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FERNANDA THAÍS VIEIRA RUBIO

Utilization of brewer's spent yeast as a bio-vehicle for incorporation and protection of bioactive compounds

Pirassununga
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Doctoral thesis presented to Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, for obtention of the title of Doctor in Science.

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Advisor: Prof. Dr. Carmen Sílvia Fávaro Trindade

Co-Advisor: Prof. Dr. Charles Windson Isidoro Haminiuk

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Dedicated to my mother, who is my example of strength and humility. I am everything I am because you taught me how to be a better person.

BIOGRAFY

Fernanda Thaís Vieira Rubio was born in Sorocaba, in the interior of São Paulo - Brazil, on September 24, 1991. She graduated in Food Engineering in 2014 at Universidade Tecnológica Federal do Paraná, in Campo Mourão – PR. During her undergraduate course, she helped funding an English Conversation Group, a project whose purpose was to practice the conversation in English. In 2012, she received a one-year scholarship to assist the teacher in the discipline of mechanics of fluids. In 2013, she received a scientific initiation scholarship from Fundação Araucária, for one year, and she worked with extraction of bioactives from residual sources and biosorption.

In 2015, she entered the Master's degree in Food Technology at Universidade Tecnológica Federal do Paraná, in Campo Mourão – PR. She received a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and worked with biosorption of phenolic compounds from grape pomace in yeasts *Saccharomyces cerevisiae*. The process of yeast modification and enrichment reported in her dissertation was patented and was categorized as a green patent.

In 2017, she entered the Doctorate at Faculdade de Zootecnia e Engenharia de Alimentos, at Universidade de São Paulo, and received a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). During her doctorate, Fernanda deepened even more her studies approaching yeasts and their utilization for incorporation of compounds.

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RESUMO

RUBIO, F. T. V. **Utilização de levedura residual de cervejaria como bio-veículo para incorporação e proteção de compostos bioativos**. 2021. 189 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

O descarte de resíduos e coprodutos da agricultura e da indústria alimentícia é, sem dúvida, uma grande preocupação, especialmente porque esses materiais podem representar fontes valiosas de compostos bioativos, fibras dietéticas e nutrientes, que podem ser reutilizados na produção de novos aditivos ou produtos. Nesse contexto, bagaços de uva, subprodutos do despolpamento de jabuticaba, cascas de abóbora e leveduras são exemplos de subprodutos que apresentam valor nutricional potencial e foram utilizados neste trabalho. O bagaço de uva e os subprodutos da jabuticaba são ricos em compostos fenólicos, tais como as antocianinas, as cascas de abóbora representam uma fonte rica em carotenoides e as leveduras contêm proteínas, vitaminas do complexo B e carboidratos e têm sido utilizadas em aplicações alimentícias. O objetivo principal deste trabalho foi aproveitar a morfologia de cápsula pré-formada das leveduras *Saccharomyces cerevisiae* para incorporar compostos bioativos usando a técnica de *spray drying*, associando o valor nutricional da levedura às propriedades benéficas à saúde dos compostos bioativos. Em geral, as microcápsulas à base de levedura produzidas neste trabalho apresentaram baixos valores de atividade de água, umidade e higroscopicidade, garantindo estabilidade física e microbiológica aos pós. A análise de espectroscopia no infravermelho médio com reflectância total atenuada mostrou diferenças significativas entre as leveduras antes e após a encapsulação dos bioativos, sugerindo o enriquecimento da levedura. A microscopia confocal de varredura a laser ajudou a entender a distribuição dos bioativos por toda a célula após a encapsulação, confirmando a presença de compostos no interior das leveduras. A estabilidade dos compostos durante o armazenamento foi maior para os compostos fenólicos em comparação com os carotenóides. A liberação dos compostos incorporados em leveduras foi avaliada durante a digestão gastrointestinal *in vitro* e as micropartículas liberaram gradativamente os compostos, protegendo-os das condições gástricas e liberando-os em maior teor na fase intestinal, o que tem efeito

positivo para a bioacessibilidade dos compostos. As leveduras enriquecidas foram utilizadas como potencial corante natural em iogurtes e conferiram cor ao produto. Os iogurtes enriquecidos foram bem aceitos pelos consumidores. Além de propor a utilização de coprodutos industriais, este trabalho contribui com avanços científicos ao abordar a utilização de leveduras como veículos inovadores e de base biológica para incorporação de diferentes compostos e trazer novas perspectivas para a utilização de leveduras enriquecidas.

Palavras-chave: *spray-drying*, compostos fenólicos, carotenoides, coprodutos, biossorção, *Saccharomyces cerevisiae*

ABSTRACT

RUBIO, F. T. V. **Utilization of brewer's spent yeast as a bio-vehicle for incorporation and protection of bioactive compounds.** 2021. 189 p. PhD thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

The disposal of wastes and byproducts from agriculture and food industry is undoubtedly of a great concern, especially because these materials could represent valuable sources of bioactive compounds, dietary fibers and nutrients, which can be reused in the production of novel additives or products. In this context, grape pomaces, jabuticaba byproducts, pumpkin peels and yeasts are examples of byproducts that present potential nutritional value and were used in this work. Grape pomaces and byproducts of jabuticaba pulping are rich in phenolic compounds, such as anthocyanins, pumpkin peels represent a carotenoid-rich source and yeasts contain proteins, complex B vitamins and carbohydrates and have been used for food applications. The main objective of this work was taking advantage of the pre-formed capsule morphology of yeasts *Saccharomyces cerevisiae* to incorporate bioactive compounds using the spray drying technology, associating the nutritional value of yeast with the health-benefit properties of the bioactive compounds. In general, yeast-based microcapsules produced in this work presented low values of water activity, moisture content and hygroscopicity, assuring a physical and microbiological stability for the powders. MIR-ATR analysis showed significant differences between yeasts before and after encapsulation of bioactives, suggesting the yeast loading. Confocal laser scanning microscopy helped to understand the distribution of the bioactives all over the cell after encapsulation, confirming the presence of compounds inside the yeasts. Compounds stability during storage was higher for phenolic compounds in comparison to carotenoids. The release of the compounds incorporated in yeasts was evaluated during an *in vitro* gastrointestinal digestion and the microparticles released gradually the compounds, protecting them from gastric conditions and releasing them in higher content in the intestinal phase, which has a positive effect for the compounds bioaccessibility. Enriched yeasts were used as potential natural colorant in yogurts and conferred color to the product. Enriched yogurts were well accepted by consumers. In addition to proposing the

utilization of industrial byproducts, this work contributes to scientific advances by addressing the use of yeasts as innovative and bio-based vehicles for incorporation of different compounds and approaching new perspectives for enriched yeast utilization.

Keywords: spray-drying, phenolic compounds, carotenoids, byproducts, biosorption, *Saccharomyces cerevisiae*

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SUMMARY

1 INTRODUCTION

Over the last decades, within the large field of food science and technology, there is an emergent and growing interest in the valorization of bioresidues (MARTINS; FERREIRA, 2017). Industrial and agricultural sectors produce large amounts of post-harvest losses and processing byproducts and wastes, representing a significant disposal problem for the industry (COELHO et al., 2020). These residues have an important role in the production of animal feed and also in the development of novel sustainable functional foods since they may present bioactive compounds and nutritional value (COMUNIAN; SILVA; SOUZA, 2021). Several studies on fruit and vegetable byproducts, in the form of peels, seeds, flower, leaf, stem, pomace and extracts, have found a wide variety of bioactive compounds, such as phenolic acids, flavonoids, anthocyanins, carotenoids and vitamin C. However, their common instability under normal food processing and storage conditions limits their use (MARCILLO-PARRA et al., 2021).

To overcome such limitation, microencapsulation is a technological solution that optimizes the preservation of active ingredients in raw materials and in foods during processing and storage. The process involves the entrapment of the functional components within one or more classes of carrier material to produce a microparticle, typically with a diameter in the microns scale (YE; GEORGES; SELOMULYA, 2018). Among the encapsulation techniques, spray drying is a low-cost production technology commonly used on an industrial scale and, compared to other methodologies, it has attractive advantages of producing microparticles in a relatively simple and continuous operation (BAKRY et al., 2016). In addition, spray drying is appropriate for heat-sensitive food ingredients due to its short processing time (ETZBACH et al., 2020). The water removal by spray drying helps to obtain a product with specific functional properties, avoids many of the risk of chemical and biological degradation and reduces the total storage and transport costs (BAKRY et al., 2016).

Extracts from vegetable and fruit sources may present low glass transition temperature, along with the presence of sugars, and the conversion of these extracts into powders is often accompanied by problems of low yield and stickiness due to the hygroscopicity and thermoplasticity of the products promoted by the humidity and temperature of the drying air. Therefore, spray drying technology frequently requires the addition of carrier agents to increase the glass transition temperature of the feed

(ETZBACH et al., 2020). The choice of a suitable wall material for compounds encapsulation is of great importance, once the encapsulant agent plays a vital role during the process of encapsulation, acting as a physical barrier, extending the shelf life of the encapsulate, improving the miscibility and offering a controlled release of the core material (FU et al., 2021). In food industry, several wall materials are applied, such as proteins, lipids, starches, phospholipids, waxes and cells (DADKHODAZADE et al., 2021). However, the cost and commercial availability of certain kinds of materials may be limiting for their utilization.

Saccharomyces cerevisiae yeast cells and its cell wall compounds have been currently studied as very promising carrier materials for incorporation of bioactive compounds (NGUYEN et al., 2018; YOUNG; DEA; NITIN, 2017), probiotics (MOKHTARI et al., 2017; MOKHTARI; JAFARI; KHOMEIRI, 2017), oils (FU et al., 2021; KAVOSI et al., 2018) and flavors (ERRENST; PETERMANN; KILZER, 2021; SULTANA et al., 2017). The methods for incorporation of compounds using yeasts are relatively simple and does not require any additives. Another advantage in using yeasts for encapsulation is that the yeast cell membrane can act as a liposome for the encapsulation of both hydrophobic and hydrophilic molecules (FU et al., 2021). There are several works reporting that enriched yeasts *Saccharomyces cerevisiae* act as a delivery system for incorporated compounds, showing a protective action during *in vitro* gastrointestinal digestion and increasing their bioaccessibility (FU et al., 2021; RIBEIRO et al., 2021; RUBIO et al., 2017). Moreover, yeasts are the second major waste from brewery process (FERREIRA et al., 2010), representing an underutilized byproduct of the brewing industry (JAEGER et al., 2020), and can be recovered as a low-cost material abundantly available. Besides the well-known utilization of yeasts in bakery and brewing industries, *Saccharomyces cerevisiae* is generally recognized as safe (GRAS) and it is already used in human food formulations (DADKHODAZADE et al., 2021), which facilitates their application.

Therefore, the main objective of this work was the use of brewer's spent yeast (BSY), a prevalent byproduct of the brewing industry, as a source of *Saccharomyces cerevisiae*, to incorporate bioactive compounds, associating the nutritional value of yeast with the health-benefit properties of the bioactive compounds.

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2 CHAPTER 1 – VALORIZATION OF INDUSTRIAL BYPRODUCTS BY ASSOCIATING WASTE BREWERY YEASTS AS A BIO-VEHICLE AND BIOACTIVE COMPOUNDS

2 CHAPTER 1 – VALORIZATION OF INDUSTRIAL BYPRODUCTS BY ASSOCIATING WASTE BREWERY YEASTS AS A BIO-VEHICLE AND BIOACTIVE COMPOUNDS

2.1 INDUSTRIAL BYPRODUCTS

2.1.1 Grape pomace

Grapes are edible berries and represent a popular crop all over the world, for fresh consumption, for pressing and utilization in wine production (SIROHI et al., 2020; TSALI; GOULA, 2018) or for the production of several other products, such as jam, juice, jelly, vinegar and seed oil (KANDYLIS; DIMITRELLOU; MOSCHAKIS, 2021). The main categories of grapes are three, namely wine grapes, table grapes and dried grapes (KANDYLIS; DIMITRELLOU; MOSCHAKIS, 2021). In 2018, the world production of grapes was 77.8 million tons, of which 57% were wine grapes, 36% were table grapes and 7% dried grapes (OIV, 2019). In Brazil, the wine production is also of enormous socioeconomic importance for the country. According to Embrapa (Brazilian Agricultural Research Corporation), in 2019, 1,445,705 tons of grapes were produced and Rio Grande do Sul was responsible for about 90% of the national production of grapes for processing. Around 698 thousand tons of produced grapes were destined for the production of juice and wine (PEREIRA et al., 2020).

Considering that grapes represent a significant culture and winemaking is really important economically, the quantity of byproducts generated and their destination are of a major concern. Although the amount of residue generated depends on the grape cultivar and the pressing and fermentation processes, residues represent, in general, 20-30% of the original grape weight (BERES et al., 2017). After winemaking, more specifically, it is estimated that about 30 kg of residues are generated for each 100 L of wine produced (BERES et al., 2019). The resultant solid byproduct generated from mechanical pressing of grapes is denominated grape pomace and consists of seeds, some parts of stems and mainly peels (SIROHI et al., 2020). Seeds are essentially constituted by fiber, essential oil, protein and phenolic compounds, such as tannins. Peels are rich in anthocyanins and stems represent a great source of tannic compounds with high nutraceutical and pharmacological potential (ANDRADE et al., 2019). Grape pomace is usually used for animal feed (BERES et al., 2019) and also can be recovered for the production of

compost with high added value (BARBOSA et al., 2018). Furthermore, it is reported the utilization of grape pomace for the production of edible acids (tartaric, malic and citric acids), ethanol, dietary fiber, grape seed oil and, not less important, grape pomace is admitted as a recognized source of phenolic compounds, including flavonoids, anthocyanins and phenolic acids (ILYAS et al., 2021).

During grapes processing for winemaking, pulps, skins, seeds and stems are usually in contact with the fermenting wine for several days and are subjected to a mild and prolonged ethanolic extraction, which provides red wines with high contents of phenolic compounds. Nevertheless, the grape pomace still contains high levels of phenolic compounds, with a higher content retained in the skin matrix (PINELO; ARNOUS; MEYER, 2006). It is reported that more than 70% of grape polyphenols remain in the pomace and it brings an opportunity for recovering them for nobler uses, such as natural colorants, preservatives and antioxidants in food products (BERES et al., 2019).

Phenolic compounds are secondary metabolites widely distributed in different parts of grapes, but mainly found in grape skins. The content of phenolic compounds may vary depending on the grape variety, grape maturity, soil composition and type, climate, geographic origin, sunlight exposure, cultivation practices and exposure to diseases (COSME; PINTO; VILELA, 2018). Anthocyanins are the main chemical components responsible for red and purple pigments in red grapes and wines (SETFORD et al., 2017) and are considered as potential substitute for synthetic pigments owing to their attractive color and water solubility, which make the compound interesting for incorporation into a variety of food systems (ROCKENBACH et al., 2011). Bioactive compounds are associated with prevention of several pathophysiological processes, including cardiovascular and neurodegenerative diseases, cancers and diabetes (SALEHI et al., 2019). The most health-protective biomolecules from grapes are proanthocyanidins, anthocyanins and other flavonoids, hydroxycinnamates and stilbenes (resveratrol), that possess antioxidant, antimicrobial, anti-cancer, anti-inflammatory properties and inhibit lipid peroxidation (SALEHI et al., 2019).

