

# 3 CHAPTER 3

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## GUARANÁ (*PAULLINIA CUPANA*) BY-PRODUCT AS A SOURCE OF BIOACTIVE COMPOUNDS AND AS A NATURAL ANTIOXIDANT FOR FOOD APPLICATIONS*

*This chapter is based on the published article:

Pinho, L. S., da Silva, M. P., Thomazini, M., Cooperstone, J. L., Campanella, O. H., da Costa Rodrigues, C. E., & Favaro-Trindade, C. S. (2021). Guaraná (Paullinia cupana) by-product as a source of bioactive compounds and as a natural antioxidant for food applications. *Journal of Food Processing and Preservation*, *45*(10), e15854. (Copyright is in Attachment B)

## NOVELTY IMPACT STATEMENT

Guaraná peels are underutilized by-products that the food and pharmaceutical industries in South America could employ in the formulation of many of their products. Guaraná peels are a source of some macro and micronutrients, caffeine, theobromine, phenolic compounds, and carotenoids, all-*trans*-β-carotene, *cis*-β-carotene, and lutein. The ability to extract carotenoids was evaluated through the screening of potentially suitable solvents, as well as the extraction time and temperature. The suitability of the solvent considered the safety and toxicity of the obtained extracts and the solvent's impact on the environment. The measured antioxidant capacity of guaraná peels extracts indicated its potential valorization by using a green solvent. To the best of our knowledge, no information has been reported concerning the full characterization and chemical origin of guaraná peels pigments, which also provides a prospective multipurpose for application in functional foods.


## ABSTRACT

This study aimed to evaluate the extraction and the potential of guaraná peels extract as source of bioactive compounds and as a natural antioxidant. Guaraná peels were characterized, and different solvents and conditions of extraction were assessed using a full factorial design. The total carotenoids content was 65.9 ± 0.4 µg/g, and the major species were β-carotene and lutein. It also presented 0.60 ± 0.01 and 0.55 ± 0.01 mg/g of caffeine and theobromine, respectively. The ability to extract carotenoids followed the increasing order: hexane < ethanol < ethyl acetate < ethanol: hexane < ethanol: ethyl acetate. Carotenoid recovery increased significantly ($P < 0.05$) at 50 °C, and the extraction yield was higher over time. ABTS (60 ± 2 µmol Trolox eq. /g) and ORAC (76 ± 3 µmol Trolox eq. /g) assays indicated that guaraná peels extract has a valuable potential for application as antioxidant in food products.



**Keywords:** Extraction; solvents; lutein; β-carotene; caffeine; theobromine.

## 3.1 Introduction

Guaraná (*Paullinia cupana*) is a plant native to the Amazon region, extensively cultivated and economically important in Brazil. The seeds of guaraná with high caffeine content (Carciochi et al., 2021) and their extracts are largely used in the production of sodas, energy drinks, and dietary supplements (Schimpl et al., 2013; da Silva et al., 2017). The red-orange peel is a by-product used to produce recycled paper or often discarded, generating significant waste (FAPEAM, 2012). Since the peel accounts for approximately 30% of the fruit weight (Maravalhas, 1965), alternatives to promote the upcycling of this high volume of material are desirable. However, there is a lack of information about the macro and micro composition of guaraná peels, important for the development of valorization strategies.

The accumulation of waste from the food industry represents an environmental and economic problem (Benmeziane et al., 2018). Vegetable and fruit processing wastes, such as guaraná peels, can be sources of natural pigments. Besides, the presence of the well-known alkaloids (caffeine, theobromine and theophylline) in guaraná seeds (Carlson & Thompson, 1998; Machado et al., 2018) can be matter of interest to instigate investigations of these compounds in their agro-industrial residue. In this context, Santana et al. (2020) have reported the recovery of alkaloids and polyphenols from guaraná peels. Therefore, the identification and extraction of the bioactive compounds emerge as an approach for the reutilization of this by-product, which can be used in the development of functional foods, cosmetics, and medicines.

The extraction process must be efficient, selective, and economically viable. The solvent used, the solid:solvent ratio, and the conditions of the process, such as time and temperature influence the recovery of the compounds. The critical factors for solvents selection are solubility, price, and solvent toxicity (Saini & Keum, 2018). Considering this, the use of food-grade solvents with the least possible toxicity, such as ethanol (Food and Drug Administration, 2018), may lead to a simpler and more reliable products. Temperature can affect solubility and improve the extractability of the solvents utilized in the extraction process. In addition, processing time can influence the extraction yield of the components (Azmir et al., 2013). A balance must be achieved between solubility, price and ability to produce a residue-free extract.

Thus, the present study consists of three main sections. In the first section, the characterization of the guaraná peels was detailed. Specifically, the composition of the material, the alkaloids content, and the chemical origin of the pigments were determined. In the second section, the screening of solvents, temperature, and time were assessed to establish the optimal

conditions for the pigment extraction. In the third section, the antioxidant capacity of the ethanolic extracts was evaluated using different *in vitro* assays.

## 3.2 Material and Methods

### 3.2.1 Materials

Guaraná fruits were provided by The Executive Commission for Cocoa Cultivation Planning - CEPLAC (Taperoá, Bahia, Brazil). The peels were removed from seeds and pulp and then washed with water. The orange and red peels were dried using convection (Marconi, MA035 / 1152) at 50 °C for 18 hours (Silva et al., 2019) decreasing the initial moisture from 84.5 ± 0.3% to 9.7 ± 0.6%. A blender was used to break apart dehydrated peels to convert them into a guaraná peel powder. This powder was milled, and Tyler series sieves (Granutest, São Paulo, Brazil) were used to obtain a fraction with an uniform particle size of a mean diameter of 1.08 ± 0.05 mm. The samples were then stored under dark at -20 °C until analysis.

Absolute ethanol (CAS 64-17-5), hexane (CAS 110-54-3), and ethyl acetate (CAS 141-78-6) with purity ≥ 98.0% were used and purchased from Synth (Diadema, Brazil). β-Carotene (CAS 7235-40-7), lutein (CAS 127-40-2), caffeine (CAS 58-08-2) and theobromine (CAS 83-67-0) standards were purchased from Sigma-Aldrich (Saint Louis, USA), and analysis solvents ethanol, hexane, methanol (CAS 67-56-1) , acetonitrile (75-05-8) and acetone (CAS 67-64-1) were from Fisher Scientific.

### 3.2.2 Proximate analysis and chemical composition of dried guaraná peel

pH was determined in a suspension resulting from mixing 10 g of dried guaraná peel with 10 mL of deionized water, using a pH meter (Marte MB10, São Paulo, Brazil). The sample water activity was measured utilizing Aqua Lab Series 3 (Pullman, WA, USA). The moisture content of the samples was measured by drying the sample at 105 ± 2 °C until reaching constant weight (AOAC, 2006). Dietary fiber content was determined using the multi-enzyme gravimetric method (Asp et al., 1983). Lipid, protein, and ash contents were quantified according to the Association of Official Analytical Chemists methods (AOAC, 1995). Macro-minerals (Ca, K, and Mg) and micro-minerals (Cu, Fe, Mn, and Zn) were estimated by nitro-perchloric digestion (Asp et al., 1983). All analyses were performed in triplicate.

### 3.2.3 Determination of alkaloids

Alkaloids (caffeine, theobromine and theophylline) were investigated in guaraná peels ethanolic extract using High-Performance Liquid Chromatography equipped with an UV/VIS diode array detector (HPLC-DAD - Shimadzu, Prominience, Kyoto, Japan), and column C18 (Ascentis, Supelco, 250 x 4.6 mm, particle size of 5 µm), maintained at 30 °C.

The extract was prepared in an ratio 1:10 of guaraná peel powder:ethanol, under agitation with a magnetic stirrer, at 50 °C, for 4 h. Afterwards, it was filtered through 0.45 µm nylon filter. The compounds quantification was performed by calibration curves plotting for each alkaloid using caffeine (1 – 600 µg/mL) and theobromine (1 – 600 µg/mL) standards. The procedure was conducted following ISO 20481:2008, using methanol–water–acetonitrile (20:75:5, v/v/v) as the mobile phase operating in isocratic mode for 12 minutes, at a flow rate of 1 mL/min, and sample injection volume of 10 µL. The detection of alkaloids was carried out at wavelength 273 nm. The data were collected and analyzed using software LC Solution, version 1.21. Results were expressed as mg/g guaraná peels.

### 3.2.4 Carotenoid profile

Carotenoid extraction was carried out following Kopec et al. (2014), with some modifications according the solvents used. Sequential extractions were performed using methanol, and a mixture of ethanol:acetone:hexane (1:1:1, v/v).

Methanol (5 mL) was added to a glass tube containing 0.2 g of dried guaraná peels. After ultrasonication, the mixture was centrifuged for 10 minutes at 4000 x *g*. The methanol layer was removed and retained. The ethanol:acetone:hexane mixture (1:1:1, v/v) was added to the same tube containing the extracted guaraná peels. After ultrasonication, the mixture was centrifuged for 10 minutes at 4000 x *g*, and the supernatant decanted and combined with the methanol. To promote phase separation 5 mL of distilled water was added.

The extract layer was brought up to 25 mL, and 5 mL of extract was dried under nitrogen gas. Extracts were redissolved in 300 µL of methanol:methyl tert-butyl ether (MTBE) (1:1, v/v). The extracts were separated and analyzed using an Agilent 1260 ultra-high performance liquid chromatograph with a diode array detector (UHPLC-DAD), using a C30 column (YMC Inc., 4.6 x 250 mm, 3 µm). The solvent system was composed by mobile phase A: 60% methanol, 35% MTBE, 3% water, 2% aqueous ammonium acetate (2% w/v), and mobile phase

B: 78% MTBE, 20% methanol, 2% aqueous ammonium acetate (2% w/v). The elution program was performed as the following procedure: 0% B to 35.6% B in 9 min, to 100% B along the next 6.5 min, maintaining for 3.5 min at 100% B, and equilibrate for 3.5 min at initial conditions, flowing at 1.3 mL/min (Cooperstone et al., 2015).

Retention times and UV-vis spectra were compared with authentic standards using previously published methods (Cooperstone et al., 2015). For peaks that did not match exactly the authentic standards, identities were annotated using an Agilent 1290 UHPLC interfaced with a 6545 quadrupole time of flight mass spectrometer (QTOF-MS), operated with atmospheric pressure chemical ionization in positive ionization mode. Relative retention time, UV-vis spectra, and accurate mass (< 10 ppm) were used to determine identity.

### 3.2.5 Screening of potential solvents

The extraction capacity of pure solvents and mixed solvents was evaluated based on experiments, analyzing the total carotenoid content in each condition, as well as the time required to obtain the maximum content. The carotenoid extraction was carried out by maceration, in a shaker at 25 and 50°C, with a 280 rpm stirrer speed, in constant agitation, under dark conditions. The ratio considered for the extraction was 1:10 (powder:solvent), and it was chosen from previous studies, to guarantee that the mass of guaraná peels was completely immersed in the solvent during the extraction. Hexane, ethanol, ethyl acetate, and the mixtures, ethanol:hexane (1:1, v/v) and ethanol:ethyl acetate (1:1, v/v) were used for the screening of solvents (Strati & Oreopoulou, 2011a; Ishida & Chapman, 2009; Sachindra et al., 2006).

Total carotenoid content was determined using a UV-visible spectrophotometer (Thermo Scientific, Genesys 10S), at a wavelength of 450 nm, after 2, 4, 6, 8, and 24 hours of extraction. Each sample was dried and brought up in hexane. The time interval for quantification of carotenoids was established in preliminary experiments of extraction kinetics. Calibration curves were prepared with β-carotene standard and the results were expressed as μg β-carotene/g guaraná peels (Rodriguez-amaya, 2001).

#### 3.2.5.1 Hansen solubility parameters (HSPs) and distance (Ra)

Hansen's theory has been used as a predictive tool to estimate the solubility of key compounds from the carotenoid class in the preselected solvents (Hansen, 2004). The Hansen

solubility parameters (HSPs) are based on the contribution from different interactions of solute and solvent in a system. The total solubility parameter ($\delta_T$), expressed in units of $(MPa)^{1/2}$ was determined according to Eq. (3), where $\delta_D$ indicates non-polar interactions, $\delta_P$, for molecular dipole interactions, and $\delta_H$, for hydrogen bonds interactions (Hansen, 2004).

$$\delta_T{}^2 = \delta_D{}^2 + \delta_P{}^2 + \delta_H{}^2 \quad (3)$$

The HSPs of mixtures of solvents can be calculated from the mass fractions ($w_i$) of each pure solvent and its respective solubility parameter according to Eq. (4). The equation estimating $\delta_K^{Mixture}$ applies to the parameters $\delta_D$, $\delta_P$ and $\delta_H$ for the mixture of solvents, $\delta_{Ki}$ is the corresponding parameter for the pure solvent $i$, and $w_i$ is the mass fraction of solvent $i$ in the mixture.

$$\delta_K^{Mixture} = \sum_{i=1}^{i=n} \delta_{Ki} w_i \quad (4)$$

HSPs values were obtained from Barton (1983) for ethanol, hexane, and ethyl acetate; from Ozel and Gogus (2014) for β-carotene; and from Cosby et al. (2020) for lutein.

The 'distance', Ra, measured in $(MPa)^{1/2}$, between solute ($i$) and solvent ($j$), is defined by Eq. (5), considering the solubility parameters of the components.

$$R_a{}^2 = 4\left(\delta_{Di} - \delta_{Dj}\right)^2 + \left(\delta_{Pi} - \delta_{Pj}\right)^2 + \left(\delta_{Hi} - \delta_{Hj}\right)^2 \quad (5)$$

Solute-solvent distance (Ra) presents as a simple way to visualize suitable solvents for the determined solute. Low values for Ra denote a high efficiency of the solvent.

### 3.2.6 Characterization of ethanolic extract

After analyzing the results of the extraction trials, ethanol was chosen as the solvent for an additional study, given its purity, low toxicity, biodegradability, and ability to be obtained from renewable sources. Extracts obtained at 50 °C and 4 hours of extraction were characterized by the Bligh-Dyer methodology (Bligh & Dyer, 1959), proteins by Kjeldahl (AOAC, 1995), and moisture by the Karl Fischer titration method (Metrohm, 787 KF Tritino), based on the ASTM standard (ASTM, 2001). The extract was also evaluated for total phenolics and its antioxidant capacity using 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), oxygen radical absorbance capacity (ORAC), ferric reducing ability of plasma (FRAP), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays, which are described below.

### 3.2.6.1 Total phenolics

The total phenolic content in guaraná peels extract was quantified by the Folin-Ciocalteu method (Singleton et al.,1965), with minor modifications. 250 µL of the ethanolic extract, 250 µL of the Folin-Ciocalteu reagent (Dinamica, Brazil), and 2 mL of distilled water were added to a glass vial. Following 3 minutes in the dark, 250 µL of a saturated sodium carbonate solution was added. The absorbance was evaluated at 750 nm utilizing a spectrophotometer (Thermo, Genesys 10S), after 30 minutes in a water bath at 37 °C away from light. The reference standard was gallic acid (Acros Organics, Belgium) used to create a standard curve in a range of concentrations of 1 – 5 µg/mL. Results were expressed as mg of gallic acid equivalent (GAE)/g of sample.

### 3.2.6.2 Antioxidant capacity assays

The ABTS assay was carried out according to Rufino et al. (2010). Initially, the reaction between the 7 mM ABTS and 145 mM potassium persulfate was induced to produce the $ABTS^{+}$ stock solution. The mixture remained at room temperature and in the dark for 12-16 h. This solution was adjusted with ethanol to an absorbance of 0.70 at 734 nm. Following, 30 µL of sample/ standard Trolox was added to 3 mL of ABTS solution, and the absorbances were checked 6 min after mixing. Ethanolic solutions of known concentrations of Trolox were used as the standard curve in the range of concentrations (100-2000 µmol Trolox equivalent/g).

ORAC method was conducted according to Ou et al. (2001). In a microplate (96 cells, Greiner Bio-One) 150 µL of fluorescein solution and aliquots of the diluted extracts (25 µL) were added. The microplate was incubated at 37 °C for 10 minutes in a spectrofluorimeter (BMG Labtech, FLUOstar OPTIMA, Offenburg, Germany). After incubation, 25 µL of AAPH solution (2.2 '- Azobis (2-methylpropionamidino) dihydrochloride 152 mM) was added. Fluorescence decay was measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm, at 1-minute intervals, for 120 minutes. For this analysis, ethanolic solutions with established concentrations of Trolox were prepared for the standard curve (12.5-200 µmol equivalent of Trolox/g).

The FRAP assay was carried out following Benzie & Strain (1996). The FRAP solution was prepared using 250 mL of acetate buffer (300 mM, 1.6% acetic acid, pH 3.6), 25 mL of solution 2,4,6-Tripyridyl-S-Triazine (10 mM, diluted in 40 mM HCl) and 25 mL of $FeCl_3$ (20

mM). The extract (0.1 mL) were mixed with 2.9 mL of FRAP solution. The solutions were homogenized (IKA, Vortex 1 V1, Germany), and kept at 37 °C (Water bath, Marconi, MA 159, Brazil), for 30 minutes in the dark. After this period, a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, USA) was used to determinate the absorbance of the samples at 593 nm. A standard curve (2.5-25 µmol equivalent of Trolox/g) was plotted.

The DPPH assay was performed according to the description of Brand-Williams, Cuvelier & Berset (1995). The DPPH solution was prepared with methanol containing 0.06 mM DPPH. The aliquot of 100 µL of guaraná extract was added to 3.9 mL of DPPH solution. The decrease of absorbance at wavelength of 515 nm was measured at 1-minute intervals during the initial 10 minutes and subsequently at 5-minute intervals until maintenance. The measurement of absorbance readings lasted 30 min. The standard curve was prepared with Trolox (20-140 µmol equivalent of Trolox/g) and results were expressed as µmol Trolox equivalent/g sample.

### 3.2.7 Statistical analysis

Data was analyzed statistically using the statistical program SPSS Statistics, with $P < 0.05$ considered significant, by analysis of variance ANOVA. A three-way ANOVA was performed to test the effect of solvent, time, temperature, and their two and three way interactions and Duncan's test for comparing means.

## 3.3 Results and Discussion

### 3.3.1 Proximate analysis and chemical composition of guaraná peels

Table 3 presents the chemical composition of guaraná peels.

Guaraná peels has medium acidity, which combined with its low moisture and water activity is able to ensure microbiological stability and avoid reactions that can affect its perishability. Fresh guaraná peels had moisture of 84.5g/100g, demonstrating the importance of the drying process for avoiding enzymatic activity that may affect the chemical stability of bioactive compounds.

**Table 3.** pH, water activity, composition, and mineral contents of guaraná peels

| **Parameters** | **Values (means ± SD)** † |
|---|---|
| pH | 5.4 ± 0.0 |
| Water activity | 0.5 ± 0.0 |
| Moisture (g/100g) | 9.7 ± 0.6 |
| Carbohydrates (g/100g) | 85.5 ± 0.1 |
| Lipids (g/100g) | 1.7 ± 0.1 |
| Protein (g/100g) | 2.3 ± 0.3 |
| Ash (g/100g) | 0.8 ± 0.1 |
| K (g/kg) | 21.0 ± 1.0 |
| Ca (g/kg) | 4.6 ± 0.5 |
| Mg (g/kg) | 3.3 ± 0.2 |
| P (g/kg) | 0.9 ± 0.1 |
| Na (g/kg) | nd |
| Cu (mg/kg) | 14.0 ±1.0 |
| Fe (mg/kg) | 137.0 ± 9.0 |
| Mn (mg/kg) | 23.0 ± 3.0 |
| Zn (mg/kg) | 20.0 ± 4.0 |
| Caffeine (mg/g) | 0.6 ± 0.0 |
| Theobromine (mg/g) | 0.5 ± 0.0 |

† d.w. – dry weight basis; nd – Not detected

Reference: Own source.

Carbohydrates are the major component of guaraná peels. The content of insoluble fiber in guaraná peels was 21.6 g/100g. Dias et al. (2020) determined the chemical composition of fruit peels, and found that fiber was the major component present in orange and passion fruit peels. Furthermore, Ajila et al. (2010) reported 32.1 g/100 of insoluble fiber in mango peels, which was higher than the content found in guaraná peels.

Guaraná peels had lower concentrations of lipid (1.7 g/100g) and protein (2.3 g/100g) than those reported by Romelle et al. (2016). They studied the composition of selected fruit peels, which ranged from 3.36 to 12.61 g lipid/100g dry peel and 2.8 to 18.06 g protein/100g dry peel. Mallek-Ayadi et al. (2017) found the lipid (2.1 g/100g dry peel), protein (7.5 g/100g

dry peel), and ash (3.7 g/100g dry peel) contents of melon peels, to be higher than in guaraná peels, although, comparatively, the differences are subtle.

The elevated content of minerals in guaraná peels indicates that this material can be utilized to produce supplements and nutraceuticals. Among macro-minerals, guaraná peels showed the highest content of potassium, which acts as a regulator of physiological body processes (Lindinger & Cairns, 2021). Potassium is a mineral, which together with sodium and chlorine are typical electrolytes found in the body, in addition to being one of the most important elements of cell cytoplasm (Mulkidjanian et al., 2012).

Singh et al. (2016) reported the composition of some fruit peels minerals and their content varied from 1.3 to 4.4 g K/kg, 0.5 to 3.2 g Ca/kg, and 0.5 to 1.2 g Mg/kg. Regarding micro-minerals, the authors reported contents ranging from 1.8 to 2.1 mg Cu/kg, 2.1 to 31.9 mg Fe/kg, 1.7 to 3.3 mg Mn/kg, and 1.3 to 9.8 mg Zn/kg. Comparing those results with the current study (Table 3), guaraná peels had higher concentrations of the same minerals.

Furthermore, several parameters may influence the mineral composition of guaraná peels, such as the environmental/soil conditions, and the ripening process. Benmeziane et al. (2018) reported that wastes of cantaloupe processing had similar levels of potassium, but guaraná peels showed a 5-fold higher level of iron.

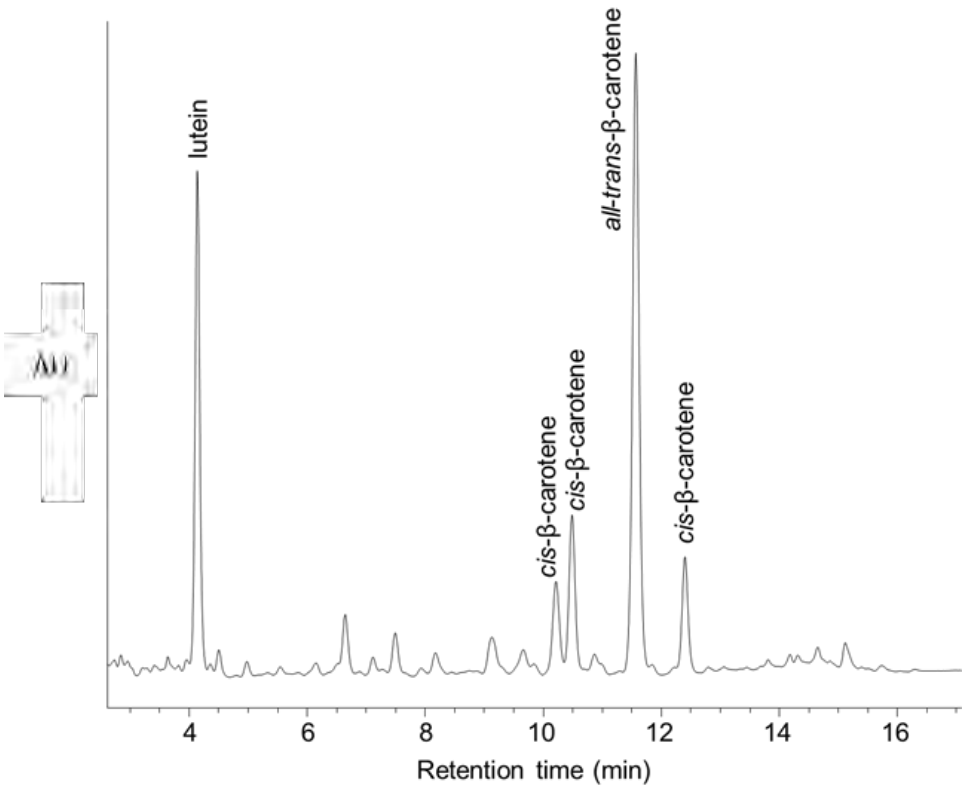
Comparing the values found in this study with those found by Gondim et al. (2005), guaraná peels had higher levels of all minerals than avocado, banana, melon, papaya, passion fruit, and pineapple peels. It is worth mentioning the exception to the levels of calcium and zinc determined in tangerine peels (4.8 g Ca/kg and 28.3 mg Zn/ kg, respectively), which were higher than those found in the current work.

The caffeine (0.60 ± 0.01 mg/g) and theobromine (0.55 ± 0.01mg/g) contents in guaraná peels were determined using HPLC-DAD analysis. Theophylline was not detected in the sample. Caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) are purine alkaloids, which are commonly extracted using water, organic solvent, or their combination (Nguyen & Nguyen, 2017). Since the raw material was washed with water at the reception, the actual concentration of caffeine and theobromine may be affected. The health properties of these bioactive compounds are largely evidenced and associated with stimulant effects, improvement of cognitive functions, and anti-obesity activity (Carrageta et al., 2018, Santana et al., 2018), demonstrating the potential benefits of the exploration of guaraná peels.

### 3.3.2 Carotenoid profile

It was determined that the pigments imparting the red-orange color to guaraná peels are carotenoids. A LC chromatogram extract at 450 nm shows the major carotenoid peaks found in guaraná peels. As illustrated in Figure 10 among the peaks separated, 2 were confirmed with authentic standards, and an additional 3 were identified according to the UV-vis spectra, relative retention time using standards, and accurate mass (< 10 ppm) using a QTOF.

**Figure 10.** UHPLC-DAD chromatogram of lipophilic extract of guaraná peels, extracted at 450 nm




Reference: Own source.

Chromatograms of saponified and unsaponified guaraná peels extracts were compared, with no noticeable increase in xanthophyll peaks in after saponification, indicating that in guaraná peels most xanthophylls are in a free form. Thereby, the chromatogram in Figure 10 represents the carotenoids profile of the unsaponified extract.

The guaraná peels displayed total carotenoid content of 65.90 ± 0.4 μg/g, obtained by the sum of the individual carotenoids determined by the HPLC. All-*trans*-β-carotene was the main carotenoid with 30.8 ± 0.2 μg/g, followed by *cis*-β-carotenes (17.8 ± 0.01 μg/g) and lutein (17.3 ± 0.2 μg/g). A higher content of β-carotene was recovered in the *trans* form compared to the *cis* form, as the ratio All-*trans*-β-carotene/*cis* isomer was approximately 2:1. This corroborates with the fact that most carotenoids are found in nature in *trans* (*Z*) configuration. Furthermore, considering that guaraná peels were dried, the heating may have induced the isomerization of carotenoids. *Trans* double bonds are susceptible to isomerization, leading to *cis* (*E*) conformation (Honda et al., 2020).

Previous work has suggested that lutein has may impart benefits against age-related macular degeneration (Koushan et al., 2013). β-Carotene, the most potent provitamin A carotenoid plays an important role in metabolic processes of the human body (Perera & Yen, 2007). In this regard, the isomeric forms of β-carotene in guaraná peels, all-*trans*-β-carotene, and *cis*-β-carotene, can contribute to vitamin A requirements, although the bioequivalency of *cis*-form is 50% lower than *trans*-form (Sweeney & Marsh, 1973).

Noronha Matos et al. (2019) reported the preponderance of β-carotene in tucumã and peach palm peels. Besides, all-*trans*-β-carotene was the main carotenoid found in mango peels (Del Pilar Sánchez-Camargo et al., 2019), and lutein, α-carotene, and β-carotene were main carotenoids in banana peels (Fu et al., 2018). Analyzing fruits from the Amazon region, De Rosso & Mercadante (2007) evaluated the carotenoid profile of several fruits and found all-*trans*-β-carotene as the major carotenoid present. The authors also reported that lutein was identified in buriti, marimari, palm oil, physalis, and tucumã.

Although chemical synthesis is still the main route to produce additives and supplements for foods and pharmaceutical products, studies to obtain carotenoids from natural sources are becoming more frequent (Britton et al., 2009). However, when a natural extract is being obtained from a complex matrix, there are challenges to be investigated such as possibilities of carotenoid recovery, as well as the bioactive potential after the extraction. Considering the valuable composition present in guaraná peels, this study also incorporates carotenoid extraction, and its antioxidant capacity of the extracts, aspects that are essential for future application of these phytochemicals.

### 3.3.3 Screening of potential solvents

The extraction process of bioactive compounds with organic solvents is one of the most used techniques, immersing a solid within a solvent system. It is important to consider miscibility with the soluble compounds and the ability to remove compounds of interest. The extractability of carotenoids from guaraná peels with different solvents, temperatures, and times are described and results illustrated in Table 4 and Figure 11.

**Table 4.** *P*-value of different parameters of extraction on the total carotenoid content using a three-way ANOVA model

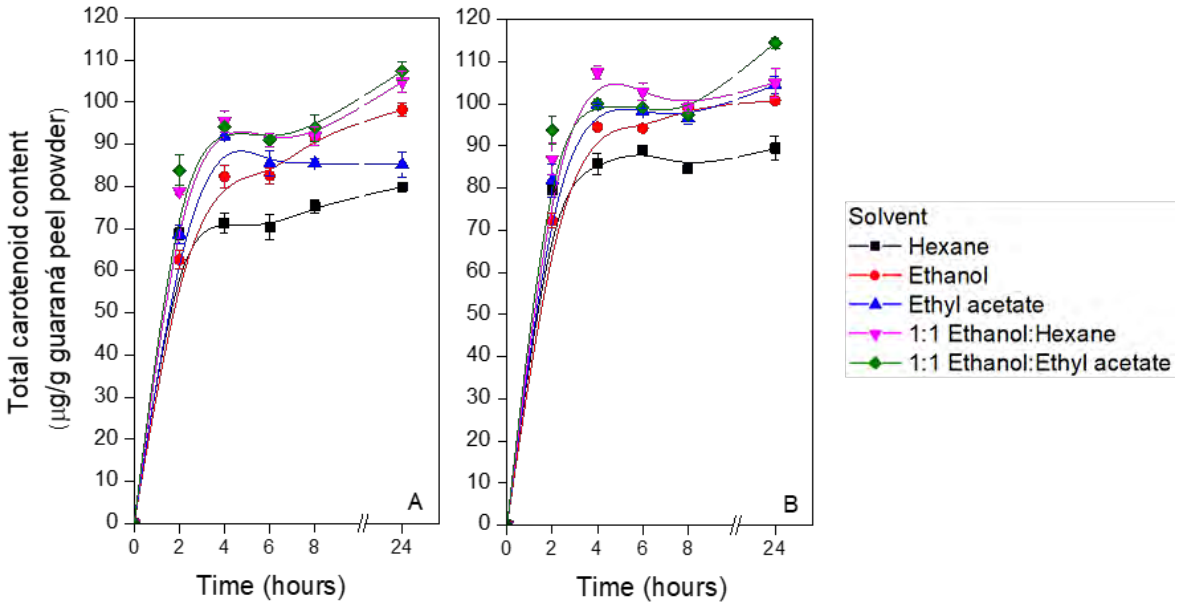
| **Parameters** | ***P*-value** |
|---|---|
| Solvent | < 0.001 |
| Time | < 0.001 |
| Temperature | < 0.001 |
| Temperature * Solvent | 0.012 |
| Temperature * Time | 0.022 |
| Solvent * Time | < 0.001 |
| Solvent * Time * Temperature | 0.072 |

Reference: Own source.

A three-way analysis of variance (ANOVA) was carried out to test the effect of each variable on the extractability of carotenoids. The resulting *P*-values are shown in Table 4. The overall effects were significant ($P < 0.05$) for all model factors and two-way interactions, except for the three-way interaction of temperature, solvent and time. These results show that each of factors, and their combinations significantly affected the extraction of carotenoids.

Extraction is the stage that defines the recovered content of bioactive compounds in materials. However, the extraction efficiency depends on time, temperature, and solute-solvent interaction (Azmir et al., 2013). Specifically, concerning carotenoid extraction, changes in pH, heating, exposure to light, as well as the presence of oxygen and oxidizing agents can promote their degradation and influence the recovery yield (Janiszewska-turak, 2017). Thus, factors such as the presence of light, oxygen, agitation, and the size of the guaraná peels particles were considered to maximize the recovery of compounds of interest during extraction.

**Figure 11.** Kinetics of extraction of total carotenoids from guaraná peels using different solvents at (A) 25 °C and (B) 50 °C. ■, Hexane; ●, Ethanol; ▲, Ethyl acetate; ▼, Ethanol:Hexane; ◆, Ethanol: Ethyl acetate. (—) spline fitting




Reference: Own source.

### 3.3.3.1 Effect of solvents

Carotenoids are classified according to polarity: carotenes (nonpolar), and xanthophylls (more polar than carotenes). The efficiency of conventional extraction can be attributed to the relative polarities of the solvents used, for example, hexane (0.009), ethyl acetate (0.228), and ethanol (0.654), comparing to water (1.0), where the numbers next to each solvent indicate their relative polarities (Reichardt, 2003). According to Craft & Soares (1992), different organic solvents act giving to the carotenoid polarity range. They reported the solubility of lutein and β-carotene in several solvents based on the relative absorptivities and maximum absorption wavelength of these carotenoids. Hexane was the least soluble solvent for lutein, and acetonitrile and methanol, for β-carotene.

In Figure 11, higher total carotenoid content was obtained with the mixtures ethanol:hexane and ethanol:ethyl acetate rather than pure ethanol, hexane, and ethyl acetate solvents. The combination of polar solvents, as ethanol, with non-polar solvents, e.g. hexane, may have favored the increased solubility of β-carotene. Pure polar solvents, on the other hand,

increased the solubilization of more polar carotenoids (lutein), in agreement with results from Strati & Oreopoulou (2011b).

It is possible to notice that the mixture of the solvents leads to an improvement in the extraction of β-carotene compared to ethanol, and an increase in the extraction of lutein in comparison to hexane. These results are also reflected in the calculated Hansen solubility parameters (Table 5). From an experimental standpoint, according to Benmeziane et al. (2018), the polarity of solvents can promote the enhancement of the permeability of the cell walls, where carotenoids are localized. Regarding the properties of solvents, hexane had a higher affinity with β-carotene compared to lutein, and this may be associated with the number of hexane carbons and the chemical structure of β-carotene (Alvarenga et al., 2020; Scharlack et al., 2017). This characteristic can favor an increase in the solubility and, thus, lead to a superior extractability. Conversely, polar solvents, ethanol and ethyl acetate, interacted more easily with lutein, and this is due to the hydroxyl and carbonyl groups in the chemical structure of the solvents, respectively, and the hydroxyl present in the lutein structure.

**Table 5.** Hansen solubility parameters and distances (Ra) between β-carotene, lutein, and solvents, at 25 °C

| **Solvents** | $\delta_D$ **(MPa)$^{1/2}$** | $\delta_P$ **(MPa)$^{1/2}$** | $\delta_H$ **(MPa)$^{1/2}$** | $\delta_T$ **(MPa)$^{1/2}$** | **Ra β-carotene** | **Ra Lutein** |
|---|---|---|---|---|---|---|
| Hex[a] | 14.90 | 0.00 | 0.00 | 14.90 | 5.3 | 8.7 |
| Etha[a] | 15.80 | 8.80 | 19.40 | 26.52 | 19.7 | 13.0 |
| EtAce[a] | 15.80 | 5.30 | 7.20 | 18.15 | 7.8 | 3.9 |
| Etha:Hex | 15.40 | 4.84 | 10.67 | 19.35 | 10.6 | 3.8 |
| Etha:EtAce | 15.80 | 6.95 | 12.93 | 21.57 | 13.2 | 6.9 |
| **Solute** | | | | | | |
| β-carotene[b] | 17.40 | 0.80 | 1.70 | 17.50 | | |
| Lutein[c] | 15.40 | 1.80 | 8.47 | 17.53 | | |

[a] Barton (1983). [b] Ozel and Gogus (2014). [c] Cosby et al. (2020)

Hex= Hexane; Etha= Ethanol; EtAce= Ethyl Acetate; Etha:Hex= Ethanol:Hexane; Etha:EtAce= Ethanol:Ethyl Acetate; $\delta_D$= dispersion solubility parameter; $\delta_P$= polar solubility parameter; $\delta_H$= hydrogen bonding solubility parameter; $\delta_T$= total solubility parameter; Ra= distance.

The $\delta_P$ and $\delta_H$ parameters calculated for hexane were closer to the parameters calculated for β-carotene than those for lutein. This is also reflected in the calculated values of Ra for β-carotene, which were lower for hexane, when compared to those calculated for the other solvents. Alvarenga et al. (2020) reported that solvents characterized by hydrocarbon chains showed HSP values close to the β-carotene parameters, specifically considering the $\delta_P$ and $\delta_H$ parameters. Conversely, ethyl acetate and ethanol:hexane, had $\delta_H$ values closest to the lutein value. Therefore, there are mostly interactions via hydrogen bonds between them, resulting in lower values of Ra between lutein and the ethyl acetate and ethanol:hexane solvents, compared to the others utilized in the research.

Yara-Varón et al. (2016) examined the predictive solubility of carotenoids in different solvents at 65°C. Considering their results, among the pure solvents utilized in the recovery of guaraná peels carotenoids, hexane exhibited a 100% probability of complete solubilization of β-carotene, and only 4.3% for lutein; ethanol exhibited a 2.5% probability to solubilize β-carotene and 100% for lutein; ethyl acetate showed 100% for both carotenoids. Taking into account the different carotenoids present in the guaraná peels, as well as those solubility parameters and the synergy among solvents, the mixtures ethanol:hexane and ethanol:ethyl acetate displayed to have the maximum recovery of the total carotenoid content during the extraction process. Overall, based on these results, the polarity of solvents played a crucial role in the yield of carotenoid content and extraction time.

#### 3.3.3.2 Effect of time

Extractability of carotenoids increased with longer times, and was at its minimum for all solvents and temperatures at 2 hours (Figure 11). The increase in solute-solvent interactions at high temperature over time, favor a decrease in the surface tension of the solvent, which favors the extraction of the solute (Mustafa & Turner, 2011). One of the conclusions from the present study is that the extraction rate depend on the solvent used, as well as on the process temperature.

In the first 2 hours, the total carotenoid content consists of the compounds extracted mainly from the surface of the guaraná peels particles. After the soaking period, in which the solvents impregnate part of the matrix, the system displayed a high concentration for 4 hours of extraction. This result is indicated by the inflection point observed in Figure 11. The oscillation in the data observed at 6-8 hours extraction is mainly due to experimental

uncertainties. The increment of the total carotenoid content in the extract phase may be attributed to the internal diffusion of the solvent and dissolution of the solute present inside the particles, i.e., due to an improved mass transfer, over time.

Indeed, the increment of residence time was a parameter that favored further mass transfer through the sample and induced a positive effect on the recovery of the bioactive compounds. Moreover, there was a tendency to reach higher concentrations after 24 h extraction. However and from a practical and economic standpoint, the extraction time should be set between 4-6 h to reduce energy costs and the negative effects arising from extended processing times.

#### 3.3.3.3 Effect of temperature

Regarding the impact of the extraction temperature, the recovery of carotenoids obtained at 50 °C was higher ($P < 0.05$) than the extraction at 25 °C. Although heat can promote degradation and isomerization of carotenoids, it can also promote the extraction of these compounds (Strati & Oreopoulou, 2011a; Shi et al., 2008). The increase of temperature can improve the solubility, as well as the solvent diffusion rate into the guaraná peels, due to decreased viscosity of the solvent during the process.

Sánchez-Camargo et al. (2019) reported that the temperature can influence the solubility parameters, depending on the solvent. The increase in temperature increased the extractability of the carotenoids for all different solvents. In this case, the temperature effect was positive, as the moderate heating at 50 °C did not lead to the degradation of the carotenoids, regardless of being thermosensitive components.

Ethanol performed close to the best solvent systems tested, besides, it is recognized as safe and obtained from renewable sources. There is a current trend to extract bioactive compounds from by-products through green extraction. Therefore, ethanol was chosen for an additional study with a view on industrial application, mainly motivated by the utilization of an environmentally friendly solvent (Chemat et al., 2012).

### 3.3.4 Guaraná peel ethanolic extract characterization

Since the evidence indicates that carotenoids provide health benefits, guaraná peels ethanolic extract could be a material of interest for application in food products. Considering

its potential use, this extract was characterized, regarding its composition and antioxidant properties. As expected, the moisture content of guaraná peels ethanolic extract (1.35 ± 0.01 g/100g) was low. It was derived from remaining moisture in the dried peels (Table 3). The lipid and protein content, were 1.93 ± 0.03 g/100g and 0.34 ± 0.03 g/100g, respectively. According to Mhemdi et al. (2016), the polarity of ethanol favors the recovery of polar composites, such as water and proteins.

The ethanolic extract of guaraná peels had total phenolic content of 8.56 mg GAE/g, and this component can perform an important role in neutralizing free radicals. The phenolic groups can serve as a source of hydrogen atoms from hydroxyl terminals or can act as metal chelator, avoiding oxidation reaction in a medium. However, other substances can reduce Folin−Ciocalteau's reagent, as well as phenolics, such as reducing sugars and proteins (Ikawa et al., 2003). Consequently, this result can also be attributed to the presence of these components, which may overestimate the phenolic content in the ethanolic extract.

The results in the present study were higher than those described by Gómez- Mejía et al. (2019). They demonstrated the phenolic compounds content of orange (3.9 mg GAE/ g), lemon (5.9 mg GAE/ g) and clementine (5.5 mg GAE/ g) peels, from extractions with ethanol:water solution. Babbar et al. (2011) reported 3.8, 17.5 and 24.6 mg GAE/ g of the total phenolic content in banana, kinnow and lychee peels, respectively, obtained with methanolic extraction.

The phenolic compounds and carotenoids present in guaraná peels contribute to the antioxidant capacity of foods. Those components have distinct mechanisms of action (Moo-Huchin et al., 2014). For this reason, to determine the antioxidant potential of the ethanolic extract, it was essential to evaluate this property based on methods that may better associate to those different mechanisms, even when the pathways are not completely clarified. Table 6 shows the antioxidant capacity of the ethanolic extract of guaraná peels, expressed as Trolox Equivalents (TE, µmol Trolox/ g sample) by the ABTS, ORAC, FRAP, and DPPH assays. These methods are generally used to investigate the antioxidant ability of food *in vitro*.

The extraction conditions selected for obtaining ethanolic extracts for further analysis were (i) the temperature with better carotenoid extractability, which was 50 °C; and (ii) the extraction time, which was 4h. These experimental conditions impacted the antioxidant properties, of the extracts considering the high carotenoid content in addition to the phenolic compounds recovered under these conditions. Similarly, Mokrani & Madani (2016) reported that the antioxidant properties of peach fruit were influenced by the high content of bioactive

compounds recovered, as a consequence of the temperature and time conditions of the extraction process.

**Table 6.** Antioxidant capacity of ethanolic extract by ABTS, ORAC, FRAP, and DPPH assays from guaraná peel powder

| **Assays** | **Antioxidant capacity (μmol Trolox equivalent/g guaraná peel powder)** |
|---|---|
| ABTS | 62 ± 2 |
| ORAC | 76 ± 3 |
| FRAP | 1.16 ± 0.02 |
| DPPH | 3.0 ± 0.1 |

Reference:Own source.

The presence of compounds in guaraná peels extract contributed to the capacity to quench ABTS radicals. This mechanism is based on the single-electron transfer reaction, which is displayed through a color variation as the oxidant is reduced. The experiments was performed at a wavelength of 744 nm that is outside of the spectra of carotenoids and phenolics. Hence, the presence of antioxidant residues in the analyzed system did not influence the estimation of ABTS concentration. In agreement with these results, Re (1999) reported the high efficiency of this assay to assess the antioxidant capacity of carotenoids. By comparing the results obtained with other fruit peels, Babbar et al. (2011) reported the antioxidant activity of banana, kinnow and lychee peels extracts obtained by the ABTS method as 22.7, 206.8 and 145.7 μmol Trolox equivalent/ g, respectively. Also, when assessing the antioxidant activity of 11 tropical fruits from Mexico (Can-Cauich et al., 2017), guaraná peels extract exhibit a higher antioxidant capacity than dragon fruit, white and black sapote, mamoncillo, custard apple, and sapodilla.

The ORAC assay depends on the ability of the antioxidants present in the guaraná peels ethanolic extract to inhibit the degradation of fluorescein caused by the radical of 2,2′-azobis (2-amidinopropane) (APPH). This technique is especially useful for extracts consisting of multiple components, and when different reaction mechanisms are involved (Huang et al., 2005). The ORAC values obtained in our study was higher than the range reported by Thaipong et al. (2006) for guava fruit (18-32 μmol TE/ g); but, it was lower than the result obtained by Batista et al. (2017) for red-jumbo peels (135.47 μmol TE/ g).

It is important to mention that the United States Department of Agriculture (USDA) disregarded the ORAC Database for selected foods. The removal of this information was based on the fact that antioxidant capacity values obtained through ORAC assay can not be associated with the effects of certain bioactive compounds on human health (USDA, 2010). However, the *in vitro* analytical method may be used as a measurement tool for antioxidant capacity. Thus, considering the exploratory process, this test was performed as an indicator of the antioxidant potential and complement data for research.

The result found for the antioxidant activity by the FRAP assay was relatively low when compared to the results obtained by Sethi et al. (2020), which FRAP values ranged from 50-192 µmol Trolox equivalent/ g of apples peels. According to Müller et al. (2011), FRAP activity is affected by the presence of conjugated double bonds (CDBs). In acyclic carotenoids with enough CDBs, the orbital overlap with that of the chromophore favoring the formation of more stable radicals with significant activity to reduce $Fe^{+3}$ into $Fe^{+2}$. Nevertheless, the presence of β-ionone rings on both sides of all-*trans*-β-carotene, *cis*-β-carotene, and lutein major carotenoids found in guaraná peels, possibly prevented these components from reacting with the di-TPTZ ferric complex. This issue was also reported by Wang et al. (2010) and Wojdyło et al. (2018).

The antioxidant capacity of the ethanolic extracts measured by the DPPH assay showed an average value of 3.00 µmol TE/ g. Carotenoids are not able to eliminate the DPPH radicals. Also, the activity was evaluated at 515 nm, and some carotenoids might present an absorption spectrum that coincides with that of DPPH, interfering with the absorbance measurements. Thus, the composition of guaraná peels ethanolic extract may affect the measurement of DPPH free radical-scavenging, besides the ionone rings of carotenoids structure can reduce the resonance effect.

The use of ethanol as a solvent can extract hydrophilic compounds, such as phenolics, in addition to carotenoids, and they can act synergistically. Regarding these compounds, it was possible to compare antioxidant activity determined by DPPH for guaraná peels with the results demonstrated by Can-Cauich et al. (2017), which ranged from 1.6 to 483.9 µmol TE/ g for methanolic extracts obtained from dragon fruit and green sugar apple peels, respectively.

Considering other materials rich in bioactive compounds, the antioxidant capacity of coffee by-products was higher compared to guaraná peels. The authors reported values ranging from 387 - 1083 µmol TE/g and 387 - 1454 µmol TE/g using the ABTS and FRAP assays, respectively applied to the main fractions of the residue generated (Jiménez-Zamora et al.,

2015). It was cited that the antioxidant capacity of the material could be associated with melanoidins and phenolic content. Similarly, the antioxidant potential of cocoa by-products was investigated by Martínez et al. (2012). Values ranging from 2.48 - 22.93 μmol TE/g by the ABTS method, 0.67 - 4.69 μmol TE/g by the FRAP method, and 1.57 – 33.93 μmol TE/g by the DPPH method were reported. Regarding FRAP and DPPH assays, these results are similar to those obtained in this study. However, by using the ABTS test, guaraná peels had 3 – 8-fold higher values than the cocoa by-products. In this case, their antioxidant capacity was attributed to polyphenolic compounds, including catechin, epicatechin, and procyanidin B2. Conversely, Okiyama et al. (2018) reported higher results for the antioxidant capacity of the dried cocoa shell when compared to guaraná peels, with values ranging from 47 – 84 μmol TE/g determined by the FRAP assay and 36 - 64 μmol TE/g by the DPPH assay. The authors associated the antioxidant potential with the total flavanols content present in the sample.

The results obtained by the ABTS and ORAC tests indicated that the guaraná peels have compounds that convert free radicals into stable products. Likewise, Cândido et al. (2015) reported that the ORAC and ABTS methods can provide better estimates of antioxidant capacity for food matrices with a high content of carotenoids. On the other hand, the results obtained by FRAP and DPPH tests suggested that the techniques are not adequate to determine the antioxidant potential of bioactive components of guaraná peels ethanolic extracts. It is worth mentioning that the antioxidant potential of a fruit matrix has different activities according to the method used.

## 3.4 Conclusions

The composition of guaraná peels includes insoluble fibers, macro- (Ca, K, and Mg) and micro- (Cu, Fe, Mn, and Zn) minerals, caffeine, theobromine, phenolic compounds, and carotenoids, all-*trans*-β-carotene, *cis*-β-carotene, and lutein. Carotenoid extractability was modified by the choice of the solvent, and by adjusting time and temperature during the process. The extraction using ethyl acetate-ethanol, for 24 hours at 50 °C was the better condition for achieving the highest recovery of carotenoids, considering the synergistic effect among solvents.

However, ethanol performed close to the mixtures and was used as the solvent for further analysis, considering the low toxicity, which allows obtaining a safe extract for food applications. The ethanolic extract of guaraná peels showed a high antioxidant capacity

determined by the ORAC and ABTS assays. The obtained data highlight the importance of recovering these bioactive compounds from a currently underutilized by-product, which has a great antioxidant property. Furthermore, our findings support the use of guaraná peel extracts with demonstrated antioxidant potential for further developments in functional foods.

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**References**

A.O.A.C. *Official methods of analysis*, 18th ed. Maryland: Association of Official Analytical Chemists, 2006.

A.O.A.C. *Official methods of analysis*, 16th ed. Washington: Association of Official Analytical Chemists, 1995.

Ajila, C. M., Aalami, M., Leelavathi, K., & Rao, U. P. (2010). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science & Emerging Technologies*, 11(1), 219-224. https://doi.org/10.1016/j.ifset.2009.10.004

Alvarenga, G. L., Cuevas, M. S., Capellini, M. C., Crevellin, E. J., de Moraes, L. A. B., & Rodrigues, C. E. da C. (2020). Extraction of carotenoid-rich palm pressed fiber oil using mixtures of hydrocarbons and short chain alcohols. *Food Research International*, *128*(October 2019), 108810. https://doi.org/10.1016/j.foodres.2019.108810

American Society for Testing & Materials – ASTM. (2001) ASTM E 203: *Standard Test Method for Water Using Volumetric Karl Fischer Tritation*. Annual Book of ASTM Standards, v. 08.01. United States.

Asp, N. G., Johansson, C. G., Hallmer, H., & Siljeström, M. (1983). Rapid Enzymatic Assay of Insoluble and Soluble Dietary Fiber. *Journal of Agricultural and Food Chemistry*, sch*31*(3), 476–482. https://doi.org/10.1021/jf00117a003

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, *44*(1), 391–396. https://doi.org/10.1016/j.foodres.2010.10.001

Barton, A. F. M. (1983). Handbook of solubility parameters end other cohesion parameters. Boca Raton: CRC Press.

Batista, Â. G., da Silva, J. K., Betim Cazarin, C. B., Biasoto, A. C. T., Sawaya, A. C. H. F., Prado, M. A., & Maróstica Júnior, M. R. (2017). Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves. *LWT - Food Science and Technology*, *76*, 284–291. https://doi.org/10.1016/j.lwt.2016.05.013

Benmeziane, A., Boulekbache-Makhlouf, L., Mapelli-Brahm, P., Khaled Khodja, N., Remini, H., Madani, K., & Meléndez-Martínez, A. J. (2018). Extraction of carotenoids from cantaloupe waste and determination of its mineral composition. *Food Research International*, *111*(May), 391–398. https://doi.org/10.1016/j.foodres.2018.05.044

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*, 70–76. https://doi.org/10.1006/abio.1996.0292

Bligh E G & Dyer W J. A. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, *37*, 911–917. https://doi.org/10.1093/nq/s9-III.75.427-d

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *The Microflown E-Book*, *28*, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2009). *Carotenoids.* Volume 5: Nutirition and Health.