Therefore, considering the importance of the grape pomace, extensive efforts have to be devoted to explore the extraction of phenolic compounds and apply them as dietary supplements or food colorants.

2.1.2 Jabuticaba byproducts

Jabuticaba [*Myrciaria cauliflora* (Mart.) O. Berg] is a dark-colored Brazilian berry belonging to *Myrtaceae* family and native from Brazilian Atlantic rainforest (CARVALHO; CONTE-JUNIOR, 2021; INADA et al., 2021; WU; LONG; KENNELLY, 2013) but also widely growing in the whole country (ALBUQUERQUE et al., 2020). Flowers born directly on the trunks and branches of the trees and fruits mature rapidly within 40 and 60 days (WU; LONG; KENNELLY, 2013). Jabuticaba presents a white and gelatinous pulp with sweet and astringent taste due to its high content of sugars and acids, appreciated for *in natura* consumption or as fruit pulp preparation (ALBUQUERQUE et al., 2020). The pericarp color ranging from red to dark-purple and black indicates a high content of phenolic compounds, especially anthocyanins and ellagitannins (INADA et al., 2021).

Due to its extreme perishability, jabuticaba has a difficult commercialization and short stability after harvesting, mainly as a consequence of the high content of water, sugars and other constituents (TARONE et al., 2021). The fruit spoils easily, leading to rapid changes in appearance arising from the loss of water, physiological and microbiological deterioration and pulp fermentation (WU; LONG; KENNELLY, 2013). For this reason, jabuticaba is usually processed into a wide range of products, such as jams, liquors, dairy and alcoholic beverages, ice creams, syrups and juices. Nevertheless, the jabuticaba processing chain generates considerable amounts of waste byproducts, consisting of peel and seeds, representing up to 50% of the total processed volume (TARONE et al., 2021). However, peels and seeds are the fruit fractions where phenolic compounds are mainly concentrated (INADA et al., 2021), which makes interesting the recovery of those byproducts to explore the bioactive compounds.

Anthocyanins have been described as the most abundant phenolic compound found in jabuticaba, with contents of up to 3222 mg/100 g of peel. Cyanidin-3-O-glucoside and delphinidin-3-O-glucoside were reported as the main anthocyanins found in jabuticaba, following the presence of other anthocyanins present in lower content, such pelargonidin-3-O-glucoside, peonidin-3-O-glucoside, peonidin, cyanidin, delphinidin and malvidin (INADA et al., 2021). In addition, gallic acid, flavonols derived from quercetin and myricetin and derivatives of ellagic acid can be detected in jabuticaba peels (NEVES et al., 2021).

Interesting *in vitro* and *in vivo* studies have reported beneficial health effects of jabuticaba, mainly associated with its composition of bioactive compounds. Among the effects, authors reported antimicrobial (ALBUQUERQUE et al., 2020; FIDELIS et al., 2020), antitumor (ALBUQUERQUE et al., 2020; FIDELIS et al., 2020; HOLKEM et al., 2021) and anti-inflammatory activities (ALBUQUERQUE et al., 2020; LAMAS et al., 2018; ZHAO et al., 2019) and antioxidant properties (DA SILVA-MAIA et al., 2019; FIDELIS et al., 2020; MANNINO et al., 2020). Therefore, considering the significant diversity of bioactive compounds found in jabuticaba and their health effects, the development of ingredients and high-value added products from jabuticaba byproducts can contribute to the diversification of food products, nutrients and functional compounds consumed by the population (FIDELIS et al., 2021).

2.1.3 Pumpkin peels

Pumpkins are vegetables belonging to the genus *Cucurbita* and family *Cucurbitaceae*, highlighting the species *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata* and *Cucurbita mixta*, which are classified according to the texture and shape of their stems (ASSOUS; SAAD; DYAB, 2014; SHI et al., 2010, 2013). Different species of pumpkins are widely cultivated and consumed worldwide (SONG et al., 2017). The species *Cucurbita moschata*, more specifically, has been cultivated for more than 5 thousand years throughout Latin America, according to archaeological records (BARBIERI; STUMPF, 2008).

The world production of pumpkins in 2016 was 26,486,616 tons, 857,150 tons only in South America (FAO, 2016). In Brazil, according to the last agricultural census of the Brazilian Institute of Geography and Statistics (“Instituto Brasileiro de Geografia e Estatística” - IBGE), in 2006, the Southeast region was the largest producer of pumpkins in the country, accounting for 53% of production, followed by the Northeast (24%), South (17%), Midwest (3%) and North (3%).

Regarding the use of pumpkin in human food, both fruits and seeds are appreciated worldwide, depending on regional customs. Pumpkin is commonly consumed cooked, roasted or as a component in salads, jams, sweets, soups and pies (BARBIERI; STUMPF, 2008; KONOPACKA et al., 2010). The species *Cucurbita moschata* is, along with *Cucurbita maxima*, the most used in the production of sweets (BARBIERI; STUMPF, 2008). Dehydrated pumpkin slices are also consumed as

snacks, as well as pumpkin flour can be used in the preparation of breakfast cereals, dietary foods, formulated for people with special needs, and traditional products such as cookies and cakes (SEREMET (CECLU) et al., 2016).

The flesh color of different pumpkin varieties, which varies from intense yellow to orange, reveals the presence of important bioactive compounds: carotenoids such as α-carotene, β-cryptoxanthin, lutein, zeaxanthin and, mainly, β-carotene. The importance of this last one is because it is a precursor of vitamin A and, consequently, it is a possible supplement for populations in areas where vitamin A deficiency - recurrent mainly in women and children - is a serious public health problem (DE CARVALHO et al., 2012), identified as a common cause of infant mortality, blindness and impaired embryonic development (SEO et al., 2005). The polysaccharides present in the pumpkin also show important health benefits, since they are able to decrease the levels of total cholesterol and low-density lipoproteins (LDL), while they increase the levels of high-density lipoproteins (HDL) and are related to diabetes control (LIU et al., 2018). In addition, they demonstrated the ability to increase plasma insulin, in a study conducted with normal and diabetic rats (XIA; WANG, 2006).

In addition to the pumpkin flesh, the seeds are also commonly accepted in food, mainly as snack food, and can be consumed mainly roasted, salted or not, or used as a culinary ingredient in the preparation of breads, cereals, salads and cakes. The seeds were, for many years, used for pharmaceutical purposes, acting as a vermifuge. The oil extracted from pumpkin seeds also gains particular attention not only because it is an edible oil, but also because it is considered nutraceutical (NAWIRSKA-OLSZAŃSKA et al., 2013; XANTHOPOULOU et al., 2009). Recent studies report the presence of vitamin E, carotenoids (DURANTE et al., 2014), palmitic, stearic, oleic and linoleic fatty acids (POTOČNIK; KOŠIR, 2017) and phenolic compounds, such as vanillic, caffeic, trans-cinnamic and coumaric acids (POTOČNIK; RAK CIZEJ; KOŠIR, 2018).

Despite the use of most pumpkin components, the main problem resulting from vegetable processing still is the generation of waste, especially peels. The use of these byproducts can be an alternative of great interest to some industries, as it contributes to the production of new products and minimizes the generation of agro-industrial waste. Song et al. (2017) report that, although pumpkin peels are not edible, they present α-carotene, zeaxanthin and β-carotene, which represent a

potential application in the food industry. Another use for the peels, reported in the literature, is the obtention of pectin, of great technological importance as a thickening, gelling and stabilizing agent (HAMED; ELKHEDIR; MUSTAFA, 2017; JUN et al., 2006). Thus, pumpkin peels can be characterized as a low-cost and little-explored source of compounds of great nutritional and technological importance. In addition, the application of pumpkin waste is important to convert abundant material into value-added products, presenting benefits for consumers and the food industry (SHI et al., 2013).

2.2 BIOACTIVE COMPOUNDS

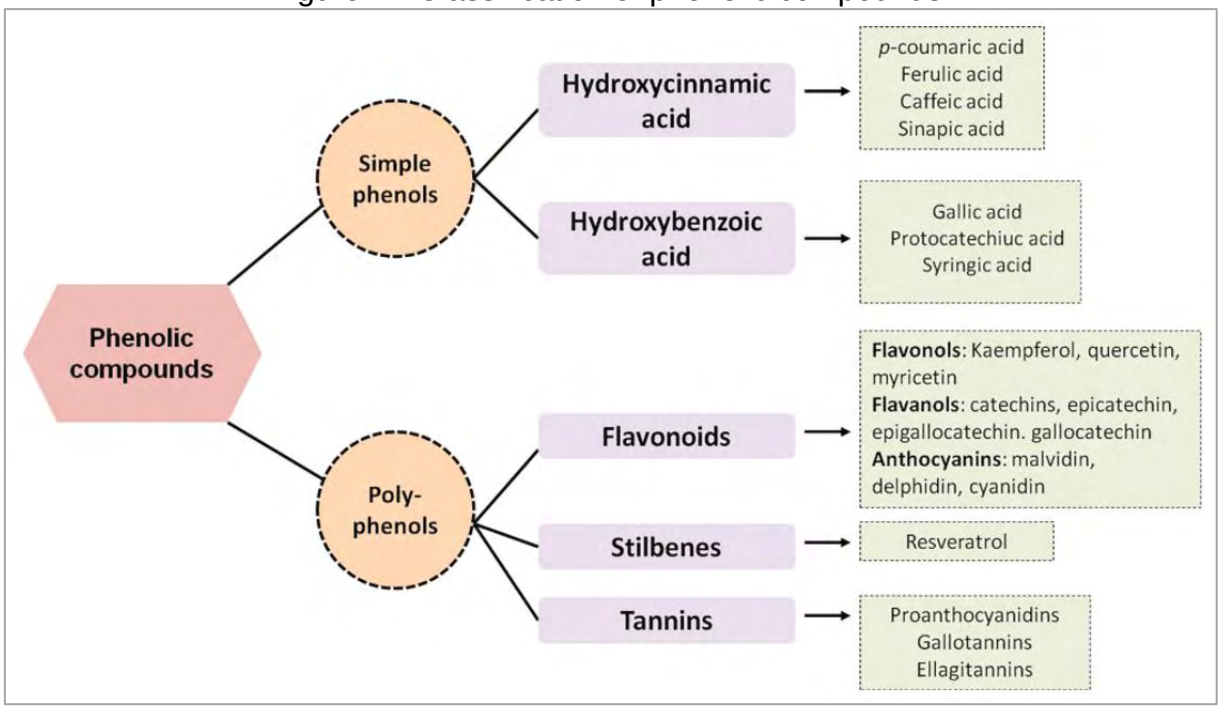
2.2.1 Phenolic compounds

Phenolic compounds are one of the most important classes of bioactive compounds which refer to thousands of functional molecules from several plant species (GARAVAND et al., 2021). They represent the most common secondary plant metabolite synthesized by plants during normal metabolic processes or as a defense mechanism, responding against certain environmental conditions including temperature, UV-radiation, infections and wounds (ALARA; ABDURAHMAN; UKAEGBU, 2021). These compounds are referred as secondary metabolites since they are not directly correlated with growth and development functions of the plant tissue, and they are normally retained in specific tissues and organs and at particular stages of development of the plant (DIAS et al., 2016). Phenolic compounds can be found mainly in fruits, legumes, vegetables, tea, wine and coffee. They are responsible for the bitterness of fruits due to their interaction with salivary glycoprotein and can also add color to many fruits and vegetables (ALARA; ABDURAHMAN; UKAEGBU, 2021). There are many different phenolic compounds in terms of structure and, by consequence, they exert several different biological activities depending also on the concentration in which they are consumed (DIAS et al., 2016).

Regarding to the structure, phenolic compounds contain an aromatic ring bearing one or more hydroxyl substituents and range from elementary single-phenolic molecules to highly polymerized compounds (BERES et al., 2017). They are classified in several subclasses, as shown in Figure 1, being flavonoids and tannins

two of the most common and largest classes (ANDRADE et al., 2019).

Figure 1 - Classification of phenolic compounds



Reference: Extracted from Sirohi et al. (2020)

Hydroxycinnamic and hydroxybenzoic acids are classified as phenolic acids, represent classes of phenolic compounds and are characterized by the presence of one functional carboxylic acid group. Hydroxycinnamic acids are mostly common in foods compared to hydroxybenzoic acids (SIROHI et al., 2020). The most popular hydroxycinnamates are ferulic acids, normally covalently linked to plant cell walls and mostly found in insoluble form in cereal brans. Another example is caffeic acid, which is the main phenolic of coffee and is found esterified with chlorogenic acid. Common hydroxybenzoic acids include gallic, vanillic, ellagic and syringic acids (GARAVAND et al., 2021).

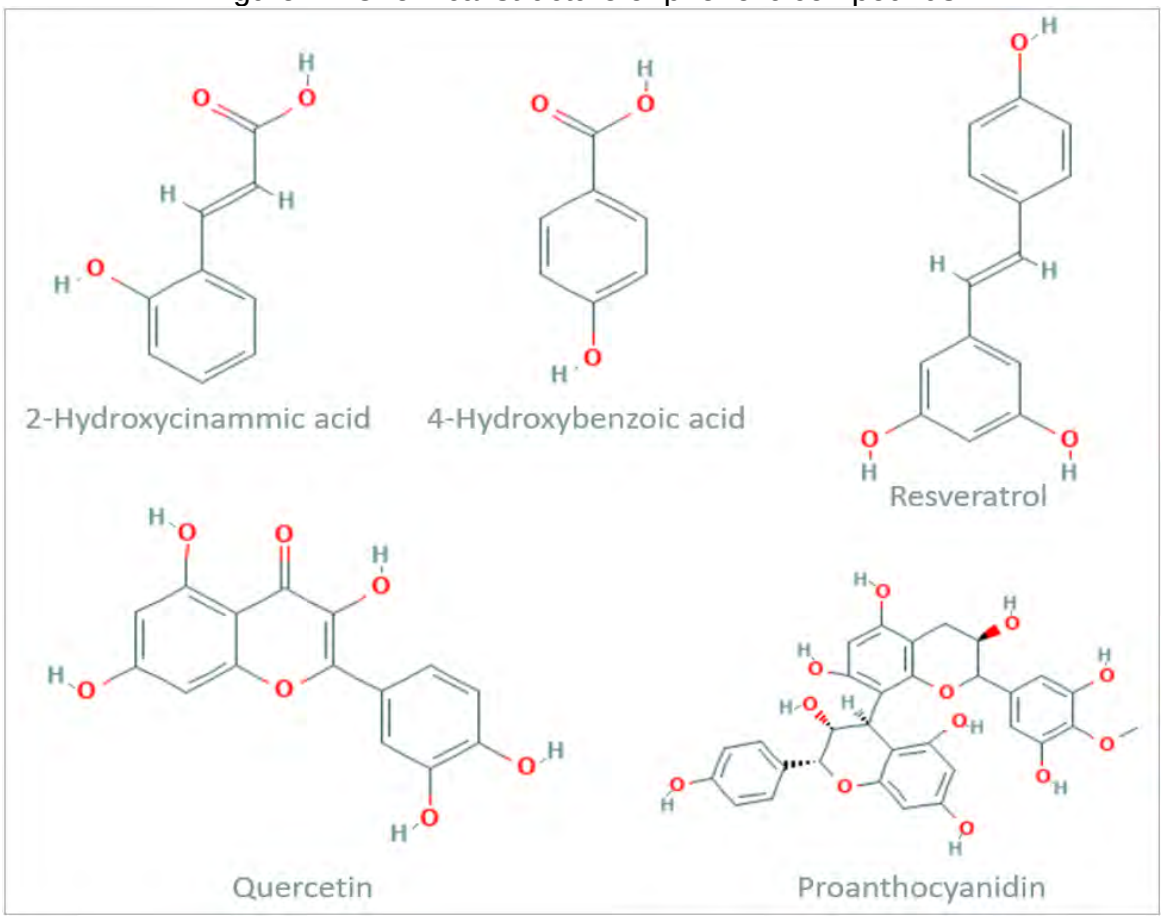
Flavonoids are the major polyphenols in human diet. Structurally, flavonoids are composed by a flavan nucleus with 15 carbon atoms arranged in 3 rings. There are seven subgroups of flavonoids: flavones, flavanones, flavanols (or flavan-3-ols), flavonols, anthocyanins, isoflavones and chalcones. They differ according to the quantity and arrangement of the hydroxyl (-OH) groups in addition to the pattern and degree of hydroxylation, prenylation, glycosylation or methoxylation (ALARA; ABDURAHMAN; UKAEGBU, 2021; GARAVAND et al., 2021). Flavonoids present in food are typically glycosylated with glucose or rhamnose, and rarely with galactose,

arabinose, xylose and glucuronic acid (GARAVAND et al., 2021).

Stilbenes are characterized by two benzene rings and can be classified into monomeric and oligomeric stilbenes. They show limited distribution among plant species (CORSO et al., 2020). One of the most important stilbene is resveratrol, which is found in grapes, wine and in grape pomaces, and its concentration varies according to the maturation stage and fruit variety (BERES et al., 2017).

Tannins can be subdivided into hydrolysable and condensed tannins. Hydrolysable tannins contain a central glucose core in an esterified form with gallic acid. Condensed tannins are oligomers or polymers of flavan-3-ol bonded via interflavan carbon bond. They are called proanthocyanidins because when heated in acid alcohol solution, they can be degraded into anthocyanidins via acid-catalyzed oxidation process (ALARA; ABDURAHMAN; UKAEGBU, 2021). As an example, cinnamon and grape seed are great sources of this kind of compound (HOLKEM; FAVARO-TRINDADE; LACROIX, 2020). Figure 2 shows the chemical structure of different classes of phenolic compounds.

Figure 2 - Chemical structure of phenolic compounds



Reference: PubChem

Ageing-related degenerative diseases, such as cancers, metabolic syndromes and neurodegenerative disorders represent a growing economic burden and global public health concern. Ageing and ageing-related diseases are associated with a progressive accumulation of damaged macromolecules (particularly proteins) and cellular organelles over life and many factors can contribute to the acceleration of these processes, such as genetic, metabolic and environmental (lifestyle, including diet, smoking, stress, sedentarism) factors, whereas this latter may be fully controlled. Human diet, for instance, can be easily driven towards bioactive compounds-rich foods intake (ARRUDA et al., 2020). In this context, the potential therapeutic effects of phenolic compounds in preventing several diseases have attracted great attention from the scientific community. The biological properties of phenolic compounds include antioxidant and anti-inflammatory activities, reduction of oxidative stress, risk of obesity and metabolic syndrome-related disorders. Their biological properties are mainly attributed to sequestering or inhibiting reactive oxygen and nitrogen species and transferring electrons to free radicals. Phenolic compounds could act as free radical scavengers and terminate the radical chain reactions during the oxidation of unsaturated fats (VARGAS-RAMELLA et al., 2021).

Nevertheless, the incorporation of phenolics into food products has to overcome some technological challenges. Once extracted from their natural sources, alongside with the poor stability, phenolic compounds are sensitive to pH variation, oxygen, heat, light, alkalinity and enzymatic activities, leading to a low bioaccessibility (AIZPURUA-OLAIZOLA et al., 2016; GARAVAND et al., 2021). The health-promoting potentials of phenolics can be limited due to their poor water dispersibility, chemical and enzymatic breakdown, instability under alkaline conditions, volatile nature, low intestinal permeability and poor metabolism digestion. Therefore, it is of great importance to provide solutions to maintain the integrity of these compounds (GARAVAND et al., 2021).

2.2.1.1 Anthocyanins

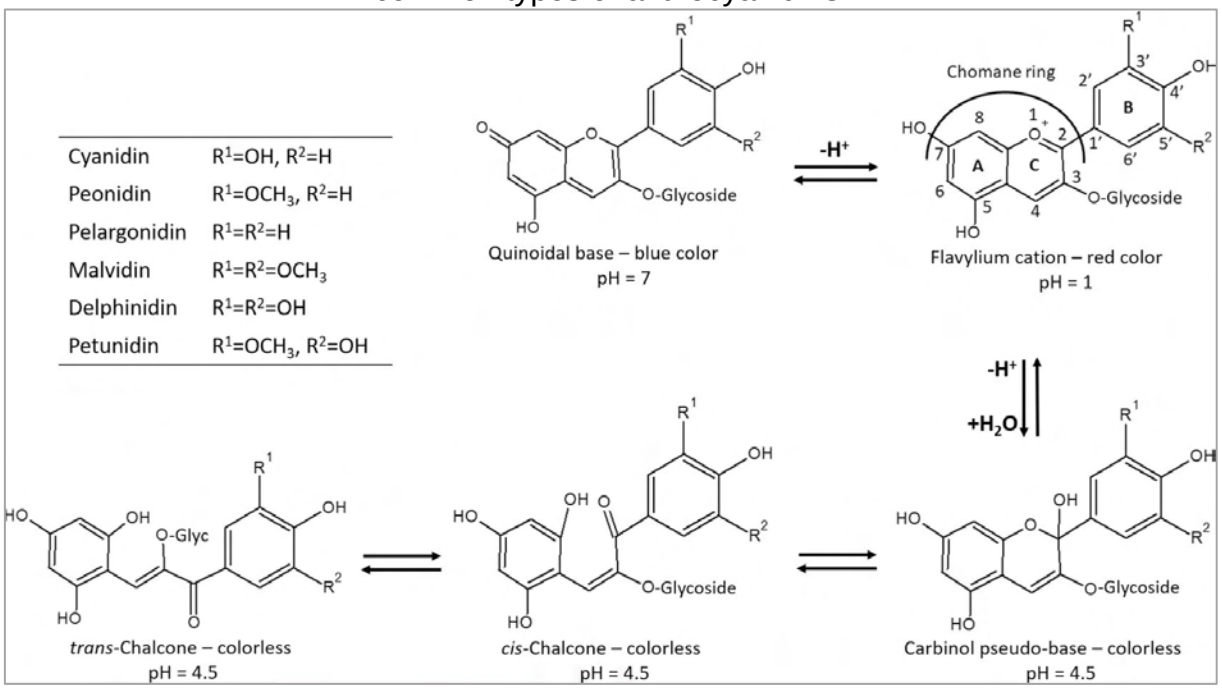
Anthocyanins are the commonest studied flavonoids present in several foods acting as water-soluble phytopigments, accounting for the purple, red, pink, orange or cyan colors and blue hues, depending on the environmental pH (ALARA; ABDURAHMAN; UKAEGBU, 2021; TARONE; CAZARIN; MAROSTICA JUNIOR,

2020). These pigments can be found in most flowers and fruits, but are also present in all types of vascular plants and on any plant tissue, which contributes to their wide distribution in the human diet through plant-based foods. Red and black berries and dark-colored vegetables, such as red onion, red radish, black bean, eggplant, purple corn, red cabbage and purple sweet potato are examples of edible sources of anthocyanins (TARONE; CAZARIN; MAROSTICA JUNIOR, 2020). The production of this pigment is highly influenced by the amount of light incident on plant tissues, once these compounds act as light adsorbers and, thus, protect the cells from adverse effects. Its production is also achieved by other type of physical elicitation, such as temperature and pH, but also through precursor feeding (DIAS et al., 2016).

Structurally, anthocyanins are composed of two aromatic rings (indicated in Figure 3 as A and B) linked by three carbons by means of an oxygenated heterocycle (structure C). The first aromatic ring (A) linked to this oxygenated heterocycle forms a chromane ring, which is associated with the aromatic properties of anthocyanins. Anthocyanins are found linked to one or several sugars, and the presence of these sugars and hydroxyl groups is responsible for the solubility of these compounds in water, ethanol and, in minor part, in methanol (TARONE; CAZARIN; MAROSTICA JUNIOR, 2020). When anthocyanins are not conjugated with sugars, they represent anthocyanidins, that is, anthocyanidins are the sugar-free counterparts of anthocyanins. More than 20 anthocyanidins are known, but only six are prevalent in plants: cyanidin, peonidin, pelargonidin, malvidin, delphinidin and petunidin, which differ according to the radicals linked to the basic structure (BELWAL et al., 2020).

Anthocyanins colors can vary from the blue when the B ring possesses more hydroxyl groups to the red when it possesses more methoxyl groups (TARONE; CAZARIN; MAROSTICA JUNIOR, 2020). For example, cyanidin, delphinidin and pelargonidin show red to magenta, violet to blue and orange to red color hues, respectively (BELWAL et al., 2020). Under pH conditions existing in plants, food and the digestive tract (pH 2 to 8), anthocyanins occur in a mixture of colored and colorless forms in equilibrium through proton transfer, hydration, dehydration and isomerization reactions. Besides the influence of these chemical species on color expression and the stability of the anthocyanins, this understanding is crucial for interpreting their interactions with other food components and biological molecules (such as digestive enzymes or biliary acids) or the ability to cross membranes, aspects that are still not well understood and determine their effects on the organism (ALVAREZ-SUAREZ et al., 2021).

Figure 3 - Anthocyanin skeleton, colored and colorless structures and the six most common types of anthocyanidins



Reference: Tarone; Cazarin; Marostica Junior (2020)

Research involving anthocyanins sources has steadily grown whenever evidence about their potential benefits in human health has accumulated, and novel applications, such as colorants or putative bioactives for the food, pharmaceutical and cosmetic industries, have been developed (ALVAREZ-SUAREZ et al., 2021). However, the anthocyanins low stability and the susceptibility to chemical transformations due to the action of agents such as light, temperature, oxygen, pH, solvents and metallic ions, makes one of the greatest focus of investigations their stabilization for use as a natural colorant in food industry (BERES et al., 2017).

2.2.2 Carotenoids

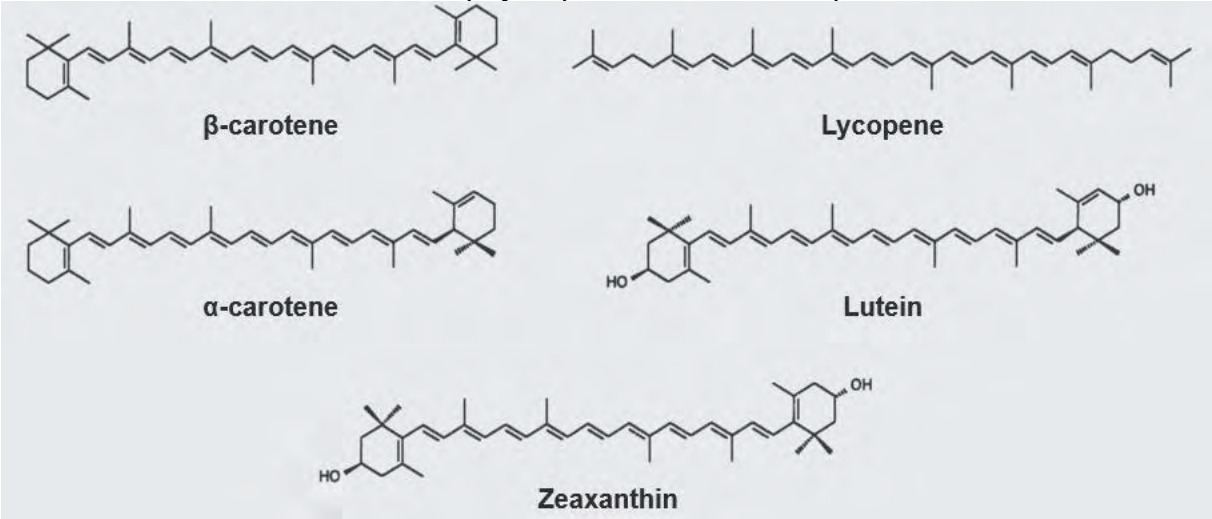
Carotenoids represent a class of fat-soluble bioactive compounds - naturally found in plants, algae and in photosynthetic bacteria - responsible for the color of a wide variety of foods, for example the colors yellow, orange and red, in various fruits and vegetables (AMORIM-CARRILHO et al., 2014; SAINI; KEUM, 2018). They are responsible, for example, for the yellow color in corn, the orange color in carrots and pumpkins and red color in tomatoes and watermelon (RODRIGUEZ-CONCEPCION et al., 2018). In photosynthetic organisms, carotenoids are responsible for protection

against photooxidation damage and, in non-phototrophic organisms, they protect during growth exposed to light and air (MAROVA et al., 2012). As plants are able to synthesize carotenoids, their composition is enriched with the presence of biosynthetic precursors, in addition to some compounds from which the main carotenoids are derived. In plants, carotenoids can be found esterified with fatty acids or not. Carotenoids can also be found in some animals, however, they are not biosynthesized, they are obtained through food and can accumulate, with or without any change in their structure (RIBEIRO et al., 2018; RODRIGUEZ-AMAYA, 2001; SAINI; KEUM, 2018).

Several pre- and post-harvest factors can influence the concentration and types of carotenoids present in food, depending, for example, on the genotype, the time of harvest, the cultivation method and climatic conditions during the development of the plant (SAINI; NILE; PARK, 2015). An important factor in the formation of carotenoids is the maturation or ripening of fruits and vegetables. When the food matures, chlorophyll - which often masks the color of carotenoids - decomposes and carotenogenesis increases, accompanied by the transformation of chloroplasts into chromoplast. The exception is when some fruits maintain their green color even after ripening, or when the color occurs mainly due to the presence of anthocyanins, thus, there is a decrease in the concentration of carotenoids during fruit ripening (RODRIGUEZ-AMAYA, 2001; SUN et al., 2017). In addition, different parts of the same food can have different concentrations and types of carotenoids (SAINI; KEUM, 2018). As an example, it is reported that the tissues - such as the peels - of fruits are generally richer in carotenoids, compared to the pulp (MARTINS; FERREIRA, 2017).