Can-Cauich, C. A., Sauri-Duch, E., Betancur-Ancona, D., Chel-Guerrero, L., González-Aguilar, G. A., Cuevas-Glory, L. F., Pérez-Pacheco, E., & Moo-Huchin, V. M. (2017). Tropical fruit peel powders as functional ingredients: Evaluation of their bioactive compounds and antioxidant activity. *Journal of Functional Foods*, *37*, 501–506. https://doi.org/10.1016/j.jff.2017.08.028

Cândido, T. L. N., Silva, M. R., & Agostini-Costa, T. S. (2015). Bioactive compounds and antioxidant capacity of buriti (Mauritia flexuosa L.f.) from the Cerrado and Amazon biomes. *Food Chemistry*, *177*, 313–319. https://doi.org/10.1016/j.foodchem.2015.01.041

Carciochi, R. A., Dieu, V., Vauchel, P., Pradal, D., & Dimitrov, K. (2021). Reduction of environmental impacts of caffeine extraction from guarana by using ultrasound assistance. *Food and Bioproducts Processing.* https://doi.org/10.1016/j.fbp.2021.02.014

Carlson, M., & Thompson, R. D. (1998). Liquid chromatographic determination of methylxanthines and catechins in herbal preparations containing guaraná. *Journal of AOAC International*, 81(4), 691-701.

Carrageta, D. F., Dias, T. R., Alves, M. G., Oliveira, P. F., Monteiro, M. P., & Silva, B. M. (2018). Anti-obesity potential of natural methylxanthines. *Journal of Functional Foods*, 43, 84-94. https://doi.org/10.1016/j.jff.2018.02.001

Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, *13*(7), 8615–8627. https://doi.org/10.3390/ijms13078615

Cooperstone, J. L., Ralston, R. A., Riedl, K. M., Haufe, T. C., Schweiggert, R. M., King, S. A., Timmers, C. D., Francis, D. M., Lesinski, G. B., Clinton, S. K., & Schwartz, S. J. (2015). Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. *Molecular Nutrition and Food Research*, *59*(4), 658–669. https://doi.org/10.1002/mnfr.201400658

Cosby, L. E., Lee, K. H., Knobloch, T. J., Weghorst, C. M., & Winter, J. O. (2020). Comparative encapsulation efficiency of lutein in micelles synthesized via batch and high throughput methods. *International Journal of Nanomedicine*, *15*, 8217–8230. https://doi.org/10.2147/IJN.S259202

Craft, N. E., & Soares, J. H. (1992). Relative solubility, stability, and absorptivity of lutein and. beta.-carotene in organic solvents. *Journal of Agricultural and Food Chemistry*, 40(3), 431-434.

da Silva, G. S., Canuto, K. M., Ribeiro, P. R. V., de Brito, E. S., Nascimento, M. M., Zocolo, G. J., Coutinho, J. P., & de Jesus, R. M. (2017). Chemical profiling of guaraná seeds (Paullinia cupana) from different geographical origins using UPLC-QTOF-MS combined with chemometrics. *Food Research International*, *102*(September), 700–709. https://doi.org/10.1016/j.foodres.2017.09.055

De Rosso, V. V., & Mercadante, A. Z. (2007). Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *Journal of Agricultural and Food Chemistry*, *55*(13), 5062–5072. https://doi.org/10.1021/jf0705421

Del Pilar Sanchez-Camargo, A., Gutierrez, L. F., Vargas, S. M., Martinez-Correa, H. A., Parada-Alfonso, F., & Narvaez-Cuenca, C. E. (2019). Valorisation of mango peel: Proximate composition, supercritical fluid extraction of carotenoids, and application as an antioxidant additive for an edible oil. *The Journal of Supercritical Fluids*, 152, 104574. https://doi.org/10.1016/j.supflu.2019.104574

Dias, P. G. I., Sajiwanie, J. W. A., & Rathnayaka, R. M. U. S. K. (2020). Chemical Composition, Physicochemical and Technological Properties of Selected Fruit Peels as a Potential Food Source. *International Journal of Fruit Science*, *00*(00), 1–12. https://doi.org/10.1080/15538362.2020.1717402

FAPEAM. (2012). Empresa inova e reaproveita resíduo do guaraná. Retrieved from www.fapeam.am.gov.br

Food and Drug Administration. (2018). Q3C — Tables and List. Guidance for Industry. Retrieved from https://www.fda.gov/downloads/drugs/guidances/ucm073395.pdf.

Fu, X., Cheng, S., Liao, Y., Huang, B., Du, B., Zeng, W., Jianga, Y., Duan, X., & Yang, Z. (2018). Comparative analysis of pigments in red and yellow banana fruit. *Food Chemistry, 239*, 1009-1018. https://doi.org/10.1016/j.foodchem.2017.07.046

Gómez-Mejía, E., Rosales-Conrado, N., León-González, M. E., & Madrid, Y. (2019). Citrus peels waste as a source of value-added compounds: Extraction and quantification of bioactive polyphenols. *Food Chemistry*, 295, 289-299. https://doi.org/10.1016/j.foodchem.2019.05.136

Gondim, J. A. M., Moura, M. D. F. V, Dantas, A. S., Medeiros, R. L. S., & Santos, K. M. (2005). Composição Centesimal e de minerais em Cascas de frutas. *Food Science and Technology*, *25*(4), 825–827. https://doi.org/10.1590/S0101-20612005000400032

Hansen, C. M. (2004). 50 Years with solubility parameters - Past and future. *Progress in Organic Coatings*, *51*(1), 77–84. https://doi.org/10.1016/j.porgcoat.2004.05.004

Honda, M., Kageyama, H., Hibino, T., Ichihashi, K., Takada, W., & Goto, M. (2020). Isomerization of commercially important carotenoids (lycopene, β-carotene, and astaxanthin) by natural catalysts: Isothiocyanates and polysulfides. *Journal of Agricultural and Food Chemistry*, 68(10), 3228-3237. https://doi.org/10.1021/acs.jafc.0c00316

Huang, D., Boxin, O. U., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856. https://doi.org/10.1021/jf030723c

Ikawa, M., Schaper, T. D., Dollard, C. A., & Sasner, J. J. (2003). Utilization of Folin− Ciocalteu phenol reagent for the detection of certain nitrogen compounds. *Journal of Agricultural and Food Chemistry*, 51(7), 1811-1815. https://doi.org/10.1021/jf021099r

Ishida, B. K., & Chapman, M. H. (2009). Carotenoid extraction from plants using a novel, environmentally friendly solvent. *Journal of Agricultural and Food Chemistry*, *57*(3), 1051–1059. https://doi.org/10.1021/jf8026292

ISO 20481:2008, Coffee and coffee products—determination of the caffeine content using high performance liquid chromatography (HPLC)—reference method.

Janiszewska-turak, E. (2017). Carotenoids microencapsulation by spray drying method and supercritical micronization. *Food Research International*, *99*, 891–901. https://doi.org/10.1016/j.foodres.2017.02.001

Jiménez-Zamora, A., Pastoriza, S., & Rufián-Henares, J. A. (2015). Revalorization of coffee by-products. Prebiotic, antimicrobial and antioxidant properties. *LWT-Food Science and Technology*, 61(1), 12-18. https://doi.org/10.1016/j.lwt.2014.11.031

Kopec, R. E., Cooperstone, J. L., Schweiggert, R. M., Young, G. S., Harrison, E. H., Francis, D. M., Clinton, S. K., & Schwartz, S. J. (2014). Avocado consumption enhances human postprandial provitamin a absorption and conversion from a novel high-β-carotene tomato sauce and from carrots. *Journal of Nutrition*, *144*(8), 1158–1166. https://doi.org/10.3945/jn.113.187674

Koushan, K., Rusovici, R., Li, W., Ferguson, L. R., & Chalam, K. V. (2013). The role of lutein in eye-related disease. *Nutrients*, *5*(5), 1823–1839. https://doi.org/10.3390/nu5051823

Lindinger, M. I., & Cairns, S. P. (2021). Regulation of muscle potassium: exercise performance, fatigue and health implications. *European Journal of Applied Physiology*, 1-28. https://doi.org/10.1007/s00421-020-04546-8

Machado, K. N., de Freitas, A. A., Cunha, L. H., Faraco, A. A. G., de Pádua, R. M., Braga, F. C., ... & Castilho, R. O. (2018). A rapid simultaneous determination of methylxanthines and proanthocyanidins in Brazilian guaraná (*Paullinia cupana Kunth.*). *Food Chemistry*, 239, 180-188. https://doi.org/10.1016/j.foodchem.2017.06.089

Mallek-Ayadi, S., Bahloul, N., & Kechaou, N. (2017). Characterization, phenolic compounds and functional properties of Cucumis melo L. peels. *Food Chemistry*, *221*, 1691-1697. https://doi.org/10.1016/j.foodchem.2016.10.117

Maravalhas, N., Estudos sobre o guaraná e outras plantas produtoras de cafeína. (1965). Publicação n° 10 (Química) Instituto National de Pesquisas da Amazonia (INPA), Manaus.

Martínez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2012). Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products. *Food Research International*, 49(1), 39-45. https://doi.org/10.1016/j.foodres.2012.08.005

Mhemdi, H., Koubaa, M., El Majid, A., & Vorobiev, E. (2016). Solute and gas assisted mechanical expression for green oil recovery from rapeseed hulls. *Industrial Crops and Products*, *92*, 300–307. https://doi.org/10.1016/j.indcrop.2016.08.015

Mokrani, A., & Madani, K. (2016). Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit. *Separation and Purification Technology*, *162*, 68-76. https://doi.org/10.1016/j.seppur.2016.01.043

Moo-Huchin, V. M., Estrada-Mota, I., Estrada-León, R., Cuevas-Glory, L., Ortiz-Vázquez, E., De Lourdes Vargas Y Vargas, M., Betancur-Ancona, D., & Sauri-Duch, E. (2014). Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chemistry*, *152*, 508–515. https://doi.org/10.1016/j.foodchem.2013.12.013

Mulkidjanian, A. Y., Bychkov, A. Y., Dibrova, D. V., Galperin, M. Y., & Koonin, E. V. (2012). Origin of first cells at terrestrial, anoxic geothermal fields. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(14). https://doi.org/10.1073/pnas.1117774109

Müller, L., Fröhlich, K., & Böhm, V. (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. *Food Chemistry*, *129*(1), 139–148. https://doi.org/10.1016/j.foodchem.2011.04.045

Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta*, *703*(1), 8–18. https://doi.org/10.1016/j.aca.2011.07.018

Nguyen, V. T., & Nguyen, N. H. (2017). Proximate composition, extraction, and purification of theobromine from cacao pod husk (Theobroma cacao L.). *Technologies*, 5(2), 14. https://doi.org/10.3390/technologies5020014

Noronha Matos, K. A., Praia Lima, D., Pereira Barbosa, A. P., Zerlotti Mercadante, A., & Campos Chisté, R. (2019). Peels of tucumã (Astrocaryum vulgare) and peach palm (Bactris gasipaes) are by-products classified as very high carotenoid sources. *Food Chemistry*, *272*(April 2018), 216–221. https://doi.org/10.1016/j.foodchem.2018.08.053

Obulesu, M., Dowlathabad, M. R., & Bramhachari, P. V. (2011). Carotenoids and Alzheimer's Disease: An insight into therapeutic role of retinoids in animal models. *Neurochemistry International*, *59*(5), 535–541. https://doi.org/10.1016/j.neuint.2011.04.004

Okiyama, D. C., Soares, I. D., Cuevas, M. S., Crevelin, E. J., Moraes, L. A., Melo, M. P., ... & Rodrigues, C. E. (2018). Pressurized liquid extraction of flavanols and alkaloids from cocoa bean shell using ethanol as solvent. *Food Research International*, 114, 20-29. https://doi.org/10.1016/j.foodres.2018.07.055

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, *49*(10), 4619–4626. https://doi.org/10.1021/jf010586o

Ozel, M. Z., & Gogus, F. (2014). Subcritical water as a green solvent for plant extraction. In F. Chemat, & M. A. Vian (Eds.). *Alternative Solvents for Natural Products Extraction*. Berlin Heidelberg: Springer-Verlag.

Perera, C. O., & Yen, G. M. (2007). Functional properties of carotenoids in human health. *International Journal of Food Properties*, *10*(2), 201–230. https://doi.org/10.1080/10942910601045271

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231–1237.

Reichardt, C., Solvents and Solvent Effects in Organic Chemistry, Weinheim: Wiley-VCH, 2003, 630 p.

Rodriguez-amaya, D. B. (2001). A Guide To Analysis in Foods. In *Life Sciences*.

Romelle, F. D., Rani, A., & Manohar, R. S. (2016). Chemical composition of some selected fruit peels. *European Journal of Food Science and Technology*, *4*(4), 12-21.

Rufino, M. do S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996–1002. https://doi.org/10.1016/j.foodchem.2010.01.037

Sachindra, N. M., Bhaskar, N., & Mahendrakar, N. S. (2006). Recovery of carotenoids from shrimp waste in organic solvents. *Waste Management*, *26*(10), 1092–1098. https://doi.org/10.1016/j.wasman.2005.07.002

Saini, R. K., & Keum, Y.-S. (2018). Carotenoid extraction methods: A review of recent developments. *Food Chemistry*, *240*, 90–103. https://doi.org/10.1016/j.foodchem.2017.07.099

Santana, A. L., & Macedo, G. A. (2018). Health and technological aspects of methylxanthines and polyphenols from guaraná: A review. *Journal of Functional Foods*, 47, 457-468. https://doi.org/10.1016/j.jff.2018.05.048

Santana, A. L., Zanini, J. A., & Macedo, G. A. (2020). Dispersion-assisted extraction of guarana processing wastes on the obtaining of polyphenols and alkaloids. *Journal of Food Process Engineering*, 43(4), e13381. https://doi.org/10.1111/jfpe.13381

Sánchez-Camargo, A. del P., Bueno, M., Parada-Alfonso, F., Cifuentes, A., & Ibáñez, E. (2019). Hansen solubility parameters for selection of green extraction solvents. *TrAC - Trends in Analytical Chemistry*, *118*, 227–237. https://doi.org/10.1016/j.trac.2019.05.046

Scharlack, N. K., Aracava, K. K., & Rodrigues, C. E. (2017). Effect of the type and level of hydration of alcoholic solvents on the simultaneous extraction of oil and chlorogenic acids from sunflower seed press cake. *Journal of the Science of Food and Agriculture*, 97(13), 4612-4620. https://doi.org/10.1002/jsfa.8331

Schimpl, F. C., Da Silva, J. F., Gonçalves, J. F. D. C., & Mazzafera, P. (2013). benza*Journal of Ethnopharmacology*, *150*(1), 14–31. https://doi.org/10.1016/j.jep.2013.08.023

Sethi, S., Joshi, A., Arora, B., Bhowmik, A., Sharma, R. R., & Kumar, P. (2020). Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. *European Food Research and Technology*, *246*(3), 591-598. https://doi.org/10.1007/s00217-020-03432-z

Shi, J., Dai, Y., Kakuda, Y., Mittal, G., & Xue, S. J. (2008). Effect of heating and exposure to light on the stability of lycopene in tomato purée. *Food Control*, 19(5), 514-520. https://doi.org/10.1016/j.foodcont.2007.06.002

Silva, M. P., Thomazini, M., Holkem, A. T., Pinho, L. S., Genovese, M. I., & Fávaro-Trindade, C. S. (2019). Production and characterization of solid lipid microparticles loaded with guaraná (Paullinia cupana) seed extract. *Food Research International*, *123*(December 2018), 144–152. https://doi.org/10.1016/j.foodres.2019.04.055

Singh, J. P., Kaur, A., Shevkani, K., & Singh, N. (2016). Composition, bioactive compounds and antioxidant activity of common Indian fruits and vegetables. *Journal of Food Science and Technology*, *53*(11), 4056–4066. https://doi.org/10.1007/s13197-016-2412-8

Singleton, V. L., Rossi, J. A., & Jr, J. (1965). Colorimetry of Total Phenolics With Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.

Strati, I. F., & Oreopoulou, V. (2011a). Effect of extraction parameters on the carotenoid recovery from tomato waste. *International Journal of Food Science and Technology*, *46*(1), 23–29. https://doi.org/10.1111/j.1365-2621.2010.02496.x

Strati, I. F., & Oreopoulou, V. (2011b). Process optimisation for recovery of carotenoids from tomato waste. *Food Chemistry*, *129*(3), 747–752. https://doi.org/10.1016/j.foodchem.2011.05.015

Sweeney, J. P., & Marsh, A. C. (1973). Liver storage of vitamin A in rats fed carotene stereoisomers. *The Journal of Nutrition*, *103*(1), 20–25. https://doi.org/10.1093/jn/103.1.20

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*(6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003

USDA. Oxygen radical absorbance capacity (ORAC) of selected foods, release 2. 2010 [cited 2021 Jun 1]. Retrieved from: https://naldc.nal.usda.gov/download/43336/PDF

Wang, C. C., Chang, S. C., Inbaraj, B. S., & Chen, B. H. (2010). Isolation of carotenoids, flavonoids and polysaccharides from Lycium barbarum L. and evaluation of antioxidant activity. *Food Chemistry*, *120*(1), 184–192. https://doi.org/10.1016/j.foodchem.2009.10.005

Wojdyło, A., Nowicka, P., & Bąbelewski, P. (2018). Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *Journal of Functional Foods*, *48*(April), 632–642. https://doi.org/10.1016/j.jff.2018.07.061

Yara-Varón, E., Fabiano-Tixier, A. S., Balcells, M., Canela-Garayoa, R., Bily, A., & Chemat, F. (2016). Is it possible to substitute hexane with green solvents for extraction of carotenoids? A theoretical versus experimental solubility study. *RSC Advances*, *6*(33), 27750–27759. https://doi.org/10.1039/c6ra03016e

# 4 CHAPTER 4

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## ENCAPSULATION OF CAROTENOIDS FROM GUARANÁ (*PAULLINIA CUPANA*) BY-PRODUCT BY COMBINATION OF SPRAY DRYING AND CHILLING

**ABSTRACT**

Guaraná by-products are rich in carotenoids featured with strong antioxidant capacity and health-promoting benefits. However, these compounds are highly susceptible to oxidation and isomerization, which limits their applications in foods. This research aimed to encapsulate carotenoid-rich extract from reddish guaraná peels by spray drying (SD), chilling (SC), and their combination (SDC) to form microparticles. The physicochemical properties of the formed microparticles were characterized and their storage stability was evaluated over 90 days. Water activity values of SD microparticles increased during storage, whereas SC and SDC microparticles were more stable. Besides, microparticles exhibited color variation and size increase over time. Carotenoid degradation was better described by a first-order kinetics for most treatments. Considering the higher carotenoid content and its stability, a formulation from each process was selected to further analysis. Scanning electron micrographs revealed spherical shape and absence of cracks on the microparticles' surface, as well as the size heterogeneity. SD increased the oxidative stability of carotenoid-rich extract by at least 52-fold, SC by 3-fold, and SDC by 545-fold. The analysis of thermograms suggested that the carrier and process of encapsulation influence the powder's thermal behavior. Water sorption data from Dynamic Vapor Sorption (DVS) of SDC microparticles depended on the blend of carrier agents (gum arabic and hydrogenated vegetable fat) during the process. The innovation proposed for carotenoid encapsulation by the combination of spray drying and spray chilling has beneficial aspects from a technological perspective, which could be applied as a promising alternative to protect bioactive compounds.

**Keywords:** Microencapsulation; storage stability; carotenoid degradation; gum arabic; hydrogenated vegetable fat.

## 4.1 Introduction

The use of by-products from agro-industrial processes has been growing over recent years as a strategy to valorize them and reduce environmental threatens. Fruit and vegetable peels, seeds, and pomace are all by-products of their processing. They display great potential as starting materials for extraction of bioactive compounds (Da Silva et al., 2020). One of the more representative is guaraná, a Brazilian plant native to the Amazon basin, whose seeds and peels

are rich in alkaloids (caffeine, theobromine, and theophylline), polyphenols (catechin, epicatechin, and epicatechin gallate), and carotenoids ($\beta$ -carotene and lutein) (Santana, Zanini, & Macedo, 2020; Pinho et al., 2021).

Carotenoids are natural pigments present in plants and other photosynthetic organisms, and often used as food colorants. They also possess antioxidant capacity, and has been demonstrated to act as an anticancer agent, immune response stimulant, and pro-vitamin A activity promoter (During & Harrison, 2007; Liu et al., 2014; Chuyen & Eun, 2017; Milani, Basirnejad, Shahbazi, & Bolhassani, 2017; Medeiros et al., 2019; Cano, Gómez-Maqueo, García-Cayuela, & Welti-Chanes, 2017; Fraser & Bramley, 2004). However, carotenoids are easily oxidized or isomerized in the presence of oxygen, light, metals, and heat during processing and storage, leading to reduced color and bioactivity (Rodriguez-Amaya, 2001).

Encapsulation is the most widespread approach to preserve the bioactivity of oxygen- and light-sensitive compounds, mask unpleasant tastes, and control releases (Sartori, Consoli, Hubinger, & Menegalli, 2015). A process commonly used for the encapsulation of carotenoids is spray drying (Troya, Tupuna-Yerovi, & Ruales, 2018; Anthero, Bezerra, Comunian, Procópio, & Hubinger, 2020; Santos, Rubio, de Carvalho Balieiro, Thomazini, & Favaro-Trindade, 2021). This process turns a liquid feed material, which is composed of an ingredient (core) and a carrier agent, into powder followed by the liquid atomization and high-temperature drying. The efficiency of encapsulation in spray drying depends mainly on the types of carrier and core materials, and the operating conditions (Islam, Kitamura, Yamano, & Kitamura, 2016). Water-soluble polymers such as modified starches, whey protein, maltodextrin, and gum arabic are the well-known and used carrier materials (Selim, Tsimidou, & Biliaderis., 2000; Souza et al. 2018; Carneiro, Tonon, Grosso, & Hubinger, 2013). Among them, gum arabic is extensively employed for the spray-drying of bioactive compounds, due to its emulsification capacity, high solubility, low viscosity, non-toxicity, and ideal retention properties.

Spray chilling is an alternative for stabilizing sensitive compounds such as carotenoids. Unlike spray drying that operates at high temperatures, the formation of lipid microparticles trapping bioactive compounds by spray chilling is at low temperatures. This would induce less heat resulting in a smaller extent of degradation of these compounds (Okuro, de Matos Junior, & Favaro-Trindade, 2013; Chambi, Alvim, Barrera-Arellano, & Grosso, 2008; Sillick & Gregson, 2012). However, the technique has relatively low encapsulation efficiency and degradation of lipid carrier may occur. Different types of materials, such as hydrogenated vegetable fat, fatty acids and/or waxes are used as encapsulating agents to produce solid lipid

microparticles by spray chilling. Due to its lower price and high availability, hydrogenated vegetable fat is more appropriate than other lipids for this application (Zuidam & Shimoni, 2010; Consoli, Grimaldi, Sartori, Menegalli, & Hubinger, 2016).

The combination of spray drying and chilling has been shown to better preserve bioactive components considering the presence of double-wall coverage. For example, Arslan-Tontul & Erbas (2017) observed higher gastric and thermal resistance of the particles containing prebiotics produced by the combination of spray drying and chilling than processing individually. Fadini et al. (2018) reported similar findings on the protection of functional oils when using the combined technologies. However, whether this applies to carotenoids remains unknown. The current study aimed to produce and compare microparticles containing carotenoids-rich extract from guaraná peels by spray drying, chilling, and their combination. The microparticles were monitored during storage in terms of water activity, size, color parameters and carotenoid stability. The physicochemical properties of microparticles including morphology, water sorption, thermal, and oxidative stability of carotenoids were also measured.

## 4.2 Material and Methods

### 4.2.1 2.1 Materials

Guaraná fruits were provided by the Executive Commission of the Rural Economic Recuperation Plan in Cacao (Taperoá, Bahia, Brazil). Gum arabic (Acacia gum - Nexira, Brazil) was used as the carrier in the spray drying process. Microparticles obtained by spray chilling were produced using Al Home P54 hydrogenated vegetable fat with a melting point of 54 °C (Cargill, Itumbiara, Goiás, Brazil) as carrier material. Microparticles obtained by the combination of spray drying and chilling were prepared using gum arabic as the first wall material, and the hydrogenated vegetable fat as the second coating. Ethanol (purity ≥ 99%) was purchased from Êxodo Científica (Sumaré, Brazil). Petroleum ether (30-70 fraction) and magnesium chloride ($MgCl_2.6H_2O$) were acquired from Synth (Diadema, Brazil).

### 4.2.2 Production of carotenoid-rich extract from guaraná peels

Guaraná fruits were supplied by The Executive Commission for Cocoa Cultivation Planning - CEPLAC (Taperoá, Bahia, Brazil). The peels were detached from seeds and pulp and then washed with water. The guaraná peels were dried in a convective oven (Marconi, MA035/1152) at 50 °C for 18 h and stored at −20 °C until further analysis. To extract carotenoids, the peels were mixed with ethanol in a ratio of 1:10 (peel: solvent, w/v). The mixture was shaken (Orbital Shaker Marconi, MA420, Piracicaba, SP) for 4 h at 50 °C and centrifuged at 7,168×$g$ for 10 min (Pinho et al., 2021). Sunflower oil was added to the supernatant in a final concentration of 3% and mixed to minimize the carotenoid degradation observed during preliminary experiments. The final concentration of 3% of the oil was selected considering the liquid-liquid equilibrium for the system composed of sunflower oil and ethanol (Cuevas, Rodrigues, Gomes, & Meirelles, 2010). Afterwards, the material was concentrated using a rotary evaporator (TE-211 Tecnal, Piracicaba, Brazil) at 48 ± 2°C to 20 % of the initial volume. The concentrate was named as "carotenoid-rich extract".