Regarding the chemical structure, carotenoids are considered isoprenoids, formed by several repeated units of groups of five carbons and by a polyunsaturated chain. Depending on the presence or absence of rings in their structure, they can be called cyclic or acyclic carotenoids, respectively. Still according to the chemical composition, carotenoids formed exclusively by carbon and hydrogen atoms are classified as carotenes and, when they have oxygenated groups, such as carboxyls and hydroxyls, they are known as xanthophylls (RODRIGUEZ-CONCEPCION et al., 2018). In Figure 4, the chemical structures of some of the main carotenoids found in food are represented.

Figure 4 - Chemical structure of carotenes (β-carotene, α-carotene e lycopene) and xanthophylls (lutein e zeaxanthin)



Reference: Extracted from Rodriguez-Amaya; Kimura (2004)

Most carotenoids are formed by eight units of C_5 isoprenoids, that is, they contain 40 carbon atoms (C_{40}), usually biosynthesized by the condensation of two C_{20} chains (SAINI; KEUM, 2018). Although C_{40} carotenoids are the most abundant in nature, it is also possible to find smaller (C_{30}) or larger (C_{45} or C_{50}) carotenoids. Furthermore, carotenoids can be cleaved and lose fragments of their polymeric chain, at the beginning or at the end of the molecule, generating chains called apocarotenoids (RODRIGUEZ-CONCEPCION et al., 2018), such as A vitamin (retinol), retinoic acid and bixin (SAINI; NILE; PARK, 2015). Carotenoids can also be found associated with other molecules, such as fatty acids, sugars or proteins (RODRIGUEZ-CONCEPCION et al., 2018).

Due to the presence of unsaturations in carotenoid molecules, both cis and trans isomers can be found. Natural carotenoids appear mostly in the trans configuration, which, in general, gives greater stability to the compounds. When carotenoids are exposed to light or heating, isomerization may occur and compounds that previously had a trans configuration may have their optical isomerism altered. However, some cis carotenoids can occur naturally, without interference from external conditions (RODRIGUEZ-CONCEPCION et al., 2018).

During the processing and storage of fruits and vegetables, carotenoid unsaturation makes them susceptible to isomerization and oxidation, and the consequence is the loss of color and biological activity and the formation of volatile compounds that cause desirable taste or not in some foods. Oxidation is the main

cause of large losses in the content of carotenoids and can occur due to the presence of oxygen, metals, enzymes, unsaturated lipids, prooxidants, exposure to light, severity of the treatment applied and storage conditions. The sequence of steps in oxidation begins with the formation of apocarotenoids, formation of low molecular weight compounds and, as a final consequence, the loss of color and biological activity. Each carotenoid has a different susceptibility to oxidation. Heating promotes trans-cis isomerization. Both reactions can occur during any stage of preparation, processing and storage of food (RODRIGUEZ-AMAYA, 2001).

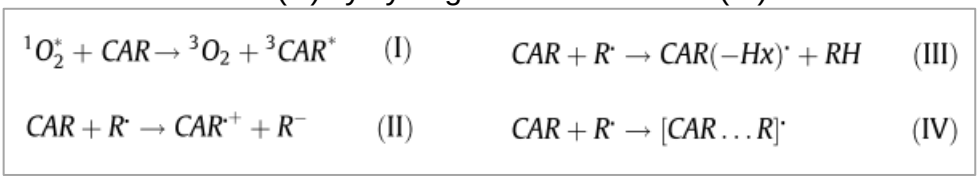
At least 40 of the 700 existing carotenoids are ingested in the human diet through fruits and vegetables, of which few have been extensively studied. Considering the importance of these species in reducing the risk of developing various disorders in human health, there is still a need to study new carotenoid molecules and relate them to their food sources and biological effects (MONEGO; DA ROSA; DO NASCIMENTO, 2017; RIBEIRO et al., 2018).

2.2.2.1 Importance of carotenoids

The coloring of foods plays an important role in consumer choice, preference and also as an indicator of sensory quality and, therefore, the use of colorants in the food industry is wide and continues to rise. The application of artificial pigments in foods, on the other hand, is a frequent concern because some are considered harmful to health. Thus, there is an increasing attempt to replace artificial sources of colors with natural pigments (BENMEZIANE et al., 2018). Carotenoids are the most widely used natural pigments in the food industry as functional ingredients or colorants (SHI et al., 2010), mainly β-carotene, lycopene, capsanthin, lutein and bixin, the best known in the food industry (JANISZEWSKA-TURAK, 2017). In addition to the importance of carotenoids from a technological point of view, there is growing interest in the search for natural sources of carotenoids and biotechnological processes for obtaining them for using as natural antioxidants, since they have a strong relationship with the reduction or prevention of chronic diseases, aging and pathologies in various stages (MAROVA et al., 2012), often related to oxidative stress, caused by the attack of free radicals on cells in the body. The antioxidant activity of carotenoids is precisely related to their ability to react with free radicals (SAMANTA; CHAUDHURI; DUTTA, 2016).

Carotenoid molecules interact with singlet oxygen primarily through direct energy transfer. Since the carotenoid molecule is not very reactive, it easily dissipates its excitation energy, quickly returning to its level of energy of fundamental state. The same molecule can repeat the process several times, which is of great advantage compared to other species with antiradical activity. Carotenoids can also scavenge free radicals simply by donating or obtaining unpaired electrons and, thus, they are able to prevent damages caused by oxidation or reduction of free radicals. Another mechanism that can occur between carotenoids and oxidizing agents is the donation of hydrogen by the bioactive compound and formation of adducts with the radicals, forming a single reaction product (MONEGO; DA ROSA; DO NASCIMENTO, 2017). The antioxidant efficacy of carotenoids in biological systems depends on their structure, sites of action, concentration and potential interaction with other carotenoids (NIRANJANA et al., 2015). Figure 5 shows the antioxidant mechanism of carotenoids.

Figure 5 - Reaction mechanisms of carotenoids with (I) singlet oxygen, (II) free radicals by electron donation and (III) by hydrogen abstraction and (IV) adduct formation



Where CAR represents the carotenoids, Hx stands for the abstracted hydrogen and [CAR…R]$^{\cdot}$ is the adduct formed.
Reference: Adapted from Monego; da Rosa; do Nascimento (2017)

In addition to the antioxidant activity, carotenoids are constantly related to a provitamin A function, decreased macular degeneration, improved cardiovascular function, maintenance of immune functions and limitation of abnormal cell growth, having a potent anti-cancer action (EGGERSDORFER; WYSS, 2018). Of all the carotenoids found in nature, approximately 50 are precursors of vitamin A (retinol) and, among them, the three most important in foods are β-carotene, α-carotene and β-cryptoxanthin (AMORIM-CARRILHO et al., 2014). The bioconversion efficiency of carotenoid to retinol depends directly on the source of the bioactive provitamin (KULCZYŃSKI et al., 2017). Vitamin A deficiency interferes with healthy human growth, development and visual adaptation to the dark, causing progressive blindness, increased infant morbidity and mortality and decreased immune response in children, lactating and pregnant (DE MOURA; MILOFF; BOY, 2015;

GEBREMESKEL et al., 2018). In the last 20 years, vitamin A supplementation has been introduced as a short-term intervention (DE MOURA; MILOFF; BOY, 2015) and the biofortification of some foods has also been studied. In Brazil, the interest in pumpkin biofortification is growing due to its significant content of β-carotene (DE CARVALHO et al., 2012).

Studies reveal the role of carotenoids in the antiproliferative activity of cultures of cancer cells in human colon (CASTRO-PUYANA et al., 2017) and the reduction of the viability of cervical and lung cancer cells (SAINI et al., 2018). Carotenoids also can prevent or reduce anti-inflammatory process of tissues when associated with lipoproteins (HOWARD; THURNHAM, 2017) and, as inflammatory inhibitors, carotenoids can decrease the risk of development of atherosclerosis (XU et al., 2012). Therefore, it is evident the importance of studies that make possible the extraction of carotenoids, mainly of byproducts that would no longer be used, and there are still many possible applications to be explored.

2.3 YEASTS AND THEIR UTILIZATION AS BIO-VEHICLE

Within the great area of food science and technology, the valorization of bio-wastes is an area that has been standing out. Large amounts of waste and byproducts are generated daily and most of them represent an important source of bioactive substances, with promising possibilities for food and pharmaceutical applications (MARTINS; FERREIRA, 2017). The byproducts of the fermentation industry, for example, are generated in large quantities and, therefore, have received increasing attention mainly for an economic reason (WANG; CHEN, 2006). Beer production involves the generation of several residues, such as malt, hops and yeasts, which represent the second largest byproduct in this sector. Brewer’s spent yeast is also known as residual yeast and is a prevalent byproduct of the brewing industry, created when the yeast used in fermentations is no longer useful. The estimative is that 15 to 18 tons of residual yeast are produced per 10,000 hL of finished beer (JAEGER et al., 2020).

Yeasts are unicellular fungus and eukaryotic microorganisms, with an organized nucleus, surrounded by a membrane, and several organelles (WANG; CHEN, 2009). Industries use yeasts as the main microorganism in the manufacture of biotechnological products, as they exceed the production capacity and yield

compared to any other group of industrial microorganisms (PEREYRA et al., 2018). In fermentative processes, yeasts are responsible for converting sugars from grains to alcohol and carbon dioxide. They also produce compounds such as organic acids, esters, aldehydes and ketones, which play an essential role in the sensory profile of beverages (FERREIRA et al., 2010). At the end of the fermentation, yeasts are separated from the bulk by a process known as flocculation. Good flocculation properties are essential for an industrial strain, once yeasts can be reused multiple times during the brewing process. A small amount of yeast from the previous fermentation is used to start the next fermentation in a process known as re-pitching (JAEGER et al., 2020).

Yeasts can also have valuable application as a raw material for different uses: in biotechnology (in other fermentative processes for the production of ethanol), as a substrate for the cultivation of microorganisms or simply in the extraction of compounds (FERREIRA et al., 2010). Among the yeast species, some of them are more studied, including *Saccharomyces pastorianus* (MARSON et al., 2020; VÉLEZ-ERAZO et al., 2021), *Saccharomyces cerevisiae* (baker's and brewer's yeast), *Saccharomyces bayanus* (wine yeast), *Candida utilis*, *Kluyveromyces fragilis* (dairy yeast), *Torulopsis lipofera*, *Endomyces vernalis* and *Cryptococcus curvatus* (oleaginous yeast) (MOGHADAM; KHAMENEH; FAZLY BAZZAZ, 2019).

Saccharomyces cerevisiae, specifically, is used extensively in biotechnological processes because it is generally recognized as safe (GRAS) and also because it is suitable for large-scale operations. Due to several organelles, unlike prokaryotic microorganisms, yeast offers different means and compartments for the biosynthesis of materials. Furthermore, it is tolerant to extreme industrial conditions (LIAN; MISHRA; ZHAO, 2018). Yeast cell walls are mainly composed of glycoproteins and polysaccharides, including glucan and chitin. Chitin provides a tensile strength and plays an important role in the structural integrity of the cell wall and glucan, which is another structural polysaccharide, accounts to 50-60% of the dry weight of the cell wall (MOGHADAM; KHAMENEH; FAZLY BAZZAZ, 2019).

Brewer's spent yeasts are mainly used for animal feed and represent an excellent source of protein for swine and ruminant (FERREIRA et al., 2010). However, despite their limited utilization, residual yeasts have an important nutritional composition, since they are composed of proteins (of superior quality to soy protein, for example), carbohydrates, minerals (such as Ca, P, K, Mg and Fe), lipids and

complex B vitamins (AMORIM et al., 2016). Yeast biomass can be used in food industry to produce yeast protein concentrates and isolates while still retain their functional properties and nutritive values. Brewer's yeast products are usually found in the form of powders, flakes or tablets, which can be sprinkled on food, mixed with milk, juices and soups (FERREIRA et al., 2010). In addition to the nutritional importance of its components, yeasts (mainly *Saccharomyces* spp) can be used as probiotic and prebiotic agents in the prevention and treatment of intestinal, nutritional and toxicological disorders. The probiotic action is related to its ability to cross the gastrointestinal tract and maintain and restore the intestinal flora (PEREYRA et al., 2018).

Besides the presence of yeasts in human nutrition, their low cost and health benefits, the non-thermally decomposable properties of yeasts also have made them an emergent and attractive encapsulation wall material (biological vehicle) for the food (MOKHTARI; JAFARI; KHOMEIRI, 2017) and pharmaceutical industries. Because of the phospholipidic membrane of yeasts, they can behave as a liposome and interact with both polar and nonpolar molecules (PARAMERA; KONTELES; KARATHANOS, 2011a). Moreover, the presence of functional groups - mainly carboxyls, hydroxyls, sulfhydryl and amine groups - present on the surface of yeast cells, is also an important factor in determining the nature of the interactions and in the use of the microorganism for incorporation or uptake of substances (JILANI et al., 2016). The rigid yeast cell wall and also the inner membrane are the main cell parts responsible for their utilization as encapsulant agent (MOGHADAM; KHAMENEH; FAZLY BAZZAZ, 2019). Table 1 shows an overview of recent works (within the last 5 years) dealing with applications of yeasts *Saccharomyces cerevisiae* for incorporation of several materials using different methods.

Table 1 - Recent studies reporting the incorporation of compounds/materials into yeasts *Saccharomyces cerevisiae*

Method used for compounds/materials incorporation	Compound/material incorporated	Other information/main results	Reference
Biosorption	Phenolic compounds from olive leaf (*Olea europaea* L.)	After carrying out an *in vitro* gastrointestinal digestion, authors reported that phenolic compounds biosorbed into yeasts had higher bioaccessibility compared to free compounds.	(JILANI et al., 2016)
	Anthocyanins from grape pomace extracts	Authors reported significant changes in the intensity of the FTIR absorption spectrum of yeasts after biosorption, suggesting their enrichment with anthocyanins.	(STAFUSSA et al., 2016)
	Phenolic compounds from grape pomace extracts	Yeasts were modified by thermal and chemical processes and yeasts treated with sodium hydroxide had higher biosorption of phenolic compounds. Compounds adsorbed by alkaline yeasts presented higher bioaccessibility after *in vitro* gastrointestinal digestion comparing to compounds adsorbed by a non-treated biomass.	(RUBIO et al., 2018)
	Cholecalciferol (Vitamin D_3)	Plasmolyzed and non-plasmolyzed yeasts were loaded by biosorption and dried by spray and freeze-drying. Yeasts plasmolyzed with NaCl and spray dried showed a higher encapsulation efficiency.	(DADKHODAZADE et al., 2018)
	Anthocyanins from *Hibiscus sabdariffa* L.	Authors suggested that yeast enzymes would be responsible for the loss of anthocyanin during storage, once the percentage of color loss was lower in conditions of low enzymatic activity. Furthermore, the utilization of wet cells 10% of ethanol in the extract led to higher encapsulation efficiencies, comparing to the use of dry yeasts and aqueous extract.	(NGUYEN et al., 2018)

Biosorption	Phenolic compounds from beetroot pomace extracts	Living, plasmolyzed and non-plasmolyzed cells of *S. cerevisiae* were used for incorporation of beetroot pomace. The higher encapsulation efficiency, of 66.1%, was obtained using living yeast cells.	(VULIĆ et al., 2019)
	Flavonoids from grape pomace extracts	By means of MIR-ATR and HPLC-DAD/UV-Vis analyses and SEM micrographs, it was suggested that yeast cells were indeed enriched. After biosorption, flavonoids had high bioaccessibility.	(OLIVEIRA et al., 2019)
	Phenolic compounds from yerba mate (*Ilex paraguasiensis*)	Cells treated with NaOH had enhanced biosorption capacity. While the compounds in the crude extract showed a bioaccessibility of 16.48%, compounds released from yeasts had a bioaccessibility of 255.68%.	(RIBEIRO et al., 2019)
	Phenolic compounds from blueberry pomace (*Vaccinium ashei*)	Biosorption capacity was enhanced using ultrasound treatment, with increased acoustic energy. Authors reported that the adsorption properties of yeast biomass depend on the type of phenolic compounds.	(TAO et al., 2019)
	Phenolic compounds from Acai pulp and seeds	Authors achieved a recovery of about 20 and 60% of phenolic compounds from aqueous extracts of acai pulp and seeds, respectively, by yeasts. Results showed that biosorption was an exothermic process.	(ROSSETTO et al., 2020)
	Phenolic compounds from back tea and olive leaves	Phenolic compounds that were released from yeasts in the intestinal phase exhibited antiproliferative activity against Caco-2 cells, acting as cytostatic agents and inhibiting cell growth. Compounds of bioaccessible fractions also reduced reactive oxygen species generation.	(JILANI et al., 2020)

Biosorption	Essential oil from *Zataria multiflora* Boiss.	After yeasts loading with the oil, carvacrol and thymol contents were evaluated during a 4-weeks storage and these compounds were stable, with no contents decrease. Oil was efficiently released from cells in aqueous media.	(MOGHADAM et al., 2021)
	Phenolic compounds from white and green teas	Yeasts were chemically treated and the effect of pH on phenolic compounds biosorption was evaluated. Alkaline medium led to an improved biosorption, as well as cells treated with NaOH had higher incorporation of compounds. Biosorption also showed a positive effect on compounds bioaccessibility.	(RIBEIRO et al., 2021)
Atomization	Flavors d-limonene, ethyl hexanoate, citral and ethyl propionate	Flavors were encapsulated in yeast cells with partially extracted β-glucans. High contents of both flavors were obtained when carrying out an incubation of the mixture for 4 h at a temperature of 40 ºC, previous to the atomization with inlet temperature of 200 ºC.	(SULTANA et al., 2017)
	Flavors d-limonene and ethyl hexanoate	Authors compared the encapsulation of flavors in yeasts (with partially extracted β-glucans) and maltodextrin. The yeast powder showed a higher oxidative stability, since the formation of oxidative products of d-limonene was much higher for maltodextrin powder.	(SULTANA et al., 2018)
Emulsion	Purslane seed oil	The encapsulation of oil in non-plasmolyzed and plasmolyzed cells was investigated. Oil loaded in plasmolyzed cells were also coated with carboxy methyl cellulose to improve the oxidative stability of oil. The encapsulation efficiency was higher for plasmolyzed cells and coating (65.37%). Encapsulated oil presented enhanced oxidative stability after storage in comparison to free oil.	(KAVOSI et al., 2018)

Emulsion	Flaxseed oil	Wheat breads were prepared using nonencapsulated oil and oil encapsulated in yeast cells or β-glucan. Compared to the other samples, breads containing oil encapsulated in yeasts showed a lower peroxide index and higher α-linolenic acid value, indicating a highest protective effect against oil oxidation during and after baking.	(BEIKZADEH et al., 2020)
	Black cumin seed oil	Results of oil content, encapsulation efficiency and loading capacity were higher for plasmolyzed yeast, in comparison to non-plasmolyzed cells. In addition, plasmolyzed cells were able to preserve thymoquinone (one of the major components in the oil) bioactivity and against degradation.	(KARAMAN, 2020)
	Antarctic krill oil	The oil encapsulated in plasmolyzed cells of yeast showed remarkable oxidative stability during accelerated storage. The bioaccessibility of the encapsulated oil was higher compared to the oil in water emulsion. Furthermore, oil was released gradually by the yeasts during an *in vitro* simulated digestion, with the highest release in intestinal phase.	(FU et al., 2021)
	Oregano essential oil	Yeast cells treated by autolysis, pulsed electric field and with high pressure homogenization showed higher encapsulation of oil compared to untreated yeasts, also resulting in reduction of incubation times and temperatures. The highest diffusion coefficients were observed for homogenized cells, followed by autolyzed cells and cells treated with pulsed electric field.	(DIMOPOULOS et al., 2021)

Vacuum Infusion	Curcumin and fisetin	The vacuum-assisted encapsulation led to higher encapsulation efficiencies compared to passive diffusion. Fisetin was encapsulated in higher concentration due to the aqueous nature of the yeast cytoplasm. In contrast to yeast cell wall particles (plasmolyzed cells), native yeast cells had improved encapsulation of both fisetin and curcumin, possibly because of the presence of organelles.	(YOUNG; DEA; NITIN, 2017)
	Curcumin	Curcumin was encapsulated in native and plasmolyzed cells and by Pickering emulsion. Native cells provided significative higher oxidative stability to encapsulated curcumin, while plasmolyzed cells provided a better barrier against thermal stability to curcumin.	(YOUNG; NITIN, 2019)
	Curcumin	The results of simulated gastric and intestinal phases showed an important role of the gastric phase followed by the presence of bile salts in intestinal phase on the release of curcumin from yeast cells. Comparing to native yeast cells, plasmolyzed cells had a significant faster release in the intestinal phase.	(YOUNG; RAI; NITIN, 2020a)
Osmoporation	Curcumin and fisetin	Cells that were perturbed by 2 repeated processes of osmoporation (dehydration in osmotic pressure followed by a rapid rehydration with an iso-osmotic medium) had higher incorporation of both fisetin and curcumin. Three stages of osmoporation led to lower viability of the cells, probably because the cell dehydration followed by the rearrangement of the phospholipidic structure may not be reverted.	(DE MEDEIROS et al., 2018)

Osmoporation	Curcumin	Curcumin was encapsulated in yeasts by means of a two-stages osmoporation. In contrast to free curcumin, yeast-encapsulated curcumin had higher thermal and photochemical stability, in terms of antioxidant stability and curcumin retention. The release profile showed a release of around 62.5% of initial curcumin.	(DE MEDEIROS et al., 2019)
Gelation	*L. acidophilus* and *B. bifidum*	Cell wall compounds were used along with calcium alginate to coat particles of probiotics encapsulated firstly by a double-layer of alginate. Authors showed that the coating with cell wall compounds was able to protect *L. acidophilus* in simulated gastrointestinal digestion, but could not enhance the acid resistance for *B. bifidum* as a highly sensitive probiotic.	(MOKHTARI; JAFARI; KHOMEIRI, 2017)
	L. acidophilus and *B. bifidum*	Both probiotics were encapsulated with calcium alginate and coated with cell wall compounds and calcium alginate. These microcapsules were added to grape juice and caused changes in juices turbidity and color. Although, *L. acidophilus* had enhanced survival with application of cell wall compounds for coating probiotic microcapsules.	(MOKHTARI et al., 2017)
Concentrated powder form	Limonene	The high-pressure spray process made possible an encapsulation of flavors in yeasts under gentle conditions, such as low temperatures and inert gas atmosphere. The process led to encapsulation efficiencies up to 86.4%.	(ERRENST; PETERMANN; KILZER, 2021)

As shown in Table 1, several methods have been used to load yeast cells with some material of interest, such as biosorption, atomization, osmoporation, gelation, emulsion, vacuum-assisted incorporation and concentrated powder form. Several materials were encapsulated, including oils and probiotic bacteria. In general, yeasts chemically or physically treated before the incorporation process presented a higher capacity of loading compared to non-treated cells. In addition to the prolonged stability of the compounds loaded into cells, yeasts are also able to protect compounds during *in vitro* gastrointestinal digestions, enabling a gradual release, reaching especially the intestinal phase, which is of utmost importance. As a consequence, when compared to free materials/compounds, yeast-loaded components have higher bioaccessibility. This overview shows that yeasts are a potential vehicle to be further explored in many different applications. Atomization, for example, is a very simple technique which was poorly investigated to provide the encapsulation of some active material using yeasts *S. cerevisiae*, evidencing an interesting option to be explored.

2.4 MICROENCAPSULATION

Consumer demand for healthy and value-added food products has increased considerably and has driven the industry to apply ingredients derived from natural sources. Phenolic compounds, carotenoids, fibers, vitamins and minerals, for example, are among the natural ingredients most often applied in food products (MARTINS; FERREIRA, 2017). However, the application of natural compounds still has limitations, especially regarding their instability when exposed to physical, chemical or enzymatic conditions, which may cause degradation. An alternative to overcome the problems related to the direct use of extracts or bioactive compounds is the microencapsulation technique, to potentially deal with the protection and application of compounds (DIAS; FERREIRA; BARREIRO, 2015).

Microencapsulation is a method applied to trap or incorporate small particles of solid, drops of liquids or gases in a material, creating a microenvironment capable of controlling the interactions between the internal and external segments. Encapsulation allows the compound or material to be protected and released in a controlled manner (BETORET et al., 2011; PARAMERA; KONTELES; KARATHANOS, 2011b). In addition to protecting the compound of interest,

encapsulation can decrease volatility, hygroscopicity and reactivity (FAVARO-TRINDADE et al., 2010), can mask unwanted odors and tastes, potentiate flavors (PAULO; SANTOS, 2017) and increase bioavailability of the encapsulated material (AIZPURUA-OLAIZOLA et al., 2016).

Microencapsulation was first addressed in the 50s by Green and Schleicher, who patented the process of preparing ink capsules produced by complex coacervation using gelatin and gum arabic. The microparticles were developed to coat paper and transfer information written on the front sheet to the bottom sheet, without the need to use carbon paper that dirties the hands of those who writes with it. The carbonless copy sheet is one of the main commercial applications of microencapsulation (DUBEY; SHAMI; RAO, 2009; GREEN; SCHLEICHER, 1957; PAULO; SANTOS, 2017). Currently, the microencapsulation technique has been explored by textile, biomedical, agricultural, electronic and mainly cosmetic, pharmaceutical and food industries (PAULO; SANTOS, 2017).

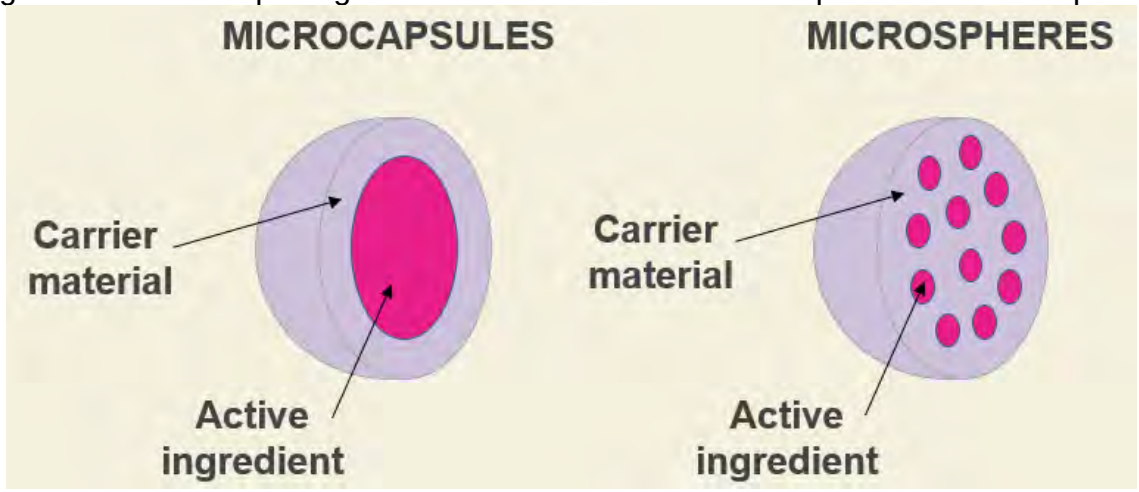
In the last two areas, several scientific works are reported, involving the microencapsulation of compounds such as ascorbic acid (CIAN et al., 2021; ZHONG; TAN; LANGRISH, 2019), curcumin (MEENA et al., 2021; YOUNG; RAI; NITIN, 2020b), anthocyanins (NGUYEN et al., 2018; ROSA et al., 2019), phenolic compounds (BALLESTEROS et al., 2017; SILVA et al., 2021), carotenoids (SANTOS et al., 2021a; TUPUNA-YEROVI et al., 2020), oils (FU et al., 2021; SHAMAEI et al., 2017) and probiotics (ARSLAN-TONTUL; ERBAS, 2017; GOMEZ-MASCARAQUE et al., 2016).

The main materials used to encapsulate bioactive compounds or other compounds of food applicability are i) water-soluble polymers: gums, alginate, chitosan, pectin, whey, milk and soybean proteins, polyethylene glycol; ii) non-polymeric water-soluble materials: cyclodextrin, maltodextrin, inulin, lactose; iii) non-water-soluble polymers: starch, casein, poly(ε-caprolactone), vaseline; iv) sucrose, lecithin, stearic acid and wax (DIAS; FERREIRA; BARREIRO, 2015). The appropriate material must be chosen based on the encapsulation process used, the type of compound to be protected and the functionality of the final application (PARAMERA; KONTELES; KARATHANOS, 2011b). Furthermore, the ideal encapsulant must be recognized as safe for consumption (GRAS - generally recognized as safe), must be biodegradable, efficient in the protection barrier between the nucleus and the surrounding environment (DIAS; FERREIRA; BARREIRO, 2015), biocompatible, non-

toxic and low cost (YE; GEORGES; SELOMULYA, 2018).

Regarding nomenclature, the encapsulated substance is known as core material, core active, fill payload or internal phase, and it is surrounded by an external phase, which can be named as coating material, encapsulant agent, carrier, shell, capsule, membrane, packaging material or wall material (EUN et al., 2019). The most common products resulting from microencapsulation are called microparticles, which can be differentiated between microcapsules and microspheres, depending on their morphology or internal structure (PAULO; SANTOS, 2017), with sizes ranging from 1 to 1000 µm (DIAS; FERREIRA; BARREIRO, 2015). In the microcapsule, the active compound is surrounded by a membrane, while in the microsphere, the compound is dispersed or microencapsulated in a heterogeneous way in the wall material (Figure 6) (KUANG; OLIVEIRA; CREAN, 2010).