### 4.2.3 Production of microparticles from the carotenoid-rich extract

Carotenoid-rich extracts were mixed with carrier materials at different proportions for particle production by spray drying and chilling. Microparticles obtained by the combination of spray drying and chilling were prepared using spray-dried microparticles as core and hydrogenated vegetable fat as carrier material as shown in Table 7.

Microparticles obtained by spray drying, chilling, and the combination of spray drying and chilling are shown in Attachment C.

#### 4.2.3.1 Microparticles obtained by Spray Drying (SD)

An emulsion was prepared by the mixing of carotenoid-rich extract and gum arabic solution (20%), v/v, using Ultra-Turrax ® IKA T25 (Labotechnic, Staufen, Germany) at 11,200×$g$ for 3 min. The emulsion was atomized according to Rocha, Fávaro-Trindade, & Grosso (2012) with slight modifications on the drying temperature. A spray dryer (Model MSD 1.0, Labmaq do Brasil, Ribeirão Preto, Brazil) equipped with a spray nozzle of 1.2 mm was used, with an inlet air temperature of 140 °C, an air speed of 2.5 m/s, a feed flow of 10 mL/min and an air pressure of 8.4 kgf/cm$^2$. During the drying procedure, the emulsion was kept under magnetic stirring.

#### 4.2.3.2 Microparticles obtained by Spray Chilling (SC)

Solid lipid microparticles were produced by spray chilling according to Pelissari et al. (2016). A dispersion was prepared from different proportions of carotenoid-rich extract and high melting point hydrogenated vegetable fat, using Ultra-Turrax ® IKA T25 (Labotechnic, Staufen, Germany) 11,200×$g$ for 3 min at 64°C. The mixture was atomized using the same spray dryer equipment (Model MSD 1.0, Labmaq do Brasil, Ribeirão Preto, Brazil) coupled to a spray nozzle of 1.2 mm with a 1.0 kgf/cm$^2$ air pressure and a feed flow of 40 mL/min at 13°C.

#### 4.2.3.3 Microparticles obtained by the combination of spray drying and chilling (SDC)

SD33 formulation was firstly prepared by spray drying as described previously (Section 4.2.3.1). SDC microparticles were then produced by dispersing SD33 microparticles into hydrogenated vegetable fat (w/w) at 11,200×$g$ for 1 min at 64 °C. The atomization conditions used were the same as described previously for the spray chilling process.

**Table 7.** Formulation and composition of microparticles produced by spray drying (SD), chilling (SC), and the combination of spray drying and chilling (SDC)

| **Formulation** | **Core (%)** | **Carrier material (%)** |
|---|---|---|
| | Extract | Gum Arabic solution |
| SD20 | 20 | 80 |
| SD25 | 25 | 75 |
| SD33 | 33 | 67 |
| | Extract | Hydrogenated vegetable fat |
| SC20 | 20 | 80 |
| SC30 | 30 | 70 |
| SC40 | 40 | 60 |
| | SD33 microparticles | Hydrogenated vegetable fat |
| SDC10 | 10 | 90 |
| SDC20 | 20 | 80 |

Reference: Own source.

### 4.2.4 Characterization of microparticles and storage stability

The powders were placed in vials and kept in desiccators containing saturated magnesium chloride solution to create a storage environment of 33 ± 5 % relative humidity (RH), at 25 ± 5 °C for 90 days. Water activity, color, mean diameter, and particle size were determined at the beginning (time 0) and the end (after 90 days) of storage. The total carotenoid content of the samples was evaluated every 15 days as recommended by Tonon, Brabet, & Hubinger (2010). The analyzes were carried out under dark conditions.

#### 4.2.4.1 Water activity

Powder water activity ($a_w$) was measured utilizing Aqualab equipment (Series 3 TE - Decagon Devices, USA) at room temperature. Measurements were conducted in triplicate.

#### 4.2.4.2 Instrumental color analysis

The color parameters L (luminosity), a* (red - green) and b* (yellow - blue) of the samples were evaluated using a portable colorimeter (Mini Scan XE Plus - Hunterlab, USA). Measurements were executed in triplicate.

#### 4.2.4.3 Particle size and distribution

The particle size distribution and the mean diameters of the particles were investigated using laser diffraction equipment (SaldI-201-V, Shimadzu, Kyoto, Japan). Ethanol was used as a dispersant for microparticles obtained by spray drying, and distilled water for microparticles produced by spray chilling and their combination. Measurements were performed in triplicate.

#### 4.2.4.4 Determination of total carotenoid content in the non-encapsulated and encapsulated extract

The total carotenoid content was quantified by a spectrophotometric method as described by Rodriguez-Amaya (2001) using a UV-VIS Spectrophotometer (Genesys 10S Thermo Scientific, São Paulo/SP, Brasil) at 450 nm.

Carotenoids were extracted following Pelissari et al. (2016) with slight modifications regarding the solvents used. Carotenoids-rich extract and SD microparticles were dispersed in hexane, while SC and SDC in petroleum ether. The mixtures were agitated for 1 min and kept in an ultrasound bath (USC-1400, Unique, Indaiatuba, Brazil) for 20 min. Distilled water was then added and agitated (Multi Reax, Heidolph Instruments, Schwabach, Germany) during 2 min. They were then centrifuged at 4930×$g$ for 10 min and the supernatants (hexane or petroleum ether rich phases) were transferred to cuvettes. The total carotenoid content was determined according to the following Eq. (6):

$$C = A * V * 10^4 / Abs_{1cm}^{1\%} * m \quad (6)$$

Where $C$ (μg/g) corresponds to the total carotenoid content; $A$ (nm) is the absorbance of the sample at 450 nm; $V$ (mL) is the final volume; $Abs_{1cm}^{1\%}$ is the extinction coefficient of $\beta$-carotene, which in petroleum ether has a value of 2592 $cm^{-1}$ (Rodriguez-Amaya, 2001), and in hexane of 2560 $cm^{-1}$ (Hart & Scott, 1995); $m$ is the sample mass (g). The determinations were performed in triplicate.

#### 4.2.4.5 Encapsulation efficiency (EE)

The encapsulation efficiency (%) was calculated as a ratio between the total carotenoid content present in the microparticles ($C_0$) and the total carotenoid content in the feed materials ($C_{FM}$) before atomization, by Eq. 7 (Sartori, Consoli, Hubinger, & Menegalli, 2015; Alvim, Stein, Koury, Dantas, & Cruz, 2016). Microparticles production were conducted in triplicate.

$$EE\ (\%) = (C_0 / C_{FM}) * 100 \quad (7)$$

#### 4.2.4.6 Kinetic of carotenoid degradation

The degradation of carotenoids during storage can be modeled by kinetic equations, which describe the reactions that may occur in the system. The kinetics and the corresponding reaction rate constants $k_o$ and $k_1$ were evaluated following zero- and first-order kinetics described by Eqs. 8 and 9, for non-encapsulated and encapsulated carotenoid-rich extract. The half-life of the first order reaction was calculated according Eq. 10 (Xiao et al., 2018).

$$C_t - C_0 = -k_0 * t \quad (8)$$

$$-ln\, C_t/C_0 = k1 \cdot \mathrm{t} \quad (9)$$

$$t_{1/2} = \ln 2/k1 \quad (10)$$

Where $C_0$ (µg/g) is the initial carotenoid content (time 0), $C_t$ (µg/g) is the content at the analysis time, and *t* (days) is the time. The reaction rate constants for zero $k_o$ (µg/ (g*s)) and first $k_1$ (1/s) -order were based on linear plots of ($C_t$–$C_{t,0}$) vs. *t* and ln ($C_t/C_{t,0}$) vs. *t*, respectively. The kinetics analysis was carried out using the program Origin Pro 8.5.

#### 4.2.4.7 Carotenoid retention (CR)

Carotenoid retention (%) was determined according to the following equation:

$$CR = (C_{90}/C_0) \times 100 \quad (11)$$

Where $C_{90}$ (µg/g) is the carotenoid concentration after 90 days of storage, whereas $C_0$ (µg/g) is the initial carotenoid concentration in the microparticles.

### 4.2.5 Analysis of selected microparticles

To further analyze the characteristics of the microparticles, three formulations were selected. SD33, SC40, and SDC20 were chosen mainly based on results of the total carotenoid content and reasonable stability during storage. They were evaluated in terms of their morphology, oxidative stability, thermal properties, and water vapor sorption as described in the following sections.

#### 4.2.5.1 Scanning electron microscopy (SEM)

The SD, SC, and SDC microparticles were placed on a double-sided carbon adhesive tape (Ted Pella Inc., Redding, EUA), they were coated with gold and analyzed using the

Benchtop Microscope Hitachi TM 300 (Tokyo, Japan) scanning electron microscope. SEM images of the powders were captured at an accelerating voltage of 15 kV.

#### 4.2.5.2 Oxidative stability

The oxidative stability of powders was conducted according to De Leonardis & Macciola (2012). Microparticles (2.0 g) were heated from 25 °C to 120 °C under 20 L/h airflow, using Rancimat equipment (model 873, Metrohm, Herisau, Switzerland). The analyses were carried out in duplicate and results were expressed as oxidation induction time.

#### 4.2.5.3 Differential scanning calorimetry (DSC)

Thermal properties of microparticles were evaluated using differential scanning calorimetry (DSC 2500, TA Instruments, New Castle, Delaware, USA.). Samples were weighed (~ 5 mg) into aluminum pans, sealed, and put into DSC. An empty pan was used as a reference. Samples were equilibrated at 25 °C for 2 min, ramped to 300 °C at a rate of 10 °C/min, isothermal for 2 min and then cooled to 25 °C at a rate of 30 °C/min (Xu et al., 2021). Data collection and analysis were conducted using Trios software (TA Instruments). Triplicates were run for each sample and the average result was shown.

#### 4.2.5.4 Dynamic vapor sorption (DVS)

The water vapor sorption of the microparticles was conducted using a Dynamic Vapor Sorption instrument (Surface Measurement Systems Ltd., Allentown, PA). The airflow in the DVS was compressed nitrogen flowing at 200 mL/min. The changes in sample mass at various relative humidity (between 0% and 90%) were recorded continuously at 25 °C using the DVS Analysis Macro V6.1 software. Each sample was run in duplicate.

### 4.2.6 Statistical analysis

Data were examined by analysis of variance (ANOVA) and Tukey's test, at the 5% level significance, using the statistical program SAS (Statistic Analysis System) version 9.2.

## 4.3 Results and Discussion

### 4.3.1 Characterization of microparticles and their changes during storage

#### 4.3.1.1 Water activity ($a_w$)

The $a_w$ of microparticles produced by spray drying, chilling, and their combination loaded with carotenoid-rich extract are shown in Table 8. SD microparticles had the lowest $a_w$, and this was probably due to larger water evaporation at the high inlet temperature (140 °C) during atomization. After storage, $a_w$ increased due to the particle hygroscopicity (water uptake) as consequence of gum arabic used as carrier material. In this system, the molecular mobility increased, and the powder absorbed water until reaching the equilibrium condition, at an intermediate relative humidity of 35 - 40% at 25 °C. The water activity of SD microparticles was below the minimum value of 0.60, which corresponds to a satisfactory condition to avoid microbial growth. It is worth mentioning that even during storage, these results demonstrated great stability of the samples.

SC microparticles had $a_w$ (0.80 - 0.95) higher than those of the SD and SDC microparticles (0.41 – 0.52), and they varied with formulations. These $a_w$ values measured are in agreement with the $a_w$ range reported by Labuza (1971), from 0.30 to 1.0, which is associated to the susceptibility of the highest lipid oxidation rate. Silva et al. (2018) presented similar results, with water activities ranging from 0.91 to 0.98, for solid lipid microparticles loaded with probiotics produced by spray chilling.

#### 4.3.1.2 Color parameters

Color coordinates L*, a*, and b* were examined during storage time, as an indicative parameter on the chemical stability of carotenoids encapsulated within SD, SC, and SDC microparticles (Table 8). SD20, SC20, and SDC10 microparticles showed higher luminosity, compared to the other treatments obtained by the same process. This was due to the higher content of gum arabic and/or hydrogenated vegetable fat, and lower concentration of extract/SD microparticles in their formulations. During the stability test, the lightness of the SD microparticles increased. This can be associated with carotenoid degradation, which leads to the changes in the color intensity of the microparticles. McClements (2002) reported that the increase in particle dimensions caused by aggregation and/or morphology, is likely to influence the light scattering efficiency of the samples, affecting their lightness.

Regarding the color parameter a*, all the microparticles had positive values, which implies the subtle redness of all samples. A relatively superior magnitude of a* was observed for the SD33 microparticles, indicating more redness compared to others. The formulation was composed of a high proportion of extract, and the evaporation of carrier solution during spray drying led to the concentration of carotenoid within the microparticles.

For the color parameter b*, all formulations displayed a noticeable decrease in this aspect during storage. Indeed, the intensity change of the yellow/orange color (ΔC*) of the microparticles during storage followed the ascending order: SC30=SDC10 < SC20=SDC20 < SD20=SC40 < SD25 < SD33. These findings suggest that the encapsulated carotenoids are more stable in lipid microparticles (SC and SDC) obtained by spray chilling and the combination of processes.

#### 4.3.1.3 Mean diameter and particle size distribution

Volume weighted mean diameters ($D_{4,3}$) and particle size distribution are shown in Table 8 and Figure 12, respectively. SD20 microparticles showed a significantly larger mean diameter than those estimated for the SD25 and SD33 microparticles. During storage, the size of SD microparticles had a slight increase, which can be due to their agglomeration promoted by the adhesion forces. In this system, surely moistened microparticles collided and remained bonded, creating agglomerates.

Comparing samples obtained by spray chilling, the average sizes varied over 90 days. In the spray chilling process, operating conditions such as temperature, pressure, cooling air speed, feed flow, and spray nozzle diameter may affect particle size and mean diameter. In addition, intrinsic parameters, such as the composition of lipid carrier, and the ratio of the feed matrix (bioactive ingredient:carrier) can also affect the size of the microparticles (Fávaro-Trindade, Okuro, & de Matos Jr, 2015).

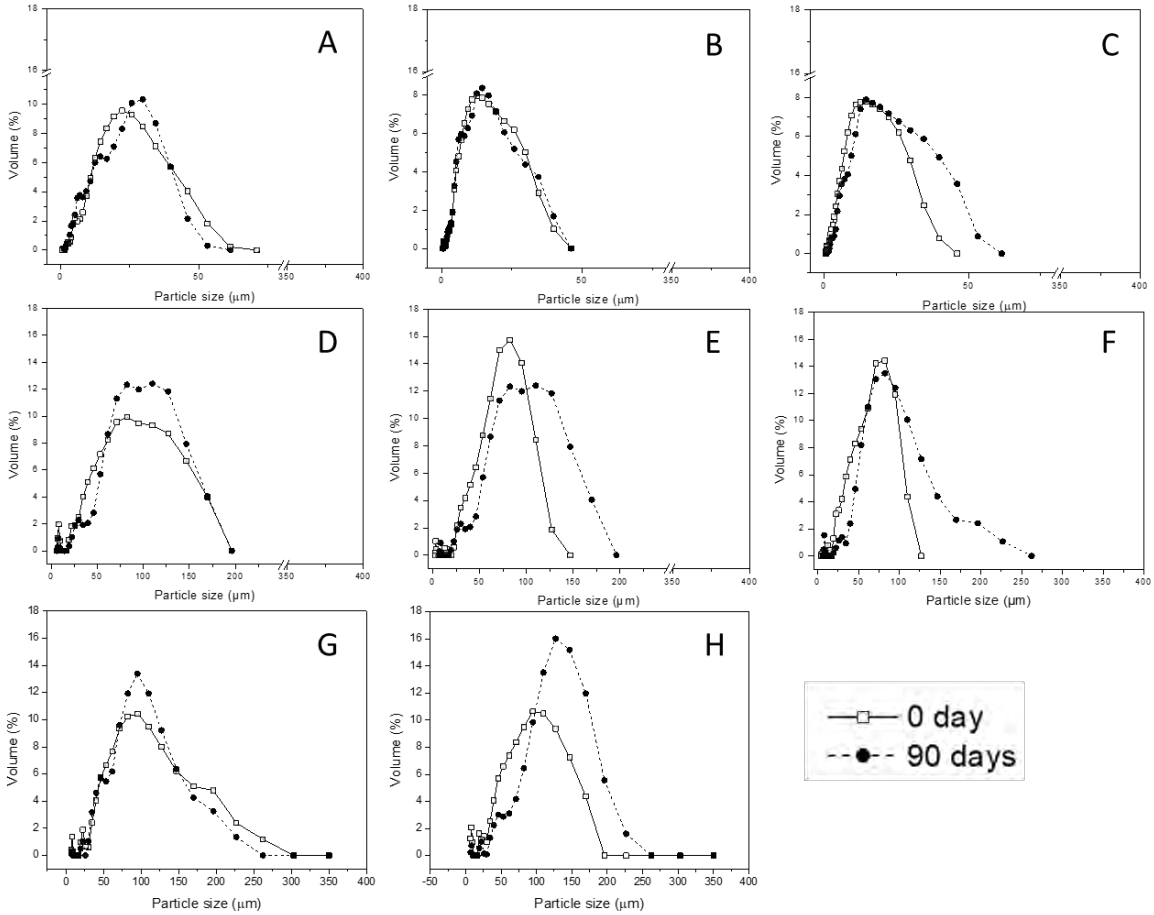
**Table 8.** Characterization of microparticles produced by spray drying (SD), chilling (SC), and their combination (SDC) before and after storage

| Response | | Time (day) | SD20 | SD25 | SD33 | SC20 | SC30 | SC40 | SDC10 | SDC20 |
|---|---|---|---|---|---|---|---|---|---|---|
| $a_w$ | | 0 | $0.132 \pm 0.004^{h,B}$ | $0.210 \pm 0.001^{f,B}$ | $0.161 \pm 0.005^{g,B}$ | $0.913 \pm 0.001^{a,B}$ | $0.872 \pm 0.002^{b,B}$ | $0.801 \pm 0.002^{c,B}$ | $0.47 \pm 0.01^{d,B}$ | $0.412 \pm 0.003^{e,B}$ |
| | | 90 | $0.456 \pm 0.002^{e,A}$ | $0.449 \pm 0.001^{e,A}$ | $0.418 \pm 0.003^{e,A}$ | $0.953 \pm 0.004^{a,A}$ | $0.897 \pm 0.006^{b,A}$ | $0.833 \pm 0.008^{c,A}$ | $0.52 \pm 0.01^{d,A}$ | $0.468 \pm 0.008^{e,A}$ |
| Color parameters | L* | 0 | $80.61 \pm 0.01^{d,B}$ | $76.44 \pm 0.01^{f,B}$ | $74.11 \pm 0.01^{h,B}$ | $84.15 \pm 0.01^{a,A}$ | $82.51 \pm 0.24^{b,A}$ | $79.20 \pm 1.82^{e,A}$ | $81.26 \pm 0.02^{c,B}$ | $74.39 \pm 0.01^{g,B}$ |
| | | 90 | $81.46 \pm 0.01^{b,A}$ | $78.80 \pm 0.01^{c,A}$ | $75.18 \pm 0.01^{f,A}$ | $76.31 \pm 0.01^{d,B}$ | $75.63 \pm 1.42^{e,B}$ | $71.01 \pm 2.29^{h,B}$ | $81.75 \pm 0.01^{a,A}$ | $75.12 \pm 0.01^{g,A}$ |
| | a* | 0 | $1.08 \pm 0.04^{d,A}$ | $2.06 \pm 0.03^{b,A}$ | $4.05 \pm 0.02^{a,A}$ | $0.79 \pm 0.02^{e,B}$ | $0.84 \pm 0.26^{e,B}$ | $1.97 \pm 016^{b,B}$ | $0.14 \pm 0.02^{f,A}$ | $1.81 \pm 0.05^{c,A}$ |
| | | 90 | $0.51 \pm 0.03^{f,B}$ | $1.04 \pm 0.02^{e,B}$ | $3.17 \pm 0.04^{a,B}$ | $1.08 \pm 0.03^{e,A}$ | $1.42 \pm 0.02^{c,A}$ | $2.29 \pm 0.07^{b,A}$ | $0.06 \pm 0.01^{g,B}$ | $1.60 \pm 0.06^{d,B}$ |
| | b* | 0 | $23.07 \pm 0.02^{g,A}$ | $33.39 \pm 0.05^{b,A}$ | $39.68 \pm 0.02^{a,A}$ | $27.29 \pm 0.05^{e,A}$ | $24.12 \pm 0.59^{f,A}$ | $30.76 \pm 1.47^{d,A}$ | $20.35 \pm 0.05^{h,A}$ | $31.04 \pm 0.08^{c,A}$ |
| | | 90 | $20.36 \pm 0.05^{g,B}$ | $29.62 \pm 0.02^{b,B}$ | $35.12 \pm 0.07^{a,B}$ | $24.07 \pm 0.09^{e,B}$ | $23.39 \pm 0.03^{f,B}$ | $25.27 \pm 0.07^{d,B}$ | $19.89 \pm 0.06^{h,B}$ | $29.02 \pm 0.10^{c,B}$ |
| Mean Diameter (μm) | | 0 | $16.2 \pm 0.3^{d,B}$ | $10.8 \pm 0.2^{e,A}$ | $10.9 \pm 0.6^{e,B}$ | $59 \pm 2^{b,B}$ | $56.6 \pm 0.4^{b,B}$ | $52 \pm 1^{c,B}$ | $73 \pm 1^{a,A}$ | $60 \pm 1^{b,B}$ |
| | | 90 | $20 \pm 1^{c,A}$ | $13 \pm 2^{c,A}$ | $15 \pm 2^{c,A}$ | $74 \pm 2^{b,A}$ | $71.3 \pm 0.8^{b,A}$ | $71.7 \pm 0.8^{b,A}$ | $75 \pm 2^{b,A}$ | $96 \pm 2^{a,A}$ |

Values are mean ± standard error (SE) (n = 3–4 analytical replicates). Different uppercase letters in a column represent a significant difference between 0 and 90 days with the response for formulations. The different lowercase letters in a row mean that there is a significant difference among formulations by the Tukey test at the 5% level of significance. SD: microparticles obtained by spray drying; SC: microparticles obtained by spray chilling; SDC: microparticles obtained by spray drying and chilling combination. Numerical suffix means the proportion of core:carrier material of each formulation.

Reference: Own source.

**Figure 12.** Particle size distribution of microparticles produced by spray drying, chilling, and their combination. A, B, and C: microparticles obtained by spray drying with 20, 25, and 33% proportion core:carrier material, respectively; D, E, F: microparticles obtained by spray chilling with 20, 30, and 40% proportion core:carrier material, respectively; G and H: microparticles obtained by spray drying and chilling combination with 10 and 20% proportion core:carrier material, respectively




Reference: Own source

SDC10 microparticles were larger than the SDC20 ones after freshly prepared. The mean diameter of the microparticles increased continuously throughout the storage ($P < 0.05$), especially for the SDC20 formulation. Microparticles produced by spray chilling are classified as matrices types, where the compound to be protected are entrapped all over the volume of the particle. In the SDC microparticles case, the structure is surrounded by hydrogenated vegetable fat (carrier) and SD microparticles (core). Regarding the observed broad range of the diameter

after storage for the SDC20 microparticles, it is hypothesized that the microparticle composition may promote the agglomeration among them.

Overall, size distribution curves of all formulations exhibited a unimodal pattern before and after storage at 25 °C. Microparticles obtained by SD showed smaller sizes compared to the SC and SDC microparticles, even though the same nozzle was used for atomization in all methods. The smaller droplet size of the emulsion in the feed material and the operational parameters, such as high temperature and pressure, might facilitate solvent evaporation and shorten coat formation time during the atomization by spray drying. In the spray chilling process, there is heat transfer between cold air and the molten feed material, in which the droplet maintains the original size formed during solidification at low temperature.

Particle size is a physical property relevant to many food applications, and it is related to the food sensory attributes of the food products when powders are incorporated (De Lara Pedroso, Thomazini, Heinemann, & Fávaro-Trindade, 2012). Hansen, Allan-Wojtas, Jin, & Paulson (2002) proposed particle size less than 100 μm would not affect the food sensory quality. According to this, the microparticles produced by spray drying, chilling, and their combination investigated in the current study had ideal sizes for practical applications. However, from a technical aspect, SD microparticles had the most applicable size for food supplementation. Besides the particle size, the composition of microparticles produced by the different methods would also affect their applications. For example, SD powders could be used to disperse and protect carotenoids in an aqueous product processing, while the SC and SDC powders could be more preferable to be used in a non-aqueous medium.

#### 4.3.1.4 Encapsulation efficiency (EE)

The EE of microparticles produced through spray drying, chilling, and their combination are shown in Table 9. The samples showed high EE, ranging from 90 to 100% for SD, 90 to 97% for SC, and 82 to 94% for the SDC techniques, which demonstrated the greater entrapment of the carotenoid content in the microparticles.

The slight carotenoids loss observed in SD samples might occur during the atomization step, in which these components possibly have adhered to the drying chamber wall during the heat processing. Whereas in SC treatments, the degradation of carotenoids may be attributed to the fact that the hydrogenated vegetable fat was kept at 64 °C during the processing time, which

involved homogenization to atomization. Although spray chilling is a technique that produces microparticles at low temperatures, it has the drawback to maintaining the fat used as a carrier in a melted status throughout processing. Additionally, the exposure to environmental conditions, such as light and oxygen, may have triggered some oxidation of the pigments.

**Table 9.** Encapsulation efficiency (%) of microparticles obtained by spray drying, chilling, and their combination

| Formulation | Encapsulation efficiency (%) |
|---|---|
| SD20 | $96 \pm 7^{ab}$ |
| SD25 | $100 \pm 2^{a}$ |
| SD33 | $90 \pm 2^{bc}$ |
| SC20 | $96 \pm 2^{ab}$ |
| SC30 | $97 \pm 1^{ab}$ |
| SC40 | $90 \pm 1^{bc}$ |
| SDC10 | $82 \pm 7^{c}$ |
| SDC20 | $94.4 \pm 0.5^{ab}$ |

SD: microparticles obtained by spray drying; SC: microparticles obtained by spray chilling; SDC: microparticles obtained by spray drying and chilling combination. Numerical suffix means the proportion of core/carrier material of each formulation. Different letter on the superscripts denotes significance among the data in the same column ($P < 0.05$).

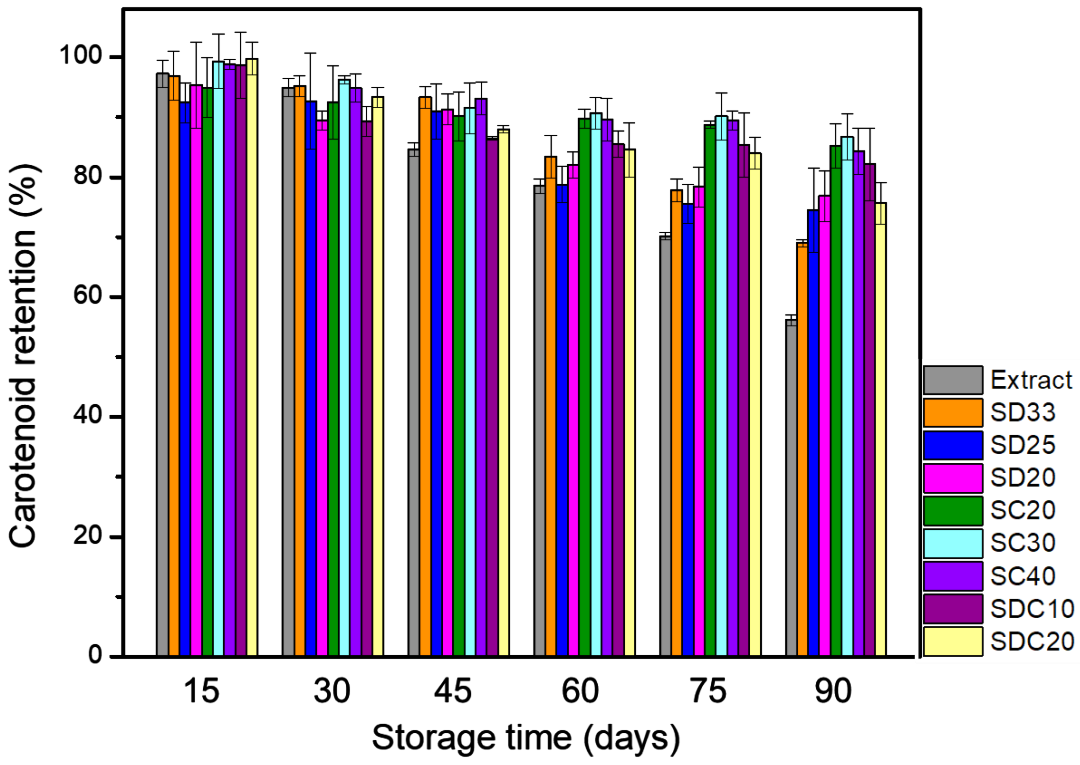
Reference: Own source

Concerning the SDC microparticles, they showed lower EE compared to the others. Lipids can exhibit three types of crystal polymorphic phases, which include alpha (α), beta-prime (β′), and beta (β), varying from the least to a more stable form (Müller, Radtke, & Wissing, 2002; Ghotra, Dyal, & Narine 2002). Thus, the carrier material can solidify in distinct crystallization patterns during the spray chilling processing. Through atomization at low temperatures via rapid cooling, the solidification of microparticles composed of gum arabic and hydrogenated vegetable fat may generate an arrangement of lipid crystals unable of trapping part of bioactive compounds. As a result, the achieved arrangement probably led to the expulsion of carotenoids and their loss during production. Similar finding was reported by Navarro-Guajardo et al. (2018).

### 4.3.2 Chemical stability of non-encapsulated and encapsulated carotenoid-rich extract

The carotenoid retention of non-encapsulated extract, SD, SC, and SDC particles upon storage is shown in Figure 13 and was calculated by Equation 6. It was observed that carotenoids were degraded to a large extent during storage, despite reasonable storage conditions and the exclusion of light. Samples obtained by SD, SC, and SDC had retentions ranging from 97 to 68%, 99 to 84%, and 99 to 75%, respectively.

**Figure 13.** Carotenoid retention (%) in non-encapsulated extract and microparticles obtained by spray drying, chilling and their combination, during 90 days of storage at 25°C. SD: microparticles obtained by spray drying; SC: microparticles obtained by spray chilling; SDC: microparticles obtained by spray drying and chilling combination. Numerical suffix means the proportion of core:carrier material of each formulation




Reference: Own source

Losses increased continuously through the 90 days storage time, although the encapsulation processes provided protection of the total carotenoids when compared to the non-encapsulated extract. However, microparticles obtained by SC and SDC displayed higher effectiveness among the three encapsulating matrices to preserve the carotenoids, which indicates that the composition of carriers impacts on the stability of those active components. This result was in agreement with the color change observed in the different powders.

The entrapment of oxygen within the carrier matrix during storage might contribute to the oxidation of the carotenoids in the powders. Besides, isomerization may also affect the stability of these compounds. Khoo, Prasad, Kong, Jiang, & Ismail (2011) and Provesi, Dias, & Amante (2011) claimed that light and temperature are the major causes of carotenoids' isomerization. Rodriguez-Amaya (2002) reported that degradation of carotenoids during storage were mainly caused by enzymatic and non-enzymatic oxidation, which correlates with the oxygen level and the carotenoid molecular structure.

The kinetics of the carotenoid degradation was investigated and is presented in Table 10. Data were fitted to the zero- and first-order kinetics based on the carotenoid content during storage. Total carotenoid retention decreased remarkably, and the degradation rate can be seen from the correlation coefficient ($R^2$) and the corresponding k values. For the zero-order model, $R^2$ ranged from 0.610 to 0.957, and the $k_0$ value varied from 0.021 to 1.558 μg/ (g*s). Reasonable values of $R^2$ (0.755 - 0.940) combined to lower $k_1$ values (0.001 - 0.006 1/s) were associated with suitable fitting, which was better achieved following a first-order kinetics. The structural characteristics of the microparticles led to different results for the kinetic parameters, among formulations obtained by spray drying, chilling, and their combination. Therefore, according to these aspects, the microparticles produced by spray chilling showed superior protective capacity, ensuring greater stability of the carotenoids over 90 days.

Similarly, some studies have described carotenoids degradation as first-order reaction (Bechoff et al., 2010; Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010; Desobry, Netto, & Labuza, 1997; Zepka, Borsarelli, da Silva, & Mercadante 2009). Song, Wang, Li, & Liu (2017) reported that a first-order kinetics was used to describe the degradation of lutein and *β*-carotene during microwave-vacuum drying. They proposed that the decrease in carotenoids content was attributed to isomerization and oxidation reactions, yielding products as *cis* isomers and epoxides. Besides, Xiao et al (2018) compared $R^2$ values for three reactions

at 25, 35, and 45°C, and revealed that the degradation of all-*trans*-lutein, *β*-cryptoxanthin, and *β*-carotene was best described by a first-order kinetics.

Taken together, the total carotenoid concentration in the microparticles was considered as an indicator to assess the stability of powders during storage, and the feasibility of using these samples for food applications. These results were used before the next steps, in which further investigation unravels more details about the formulations from the different processing.

**Table 10.** Rate constants ($k_0$ and $k_1$values), coefficients of determination ($R^2$) and half-life periods ($t_{1/2}$) for degradation of non-encapsulated and encapsulated carotenoid-rich extract obtained by different process, during storage in dark at 25°C

| **Sample** | **Zero-order** | | **First-order** | | **$t_{1/2}$ (days)** |
|---|---|---|---|---|---|
| | **$k_0$ (µg/(g*s))** | **$R^2$** | **$k_1$ (1/s)** | **$R^2$** | |
| Non encapsulated extract | 1.558 | 0.957 | 0.006 | 0.857 | 108.08 |
| SD20 | 0.089 | 0.911 | 0.003 | 0.901 | 236.93 |
| SD25 | 0.176 | 0.844 | 0.003 | 0.847 | 212.20 |
| SD33 | 0.284 | 0.805 | 0.004 | 0.783 | 168.07 |
| SC20 | 0.025 | 0.925 | 0.001 | 0.919 | 606.36 |
| SC30 | 0.035 | 0.909 | 0.001 | 0.792 | 581.55 |
| SC40 | 0.075 | 0.949 | 0.001 | 0.859 | 546.95 |
| SDC10 | 0.021 | 0.610 | 0.003 | 0.755 | 316.59 |
| SDC20 | 0.058 | 0.942 | 0.003 | 0.940 | 223.45 |

SD: microparticles obtained by spray drying; SC: microparticles obtained by spray chilling; SDC: microparticles obtained by spray drying and chilling combination. Numerical suffix means the proportion of core:carrier material of each formulation.

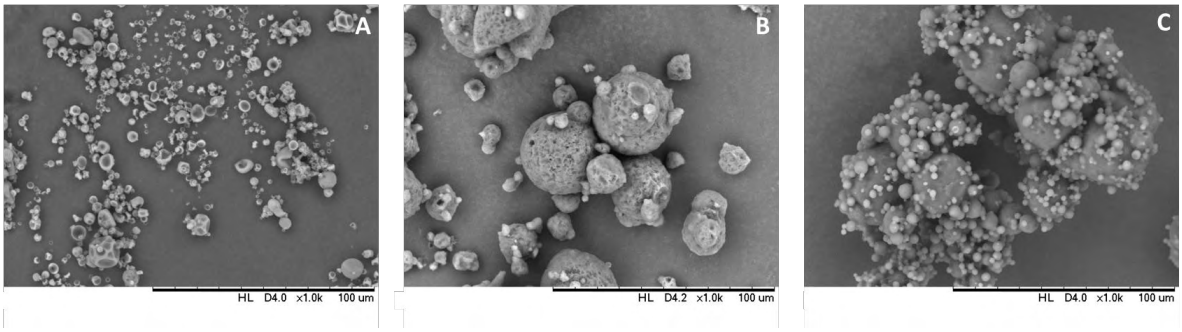
Reference: Own source.

### 4.3.3 Characterization of selected microparticles by morphology, thermal properties, and moisture sorption

#### 4.3.3.1 Scanning electron microscopy (SEM)

Scanning electron micrographs of SD33, SC40, and SDC20 microparticles loaded with carotenoids are illustrated in Figure 14. The SD33 microparticles had different sizes with irregular spherical shapes, and no cracks were found at the outer surfaces, which is important to prevent gas permeability that may affect carotenoids protection. The concavities on the surface are associated with the rapid solvent evaporation and higher pressure inside the microparticles at high temperatures during the atomization (Medina-Torres et al., 2016). According Elik, Yanık, & Göğüş (2021), temperatures range between drying inlet air and powders lead to wrinkle surface on the particle. This could be attributed to the initial expansion by the incorporation of air in the particle, followed by the contraction in the colder chamber of the equipment. Bhusari, Muzaffar, & Kumar (2014) proposed that the concavities in the microparticles containing gum arabic may be linked to the protein fraction in the molecule.

**Figure 14.** Scanning electron micrographs (1.0k × magnification) of microparticles obtained by A: spray drying (SD33), B: spray chilling (SC40) and C: Spray drying and chilling combination (SDC20). Numerical suffix means the core:carrier material ratio of selected formulation




Reference: Own source

SC40 and SDC20 microparticles showed spherical shapes with different sizes. The differences demonstrated that the addition of SD powders into the hydrogenated vegetable fat carrier affected their morphologies, mainly on the surface of the microparticles. The SC40 powders showed some agglomeration and an irregular surface with some pores. From the technological standpoint, these physical properties could cause a reduction the powders flow ability. Besides, holes on the microparticles surface can increase the exposition of carotenoids to the oxygen, reducing the powder functionality. Regarding the SDC powders, the morphological analysis showed a smooth surface with a high level of agglomeration. This may

be related to the complex carrier matrix formed by the combination of gum arabic and hydrogenated vegetable fat and its water holding capacity. The collision of microparticles might induce the agglomeration; besides, incomplete solidification of carrier material could also happen and led to the formation of partially melted microparticles favoring the agglomeration.

#### 4.3.3.2 Oxidative stability

Rancimat method is widely used to investigate the oxidative stability of oil/lipid-containing samples (Choe & Min, 2006). The technique is based on the measurement of the induction period (IP) linked to the formation of the certain components when the lipid samples are oxidized under heating (with a temperature higher than 100 °C) and under a constant aeration flow (20 L/h). In the present research, the higher the IP of the encapsulated extract compared to the non-encapsulated, the better is the stability of the carotenoids within the microparticles. The data of the non-encapsulated extracts, which contain oil in its formulation and for the selected microparticles are presented in Table 11.

The findings indicated that under the conditions applied in the test the carotenoid-rich extract is highly sensitive to oxidation. The oxidative stability improved significantly ($P < 0.05$), when microparticles prepared using different carriers were assessed in a comparative approach. It was observed that spray drying encapsulation increased the thermal stability of the extract by at least 52-fold, spray chilling by 3-fold, and their combination by 545-fold, confirming the ability of the encapsulated microparticles to protect sensitive compounds against degradation. From SD encapsulation, the microparticles obtained are composed of a resistant shell, which has limited gas transfer. Regarding SC40 microparticles, although they had a lower IP compared to the other microparticles, they showed a high level of carotenoid retention after 90 days (Figure 13). The storage conditions (25 °C and ~35% HR), did not affect their stability. However, when the Rancimat test was used on the SC40 microparticles, the drastic treatment at high temperatures (120 °C) and high flow oxygen exposure caused morphological changes, increased surface area, and exposition of the lipid matrix and carotenoids, which led to a rapid carotenoid degradation. When evaluating the effect of the microencapsulation combined techniques on oxidative stability, it was observed the lack of porosity of the wall on the surfaces of SDC20 microparticles indicating a complete coverage. This structure could have created a sturdier and dense shell matrix that reduced oxygen permeability to the microparticle core.

**Table 11.** Oxidation induction period of non-encapsulated and encapsulated carotenoid-rich extract, evaluated by Rancimat, at 120 °C

| Sample | Induction time (h) |
|---|---|
| Non encapsulated extract | $0.04 \pm 0.00^{d}$ |
| SD33 | $2.10 \pm 0.01^{b}$ |
| SC40 | $0.11 \pm 0.02^{c}$ |
| SDC20 | $21.8 \pm 0.3^{a}$ |

SD33: microparticles obtained by spray drying; SC40: microparticles obtained by spray chilling; SDC20: microparticles obtained by spray drying and chilling combination. Numerical suffix means the core/wall material ratio of the selected formulation. Values with the same lower case letter are not statistically different ($P > 0.05$). Reference: Own source.

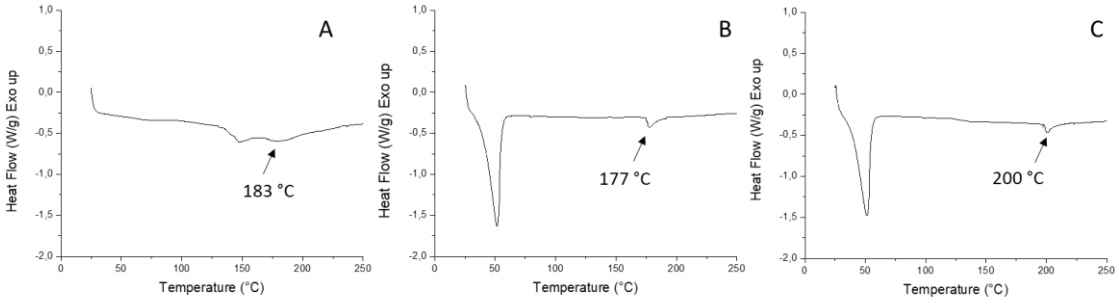
#### 4.3.3.3 Differential scanning calorimetry (DSC)

DSC is a technique used to evaluate the thermal properties of bioactive compounds in matrices, detect melting points, and record variations in the crystal structure via the displacement or disappearance of the endothermic peaks in the material analyzed (Rutz, Borges, Zambiazi, da Rosa, & da Silva, 2016). DSC curves shown in Figure 15 represent the thermal profile of the different microparticles obtained by the different used encapsulation processes. Regarding the first endothermic peak for all formulations, in the SD microparticles the transition began at 141 °C and ended at 160 °C, with a melting temperature ($T_m$) of 147°C and an enthalpy of 8.3 ± 0.6 J/g powder. The broad curve is assumed to be related to the crystallite melting of gum arabic, which agrees with the results of Mothé & Rao (2000). For the SC40 microparticles, the melting peak started at 39 °C and ended at 61 °C, having a $T_m$ of 52 °C and an enthalpy of 86 ± 2 J/g powder. For the SDC20 microparticles, the melting began at 38 °C and ended at 59 °C, with a $T_m$ of 51 °C and an enthalpy of 80 ± 2 J/g of. For both samples, SC40 and SDC20, those peaks refer to the melting range of hydrogenated vegetable fat that was used as a carrier agent.

Furthermore, among the three encapsulating processes used in this work, SD33, SC40, and SDC20 microparticles presented a second peak (highlighted in Figure 15), ranging from 177 to 200 °C. These peaks are probably associated with the carotenoid melting at high temperatures. Similarly, in DSC curve of β-carotene in a powder form, a large endothermic

process was reported at about 180 °C (Peinado, Mason, Romano, Biasioli, & Scampicchio, 2016). Besides, Sy, Gleize, Dangles, Landrier, Veyrat, & Borel (2012) described the melting point of carotenoids ranging from 175 to 195°C. Based on the thermograms, the occurrence of two peaks suggested the formation of a heterogeneous system, and the changes in thermal profile indicate interactions between carrier and core materials. The crystalline structure of microparticles suggests higher stability at room temperature and contributes to the protection of components against environmental factors (Yan & Zhang, 2014). In this system, the fusion enthalpy is related to the energy absorbed to disrupt and breakdown the crystalline lipid structure of carrier agent. Therefore, the more homogeneous of the crystalline structure, the higher is the energy absorbed to carry out the melting transition. The melting points of each microparticles presented particular values, as different process and carriers were assessed to the obtainment of powders.

**Figure 15.** Differential scanning calorimetry (DSC) thermograms of microparticles obtained by A: spray drying (SD33), B: spray chilling (SC40) and C: Spray drying and chilling combination (SDC20). Numerical suffix means the core:carrier material ratio of the selected formulation




Reference: Own source.

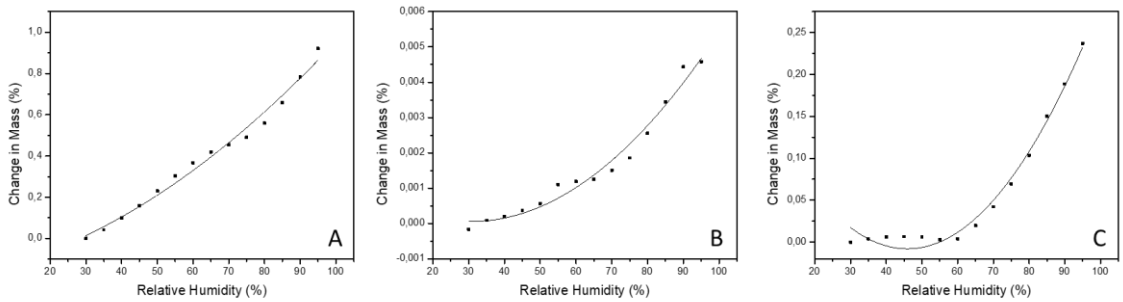
#### 4.3.3.4 Sorption Isotherms

Sorption isotherms usually express the relationship between RH and the equilibrium moisture content of the sample, at a certain temperature. This data is important in food processing activities such as packaging and storage of materials to preserve the quality of products. Figure 16 illustrates that SD33 samples showed significant changes throughout the whole range of RH used when compared to the other samples. These microparticles were

formulated with gum arabic as the carrier material, which has a composition consisting of ~98 wt% of polysaccharides and a ~2 wt % of proteinaceous material (Isobe et al., 2020). When exposed to atmospheric conditions of increasing RH the hydrophilic polysaccharide domain tend to reach a fast equilibrium moisture with the surrounding environment. The SC40 sample showed a lower increase in moisture content, even at relative 95% RH. As expected, this sample presented minimum fluctuations in moisture, as the hydrogenated vegetable fat used for microencapsulation creates a hydrophobic coating. SDC20, on the other hand, showed a slight increase in the moisture content up to 60% RH, and a large increase thereafter. The presence of gum arabic and hydrogenated vegetable fat in the microparticles possibly altered the balance of hydrophilic/hydrophobic interaction, favoring the two stages of water sorption at 25°C.

Generally, it can be seen that the behaviors of DVS curves were of type III (J shape). It is worth noting that in the SD33 microparticles, this behavior was not that obvious. The isotherm profile by DVS analysis was a consequence of the physical and/or chemical transformations that occurred during the atomization, in addition to the carrier material in the microparticles composition.

**Figure 16.** Sorption isotherms determined by using the dynamic water sorption technique of microparticles obtained by A: spray drying (SD33), B: spray chilling (SC40), and C: Spray drying and chilling combination (SDC20). Numerical suffix means the core:carrier material ratio of the selected formulation




Reference: Own source.

## 4.4 Conclusions

The production of microparticles loaded with carotenoids by spray drying, chilling, and the combination of both techniques is an effective approach to maintain the pigment stability with a high encapsulation efficiency of 82 to 100%. The mean diameter and size distribution of microparticles presented an increase after 90 days; however, even after storage, they were within the suitable scale for industrial applications.

In addition, all formulations exhibited variation in color over storage. The degradation kinetics of the carotenoids followed a first-order kinetics for all samples, presumably driven by permeation of oxygen into the core of the microparticles. In the study using selected formulations from each process, micrographs demonstrated details of the microparticles, which included their shape, e.g, with an irregular surface for the SC microparticles and spherical for the microparticles prepared with the other treatments. Micrographs also showed the SD and SDC samples with a smooth surface, and heterogeneous sizes.

Although losses in the carotenoid content were noted in the SDC particles, they displayed ideal performance regarding thermal properties, oxidative stability, and water sorption. Overall, the microencapsulation processes used in the present study not only provides practical options to improve carotenoid stability, but also explain the phenomenon behind the pigment protection. Further investigations should be conducted to evaluate the carotenoid bioavailability within the different matrices obtained from the encapsulation techniques developed in this research.

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## References


Achir, N., Randrianatoandro, V. A., Bohuon, P., Laffargue, A., & Avallone, S. (2010). Kinetic study of β-carotene and lutein degradation in oils during heat treatment. *European Journal of Lipid Science and Technology*, *112*(3), 349-361. https://doi.org/10.1002/ejlt.200900165

Alvim, I. D., Stein, M. A., Koury, I. P., Dantas, F. B. H., & Cruz, C. L. D. C. V. (2016). Comparison between the spray drying and spray chilling microparticles contain ascorbic acid in a baked product application. *LWT-Food Science and Technology*, *65*, 689-694. https://doi.org/10.1016/j.lwt.2015.08.049

Anthero, A. G. D. S., Bezerra, E. O., Comunian, T. A., Procópio, F. R., & Hubinger, M. D. (2020). Effect of modified starches and gum arabic on the stability of carotenoids in paprika oleoresin microparticles. *Drying Technology*, 1-14. https://doi.org/10.1080/07373937.2020.1844227

Arslan-Tontul, S., & Erbas, M. (2017). Single and double layered microencapsulation of probiotics by spray drying and spray chilling. *LWT*, *81*, 160-169. https://doi.org/10.1016/j.lwt.2017.03.060

Bechoff, A., Dhuique-Mayer, C., Dornier, M., Tomlins, K. I., Boulanger, R., Dufour, D., & Westby, A. (2010). Relationship between the kinetics of β-carotene degradation and formation of norisoprenoids in the storage of dried sweet potato chips. *Food Chemistry*, *121*(2), 348-357. https://doi.org/10.1016/j.foodchem.2009.12.035

Bhusari, S. N., Muzaffar, K., & Kumar, P. (2014). Effect of carrier agents on physical and microstructural properties of spray dried tamarind pulp powder. *Powder technology*, *266*, 354-364. https://doi.org/10.1016/j.powtec.2014.06.038

Cano, M. P., Gómez-Maqueo, A., García-Cayuela, T., & Welti-Chanes, J. (2017). Characterization of carotenoid profile of Spanish Sanguinos and Verdal prickly pear (Opuntia ficus-indica, spp.) tissues. *Food Chemistry*, 237, 612–622. https://doi.org/10.1016/j.foodchem.2017.05.135

Carneiro, H. C., Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering*, *115*(4), 443-451. https://doi.org/10.1016/j.jfoodeng.2012.03.033

Chambi, H. N. M., Alvim, I. D., Barrera-Arellano, D., & Grosso, C. R. F. (2008). Solid lipid microparticles containing water-soluble compounds of different molecular mass: Production, characterisation and release profiles. *Food Research International*, *41*(3), 229-236. https://doi.org/10.1016/j.foodres.2007.11.012

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, *5*(4), 169-186. https://doi.org/10.1111/j.1541-4337.2006.00009.x

Chuyen, H. Van, & Eun, J. B. (2017). Marine carotenoids: Bioactivities and potential benefits to human health. *Critical Reviews in Food Science and Nutrition*, *57(12)*, 2600–2610. https://doi.org/10.1080/10408398.2015.1063477

Consoli, L., Grimaldi, R., Sartori, T., Menegalli, F. C., & Hubinger, M. D. (2016). Gallic acid microparticles produced by spray chilling technique: Production and characterization. *LWT-Food Science and Technology*, *65*, 79-87. https://doi.org/10.1016/j.lwt.2015.07.052

Cuevas, M. S., Rodrigues, C. E., Gomes, G. B., & Meirelles, A. J. (2010). Vegetable Oils Deacidification by Solvent Extraction: Liquid− Liquid Equilibrium Data for Systems Containing Sunflower Seed Oil at 298.2 K. *Journal of Chemical & Engineering Data*, *55*(9), 3859-3862. https://doi.org/10.1021/je900791w

Da Silva, L. M. R., de Sousa, P. H. M., de Sousa Sabino, L. B., do Prado, G. M., Torres, L. B. V., Maia, G. A., ... & Ricardo, N. M. P. S. (2020). Brazilian (North and Northeast) Fruit By-Products. *Food Wastes and By-products: Nutraceutical and Health Potential*, 127-158. https://doi.org/10.1002/9781119534167.ch5

De Lara Pedroso, D., Thomazini, M., Heinemann, R. J. B., & Favaro-Trindade, C. S. (2012). Protection of Bifidobacterium lactis and Lactobacillus acidophilus by microencapsulation using spray-chilling. *International Dairy Journal*, *26*(2), 127-132. https://doi.org/10.1016/j.idairyj.2012.04.008

De Leonardis, A., & Macciola, V. (2012). Heat-oxidation stability of palm oil blended with extra virgin olive oil. *Food Chemistry*, *135*(3), 1769-1776. https://doi.org/10.1016/j.foodchem.2012.06.046

Desobry, S. A., Netto, F. M., & Labuza, T. P. (1997). Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. *Journal of Food Science*, *62*(6), 1158-1162. https://doi.org/10.1111/j.1365-2621.1997.tb12235.x

During, A., & Harrison, E. H. (2007). Mechanisms of provitamin A (carotenoid) and vitamin A (retinol) transport into and out of intestinal Caco-2 cells. *Journal of lipid research*, *48*(10), 2283-2294. https://doi.org/10.1194/jlr.M700263-JLR200

Elik, A., Yanık, D. K., & Göğüş, F. (2021). A comparative study of encapsulation of carotenoid enriched-flaxseed oil and flaxseed oil by spray freeze-drying and spray drying techniques. *LWT, 143*, 111153. https://doi.org/10.1016/j.lwt.2021.111153

Fadini, A. L., Alvim, I. D., Ribeiro, I. P., Ruzene, L. G., da Silva, L. B., Queiroz, M. B., ... & Rodrigues, R. A. F. (2018). Innovative strategy based on combined microencapsulation technologies for food application and the influence of wall material composition. *LWT*, *91*, 345-352. https://doi.org/10.1016/j.lwt.2018.01.071

Favaro-Trindade, C. S., Okuro, P. K., & de Matos Jr, F. E. (2015). 5 Encapsulation via Spray. *Handbook of encapsulation and controlled release*, 71.