Figure 6 - Inner morphological differences between microcapsules and microspheres



Reference: Adapted from Paulo & Santos (2017)

Microencapsulation methods can be classified into three groups: i) physical methods: extrusion, submerged nozzle, vibrating nozzle, spray drying, rotating disc, pan coating, air suspension, spray chilling, fluidized bed and co-crystallization; ii) chemical methods: interfacial polymerization, *in situ* polymerization; and physico-chemical methods: simple coacervation, complex coacervation, ionic gelation, liposomes, lipospheres, solvent evaporation and molecular inclusion (OZKAN et al., 2019; FAVARO-TRINDADE; PINHO; ROCHA, 2008).

It is important to mention that the choice of the microencapsulation technique must simultaneously attend to parameters such as process reproducibility, minimum

cost, quality of the formed microparticle, yield, high encapsulation efficiency and low adherence or aggregation. The production cost, despite being an important factor in the preparation of an ingredient, can be justified by adding value to the product. In addition, the possibility of increasing the scale of production to the industrial scale is also a factor to be considered (KUANG; OLIVEIRA; CREAN, 2010).

2.4.1 Spray drying technique

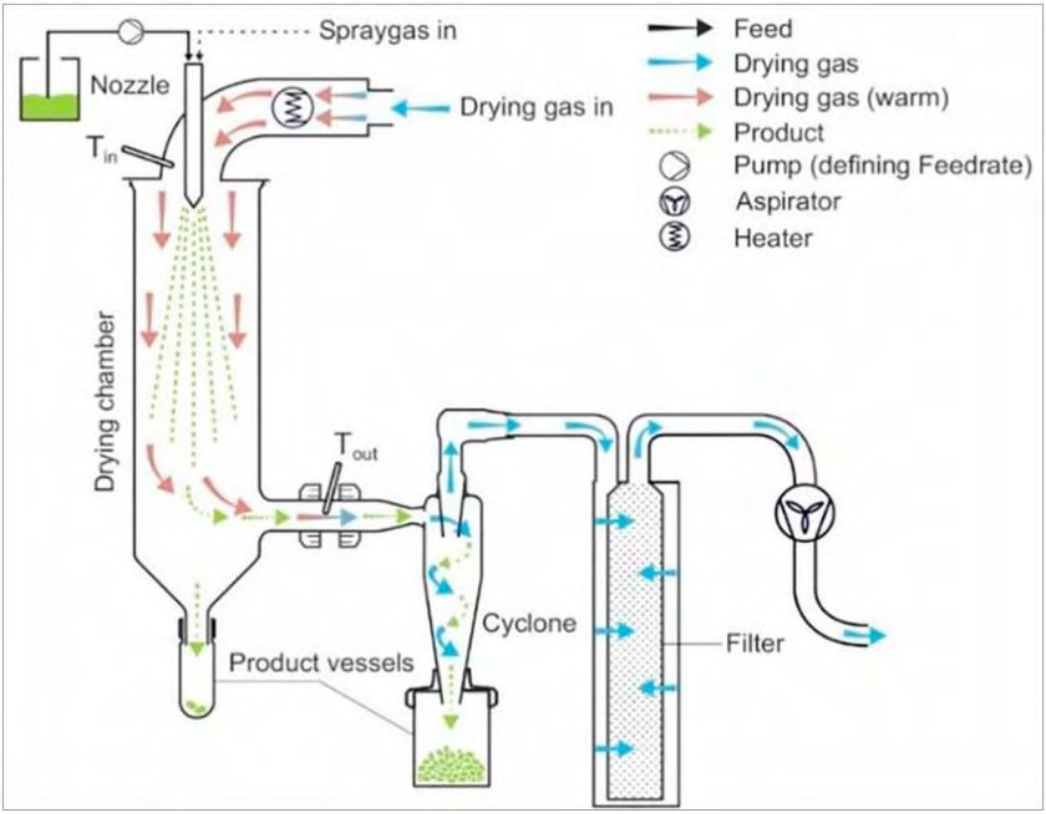
The spray drying technique is one of the most used as an encapsulation method, mainly due to the availability of the equipment, the relatively low process cost, the possibility of using a wide range of carrier materials and good final product stability (FAVARO-TRINDADE et al., 2010). Furthermore, it is a simple and fast method, of common application on larger production scales (ARPAGAUS et al., 2018) and presents advantages compared to other encapsulation processes regarding to the production of solid particles (SANTOS et al., 2021b) and because it is a continuous process (BAKRI et al., 2016).

In general, spray drying involves three stages: preparation of the suspension or emulsion, homogenization and atomization followed by drying (CASTRO-ROSAS et al., 2017). The prepared and homogenized mixture is fed into the system, passes through an atomizer, goes to the drying chamber and is distributed in very small particles in a large volume. This last step allows the atomization to maximize the contact area of the liquid fed and culminates in efficient and fast drying. The drops of the material come into contact with the hot air that enters the drying chamber and, as the temperature increases, water evaporates from the droplets. The dry particles are separated from the humid air by a cyclone and are recovered at its end, equipped with a collection container (SHISHIR; CHEN, 2017). Figure 7 shows a representation of the atomization equipment.

Compared to other methods, the temperatures applied in spray drying are higher, but the operating time is shorter. The wall material used in the encapsulation can affect the morphology, the yield, the retention of the encapsulated bioactive compound as well as its antioxidant activity (FLORES et al., 2014). The drying temperature is the most important factor in the physicochemical properties of an atomized product. Higher temperatures, for example, increase the drying rate and reduce the humidity of the particles produced. The particle size also depends on the

temperature of the incoming air. The increase in temperature culminates in faster evaporation of the water without allowing the spheres to shrink, resulting in larger microparticles. Still, generally, the faster the evaporation of water, the greater the yield and the possibility of pore formation in the particles (SHISHIR; CHEN, 2017).

Figure 7 - Flow diagram of a traditional spray dryer



Fonte: Extracted from Arpagaus et al. (2018)

2.5 CONCLUSIONS

Considering the great concern with the amount of residues generated by the food processing and knowing their valuable nutritional composition, it is of greatest importance to propose solutions and possible applications for industrial byproducts. The utilization of brewer's spent yeast (BSY) as a vehicle for incorporation of bioactive compounds, also coming from byproducts, may emerge as an innovative approach and may bring a huge range of possible applications for BSY as a functional food additive.

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3 CHAPTER 2 – UTILIZATION OF GRAPE POMACES AND BREWERY WASTE *SACCHAROMYCES CEREVISIAE* FOR THE PRODUCTION OF BIO-BASED MICROENCAPSULATED PIGMENTS[1]

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3 CHAPTER 2 - UTILIZATION OF GRAPE POMACES AND BREWERY WASTE *SACCHAROMYCES CEREVISIAE* FOR THE PRODUCTION OF BIO-BASED MICROENCAPSULATED PIGMENTS

ABSTRACT

This research approaches the utilization of brewery waste yeast *Saccharomyces cerevisiae* as a vehicle for the encapsulation and protection of phenolic compounds from Cabernet Sauvignon and Bordeaux grape pomace extracts. The main purpose of this research was to enrich the biomass of yeast to investigate its potential as a novel vehicle for further application as pigment or functional ingredient. The obtained powders presented characteristics appropriated for storage, such as low water activity (< 0.289), hygroscopicity (< 13.71 g/100 g) and moisture (< 7.10%) and particle sizes lower than the sensory perceptible (< 11.45 µm). This work proved that yeasts were loaded after spray-drying, thus, they might be considered as biocapsules. Furthermore, the bioaccessibility of encapsulated phenolic compounds from Bordeaux and Cabernet Sauvignon extracts was 34,96% and 14,25% higher compared to their respective free extracts, proving that yeasts are not only biocapsules of easy application, but also a biological material capable of protecting and delivering the compounds during gastrointestinal digestion.

Keywords: yeast, biocarrier, spray-drying, bioproducts, food ingredient, bioaccessibility.

3.1 INTRODUCTION

The increasing interest in the valorization of food and agricultural industries byproducts is a recurrent subject in the scientific literature and the importance of studies involving natural sources of bioactive compounds, such as grape pomace, is well established. Several studies highlight this winery byproduct as an attractive source of phenolic compounds (Goula, Thymiatis, & Kaderides, 2016; Nayak, Bhushan, Rosales, Turienzo, & Cortina, 2018; Peixoto et al., 2018; Rubio et al., 2018), which exert antiproliferative properties against colon cancer cells (JARA-PALACIOS et al., 2015), antioxidant (FARHADI et al., 2016; IORA et al., 2015; PEIXOTO et al., 2018), antibacterial (PEIXOTO et al., 2018; XU et al., 2016), cardioprotective (RODRIGUEZ-RODRIGUEZ et al., 2012) and skin anti-aging (WITTENAUER et al., 2015) activities.

Although the benefits and promising applications, phenolic compounds stability is a crucial aspect to consider their utilization as antioxidants and colorants in foods (BAKOWSKA-BARCZAK; KOLODZIEJCZYK, 2011; DE SOUZA et al., 2015; SOUZA et al., 2014). Phenolic compounds can be affected by pH variation, presence of metal ions, light, temperature, oxygen and enzymatic activities (AIZPURUA-OLAIZOLA et al., 2016) and, in addition to the poor long-term stability, the bioavailability and bioactivity of the potential bioactive compounds can be also altered during the exposure to different chemical, physical and biochemical conditions under gastrointestinal digestion (MOSELE et al., 2016). In this context, it is essential to apply some kind of technology to overcome these problems.

Microencapsulation is a technique widely used by food industry to protect functional food ingredients due to the low cost and flexibility (BALLESTEROS et al., 2017; SHAMAEI et al., 2017). Among microencapsulation techniques, spray drying is a cost-effective method that can often result in the formation of stable and free-flowing powders (FLORES et al., 2014) with high quality, low water activity and good storage capability (SHAMAEI et al., 2017). The quick evaporation of the solvent employed in the mixture keeps the temperature of the formed particles low and, therefore, enables the drying of heat-sensitive products without affecting their quality in a significant way (DE SOUZA et al., 2015).

Recently, there is an increasing interest in the use of yeast cells as a carrier material for encapsulation (Pham-Hoang, Romero-Guido, Phan-Thi, & Waché, 2018; Sultana et al., 2017; Young & Nitin, 2019; Young, Rai, & Nitin, 2020) because of its

structure and nutritional benefits. The cell wall of *Saccharomyces cerevisiae*, for instance, consists of β-glucans, mannoproteins and small amounts of chitin which are permeable for both hydrophilic and hydrophobic compounds (SULTANA et al., 2017). Yeasts semi-permeable membrane has a proven efficacy in protecting intracellular components from undesirable effects, such as light and oxygen, and the structure of yeast capsules is resistant to temperatures higher than 265 ºC (Paramera et al., 2011). Furthermore, the presence of *Saccharomyces cerevisiae* in human nutrition is not a novelty, once this eukaryotic structure is recognized as GRAS (generally recognized as safe), natural (MOKHTARI; JAFARI; KHOMEIRI, 2016) and represents a great source of B vitamins, proteins, nucleic acids and minerals (FERREIRA et al., 2010). *S. cerevisiae* is well known for its fermentative action in wine and beer production (CAPECE et al., 2018; SHI et al., 2019; YAN et al., 2020). After their utilization, yeasts are usually discarded as liquid effluent or used as animal feed and they lost their commercial value, what makes them a great low-cost material for reutilization.

Recent studies have proven that *Saccharomyces cerevisiae* acts like a delivery system for phenolic compounds, capable to protect them from *in vitro* gastrointestinal digestion and increase their bioaccessibility (Jilani, Cilla, Barberá, & Hamdi, 2015, 2016; V. R. Ribeiro et al., 2019; Rubio et al., 2018). Thus, the objective of this study was to enrich the biomass of waste *Saccharomyces cerevisiae* with phenolic compounds from grape pomace using the spray drying technique in order to produce a bio-based pigment or colorant with health benefits and increased stability under *in vitro* gastrointestinal digestion.

3.2 MATERIAL AND METHODS

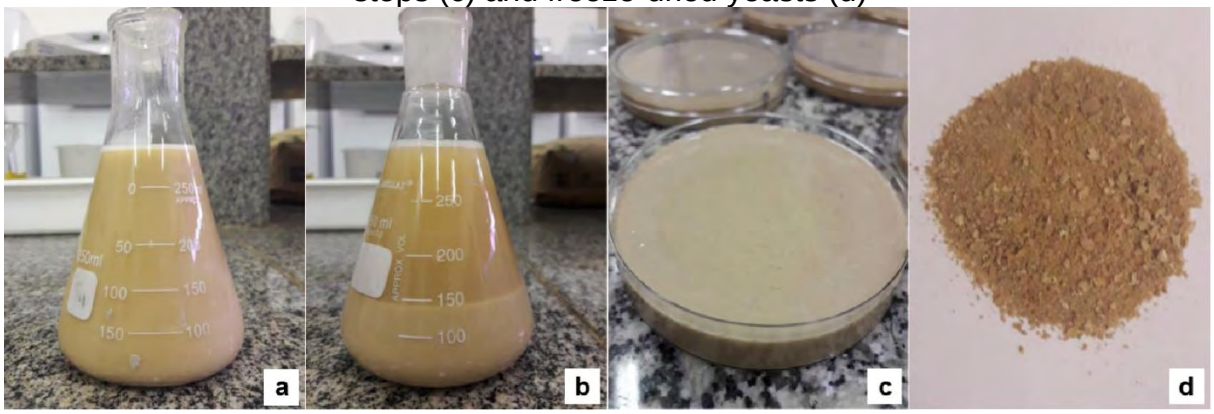
3.2.1 Residual materials

In this work, two varieties of industrial residue of grape pressing for vinification were used: grape pomaces from Cabernet Sauvignon (*Vitis vinifera*) and Bordeaux (*Vitis labrusca*) grapes, from the regions of Toledo and Marialva (PR, Brazil), respectively. Grapes were harvested in 2016 and after pressing for winemaking, their grape pomaces were kindly provided by cooperatives. The drying conditions of both pomaces are described by Rubio et al. (2018), in which work the same material was used. Samples were stored at 4 °C and protected from light.

The yeast biomass, *Saccharomyces cerevisiae* (S-33, Fermentis Safbrew), was

kindly provided by Cervejaria Campanária (Pirassununga, SP, Brazil), after its utilization twice in the Pilsen brewing. The biological material was washed with distilled water several times and it was separated from the wash water by decantation (RUBIO et al., 2018). After complete removal of beer residue, the yeast was placed in Petri dishes and frozen at – 20 °C for 24 hours. Then, the biomass was lyophilized in a Terroni freeze-dryer (model LC 1500, São Carlos, Brazil) for 48 hours, at – 20 °C and pressure of 1–0.1 kPa. Biomass was stored in polyethylene plastic bags, at – 20 ºC, for 6 months prior its utilization. Figure 8 shows each step of yeasts preparation.