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research*, *43*(3), 228-265. https://doi.org/10.1016/j.plipres.2003.10.002.

Ghotra, B. S., Dyal, S. D., & Narine, S. S. (2002). Lipid shortenings: a review. *Food Research International*, *35*(10), 1015-1048. https://doi.org/10.1016/S0963-9969(02)00163-1

Hansen, L. T., Allan-Wojtas, P. M., Jin, Y. L., & Paulson, A. T. (2002). Survival of Ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions. *Food microbiology*, *19*(1), 35-45. https://doi.org/10.1006/fmic.2001.0452

Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry*, *54*(1), 101-111. https://doi.org/10.1016/0308-8146(95)92669-B

Islam, M. Z., Kitamura, Y., Yamano, Y., & Kitamura, M. (2016). Effect of vacuum spray drying on the physicochemical properties, water sorption and glass transition phenomenon of orange juice powder. *Journal of Food Engineering*, *169*, 131-140. https://doi.org/10.1016/j.jfoodeng.2015.08.024

Isobe, N., Sagawa, N., Ono, Y., Fujisawa, S., Kimura, S., Kinoshita, K., ... & Deguchi, S. (2020). Primary structure of gum arabic and its dynamics at oil/water interface. *Carbohydrate Polymers*, *249*, 116843. https://doi.org/10.1016/j.carbpol.2020.116843

Khoo, H. E., Prasad, K. N., Kong, K. W., Jiang, Y., & Ismail, A. (2011). Carotenoids and their isomers: color pigments in fruits and vegetables. *Molecules*, *16*(2), 1710-1738. https://doi.org/10.3390/molecules16021710

Labuza, T. P. (1971). Properties of water as related to the keeping quality of foods. In *International Congress of Food Science and Technology. Proceedings*.

Liu, X. H., Yu, R. B., Liu, R., Hao, Z. X., Han, C. C., Zhu, Z. H., & Ma, L. (2014). Association between lutein and zeaxanthin status and the risk of cataract: a meta-analysis. *Nutrients*, *6*(1), 452-465. https://doi.org/10.3390/nu6010452

McClements, D. J. (2002). Theoretical prediction of emulsion color. *Advances in colloid and interface science*, *97*(1-3), 63-89. https://doi.org/10.1016/S0001-8686(01)00047-1

Medeiros, A. K. de O. C., Gomes, C. de C., Amaral, M. L. Q. de A., Medeiros, L. D. G. de, Medeiros, I., Porto, D. L., Aragão, C. F. S., Maciel, B. L. L., Morais, A. H. de A., & Passos, T. S. (2019). Nanoencapsulation improved water solubility and color stability of carotenoids extracted from Cantaloupe melon (Cucumis melo L.). *Food Chemistry*, 270(April 2018), 562–572. https://doi.org/10.1016/j.foodchem.2018.07.099

Medina-Torres, L., Santiago-Adame, R., Calderas, F., Gallegos-Infante, J. A., González-Laredo, R. F., Rocha-Guzmán, N. E., ... & Manero, O. (2016). Microencapsulation by spray drying of laurel infusions (Litsea glaucescens) with maltodextrin. *Industrial Crops and Products*, *90*, 1-8. https://doi.org/10.1016/j.indcrop.2016.06.009

Milani, A., Basirnejad, M., Shahbazi, S., & Bolhassani, A. (2017). Carotenoids: biochemistry, pharmacology and treatment. *British Journal of Pharmacology*, 174(11), 1290–1324. https://doi.org/10.1111/bph.13625

Mothé, C. G., & Rao, M. A. (2000). Thermal behavior of gum arabic in comparison with cashew gum. *Thermochimica acta*, *357*, 9-13. https://doi.org/10.1016/S0040-6031(00)00358-0

Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Nanostructured lipid matrices for improved microencapsulation of drugs. *International journal of pharmaceutics*, *242*(1-2), 121-128. https://doi.org/10.1016/S0378-5173(02)00180-1

Navarro-Guajardo, N., García-Carrillo, E. M., Espinoza-González, C., Téllez-Zablah, R., Dávila-Hernández, F., Romero-García, J., ... & Pariona, N. (2018). Candelilla wax as natural slow-release matrix for fertilizers encapsulated by spray chilling. *Journal of Renewable Materials*, 6(3), 226-236. https://doi:10.7569/JRM.2017.634164

Okuro, P. K., de Matos Junior, F. E., & Favaro-Trindade, C. S. (2013). Technological challenges for spray chilling encapsulation of functional food ingredients. *Food Technology and Biotechnology*, 51(2), 171.

Peinado, I., Mason, M., Romano, A., Biasioli, F., & Scampicchio, M. (2016). Stability of β-carotene in polyethylene oxide electrospun nanofibers. *Applied Surface Science*, *370*, 111-116. https://doi.org/10.1016/j.apsusc.2016.02.150

Pelissari, J. R., Souza, V. B., Pigoso, A. A., Tulini, F. L., Thomazini, M., Rodrigues, C. E., ... & Favaro-Trindade, C. S. (2016). Production of solid lipid microparticles loaded with lycopene by spray chilling: Structural characteristics of particles and lycopene stability. *Food and Bioproducts Processing*, *98*, 86-94. https://doi.org/10.1016/j.fbp.2015.12.006

Pinho, L. S., da Silva, M. P., Thomazini, M., Cooperstone, J. L., Campanella, O. H., da Costa Rodrigues, C. E., & Favaro-Trindade, C. S. (2021). Guaraná (Paullinia cupana) by-product as a source of bioactive compounds and as a natural antioxidant for food applications. *Journal of Food Processing and Preservation*, e15854. https://doi.org/10.1111/jfpp.15854

Provesi, J. G., Dias, C. O., & Amante, E. R. (2011). Changes in carotenoids during processing and storage of pumpkin puree. *Food Chemistry*, *128*(1), 195-202.

Rocha, G. A., Fávaro-Trindade, C. S., & Grosso, C. R. F. (2012). Microencapsulation of lycopene by spray drying: characterization, stability and application of microcapsules. *Food and Bioproducts Processing*, *90*(1), 37-42. https://doi.org/10.1016/j.fbp.2011.01.001

Rodriguez-Amaya, D. B. (2001). A guide to carotenoid analysis in foods.

Rodriguez-Amaya, D. B. (2002). Effects of processing and storage on food carotenoids. *Sight and Life Newsletter*, *3*(Special Issue), 25-35.

Rutz, J. K., Borges, C. D., Zambiazi, R. C., da Rosa, C. G., & da Silva, M. M. (2016). Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. *Food Chemistry*, *202*, 324-333. https://doi.org/10.1016/j.foodchem.2016.01.140

Santana, Á. L., Zanini, J. A., & Macedo, G. A. (2020). Dispersion-assisted extraction of guarana processing wastes on the obtaining of polyphenols and alkaloids. *Journal of Food Process Engineering*, *43*(4), e13381. https://doi.org/10.1111/jfpe.13381

Santos, P. D. F., Rubio, F. T. V., de Carvalho Balieiro, J. C., Thomazini, M., & Favaro-Trindade, C. S. (2021). Application of spray drying for production of microparticles containing the carotenoid-rich tucumã oil (*Astrocaryum vulgare Mart.). LWT*, *143*, 111106. https://doi.org/10.1016/j.lwt.2021.111106

Sartori, T., Consoli, L., Hubinger, M. D., & Menegalli, F. C. (2015). Ascorbic acid microencapsulation by spray chilling: Production and characterization. *LWT-Food Science and Technology*, *63*(1), 353-360. https://doi.org/10.1016/j.lwt.2015.03.112

Selim, K., Tsimidou, M., & Biliaderis, C. G. (2000). Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. *Food Chemistry*, *71*(2), 199-206.

Sillick, M., & Gregson, C. M. (2012). Spray chill encapsulation of flavors within anhydrous erythritol crystals. *LWT-Food Science and Technology*, *48*(1), 107-113. https://doi.org/10.1016/j.lwt.2012.02.022

Silva, M. P., Tulini, F. L., Matos-Jr, F. E., Oliveira, M. G., Thomazini, M., & Fávaro-Trindade, C. S. (2018). Application of spray chilling and electrostatic interaction to produce lipid microparticles loaded with probiotics as an alternative to improve resistance under stress conditions. *Food Hydrocolloids*, *83*, 109-117. https://doi.org/10.1016/j.foodhyd.2018.05.001

Song, J., Wang, X., Li, D., & Liu, C. (2017). Degradation kinetics of carotenoids and visual colour in pumpkin (Cucurbita maxima L.) slices during microwave-vacuum drying. *International Journal of Food Properties*, *20*(sup1), S632-S643. https://doi.org/10.1080/10942912.2017.1306553

Souza, V. B., Thomazini, M., Barrientos, M. A. E., Nalin, C. M., Ferro-Furtado, R., Genovese, M. I., & Favaro-Trindade, C. S. (2018). Functional properties and encapsulation of a proanthocyanidin-rich cinnamon extract (Cinnamomum zeylanicum) by complex coacervation using gelatin and different polysaccharides. *Food Hydrocolloids*, *77*, 297-306. https://doi.org/10.1016/j.foodhyd.2017.09.040

Sy, C., Gleize, B., Dangles, O., Landrier, J. F., Veyrat, C. C., & Borel, P. (2012). Effects of physicochemical properties of carotenoids on their bioaccessibility, intestinal cell uptake, and blood and tissue concentrations. *Molecular Nutrition & Food Research*, *56*(9), 1385-1397. https://doi.org/10.1002/mnfr.201200041

Tonon, R. V., Brabet, C., & Hubinger, M. D. (2010). Anthocyanin stability and antioxidant activity of spray-dried açai (Euterpe oleracea Mart.) juice produced with different carrier agents. *Food Research International*, *43*(3), 907-914. https://doi.org/10.1016/j.foodres.2009.12.013

Troya, D., Tupuna-Yerovi, D. S., & Ruales, J. (2018). Effects of wall materials and operating parameters on physicochemical properties, process efficiency, and total carotenoid content of microencapsulated banana passionfruit pulp (Passiflora tripartita var. mollissima) by spray-drying. *Food and Bioprocess Technology*, *11*(10), 1828-1839. https://doi.org/10.1007/s11947-018-2143-0

Xiao, Y. D., Huang, W. Y., Li, D. J., Song, J. F., Liu, C. Q., Wei, Q. Y., ... & Yang, Q. M. (2018). Thermal degradation kinetics of all-trans and cis-carotenoids in a light-induced model system. *Food Chemistry*, *239*, 360-368. https://doi.org/10.1016/j.foodchem.2017.06.107

Xu, P. X., Dai, Z. Q., Li, D. J., Liu, C. Q., Wu, C. E., & Song, J. F. (2021). Preparation, optimization, characterization, and in vitro bioaccessibility of a lutein microparticle using spray drying with β-cyclodextrin and stevioside. *Journal of Food Processing and Preservation*, *45*(1), e15032. https://doi.org/10.1111/jfpp.15032

Yan, C., & Zhang, W. (2014). Coacervation processes. In *Microencapsulation in the food industry* (pp. 125-137). Academic Press. https://doi.org/10.1016/B978-0-12-404568-2.00012-1

Zepka, L. Q., Borsarelli, C. D., da Silva, M. A. A. P., & Mercadante, A. Z. (2009). Thermal degradation kinetics of carotenoids in a cashew apple juice model and its impact on the system color. *Journal of Agricultural and Food Chemistry*, *57*(17), 7841-7845. https://doi.org/10.1021/jf900558a

Zuidam, N. J., & Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them. In *Encapsulation technologies for active food ingredients and food processing* (pp. 3-29). Springer, New York, NY.

# 5 CHAPTER 5

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## MICROENCAPSULATION OF CAROTENOID-RICH EXTRACT FROM GUARANÁ (*PAULLINIA CUPANA*) PEELS AND STUDY OF MICROPARTICLES FUNCTIONALITY THROUGH THEIR INCORPORATION INTO AN OATMEAL PASTE

**ABSTRACT**

The peels of guaraná fruit contain abundant carotenoid content, which has demonstrated health benefits. However, these compounds are unstable to certain conditions, and their application into food products can be restricted considering the processing parameters. This study aimed to encapsulate the carotenoid-rich extract from guaraná peels by spray drying (SD), characterize the microparticles, investigate their influence on the pasting properties of oatmeal paste, and evaluate the effects of temperature and shear on carotenoids stability during the preparation of this product. A rheometer with a pasting cell was used to simulate the extrusion conditions. Temperatures of 70, 80, and 90 °C and shear rates of 50 and 100 1/ s were the parameters evaluated. Microparticles with a total carotenoid content between 40 and 96 µg/g were obtained. Over the storage period, the carotenoid stability, particle size, color, moisture, and water activity varied according to the core:carrier material proportion used. Afterward, the formulation SD1:2 was selected to be incorporated in oatmeal, and the paste viscosity was influenced by the addition of this powder. β-carotene retention was higher than those of lutein subsequently the treatment. The less severe treatment involving a temperature of 70 °C and 50 1/ s shear rate exhibited better retention of total carotenoids, regardless the carotenoid-rich extract was encapsulated or non-encapsulated. In the other treatments, the thermo-mechanical stress significantly influenced the stability of the total carotenoid. These results suggest that the addition of encapsulated carotenoids to foods increase their resistance towards processing conditions, thus leading to the higher maintaince of bioactivity to develop functional foods.

**Keywords:** Mechanical stress; thermal stress; β-carotene; lutein; stability.

## 5.1 Introduction

Carotenoids are one of the major classes of pigments found in tree leaves, fruits, and vegetables. In addition, consumption of these compounds has been considered to bring health benefits. According to literature, carotenoids may act as reducing agents for cancer (Micozzi, Beecher, Taylor, & Khachik, 1990; Toma, Losardo, Vincent, & Palumbo, 1995; Kumar, Hosokawa, & Miyashita., 2013), cardiovascular diseases (Riccioni, 2009; Monroy-Ruiz, Sevilla, Carrón, Montero, 2011), and macular degeneration (Gale, Hall, Phillips, & Martyn,

2003), as well as antioxidants (Stahl & Sies, 2003) and provitamin A (Olson, 1989). Carotenoids' nutritive and coloring properties make them an ideal food additive to develop functional products with a desirable appearance.

The interest in recovering carotenoids from agro-industrial waste has increased, considering the high potential of this material to enhance the valorization of by-products. Several researchers (Chen & Tang, 1998; Ajila, Bhat, & Rao 2007; de Andrade Lima, Kestekoglou, Charalampopoulos, & Chatzifragkou 2019; De Souza Mesquita et al. 2020) have reported the extraction of carotenoids from different vegetable wastes. However, guaraná residues, generated in the production of soft drinks and food ingredient industries, are rarely explored as a possible source of carotenoids.

Carotenoids are highly susceptible to harsh external conditions, such as high temperature and shear, presence of oxygen, light, acidity, and prooxidant agents (Caris-Veyrat, Schmid, Carail, & Böhm, 2003; Boon, McClements, Weiss, & Decker 2010; Xiao et al., 2018). Encapsulation has emerged as a key step in making the incorporation of these compounds in processed foods feasible. In the food industry, spray drying is the most widely used technology for the entrapment of bioactive molecules in the form of microparticles. The technique consists of atomizing a dispersion or emulsion with the component of interest and drying adjuvants, followed by dehydrating the microparticles. This encapsulation facilitates transport, prolongs shelf-life, as well as reduces the risk of carotenoid degradation during food processing (Sagar & Kumar, 2010; Subtil et al., 2014).

The development of starchy products includes the investigation of new formulations and new processing techniques. The typical technologies used to induce gelatinization and texturization of starchy products are those that apply heat and shear simultaneously. During gelatinization, starch swells, destabilizes its crystalline structure and loses the birefringence. The starch main fractions, amylose and amylopectin, disperse and lead to the production of a paste (Ai & Jane, 2015; Wang, Xia, Wang, Ali, & Li, 2019). Once gelatinization, starch is susceptible to mechanical stress, which is applied by shear.

To simulate a pasting environment where temperature and shear are applied, a rheometer equipped with a pasting cell was used to produce oatmeal paste enriched with encapsulated carotenoids. Thus, this study aimed to prepare a carotenoid-rich extract from guaraná peels, encapsulate this extract, investigate the influence of microparticles on the pasting properties of

oatmeal, and evaluate the effect of thermo- and mechanical-stress on carotenoids stability under different conditions.

## 5.2 Material and Methods

### 5.2.1 Materials

Guaraná fruits were provided by The Executive Commission for Cocoa Cultivation Planning - CEPLAC (Taperoá, Bahia, Brazil). The fruit peels were collected, washed with water, dried in a convection oven (Marconi, MA035 / 1152) at 50 °C for 18 h (Silva et al., 2019), milled and stored under dark at -20 °C until further use.

β-carotene (CAS 7235-40-7) and lutein (CAS 127-40-2) standards were purchased from Sigma-Aldrich (Saint Louis, USA). Analytical grade ethanol (CAS 64-17-5), hexane (CAS 110-54-3), and acetone (CAS 67-64-1) were obtained from Fisher Scientific.

The microparticles rich in carotenoids produced by spray drying (SD) were prepared using gum arabic as carrier material, obtained from Nexira, Brazil. The oat flakes were purchased from the Oat Quaker Company, Columbus, USA.

### 5.2.2 Carotenoid-rich extract preparation

Carotenoid-rich extract preparation from guaraná peels was performed following Pinho et al. (2021), using ethanol as solvent, ratio 1:10 (peel: solvent, w/v), during 4 hours at 50°C. The concentration of 3% of sunflower oil was added to the extract, to reduce the carotenoid degradation detected during preliminary experiments. The final concentration was determinate considering the liquid-liquid equilibrium for the mixture composed of oil and ethanol (Cuevas, Rodrigues, Gomes, & Meirelles, 2010). As follow, the material was concentrated using a rotary evaporator (TE-211 Tecnal, Piracicaba, Brazil) at 48 ± 2°C to 20 % of the initial volume. The concentrated extract was named guaraná peel extract (GPE).

### 5.2.3 Microencapsulation by spray-drying

The formulations were prepared from the ratio 1:2, 1:3, and 1:4 of guaraná peel extract: gum arabic in aquos solution (20% w/v), v/v. An Ultra-Turrax ® IKA T25 (Labotechnic, Staufen, Germany) was used to mix the formulation at 11,200×$g$ for 3 minutes. The mixture was atomized according to Rocha et al. (2012), with modifications. The spray dryer (Model MSD 1.0, Labmaq do Brasil, Ribeirão Preto, Brazil) was used coupled with a spray nozzle of 1.2 mm, with an inlet air temperature of 100 °C, the air drying speed of 2.5 m/s, feed flow of 10 mL/min, and air pressure of 8.4 kgf/cm$^2$.

### 5.2.4 Total carotenoid content

The microparticles were blended with hexane for 1 min and ultrasonicated for 20 min using an ultrasound bath Branson 1800 (Branson Ultrasonics Corporation, Danbury, CT, USA) to extract carotenoids. Absorbances of extracts were measured at 450 nm wavelength and recorded using a UV-visible spectrophotometer (Thermo Scientific, Genesys 10S). The β-carotene standard was used for quantification, and the results were expressed as μg β-carotene/g sample (Rodriguez-Amaya, 2001).

The retention after encapsulation was calculated as a ratio of the total carotenoid amount in the microparticles to that in the feed materials before atomization, according to Equation (12)

$$Carotenoid\ rentention\ (\%) = \frac{Carotenoid\ content\ (after\ atomization)}{Carotenoid\ content\ (before\ atomization)} \times 100 \qquad (12)$$

### 5.2.5 Stability study of microparticles

The samples were placed in glass vials covered with aluminum and stored in desiccators containing saturated solutions of magnesium chloride $MgCl_2$ (relative humidity, RH of 32.8%). The desiccators were kept at a temperature of 25 °C, and the storage period lasted for 90 days under the specified conditions (Tonon, Brabet, & Hubinger, 2010). The samples were evaluated every 15 days in terms of carotenoid content and color. Particle size distribution, mean diameter, moisture content, and water activity were analyzed at the initial time and after 90 days of storage.

#### 5.2.5.1 Carotenoid degradation kinetics

The carotenoid stability was determined by comparing the total carotenoid concentration at the initial time and over the storage of encapsulated and non-encapsulated carotenoid-rich GPE. Previous studies (Hidalgo & Brandolini, 2008; Song et al., 2017) have hypothesized the first-order kinetics model to describe the reaction order of carotenoids degradation. Thereby, to investigate the stability of our samples, the degradation constant (k) and the half-life ($t_{1/2}$) were determined following the first-order kinetic, according to Equations (13) e (14).

$$\ln(Ct) / (C_0) = - kt \quad (13)$$

$$t_{1/2} = \ln(2)/k \quad (14)$$

Where C = carotenoid concentration at time t (μg/g); $C_0$ = initial carotenoid concentration (μg/g); t = time (days).

#### 5.2.5.2 Color

The color of microparticles in terms of L*-values (Brightness), a*-values, and b*-values were determined using a Hunter Lab Mini Scan XE colorimeter (Reston, Virginia, USA). Chroma (color saturation) was calculated (Equation 15), according to Minolta (1994):

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2} \quad (15)$$

The total color difference (ΔE) was calculated according to Equation 16.

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (16)$$

#### 5.2.5.3 Mean particle diameter

Mean particle diameter was performed using the SALD-201V laser diffraction particle analyzer, Shimadzu (Kyoto, Japan). Ethanol was used as a dispersing liquid. The measurements were conducted at 25 ºC, in triplicate.

#### 5.2.5.4 Moisture content and water activity

The moisture content of the microparticles was carried out in a moisture analyzer model MB 35 from Ohaus (Ohio, United States), in triplicate. The results are expressed in percentage. The determination of water activity was performed by direct reading, on an Aqualab hygrometer, model CX-2T, from Decagon Devices Inc. The readings were performed at 25 ºC, in triplicate.

### 5.2.6 Dynamic vapor sorption (DVS) of microparticles and oat flakes

The water vapor sorption values of the microparticles and oat flakes were performed at 25 °C using a dynamic vapor sorption instrument (Surface Measurement Systems Ltd., Allentown, PA). Under continuous airflow (200 mL/min), the system was pre-equilibrated at 5% Relative Humidity (RH). The samples were exposed sequentially from 30 to 95% RH. The RH transitions and mass variations of the sample were monitored continuously. The moisture sorption isotherms were determined using the DVS Analysis Macro V6.1 software.

### 5.2.7 Thermal properties of oat flakes and oat flakes containing microparticles

The thermal behavior of the oat flakes and oat flakes with microparticles described by the following parameters 'onset of gelatinization' ($T_o$); 'peak gelatinization temperature' ($T_p$); and 'enthalpy' (J/ g) was analyzed using a Multi-Cell Differential Scanning Calorimeter (MC-DSC, TA Instruments, New Castle, DE, USA) equipped with the TRIOS software (TA Instruments, New Castle, DE, USA). The samples dispersed in water (with a moisture content of 80%) were weighed into ampoules and sealed. An empty pan was used as the reference. The samples were equilibrated at 5 °C and then heated to 140 °C at a rate of 1 °C/ min. Each sample was run in triplicate and the average results are shown.

### 5.2.8 Pasting properties

The oatmeal paste was prepared using the discovery hybrid rheometer 3 (DHR-3, TA Instruments Ltd., New Castle, DE, USA) with a pasting cell geometry, to simulate operating conditions where temperature and shear are applied during the process. The experiment was conducted by mixing (i) oat flakes and distilled water (80% w/ w); (ii) oat flakes blended with microparticles (6% w/w), and distilled water (80% w/w); (iii) oat flakes, GPE (6% w/w), and distilled water (80% w/w).

The samples were prepared using the following procedure: (1) Conditioning the sample at 25 °C for 2 min; (2) Heating ramp to the selected temperatures of 70, 80 or 90 °C at 5 °C/ min with a shear rate of 50 or 100 1/ s; (3) Flow peak hold in the selected temperature and shear conditions for 120 s; (4) Cooling ramp to 25 °C at 5 °C/ min; (5) Oscillation frequency at 25 °C from 0.01 to 10 Hz with a 0.5% strain. The testing parameters and treatments are shown in Table 12.

**Table 12.** Variable parameters evaluated in the preparation of oatmeal paste in DHR-3

| **Treatments** | **Parameters** | |
|---|---|---|
| | Temperature (°C) | Shear (1/s) |
| 70/50 | 70 | 50 |
| 80/50 | 80 | 50 |
| 90/50 | 90 | 50 |
| 70/100 | 70 | 100 |
| 80/100 | 80 | 100 |
| 90/100 | 90 | 100 |

Reference: Own source.

### 5.2.9 Retention of β-carotene, lutein, and total carotenoid content in the oatmeal paste

Total carotenoid content of oatmeal paste containing encapsulated and free GPE was analyzed using spectrophotometry, as described in Section 5.2.4. The main carotenoids extracted from guaraná peels were β-carotene and lutein (Pinho et al., 2021). The contents of the incorporated carotenoids were quantified in the oatmeal paste and the raw material, by high performance liquid cromatography (HPLC), to evaluate the effect of the process on the retention

of these compounds. Carotenoid extraction was carried out following Kopec et al. (2014), with some modifications according to the solvents used. Sequential extractions were performed using methanol and the mixture of ethanol: acetone: hexane (1:1:1, v/v/v). The extract was then injected into HPLC apparatus Agilent 1260 ultra-high performance liquid chromatograph with a diode array detector (UHPLC-DAD), using a C30 column (YMC Inc., 4.6 x 250 mm, 3 μm). The solvent system utilized consisted of mobile phase A: 60% methanol, 35% MTBE, 3% water, 2% aqueous ammonium acetate (2% w/v), and mobile phase B: 78% MTBE, 20% methanol, 2% aqueous ammonium acetate (2% w/v). The HPLC elution program was carried out as the following procedure: 0% B to 35.6% B in 9 min, to 100% B during the next 6.5 min, maintaining for 3.5 min at 100% B, and equilibrate for 3.5 min at initial conditions, using a flow rate of 1.3 mL/min (Cooperstone et al., 2015).

The contents of β-carotene and lutein were calculated from their peak areas in comparison to standards with known concentration, using a calibration curve. Carotenoid retention was determined by comparing their content before and after the pasting process and expressed as a percentage.

### 5.2.10 Statistical analysis

The data were analyzed using ANOVA and Tukey test in SAS statistical software (version 8.02, Statistic Analysis System). Significant differences were defined at $P < 0.05$. All data were expressed as means ± Standard Deviation (SD). The pasting process was performed in duplicate.

## 5.3 Results and Discussion

### 5.3.1 Total carotenoid content of microparticles

Microparticles rich in carotenoids were produced by spray-drying under three formulations to assess the best treatment for further application. Total carotenoid content and retention after encapsulation are presented in Table 13. The SD1:2 treatment showed a significantly higher carotenoid content compared to the others. This difference was consistent,

since the microparticles containing more carotenoid-rich extract in the proportion core:carrier material would present higher content of carotenoid.

The protective effect of carrier material on carotenoid stability after encapsulation, the SD1:4 treatment had the lowest retention. Although this sample had the highest carrier material proportion, the temperature of atomization was not sufficiently high to evaporate the water and dry the microparticles effectively. In consequence, this resulted in the adherence of semi-moist powder to the wall of the drying chamber, leading to the additional exposition to the temperatures of the process, and degradative reactions of carotenoids.

**Table 13.** Total carotenoid content and retention in microparticles after spray drying (SD) process

| Formulations | Carotenoid content (µg/g) | Carotenoid retention (%) |
|---|---|---|
| SD1:2 | $96 \pm 1^{a}$ | $100 \pm 1^{a}$ |
| SD1:3 | $57.6 \pm 0.7^{b}$ | $99.4 \pm 0.2^{a}$ |
| SD1:4 | $40.7 \pm 0.9^{c}$ | $96.7 \pm 0.3^{b}$ |

In formulations, the name is the proportion of the core:carrier material. The results are expressed as the mean value ± standard deviation (n = 3). Different letters in the columns represent a significant difference ($P < 0.05$).

Carotenoids are sensitive compounds as well documented, and the temperature applied for the atomization can influence their stability. The high retention observed in SD1:2 and SD1:3 was probably associated with the relationship between the suitable degree of heat treatment and feed material of these formulations during the process. The finding supports our hypothesis that one of the main factors that affect carotenoid retention during encapsulation by spray drying is the core: carrier material proportion associated with the operational condition of atomization.

### 5.3.2 Stability study of microparticles during storage

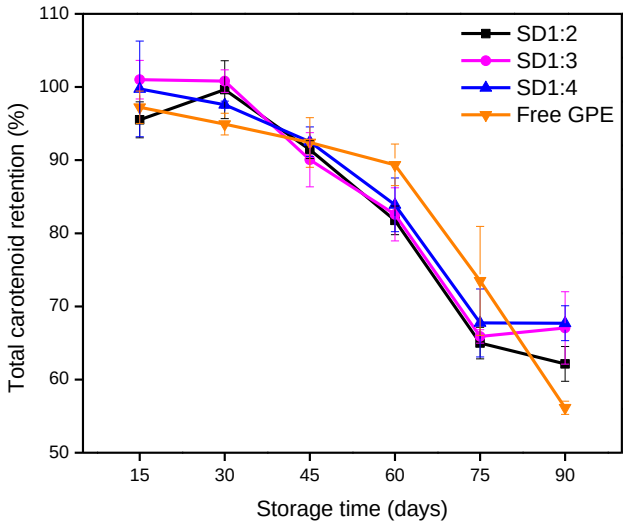
#### 5.3.2.1 Carotenoid retention

Figure 17 shows the retentions of total carotenoids in the microparticles and free GPE during storage under dark at 25 °C. Following 90 days, a carotenoid loss of 45% was found in

the free GPE, whereas only ~30% was observed in the microparticles. It is essential to highlight that free GPE considered a control contains sunflower oil, as mentioned in Section 5.2.2. The sunflower oil was added to alleviate the significant losses observed in obtaining the extract, which certainly protected the carotenoids during storage.

Regarding the microparticles, the carrier material acts as a physical barrier against environmental conditions, and the core was efficiently entrapped using gum arabic. This reduced the pronounced degradation of the active component, as observed in the free GPE. However, isomerization and/or oxidation of these compounds occured in the encapsulated samples. In this system, the high degree of carotenoids unsaturation concomitant with the presence of oxygen were the crucial reasons that contributed to their degradation over time.

**Figure 17.** Total carotenoid retention in free and encapsulated guaraná peel extract (GPE) produced by spray drying at a temperature of 100 °C. The formulations represent the proportion of the core:carrier material




Reference: Own source.

To assess the stability of carotenoids in encapsulated and non-encapsulated GPE, the first-order kinetics model was followed, and the results are described in Table 14. In a first-order mechanism, the reaction rate depends on variations in the amount of only one reactant; in this case, the reactants are the carotenoids. All microparticles showed low values for the first-order rate constant (k), which indicates a reduced degradation, also evidenced by the half-life

($t_{1/2}$). From this aspect, it can be noted that the loss of carotenoids occurred faster in the non-encapsulated extract than those in the encapsulated. The findings denote that the spray drying technique improved the stability of GPE due to the protective potential of the carrier material under storage conditions, promoting a longer shelf life. Similar results were reported when the carotenoid stability was evaluated in spray-dried samples (Rascón, Beristain, García, & Salgado 2011; Etzbach et al. 2020).

**Table 14.** Kinetic parameters for carotenoid degradation in non-encapsulated and encapsulated extract during storage

| **Sample** | **k ($s^{-1}$)** | **$t_{1/2}$ (days)** | **$R^2$** |
|---|---|---|---|
| Free extract | 0.006 | 108.082 | 0.811 |
| SD1:2 | 0.005 | 141.924 | 0.888 |
| SD1:3 | 0.005 | 152.286 | 0.897 |
| SD1:4 | 0.005 | 144.676 | 0.874 |

Formulation SD (core:carrier material ratio).

Reference: Own source.

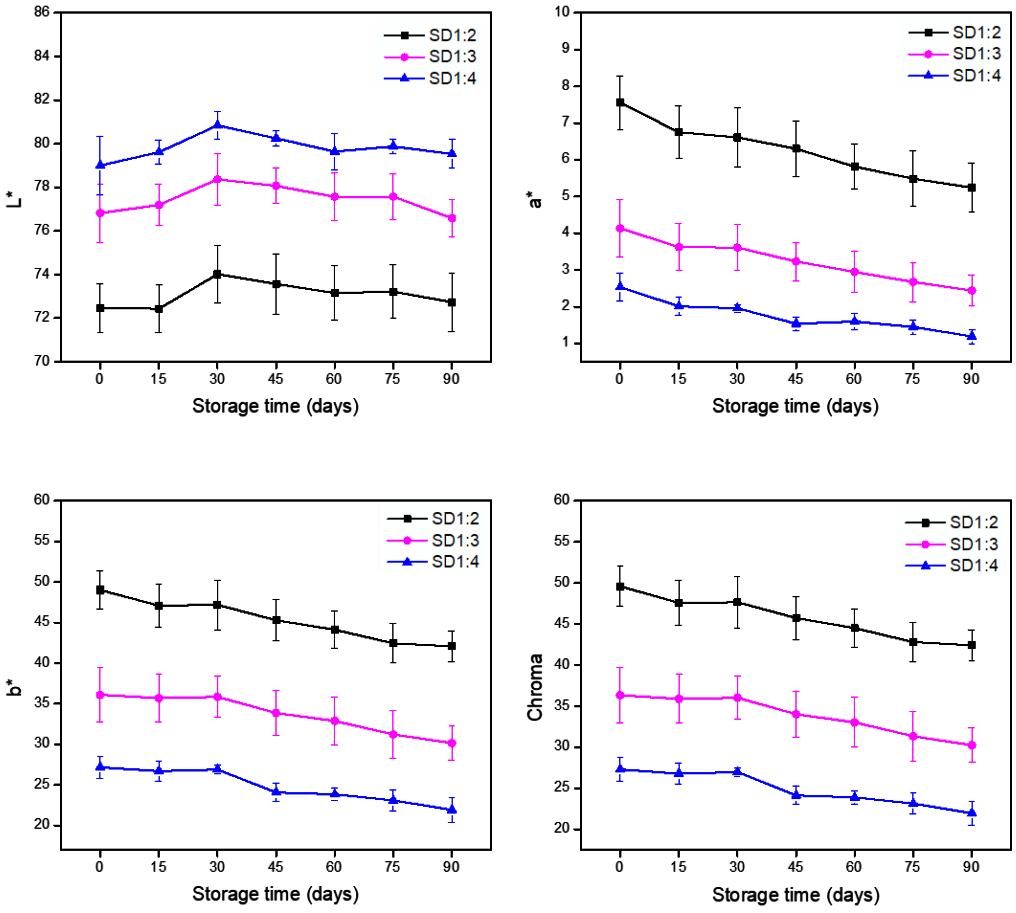
### 5.3.2.2 Color parameters

The powder's color, measured by the parameters L*, a*, b*, Chroma, and ΔE (total color difference) may indirectly indicate the degradation of carotenoids during the storage. Overall, the color characteristics of the samples were influenced by core:carrier material concentration in the feed material formulations (Figure 18). Comparing the treatments, an increase in the L* values was observed by increasing the ratio of gum arabic solution in the samples as following the order: SD14>SD1:3>SD1:2. Besides, the formulations with the highest concentration of GPE showed higher values of a* and b*, as expected. Chroma represents color saturation, which was in the first quadrant of the CIELab chart, corresponding to the vivid red-yellow color. A slight reduction of Chroma was observed in all formulations during storage, indicating loss of color intensity and degradation of the pigment, which corroborates with the results found in the stability study.

Regarding the trend for the total color difference, the expression of ΔE values for SD1:2 (7.31), SD1:3 (6.19), and SD1:4 (5.19) exhibited the overall variation between the samples at

initial time and the samples stored 90 days in the absence of light. This result is related to the degradation of carotenoids during the period, taking into account the carrier agent (gum arabic) offering stability to the component of interest. Indeed, the color variation over storage had some influence on the powder quality, considering the colorant property as well as the bioactivity capacity of these pigments.

**Figure 18.** L*, a*, b*, and Chroma parameters obtained for each formulation produced by spray drying in instrumental color analysis during storage at 25 °C. The formulations denote the proportion of the core:carrier material




Reference: Own source.

### 5.3.2.3 Mean particle diameter

Particle size is an important property for application as an ingredient in food products. The microparticle size distribution can be influenced by external and/or internal aspects. The conditions used in the spray drying procedure, such as temperature, pressure nozzle, airspeed, and feed flow are some of the external factors. Whereas, the feed material formulation and its preparation process may act as the internal factors (Favaro-Trindade, Okuro, & de Matos Jr, 2015).

Unlike formulations SD1:2 and SD1:3, SD1:4 microparticles exhibited a remarkable size variation, in which the median diameter ranged from 9 to 18 μm, over storage (Table 15). This phenomenon can be attributed to agglomeration. The hydrophilic active sites of gum arabic (carrier material) can absorb water favoring adherence properties of the samples. Due to the electrostatic effects and covalent bonds, adhesion between the wetted microparticles occurs, and the contact or collision among them can generate new agglomerate structures (Walton & Mumford, 1999; Ghosal, Indira, & Bhattacharya, 2010). Considering the higher proportion of gum arabic in SD1:4 formulation, the effect of this phenomenon was maximized and reflected in the size variation after 90 days.

**Table 15.** Mean and standard deviation of diameter sizes (μm) expressed in volume for each formulation of microparticles obtained by spray drying at 0 and 90 days of storage at 25 °C

| Time (days) | Formulations | Median diameter (μm) |
|---|---|---|
| 0 | SD1:2 | $12 \pm 1^{a}$ |
| 0 | SD1:3 | $9 \pm 2^{ab}$ |
| 0 | SD1:4 | $9 \pm 1^{b}$ |
| 90 | SD1:2 | $15 \pm 3^{a}$ |
| 90 | SD1:3 | $13 \pm 2^{a}$ |
| 90 | SD1:4 | $18 \pm 5^{a}$ |

Formulation SD (core:carrier material ratio). The results are expressed as the mean value ± standard deviation (n = 3). Different letters in the columns represent a significant difference ($P < 0.05$).

Reference: Own source.

The particle size was within the typical range for atomization, which varies from 5 to 150 μm. Their dimensions were below the value (<100 μm) of particles which have been found to cause little or no interference sensory when being added to food. Moreover, this parameter may be associated with bioavailability and solubility of the active components entrapped,

considering that the more surface area of the particle the more the bioactivity of the compounds (Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan 2013).

#### 5.3.2.4 Moisture content and Aw

Moisture and Aw are indicators of drying efficiency and are the main factors that affect powder stability. The mean values for these parameters before and after storage are shown in Table 16.

**Table 16.** Moisture and water activity (Aw) of encapsulated carotenoid-rich guaraná peel extract

| Time (days) | Formulations | Parameters | |
|---|---|---|---|
| | | Moisture (%) | Aw |
| 0 | SD1:2 | $3.9 \pm 0.4^{d}$ | $0.21 \pm 0.02^{c}$ |
| 0 | SD1:3 | $4.8 \pm 0.4^{c}$ | $0.24 \pm 0.03^{c}$ |
| 0 | SD1:4 | $4.1 \pm 0.7^{cd}$ | $0.16 \pm 0.02^{d}$ |
| 90 | SD1:2 | $6.9 \pm 0.3^{b}$ | $0.461 \pm 0.007^{b}$ |
| 90 | SD1:3 | $8.1 \pm 0.3^{a}$ | $0.479 \pm 0.006^{a}$ |
| 90 | SD1:4 | $8.2 \pm 0.1^{a}$ | $0.50 \pm 0.02^{a}$ |

Formulation SD (core:carrier material ratio). The results are expressed as the mean value ± standard deviation (n = 3). Different letters in the columns represent a significant difference ($P < 0.05$).
Reference: Own source.

After 90 days, the moisture and water activity of the powders increased significantly ($P < 0.05$). This occurred because of the water uptake by powder, during storage under a relative humidity of 33% and 25°C. However, even after this period, the samples showed low values of water activity. The moisture content and water activity can influence microbial development, in addition to enabling biochemical reactions. According to the literature, to avoid microbial growth, the water activity of products must be less than 0.60 (Chiou & Langrish, 2007; Tuyen, Nguyen, & Roach, 2010); therefore, the powder evaluated may be considered microbiologically safe. Besides, Aw values near 0.30 imply that the food products are less sensitive against non-enzymatic browning and enzymatic activities during storage (Labuza & Dugan, 1971; Álvarez-Henao et al., 2018).

The relevance of powdery ingredients lies in their large application in the food industry. The incorporation of microparticles enriched with carotenoids in a model food requires investigating the characteristics of powder in general. In this way, it is possible to achieve a suitable application considering the convenient features for processing. The findings were expected to suggest a reasonable formulation, taking into account the carotenoid content and particle behavior. Thus, the formulation SD1:2 was selected to be incorporated in oatmeal paste, to better understand the effect of operational conditions on the carotenoid stability and paste properties.

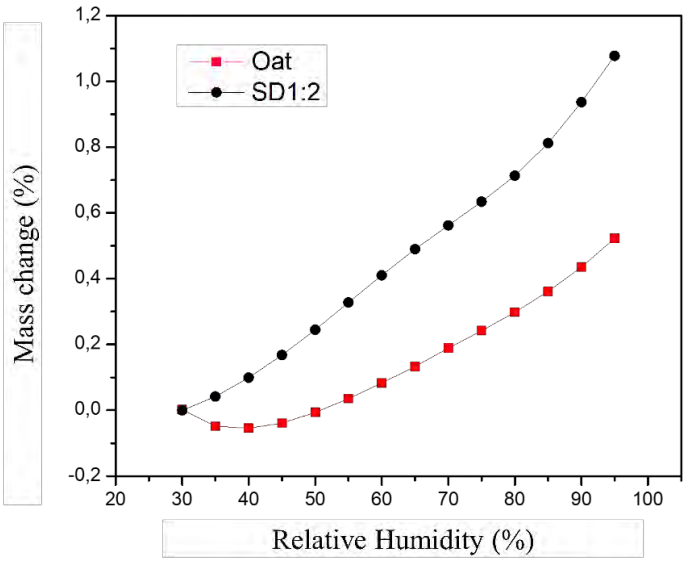
### 5.3.3 Dynamic vapor sorption (DVS) of oat flakes and microparticles

Most materials are sensitive to the presence of water vapor or moisture content in a system (Hettrich & Fanter, 2010). Investigation of interactions between water and oat flakes, as well as between water and microparticles is essential to improve the conception of the effect of the encapsulated GPE incorporation on the characteristics of oatmeal paste. As shown in Figure 19, the equilibrium moisture content of the microparticles was 5-fold higher than those of oat flakes at RH of 60%. The DVS curves for the samples were type III (non-sigmoidal).

A sorption moisture isotherm is determined by subjecting a material to different increasing relative humidity and monitoring the change in mass due to water absorption. The absorption of water depends on the number of available sites in the material capable of binding water molecules (Al-Muhtaseb, McMinn, & Magee, 2002; Steenberg & Harris, 1984).

The structural difference between oat flakes and encapsulated GPE may be correlated with their distinct absorption rates. Gum arabic is a complex polysaccharide with a highly branched structure, which facilitates polar interactions with water by hydrogen bonds, at room temperature (Gabas, Telis, Sobral, & Telis-Romero, 2007). Whereas, the oat flake comprises mainly starch and fiber. Starch contains amylose, a linear molecule, and amylopectin, a non-linear and highly branched molecule (Damodaran, Parkin, & Fennema, 2007; Brett et al., 2009). In the crystalline regions of the granule, the intermolecular interactions among the chains are very strong and the diffusion of the plasticizer like water is slow at room temperature.

**Figure 19.** Dynamic vapor sorption isotherms of particles produced by spray-drying (SD1:2) and oat flakes




Reference: Own source.

### 5.3.4 Thermal properties

The onset temperature and the peak temperature for oat flakes and oat flakes incorporated with encapsulated GPE with a moisture content of 80% are listed in Table 17. The thermal properties were significantly affected ($P < 0.05$) by the addition of microparticles, showing a decrease in the enthalpies and a slight increase in gelatinization temperatures. This indicates that in samples containing oat flakes and microparticles, the starch was less gelatinized which is likely attributed to the decreased availability of water (Díaz-Calderón et al., 2018; Eliasson, 1983).

In the system studied, microparticles and starch competitively bind water to form hydrogen bonds. As part of the water is bound to the microparticles with larger water absorption capacity, the available water for starch is reduced, which could elevate the starch gelatinization temperature due to insufficient hydration and swelling.

**Table 17.** DSC gelatinization properties of oat flakes and oat flakes enriched with encapsulated guaraná peel extract (GPE)

| Sample | Onset Temp. (°C) | Peak Temp. (°C) | Enthalpy (J/g) |
|---|---|---|---|
| Oat | $53.05 \pm 0.01^{b}$ | $60.1 \pm 0.3^{b}$ | $3.1 \pm 0.1^{a}$ |
| Oat+Encapsulated GPE | $54.3 \pm 0.6^{a}$ | $61.0 \pm 0.3^{a}$ | $2.6 \pm 0.1^{b}$ |

The results are expressed as the mean value ± standard deviation (n = 3). Different letters in the columns represent a significant difference ($P < 0.05$). Reference: Own source.

### 5.3.5 Pasting properties

The viscosity of the oat flakes starch with and without GPE were investigated during the heating-cooling procedure as described in Section 5.2.8, to evaluate their influence on the oatmeal paste properties. In Figure 20, at 70 °C or above, gelatinization of the samples was observed, showing a rapid increase in viscosity.

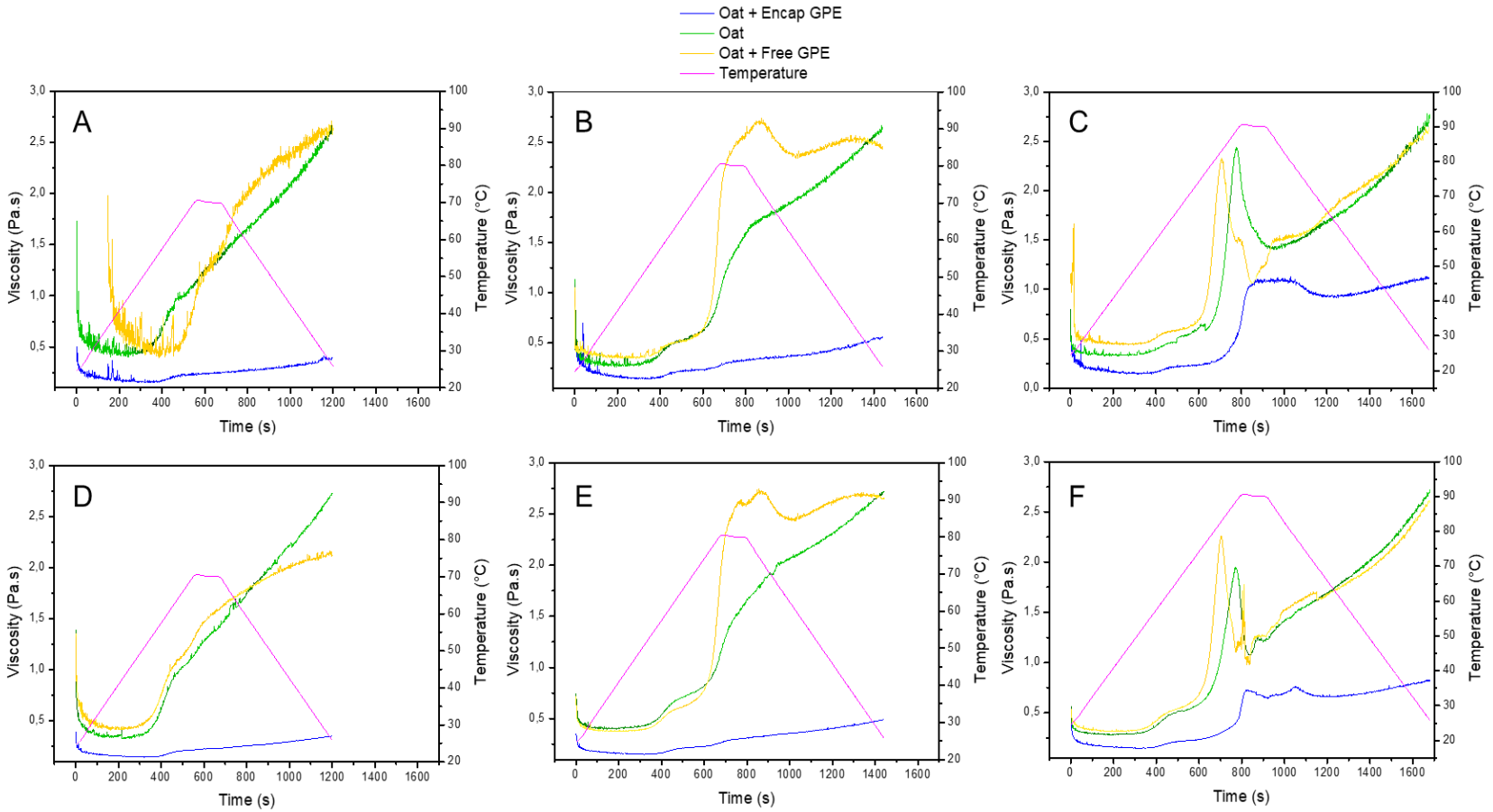
Temperature significantly affected the pasting behavior of the samples. At 70 °C and 80 °C, the viscosity of oatmeal paste with encapsulated GPE was lower than those sole oatmeal without a noticeable peak. This implies smaller extent of starch swelling/gelatinization when encapsulated GPE were present. The increase in starch viscosity originates from the swelling of starch molecules. Further heating resulted in molecular disorganization and the leaching of amylose and amylopectin into the solvent. Shear facilitated the formation of the paste by promoting non-covalent interactions between starch molecules, which can be affected by the temperature and sample composition (Punia et al., 2020; Taghizadeh et al., 2013; Wang et al., 2019).

Particles produced using gum arabic as the carrier material hindered the swelling/gelatinization of the grains. Singh, Geveke, & Yadav (2017) suggested that gum arabic might cover the surface of the starch during processing, leading to the reduced interaction among neighboring starch granules, which may control the swelling power of starch and restricted the increment of viscosity. In this case, the system would require higher temperatures to completely gelatinize the starch. Shahzad et al. (2019) reported similar findings.