Figure 8 - Yeast biomass suspended in beer residues (a), yeasts separated from distilled water by sedimentation after washing (b), wet yeasts collected after washing steps (c) and freeze-dried yeasts (d)



Reference: Elaborated by the author

3.2.2 Preparation of extracts

For the Bordeaux and Cabernet Sauvignon extracts preparation, 1 g of grape pomace was dissolved in 20 mL of 40% ethanol, according to Iora et al. (2015). After shaking (125 rpm) (Orbital Shaker Marconi, MA420, Piracicaba, SP) at 25 °C for 180 min, samples were centrifuged at 6000 rpm for 10 min in an Eppendorf 543 0R centrifuge. The final volume of extracts was concentrated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 40 °C, until the extracts were reduced to half of the initial volume. In this work, Bordeaux and Cabernet Sauvignon extracts will be called by the acronyms BE and CSE, respectively.

3.2.3 Microparticles preparation by spray drying

The mixtures of yeast-extract were prepared by suspending 5% (w/w) of dry

yeast in each extract. The flask containing the suspension was kept under magnetic stirring and room temperature (23-25 °C) during the drying procedure in order to not separate the liquid and solid phases. The solutions were atomized in a pilot scale spray dryer (model MSD 1.0, Labmaq do Brasil Ltda, Ribeirão Preto, Brazil), using a 2 mm nozzle and air flow of 65 L min^{-1}. The inlet temperature and air compressor pressure used were 130 °C and 0.2 MPa, respectively. The outlet temperature was recorded around 80 ºC. Powders were collected at the bottom of the dryer's cyclone and were stored in bottles hermetically sealed, in absence of light, at – 20 °C. Figure 9 shows the yeasts before encapsulation and the produced microparticles.

Figure 9 - Control yeast *Saccharomyces cerevisiae* (a) and powders BY and CSY obtained after encapsulation of extracts from Bordeaux and from Cabernet Sauvignon grape pomaces in yeast, respectively (b and c)

Reference: Elaborated by the author

3.2.4 Particle characterization

The particle characterization was carried out with yeasts without incorporation of extracts (named control yeasts) and with microparticles of yeasts containing the extracts from BE and CSE (called by the acronyms BY and CSY, respectively), in order to observe yeast changes after atomization and incorporation of extracts.

3.2.4.1 Moisture content

The moisture content of microparticles was measured in a moisture analyzer (MB35 Halogen, Ohaus, Switzerland) using infrared radiation and a halogen-heating lamp.

3.2.4.2 Water activity

The water activity (a_w) of the powders was measured using an Aqualab Pre Water Activity Analyzer (Decagon Devices Inc., USA) at 25 °C after stabilization of the samples at this temperature.

3.2.4.3 Hygroscopicity

Hygroscopicity measurements were carried out as described by Cai & Corke (2000), with slight modifications. Triplicates of 0.5 g of microparticles were placed in Petri dishes in a desiccator containing NaCl saturated solution (relative humidity of 79.6%). After one week of storage, samples were weighed and hygroscopicity was expressed as g of adsorbed water per 100 g of dry solids.

3.2.4.4 Particle sizing

Analysis was carried out as described by Silva et al. (2018), with modifications. Small samples of microparticles were slightly macerated with a pistil and were suspended in distilled water. The samples were immersed in an ultrasound bath and subjected to ultrasound treatment at constant frequency of 25 kHz (150 W) for 2 minutes, in an attempt to disrupt agglomerates of particles. Then, the mixture was placed in a proper cuvette, the particle size was measured using a laser diffraction particle analyzer (Shimadzu SALD-201V, Kyoto - Japan) and results were expressed as D[4,3], the mean diameter over the volume distribution.

3.2.4.5 Scanning electron microscopy (SEM)

Samples were slightly macerated, in order to separate agglomerates of yeasts and facilitate the microscope zoom, as well as being mounted on aluminum stubs using carbon adhesive tapes. The microstructure of control and encapsulated yeasts was analyzed using a scanning electron microscope (Tabletop Microscope, Hitachi TM300) and a 15 kV voltage was applied (Rubio et al., 2018).

3.2.4.6 Confocal laser scanning microscopy

Samples preparation followed the procedure described by (PHAM-HOANG et al., 2018), with some modifications. The analysis was carried out with yeasts before and after atomization, in an attempt to observe changes in yeasts outer and inner morphology. Samples were immersed in 1 µg mL^{-1} Calcofluor White M2R solution, rinsed three times in distilled water and, then, immersed in Nile Red solution (1 µg mL^{-1}) in order to stain cell walls and lipid bodies, respectively. Yeast cells were observed using a Leica TCS SP5 Confocal Microscope (Leica Microsystems, Germany). Calcofluor was excited at 405 nm and light emitted was recorded between 430 and 480 nm by a band-pass filter. Nile Red was excited at 488 nm and emission wavelengths were recorded between 515 and 645 nm.

3.2.4.7 Color

Powders were filled into a glass cell against the light source and color was measured in a colorimeter (Mini Scan XE, HunterLab, Reston, USA). The instrument was calibrated with black and white calibration plates before color measurement and the results were expressed according to the CIELAB color system (L*, a* and b*), where the L* value indicates the measure of lightness, the parameter a* is a measure of redness or greenness and b* value is a measure of the amount of yellowness or blueness (ZHANG; LI; FAN, 2019).

3.2.5 Stability test

Microencapsulated yeasts were stored at – 20 °C and total phenolic compounds (TPC) analysis was carried out every 30 days. During a period of 180 days, the extraction of encapsulated compounds was performed and TPC content was assessed in order to determine the stability of the compounds under storage conditions. Three replicates per sample were tested throughout the experiment. The half-life time ($t_{1/2}$) of encapsulated compounds was obtained by the Equations 1 and 2 (SOUZA et al., 2014), where k is the reaction rate constant, C_o is the initial TPC content and C_t is the TPC content at the reaction time t (days).

$$-\ln\frac{C_t}{C_o} = kt \quad (1)$$

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

3.2.6 Extraction of encapsulated compounds and retention

To determine phenolic compounds retention into the yeast cells, the TPC of the extracts before drying and TPC content in the microparticles have been determined. Encapsulated phenolic compounds were extracted from yeast cells by washing the microcapsules with the solvent used to prepare the extracts. For this purpose, 50 mg of each sample were mixed with 1 mL of 40% ethanol in Eppendorf micro tubes and samples were kept for 5 min. in an ultrasonic water bath (Unique, USC-1400, Indaiatuba – SP, Brazil) with 40 kHz of frequency and power of 135 W. After washing, samples were centrifuged at 6000 rpm for 5 min. The washing and centrifugation steps were repeated for sufficient times until the resultant supernatant became colorless. The extracts used for phenolic compounds quantification were the mixture of all obtained supernatants from each extraction. Retention was determined by the Equation 3.

$$Retention = \frac{TPC\ content\ in\ microparticles}{TPC\ content\ in\ extracts} \times 100 \quad (3)$$

3.2.7 Mid-infrared attenuated total reflectance (MIR-ATR)

Samples of biomasses, before and after the atomization, were characterized by Fourier transform infrared spectroscopy, in the mid-infrared region. Spectra were recorded on a Perkin Elmer spectrometer (Spectrum ONE FT-IR, Universal ATR Sampling Accessory) over the range of 4000-650 cm^{-1}, with 32 scans and 4 cm^{-1} resolution (Rubio et al., 2018).

3.2.8 *In vitro* simulated digestion

3.2.8.1 Samples preparation

The effect of a gastrointestinal digestion on free and encapsulated TPC was evaluated using the method described by Koehnlein et al. (2016) with modifications proposed by Rubio et al. (2018). After rota-evaporation, crude Bordeaux and Cabernet Sauvignon extracts were dried by lyophilization on a freeze-dryer (Terroni, model LC 1500, São Carlos, Brazil) for 24 hours. Six different samples were used: yeasts containing the extracts from BE and CSE (BY and CSY), freeze-dried extracts from BE and CSE (FDBE and FDCSE), gastric and intestinal blanks. Each sample was prepared in duplicate for gastric and intestinal phases. For BY, CSY, FDBE and FDCSE, samples were prepared suspending 1 g of the respective powder with 10 mL of distilled water.

3.2.8.2 Simulated gastrointestinal procedure

The first step was to adjust the pH of the samples to 1.2 by the addition of 5 mol L^{-1} HCl. Then, 30 mL of simulated gastric fluid (prepared with 3.2 g L^{-1} of pepsin in 0.03 mol L^{-1} NaCl solution previously adjusted to pH 1.2) was added. Samples were shaken in an orbital shaker (Marconi, MA420, Piracicaba, SP) at 150 rpm for 120 min and 37 °C in the protection of light. Afterward, samples were kept on ice for 10 min in order to stop the pepsin activity. Then, half of the samples and gastric blanks were centrifuged at 6000 rpm for 10 min, the supernatants were collected and stored at – 20 °C. The pH of the other half of the samples and intestinal blanks was adjusted to 6.0 with 1 mol L^{-1} $NaHCO_3$. After the addition of 5 mL of 120 mmol L^{-1} NaCl and 5 mL of 5 mmol L^{-1} KCl, 30 mL of freshly prepared intestinal fluid (prepared by dissolving 0.05 g of pancreatin and 0.3 g of bile salts for each 35 mL of 0.1 mol L^{-1} $NaHCO_3$ solution) was added. The mixtures were shaken for 180 min under the same incubation conditions and, then, the digests were also kept on ice for 10 min, following centrifugation and supernatants storage at – 20 °C.

3.2.8.3 Total phenolic compounds content

The TPC analysis was carried out following the Folin-Ciocalteu colorimetric

method proposed by Singleton & Rossi (1965), with a previously determined standard calibration curve (45-500 mg of Gallic Acid/L). Absorbances were recorded at 765 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, Shanghai, China) and the results of TPC content were expressed as milligrams of Gallic Acid Equivalents (GAE) per liter of extract. The bioaccessibility was calculated following the Equation 4 (Yu et al., 2019).

$$Bioaccessibility(\%) = \frac{PC_{SF}}{PC_B} \times 100 \quad (4)$$

Where PC_{SF} is the phenolic compounds content in the soluble fractions after *in vitro* digestion and PC_B is the phenolic content in the samples before digestion.

3.2.9 Statistical analysis

All analyses were performed in triplicate and results are presented in terms of mean and respective standard deviation. One-way analysis of variance (ANOVA) with post-hoc Tukey was used for comparison between means. The analysis was performed using STATISTICA 13 software (StatSoft, Tulsa, OK, USA).

MIR-ATR spectra were preprocessed through the isolation of the fingerprint area followed by a multiplicative scatter correction (MSC) algorithm. Then, a principal component analysis (PCA) was performed in the treated data, using MATLAB R2008 (The MathWorks Inc., Natick, USA).

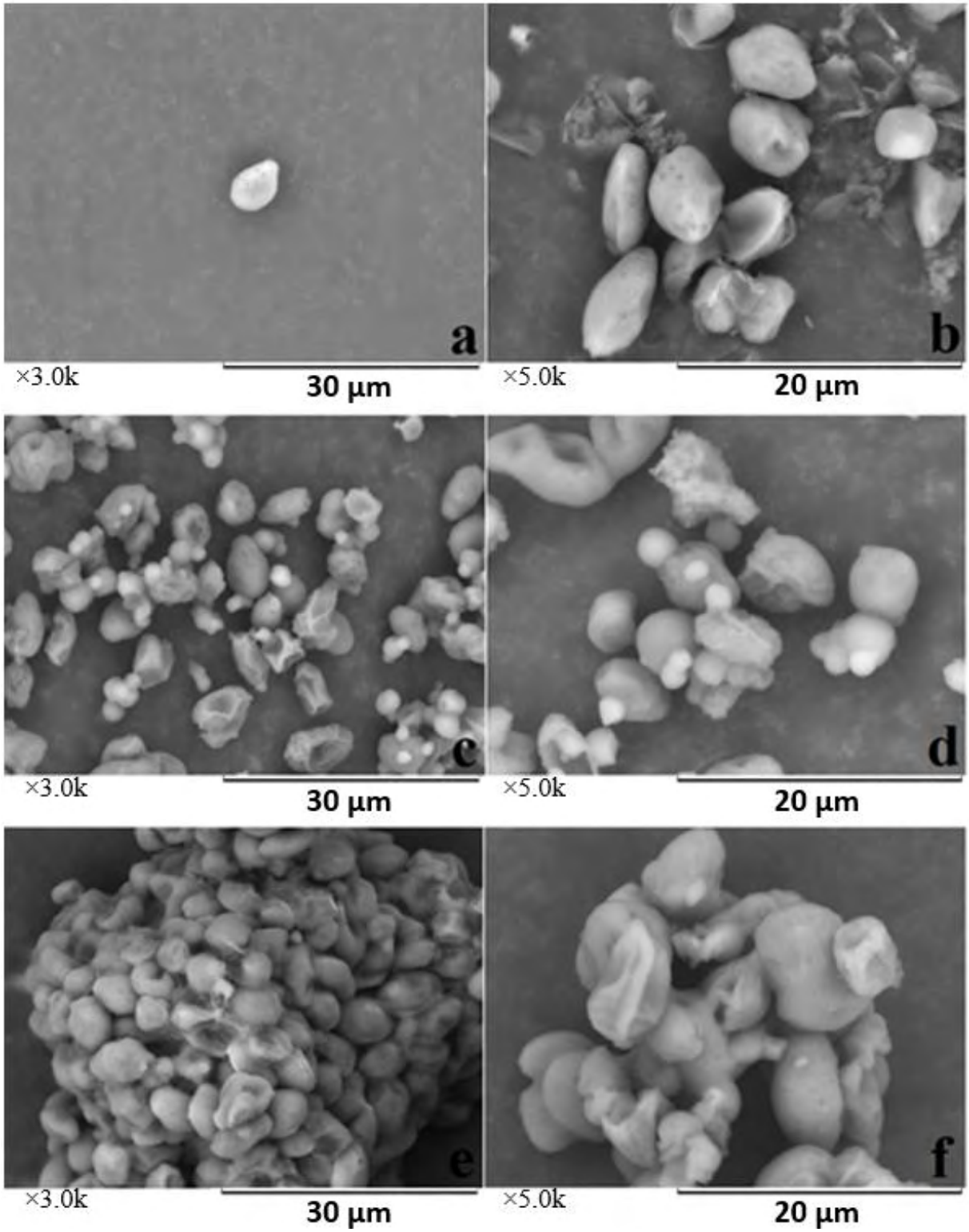
3.3 RESULTS AND DISCUSSION

3.3.1 Powders characterization

3.3.1.1 Morphology and confocal imaging

Scanning electron microscopy was applied to reveal changes in the yeasts surface and morphology after phenolic compounds encapsulation. The micrographs obtained are shown in the Figure 10.