At 80 °C, the oatmeal paste with free GPE showed significantly higher peak viscosity than the control (only oat flakes and water). This may be attributed to the presence of residual

ethanol from GPE in the starch granules, which favors maximum swelling and leads to the gelatinization of most of the starch in the system (Farrag et al., 2018). Previous researchers (Sun et al., 2021) have reported that an aqueous ethanol medium promoted the formation of hydrogen bonds with starch molecules, resulting in stronger gels compared to those prepared with pure water. Alternatively, GPE contains high amount of carotenoids. These compounds are hydrophobic, which may interact with double helices structures via hydrophobic interactions, and contributes to higher viscosity.

**Figure 20.** DHR- 3 pasting curves of oatmeal paste enriched with encapsulated GPE and free GPE. Testing conditions: (A) temperature 70 °C and shear rate 50 1/s; (B) temperature 80 °C and shear rate 50 1/s, (C) temperature 90 °C and shear rate 50 1/s; (D) temperature 70 °C and shear rate 100 1/s; (E) temperature 80 °C and shear rate 100 1/s; (F) temperature 90 °C and shear rate 100 1/s



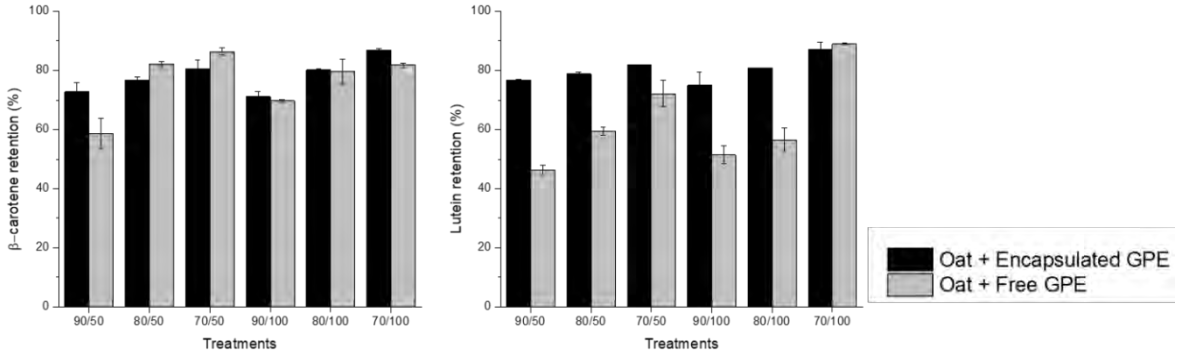

Reference: Own source.

### 5.3.6 Carotenoid retention

The formulation of foods enriched with active compounds is a matter of concern for the food industries, considering the impact of processing on its bioactivity. For this reason, the retention of β-carotene and lutein under the thermo-mechanical treatment applied during the production of oatmeal paste was investigated. The results are illustrated in Figure 21.

β-carotene showed high stability with retentions ranging from 59 – 87% under different temperature and shear rates, regardless of its status (encapsulated or not). The lutein stability was significantly higher in samples enriched with encapsulated GPE than the sample with free GPE, varying from 51 – 89%. Regarding the testing conditions, 90 °C and the shear rate 100 1/s exhibited a larger destructive effect on the carotenoids, either free or in their encapsulated treatments.

**Figure 21.** β-carotene and lutein retentions in oatmeal paste incorporated with encapsulated and free guaraná peel extract (GPE) after the process at different conditions (temperature/shear rate)




Reference: Own source.

Carotenoids are classified into carotenes and xanthophylls, in which the latter is more polar. The first group, comprising pro-vitamin A carotenoids (β-carotene), demonstrated greater stability. Higher retention of β-carotene in the aqueous system may be due to its physical stability towards environmental conditions. In contrast, the reactivity of xanthophylls, such as lutein, was highly affected during food processing by its structure, characterized by the presence of oxygen in the molecule chain. The trapped oxygen within the food matrix composed of water and oat flakes may contribute to the oxidation of the carotenoids in the free guaraná peel extract,

and as a result, this system led to the substantial loss of lutein. Therefore, the encapsulation technique reduced the degradation of this component. Dhuique-Mayer et al. (2007) assessed the stability of carotenes and xanthophylls from citrus juice upon thermal treatment, and they suggested that the former may be less reactive in a polar solvent. Interestingly, on the opposite, when the stability of β-carotene and lutein in oil (i.e. a nonpolar solvent) was investigated during heat treatment, they found that β-carotene had the highest reactivity (Achir et al., 2010).

Carotenoids have highly unsaturated chains and are easily to be oxidized. In addition, the isomerization of these compounds also affects their stability through food processing (Aman, Schieber, & Carle, 2005; Waramboi, Gidley, & Sopade, 2013). During the preparation of the oatmeal paste, extrinsic and intrinsic factors can be considered the promoters of oxidation/ isomerization reactions. Thermo-mechanical stress, the incidence of light, and the presence of oxygen may be the main external reasons contributing to the loss of carotenoids. The composition of the food matrix, the physical status of the carotenoid, and the air incorporation into the sample may be considered the internal factors affecting the active retention (Vásquez-Caicedo, Schilling, Carle, & Neidhart 2007; Rodriguez-Amaya, 2001).

Indeed, degrading reactions are promoted by the structure of the carotenoid as well as the factors mentioned previously. Similar results have been reported by other researchers (Gama & Sylos, 2007; Provesi, Dias, & Amante, 2011).

## 5.4 Conclusions

Microencapsulation of carotenoid-rich extract from guaraná peels enhanced its stability over storage. The sample SD1:2 exhibited promising features among other formulations, including high carotenoid content, suitable particle size, and intense color. The addition of microparticles rich in bioactive compounds into oatmeal paste increased the onset and peak temperature of starch but decreased its enthalpy. Besides, it reduced the viscosity of the system, whereas the opposite trend was observed for the samples with free GPE. Overall, the gelatinization of oat flakes starch was affected by the decrease of accessible water, due to the presence of the microparticles.

Testing conditions such as temperature and shear, favored heat transfer and oxygen incorporation in the system, reducing the total carotenoid content of the samples. In addition, the treatment significantly altered the lutein content, when compared to β-carotene. However, pigment loss was minimized by encapsulation.

The recovery of bioactive components from by-products, such as β-carotene (provitamin A) and lutein (recognized as an agent that prevents macular degeneration), revealed the importance of the present research on adding value to a waste of a food process encouraging sustainable development. In addition, the study of applications of these materials, as suggested in the current work, represents a suitable trend for obtaining functional foods.

## Acknowledgments

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## References

Achir, N., Randrianatoandro, V. A., Bohuon, P., Laffargue, A., & Avallone, S. (2010). Kinetic study of β-carotene and lutein degradation in oils during heat treatment. *European Journal of Lipid Science and Technology*, *112*(3), 349-361. https://doi.org/10.1002/ejlt.200900165

Álvarez-Henao, M. V., Saavedra, N., Medina, S., Cartagena, C. J., Alzate, L. M., & Londoño-Londoño, J. (2018). Microencapsulation of lutein by spray-drying: Characterization and stability analyses to promote its use as a functional ingredient. *Food Chemistry*, *256*, 181-187. https://doi.org/10.1016/j.foodchem.2018.02.059

Aman, R., Schieber, A., & Carle, R. (2005). Effects of heating and illumination on trans− cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *Journal of Agricultural and Food Chemistry*, *53*(24), 9512-9518. https://doi.org/10.1021/jf050926w

Ai, Y., & Jane, J. L. (2015). Gelatinization and rheological properties of starch. *Starch-Stärke*, *67*(3-4), 213-224. https://doi.org/10.1002/star.201400201

Ajila, C. M., Bhat, S. G., & Rao, U. P. (2007). Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry*, *102*(4), 1006-1011. https://doi.org/10.1016/j.foodchem.2006.06.036

Al-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture sorption isotherm characteristics of food products: a review. *Food and Bioproducts Processing*, *80*(2), 118-128. https://doi.org/10.1205/09603080252938753

Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. A. (2010). Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science And Nutrition*, *50*(6), 515-532. https://doi.org/10.1080/10408390802565889

Brett, B., Figueroa, M., Sandoval, A. J., Barreiro, J. A., & Müller, A. J. (2009). Moisture sorption characteristics of starchy products: oat flour and rice flour. *Food Biophysics*, *4*(3), 151-157. https://doi.org/10.1007/s11483-009-9112-0

Caris-Veyrat, C., Schmid, A., Carail, M., & Böhm, V. (2003). Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *Journal of Agricultural and Food Chemistry*, *51*(25), 7318-7325. https://doi.org/10.1021/jf034735+

Chen, B. H., & Tang, Y. C. (1998). Processing and stability of carotenoid powder from carrot pulp waste. *Journal of Agricultural and Food Chemistry*, *46*(6), 2312-2318. https://doi.org/10.1021/jf9800817

Chiou, D., & Langrish, T. A. G. (2007). Development and characterisation of novel nutraceuticals with spray drying technology. *Journal of Food Engineering*, *82*(1), 84-91. https://doi.org/10.1016/j.jfoodeng.2007.01.021

Cooperstone, J. L., Ralston, R. A., Riedl, K. M., Haufe, T. C., Schweiggert, R. M., King, S. A., Timmers, C. D., Francis, D. M., Lesinski, G. B., Clinton, S. K., & Schwartz, S. J. (2015). Enhanced bioavailability of lycopene when consumed as cis-isomers from tangerine compared to red tomato juice, a randomized, cross-over clinical trial. *Molecular Nutrition and Food Research, 59(4),* 658–669. https://doi.org/10.1002/mnfr.201400658

Cuevas, M. S., Rodrigues, C. E., Gomes, G. B., & Meirelles, A. J. (2010). Vegetable Oils Deacidification by Solvent Extraction: Liquid− Liquid Equilibrium Data for Systems Containing Sunflower Seed Oil at 298.2 K. *Journal of Chemical & Engineering Data*, 55(9), 3859-3862. https://doi.org/10.1021/je900791w

Damodaran, S., Parkin, K. L., & Fennema, O. R. (Eds.). (2007). *Fennema's food chemistry*. CRC press.

De Andrade Lima, M., Kestekoglou, I., Charalampopoulos, D., & Chatzifragkou, A. (2019). Supercritical fluid extraction of carotenoids from vegetable waste matrices. *Molecules*, *24*(3), 466. https://doi.org/10.3390/molecules24030466

De Souza Mesquita, L. M., Martins, M., Maricato, É., Nunes, C., Quinteiro, P. S., Dias, A. C., ... & Ventura, S. P. (2020). Ionic liquid-mediated recovery of carotenoids from the Bactris gasipaes fruit waste and their application in food-packaging chitosan films. *ACS Sustainable Chemistry & Engineering*, *8*(10), 4085-4095. https://doi.org/10.1021/acssuschemeng.9b06424

Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M., & Amiot, M. J. (2007). Thermal degradation of antioxidant micronutrients in citrus juice: kinetics and newly formed compounds. *Journal of Agricultural and Food Chemistry*, *55*(10), 4209-4216. https://doi.org/10.1021/jf0700529

Díaz-Calderón, P., MacNaughtan, B., Hill, S., Foster, T., Enrione, J., & Mitchell, J. (2018). Changes in gelatinisation and pasting properties of various starches (wheat, maize and waxy maize) by the addition of bacterial cellulose fibrils. *Food Hydrocolloids*, *80*, 274-280. https://doi.org/10.1016/j.foodhyd.2018.02.023

Eliasson, A. C. (1983). Differential scanning calorimetry studies on wheat starch—gluten mixtures: I. Effect of gluten on the gelatinization of wheat starch. *Journal of Cereal Science*, *1*(3), 199-205. https://doi.org/10.1016/S0733-5210(83)80021-6

Etzbach, L., Meinert, M., Faber, T., Klein, C., Schieber, A., & Weber, F. (2020). Effects of carrier agents on powder properties, stability of carotenoids, and encapsulation efficiency of goldenberry (Physalis peruviana L.) powder produced by co-current spray drying. *Current Research in Food Science*, *3*, 73-81. https://doi.org/10.1016/j.crfs.2020.03.002

Ezhilarasi, P. N., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2013). Nanoencapsulation techniques for food bioactive components: a review. *Food and Bioprocess Technology*, *6*(3), 628-647. https://doi.org/10.1007/s11947-012-0944-0

Farrag, Y., Sabando, C., Rodríguez-Llamazares, S., Bouza, R., Rojas, C., & Barral, L. (2018). Preparation of donut-shaped starch microparticles by aqueous-alcoholic treatment. *Food Chemistry*, *246*, 1-5. https://doi.org/10.1016/j.foodchem.2017.10.147

Favaro-Trindade, C. S., Okuro, P. K., & de Matos Jr, F. E. (2015). 5 Encapsulation via Spray. *Handbook of encapsulation and controlled release*, 71.

Gabas, A. L., Telis, V. R. N., Sobral, P. J. A., & Telis-Romero, J. (2007). Effect of maltodextrin and arabic gum in water vapor sorption thermodynamic properties of vacuum dried pineapple pulp powder. *Journal of Food Engineering*, *82*(2), 246-252. https://doi.org/10.1016/j.jfoodeng.2007.02.029

Gale, C. R., Hall, N. F., Phillips, D. I., & Martyn, C. N. (2003). Lutein and zeaxanthin status and risk of age-related macular degeneration. *Investigative ophthalmology & visual science*, *44*(6), 2461-2465. https://doi.org/10.1167/iovs.02-0929

Gama, J. J. T., & de Sylos, C. M. (2007). Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice. *Food Chemistry*, *100*(4), 1686-1690. https://doi.org/10.1016/j.foodchem.2005.01.062

Ghosal S., Indira T. N., & Bhattacharya S. (2010). Agglomeration of a model food powder: Effect of maltodextrin and gum Arabic dispersions on flow behavior and compacted mass. *Journal of Food Engineering*, 96(2):222-8. https://doi.org/10.1016/j.jfoodeng.2009.07.016

Hettrich, K., & Fanter, C. (2010). Novel xylan gels prepared from oat spelts. In *Macromolecular symposia* (Vol. 294, No. 2, pp. 141-150). Weinheim: WILEY-VCH Verlag. https://doi.org/10.1002/masy.200900169

Hidalgo, A., & Brandolini, A. (2008). Kinetics of carotenoids degradation during the storage of einkorn (Triticum monococcum L. ssp. monococcum) and bread wheat (Triticum aestivum L. ssp. aestivum) flours. *Journal of Agricultural and Food Chemistry, 56*(23), 11300-11305. https://doi.org/10.1021/jf802448t

Kopec, R. E., Cooperstone, J. L., Schweiggert, R. M., Young, G. S., Harrison, E. H., Francis, D. M., Clinton, S. K., & Schwartz, S. J. (2014). Avocado consumption enhances human postprandial provitamin a absorption and conversion from a novel high-β-carotene tomato sauce and from carrots. *Journal of Nutrition, 144(8),* 1158–1166. https://doi.org/10.3945/jn.113.187674

Kumar, S. R., Hosokawa, M., & Miyashita, K. (2013). Fucoxanthin: A marine carotenoid exerting anti-cancer effects by affecting multiple mechanisms. *Marine drugs, 11*(12), 5130-5147. https://doi.org/10.3390/md11125130

Kurozawa, L. E., Park, K. J., & Hubinger, M. D. (2009). Effect of maltodextrin and gum arabic on water sorption and glass transition temperature of spray dried chicken meat hydrolysate protein. *Journal of Food Engineering, 91*(2), 287-296. https://doi.org/10.1016/j.jfoodeng.2008.09.006

Labuza, T. P., & Dugan Jr, L. R. (1971). Kinetics of lipid oxidation in foods. *Critical Reviews in Food Science & Nutrition, 2*(3), 355-405. https://doi.org/10.1080/10408397109527127

Micozzi, M. S., Beecher, G. R., Taylor, P. R., & Khachik, F. (1990). Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. *JNCI: Journal of the National Cancer Institute, 82*(4), 282-285. https://doi.org/10.1093/jnci/82.4.282

Minolta Corporation. (1994). *Precise color communication: color control from feeling to instrumentation*. Minolta.

Monroy-Ruiz, J., Sevilla, M. Á., Carrón, R., & Montero, M. J. (2011). Astaxanthin-enriched-diet reduces blood pressure and improves cardiovascular parameters in spontaneously hypertensive rats. *Pharmacological Research, 63*(1), 44-50. https://doi.org/10.1016/j.phrs.2010.09.003

Olson, J. A. (1989). Provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. *The Journal of nutrition, 119*(1), 105-108. https://doi.org/10.1093/jn/119.1.105

Pinho, L. P., Silva, M. P., Thomazini, M., Cooperstone, J. L., Campanella, O., Rodrigues, C. E. C., & Fávaro-Trindade, C. S. (2021). Guaraná (*Paullinia cupana*) by-product as a source of bioactive compounds and as a natural antioxidant for food applications. *Food Processing and Preservation,* 00, e15854. https://doi.org/10.1111/jfpp.15854

Provesi, J. G., Dias, C. O., & Amante, E. R. (2011). Changes in carotenoids during processing and storage of pumpkin puree. *Food Chemistry*, *128*(1), 195-202. https://doi.org/10.1016/j.foodchem.2011.03.027

Punia, S., Sandhu, K. S., Dhull, S. B., Siroha, A. K., Purewal, S. S., Kaur, M., & Kidwai, M. K. (2020). Oat starch: Physico-chemical, morphological, rheological characteristics and its applications-A review. *International journal of Biological Macromolecules*, *154*, 493-498. https://doi.org/10.1016/j.ijbiomac.2020.03.083

Rascón, M. P., Beristain, C. I., García, H. S., & Salgado, M. A. (2011). Carotenoid retention and storage stability of spray-dried encapsulated paprika oleoresin using gum Arabic and soy protein isolate as wall materials. *LWT-Food Science and Technology*, *44*(2), 549-557. https://doi.org/10.1016/j.lwt.2010.08.021

Riccioni, G. (2009). Carotenoids and cardiovascular disease. *Current atherosclerosis reports*, *11*(6), 434-439.

Rocha, G. A., Fávaro-Trindade, C. S., & Grosso, C. R. F. (2012). Microencapsulation of lycopene by spray drying: characterization, stability and application of microcapsules. *Food and Bioproducts Processing*, 90(1), 37-42. https://doi.org/10.1016/j.fbp.2011.01.001

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*.

Sagar, V. R., & Kumar, P. S. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. *Journal of food science and technology*, *47*(1), 15-26. https://doi.org/10.1007/s13197-010-0010-8

Shahzad, S. A., Hussain, S., Mohamed, A. A., Alamri, M. S., Ibraheem, M. A., & Qasem, A. A. A. (2019). Effect of hydrocolloid gums on the pasting, thermal, rheological and textural properties of chickpea starch. *Foods*, *8*(12), 687. https://doi.org/10.3390/foods8120687

Silva, M. P., Thomazini, M., Holkem, A. T., Pinho, L. S., Genovese, M. I., & Fávaro-Trindade, C. S. (2019). Production and characterization of solid lipid microparticles loaded with guaraná (Paullinia cupana) seed extract. Foo*d Research International*, *123*, 144–152. https://doi.org/10.1016/j.foodres.2019.04.055

Singh, A., Geveke, D. J., & Yadav, M. P. (2017). Improvement of rheological, thermal and functional properties of tapioca starch by using gum arabic. *LWT*, *80*, 155-162. https://doi.org/10.1016/j.lwt.2016.07.059

Song, J., Wang, X., Li, D., & Liu, C. (2017). Degradation kinetics of carotenoids and visual colour in pumpkin (Cucurbita maxima L.) slices during microwave-vacuum drying. *International Journal of Food Properties*, *20*(sup1), S632-S643. https://doi.org/10.1080/10942912.2017.1306553

Stahl, W., & Sies, H. (2003). Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, *24*(6), 345-351. https://doi.org/10.1016/S0098-2997(03)00030-X

Steenberg, E., & Harris, P. J. (1984). Adsorption of carboxymethylcellulose, guar gum and starch onto talc, sulphides, oxides and salt type minerals. *South African Journal of Chemistry*, *37*(3), 85-90. https://doi.org/10.10520/AJA03794350_1501

Subtil, S. F., Rocha-Selmi, G. A., Thomazini, M., Trindade, M. A., Netto, F. M., & Favaro-Trindade, C. S. (2014). Effect of spray drying on the sensory and physical properties of hydrolysed casein using gum arabic as the carrier. *Journal of Food Science and Technology*, *51*(9). https://doi.org/10.1007/s13197-012-0722-z

Sun, Y., Li, F., Luan, Y., Li, P., Dong, X., Chen, M., ... & Sun, Q. (2021). Gelatinization, pasting, and rheological properties of pea starch in alcohol solution. *Food Hydrocolloids*, *112*, 106331. https://doi.org/10.1016/j.foodhyd.2020.106331

Taghizadeh, A., & Favis, B. D. (2013). Effect of high molecular weight plasticizers on the gelatinization of starch under static and shear conditions. *Carbohydrate polymers*, *92*(2), 1799-1808. https://doi.org/10.1016/j.carbpol.2012.11.018

Toma, S., Losardo, P. L., Vincent, M., & Palumbo, R. (1995). Effectiveness of beta-carotene in cancer chemoprevention. *European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP)*, *4*(3), 213-224. https://doi.org/10.1097/00008469-199506000-00002

Tonon, R. V., Brabet, C., & Hubinger, M. D. (2010). Anthocyanin stability and antioxidant activity of spray-dried açai (Euterpe oleracea Mart.) juice produced with different carrier agents. *Food Research International*, *43*(3), 907-914. https://doi.org/10.1016/j.foodres.2009.12.013

Tuyen, C. K., Nguyen, M. H., & Roach, P. D. (2010). Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (Momordica cochinchinensis) fruit aril powder. *Journal of Food Engineering*, *98*(3), 385-392. https://doi.org/10.1016/j.jfoodeng.2010.01.016

Vásquez-Caicedo, A. L., Schilling, S., Carle, R., & Neidhart, S. (2007). Impact of packaging and storage conditions on colour and β-carotene retention of pasteurised mango purée. *European Food Research and Technology*, *224*(5), 581-590. https://doi.org/10.1007/s00217-006-0339-4

Walton D. E. & Mumford C. J. (1999) Spray dried products—characterization of particle morphology. *Chemical Engineering Research and Design*, 77(1):21-38. https://doi.org/10.1205/026387699525846

Wang, J., Xia, S., Wang, B., Ali, F., & Li, X. (2019). Effect of twin-screw extrusion on gelatinization characteristics of oat powder. *Journal of Food Process Engineering, 42*(3), e13014. https://doi.org/10.1111/jfpe.13014

Waramboi, J. G., Gidley, M. J., & Sopade, P. A. (2013). Carotenoid contents of extruded and non-extruded sweetpotato flours from Papua New Guinea and Australia. *Food Chemistry, 141*(3), 1740-1746. https://doi.org/10.1016/j.foodchem.2013.04.070

Xiao, Y. D., Huang, W. Y., Li, D. J., Song, J. F., Liu, C. Q., Wei, Q. Y., ... & Yang, Q. M. (2018). Thermal degradation kinetics of all-trans and cis-carotenoids in a light-induced model system. *Food Chemistry, 239*, 360-368. https://doi.org/10.1016/j.foodchem.2017.06.107

# 6 GERAL CONCLUSION

Guaraná peel comprises insoluble fibers, macro-and micro minerals, caffeine, theobromine, phenolic compounds, and carotenoids, including all-*trans*-β-carotene, *cis*-β-carotene, and lutein. The ethanolic extract of guaraná peels exhibits a tremendous antioxidant capacity assessed by the ORAC and ABTS tests.

The encapsulation of the carotenoid-rich extract by spray drying, chilling, and combining both techniques can protect the bioactive components with high retention ranging from 70 to 98% over storage. A slight degradation of encapsulated carotenoids observed in all particles evaluated in this study was likely caused by oxidation reactions.

The resultant microparticles obtained by combining techniques displayed promising thermal properties, oxidative stability, and water sorption features. Although the spray chilled microparticles presented better retention after 90 days of storage, the spray-dried microparticles exhibited good retention, higher encapsulation efficiency and carotenoid content. Thus, these particles were selected to be applied in a food matrix model for further evaluation.

Incorporating microparticles loaded with carotenoids into oatmeal intensified starch's onset and peak temperature but reduced its enthalpy. Overall, the gelatinization of oat starch was affected lying the presence of the microparticles, primarily due to the decrease of available water in the system. The operating conditions influenced the degradation of carotenoids, and specifically, the treatment reduced the lutein content compared to β-carotene. However, pigment loss was minimized by encapsulation.

It is worth noting that the particles from each encapsulation technique can be better applied according to the food matrix to be enriched. Considering the particles obtained in the current study, all had good features for producing functional products.

In general, this study endorses the initiative to mitigate the environmental waste load by reutilizing by-products and adding value to foods. Nevertheless, it was proven that spray-drying, chilling, and combined techniques consist of robust alternatives to improve the stability of carotenoids and offer a longer shelf life. The use of gum arabic, hydrogenated vegetable fat, and their mixture as carrier materials exhibit a significant impact on the features of microparticles. The quality of food products enriched with bioactive components depends on the processing techniques and operating parameters. Investigations focused on the interactions between food ingredients upon certain processing conditions open up new insights, which disclose phenomena during the application. For this reason, more studies should be conducted dealing with the incorporation of encapsulated bioactive compounds in foods. In this suggestion, the consumers have an essential role in the acceptance of new products. In addition,

the bioaccessibility/bioavailability of these functional foods is a crucial aspect to be evaluated to validate their health benefits to the consumers.

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**ATTACHMENT C**

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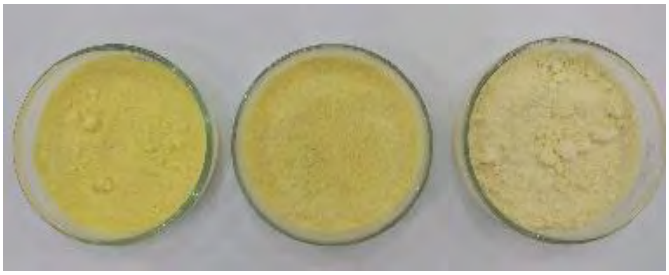
# MICROPARTICLES OBTAINED BY SPRAY DRYING, CHILLING, AND THE COMBINATION OF SPRAY DRYING AND CHILLING

Microparticles obtained by Spray Drying

Formulations: SD33 SD25 SD20

Microparticles obtained by Spray Chilling



Formulations: SC40 SC30 SC20

Microparticles obtained by Spray Drying/Chilling

Formulations: SDC20 SDC10