Figure 10 - Scanning electron microscopy micrographs of control yeast *Saccharomyces cerevisiae* (a and b); microparticles obtained after atomization of BE in yeasts (c and d); and microparticles obtained after atomization of CSE in yeasts (e and f)



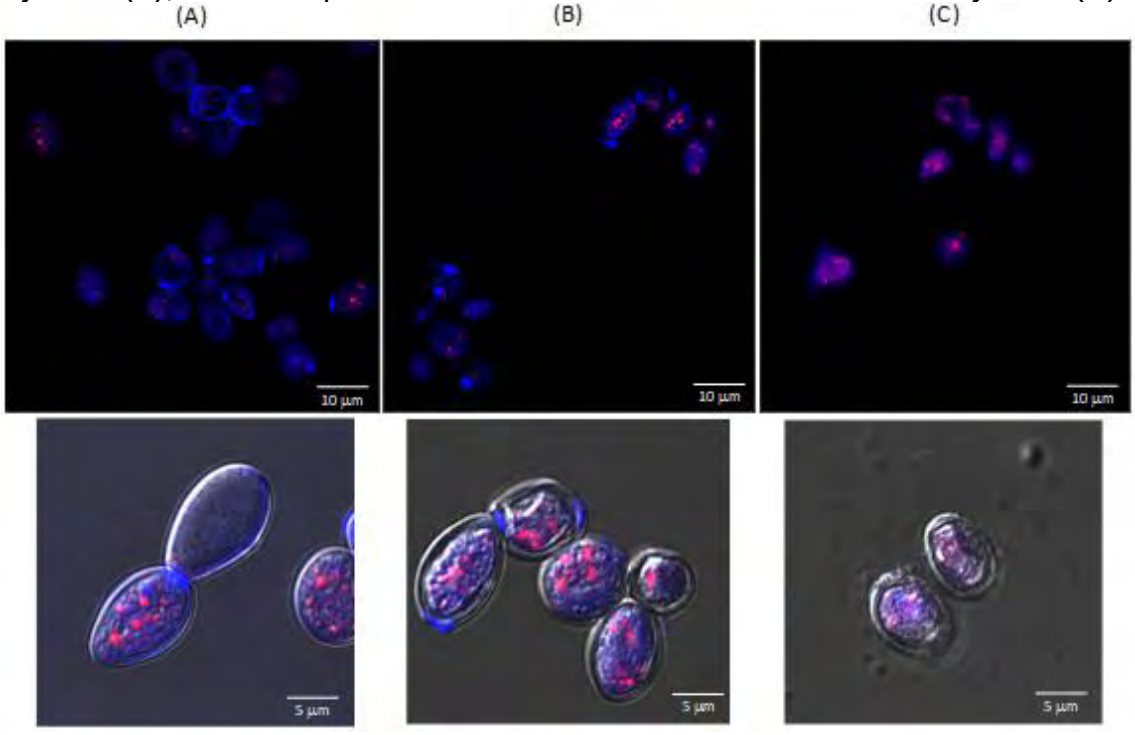
Reference: Elaborated by the author

In the Figures 10a and 10b (before atomization), yeast cells presented a typical ellipsoid shape with smooth surface. The same aspect was also reported by

Zhang, Liu, Zhang, Wang, & Zhao (2011) and Qiu, Feng, Dai, & Chang (2017). However, waste yeast cells can present damages in their structure caused by the previous utilization in the brewery fermentation and by the washing and drying processes that they were submitted before the atomization (Rubio et al., 2018). After spray-drying, in general, cells presented a more irregular surface, observed in Figures 10c, 10d, 10e and 2f. Some cells showed a shrinked shape and concavities, which can be related to the evaporation of liquid droplets during the drying process, characteristic of atomized products, as cited by Favaro-Trindade et al. (2010). Also, yeasts seem to be larger after atomization and mostly swollen cells can be observed, fact that can be explained by the loading of compounds inside the cell structure. The aggregation of microparticles can also be observed after spray-drying, in Figures 10c, 10d and mainly in 10e and 10f, which is a common characteristic of spray-dried materials. The same behavior was evidenced in works using yeasts (SULTANA et al., 2017) and other carrier materials (PAINI et al., 2015; SHAMAEI et al., 2017).

By confocal microscopy observations, the cell outer morphology was almost the same, regardless the treatment used. Under calcofluor excitation, yeast cells exhibited some fluorescence, which can be observed in blue (Figure 11).

Figure 11 - Confocal laser scanning microscopy images of control *Saccharomyces cerevisiae*, without extract (A); microparticles obtained after atomization of BE in yeasts (B); and microparticles obtained after atomization of CSY in yeasts (C)



Reference: Elaborated by the author

Saccharomyces cerevisiae cell wall is composed mainly by a high-order complexes of mannoproteins, β-1,3- and β-1,6-glucan and some amount of chitin (ORLEAN, 2012) and calcofluor is responsible for staining β-1,6-glucans and chitin. Control cells were more homogeneous, with no cell deformation and the cell wall was clearly stained by calcofluor. On the other hand, after spray-drying, the cell wall fluorescence was faint and the internal part of the cell was stained with higher intensity. Some authors evidenced the intense fluorescence at excitation 405 nm and emission 530-600 due to the presence of polyphenols inside the yeast cell (NGUELA et al., 2019). In addition, it can be observed that the cell organization was slightly perturbed after atomization of phenolic compounds. While the inner material is basically homogeneously distributed inside control cells, cells after spray-drying present some shrinkage in the inner material, as if it was unstuck from the cell membrane. The red structures that appear under Nile Red excitation are possibly lipid bodies, such as organelles. Nile Red stain fluoresces when in contact with hydrophobic components, according to Pham-Hoang et al. (2018). In control yeasts, organelles are spread all over the cell structure, whereas in cells after atomization, they appear more agglomerated. Once there was the impregnation with hydrophilic compounds, it is possible that organelles were repulsed by phenolic compounds and attracted by each other, what explains the more irregular format that they have after spray-drying and the inner liposoluble area extended, showing a more intense red area under excitation. In conclusion, all differences observed between control cells and cells after atomization might indicate the presence of phenolic compounds inside the cells.

3.3.1.2 Particle size, water activity, moisture and hygroscopicity

Independent of the extract dried using *Saccharomyces cerevisiae*, both obtained powders presented similar characteristics, interesting for application as a novel ingredient, such as a pigment or a food supplement. The average powder diameter was found to be within the range of 9.42–11.45 μm (Table 2), which is in accordance with the diameter showed by the micrographs. There was no significant difference between treatments.

Table 2 - Properties of control and spray-dried yeasts

Sample	Properties			
	Mean particle size (µm)	Moisture (%)	Water activity	Hygroscopicity (g/100 g)
CY	11.45^{a}±0,01	5.60^{c}±0.106	0.166^{b}±0.005	8.98^{b}±0.314
BY	9.69^{a}±0,30	6.40^{b}±0.145	0.289^{a}±0.007	13.70^{a}±0.001
CSY	9.42^{a}±1,70	7.10^{a}±0.247	0.286^{a}±0.011	13.71^{a}±0.692

Mean values in the same column followed by the same superscripts are not significantly different ($p > 0.05$). CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract.
Reference: Elaborated by the author

Sultana et al. (2017) encapsulated flavors (d-limonene, ethyl hexanoate, citral and ethyl propionate) in yeast cells and reported lower particle sizes, ranging between 4.8 and 9.1 µm. The authors highlight the role of the equipment nozzle structure and drying conditions on particles sizes. Lower particle sizes obtained by the authors can be mainly related to the higher inlet temperature used in their work, of 200 ºC. There are evidences that when the inlet temperature is higher, because of the faster evaporation of droplets of water, particle structure tends to be more porous (DE SOUZA et al., 2015) and the lower diameter is probably related to particle shrinkage. An issue in measuring of particle size of yeasts using laser diffraction is that cells are generally agglomerated and the base of the size distribution may be large. According to Sultana et al. (2017), the aggregation between cells might occur because of the binding properties of β-glucans, naturally present in the thick yeast cell. Although, sizes obtained in this work were within the range of 5–150 µm, expected for microparticles produced by common spray dryers (FAVARO-TRINDADE et al., 2010) and, in addition, particles with diameters below 100 µm can be incorporated into food without interfering negatively with the texture of the final product and consumers perception (COMUNIAN et al., 2017).

Table 2 also shows the values obtained for the water activity of control, varying between 0.166 and 0.289. Microparticles obtained after spray-drying presented higher water activity in comparison to control yeasts. The difference of a_w between yeasts before and after spray drying may be related mainly to the rehydration of yeasts with the extracts and posterior drying process applied. Values of water activity lower than 0.6 are within the recommended limit to assure the proper

microbiological stability of the powders and capacity for a long-time storage in suitable packaging and storage conditions (ROSA et al., 2019; SARABANDI et al., 2018).

In relation to the moisture content (Table 2), values were within the range of 5.6% and 7.1%, with significant differences between samples and lower value for control yeast. Similar values were found by Sultana et al. (2017), with values of moisture varying from 5.1% to 9.12% when using yeasts *S. cerevisiae* for the encapsulation of flavors. Low values of moisture are desirable for powders, because the increase of moisture content is frequently related to the instability of encapsulated compounds. Moisture can promote the release or diffusion of encapsulated bioactive through the encapsulation matrix to the capsule surface, where the compound is more vulnerable to oxidation or the solubility of the compound can be increased and, then, microcapsules present higher losses of the bioactive material (PARAMERA; KONTELES; KARATHANOS, 2011b; ZHENG et al., 2011).

Control yeasts present hygroscopicity of 8.98 g of water/100 g of dry matter, while yeasts after encapsulation by spray-drying show higher values of 13.70 and 13.71 g of water/100 g of dry matter. Notwithstanding using different wall material, De Souza et al. (2015) reported similar values for hygroscopicity of pigments extracted from grape byproducts and encapsulated by spray-drying with maltodextrin. The authors found values between 12.44 and 16.90 g of water/100 g of dry matter. Also, Rezende, Nogueira & Narain (2018) reported hygroscopicity values of powders produced by freeze and spray-drying of extracts from acerola pulp and residue into maltodextrin and gum arabic ranging from 9.24 to 12.46 g of water/100 g of dry matter. Lower hygroscopicity of powders facilitates their conservation and preservation of color and bioactive compounds (REZENDE; NOGUEIRA; NARAIN, 2018).

3.3.1.3 Color changes after encapsulation and the influence of storage on powder color

Based on the Figure 9, visually analyzing, while the powder obtained after encapsulation of phenolic compounds from Cabernet Sauvignon have a lighter color, the particles obtained after encapsulation of Bordeaux extracts present a more intense purple color, which may be more interesting from the point of view of an

industry whose goal is to add it to a product as a natural pigment. Table 3 shows the color parameters for control and spray-dried yeasts.

Table 3 - Color parameters L* (luminosity), a* (difference between red and green) and b* (difference between blue and yellow) for control and spray-dried yeasts

	Samples				
Parameter	CY	BYt_0	$CSYt_0$	BYt_{180}	$CSYt_{180}$
L*	55.31^{a}±0.04	35.49^{d}±0.81	49.44^{b}±1.18	30.54^{e}±0.07	44.18^{c}±0.03
a*	6.02^{d}±0.19	17.57^{a}±0.12	7.71^{c}±0.02	17.61^{a}±0.04	8.58^{b}±0.04
b*	21.76^{a}±0.40	-3.17^{d}±0.11	12.44^{b}±0.03	-2.44^{c}±0.12	12.84^{b}±0.10

Where t_0 and t_{180} represent the time zero and after 180 days, respectively. CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. Mean values in the same line followed by the same superscripts are not significantly different ($p > 0.05$).
Reference: Elaborated by the author

Control yeast parameters of L*, a* and b* differ from those obtained for yeasts after spray-drying, indicating that the color of yeast was altered by the enrichment with phenolic compounds from grape pomaces. After encapsulation of the extracts in yeasts, lower L* and b* values were observed, while a* values increased. According to Bernardes et al. (2019), normally, when the carrier material is white or light colored, the color of the added extract tends to predominate and the obtained sample will be darker, presenting lower luminosity (L*). The higher a* values reveal a deeper red and lower b* values indicate an increase in the blue color after enrichment with phenolic compounds, probably related to the incorporation of pigments such as anthocyanins, one class of the phenolic compounds and the most important pigments of vascular plants (DE SOUZA et al., 2015). The main anthocyanins found in both Cabernet Sauvignon and Bordeaux grape pomaces are peonidin-3-O-acetylglucoside, peonidin-3-O-glucoside, malvidin-3-O-acetylglucoside, malvidin-3-O-*p*-coumaroylglucoside and malvidin-3-O-glucoside, as reported by Ribeiro et al. (2015).

In relation to the color of the powders after 180 days of storage (Table 3), both BY and CSY presented a decrease in the parameter of luminosity (L*), implying in lower levels of lightness. According to Taofiq et al. (2018), the decrease in lightness may be derived from the oxidation of incorporated compounds. For BY samples, there was an increase in b* parameter from -3.17 to -2.44 and no significant

difference was observed for a* values. The evolution from negative to less negative b* values can be related to the loss of copigmentation effects accompanied by the formation of anthocyanin-derived red-orangish pigments, such as pyranoanthocyanins (TSALI; GOULA, 2018). On the contrary, there was an increase in a* values for CSY samples, from 7.71 to 8.58, and no significant difference was found for b* parameters. The formation of pigments derived from anthocyanins that stabilize the flavylium red-colored form can explain this behavior (LAGO-VANZELA et al., 2014). Likewise, Moser et al. (2017) also reported an increase in the red color component after 150 days of storage of grape juice encapsulated with a mixture of whey protein and maltodextrin.

3.3.2 Phenolic compounds retention and stability of compounds

The retention of phenolic compounds from Cabernet Sauvignon and Bordeaux grape pomaces extracts encapsulated into *Saccharomyces cerevisiae* is shown in Table 4.

Table 4 - Percentage of retention, stability and half-life time of total phenolic compounds

Sample	Retention (%)	$TPCt_0$ (mgGAE L^{-1})	$TPCt_{180}$ (mgGAE L^{-1})	$t_{1/2}$ (days)
BY	95.22	2288.09^{A}±74.34	1561.9^{B}±50.51	326.77
CSY	97.20	3560^{A}±98.97	2651.19^{B}±75.76	423.29

Where $TPCt_0$ and $TPCt_{180}$ represent the total phenolic compounds in the time zero and after 180 days of storage, respectively. $t_{1/2}$ is compounds half-life time, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. Mean values in the same line followed by the same superscripts are not significantly different ($p > 0.05$).
Reference: Elaborated by the author

The obtained values were within the range from 95.22 to 97.2%. Retentions close to 100% suggests that phenolic compounds were not affected by the temperature of 130 ºC used in the atomization process. Yet, it is known that the structure of yeast capsules can resist high temperatures up to 256 ºC (PARAMERA; KONTELES; KARATHANOS, 2011a), so, if phenolic compounds have affinity for wall components and they are able to bound into cell wall, it is possible that yeasts can also protect them from high temperatures applied in spray-drying. Due to the

amphiphilic character of its membrane structure, formed with a continuous bilayer of lipids oriented with the polar lipid heads toward outside and the nonpolar heads toward the center of the membrane (WANG; CHEN, 2009), yeast presents a great affinity for both hydrophobic and hydrophilic compounds, such as phenolic compounds. This affinity can increase the accessibility of phenolic extract to the diffusion process. Moreover, as yeasts are probable to agglomerate in consequence of β-glucans presence, bioactives can be entrapped in the interlacing formed between cells.

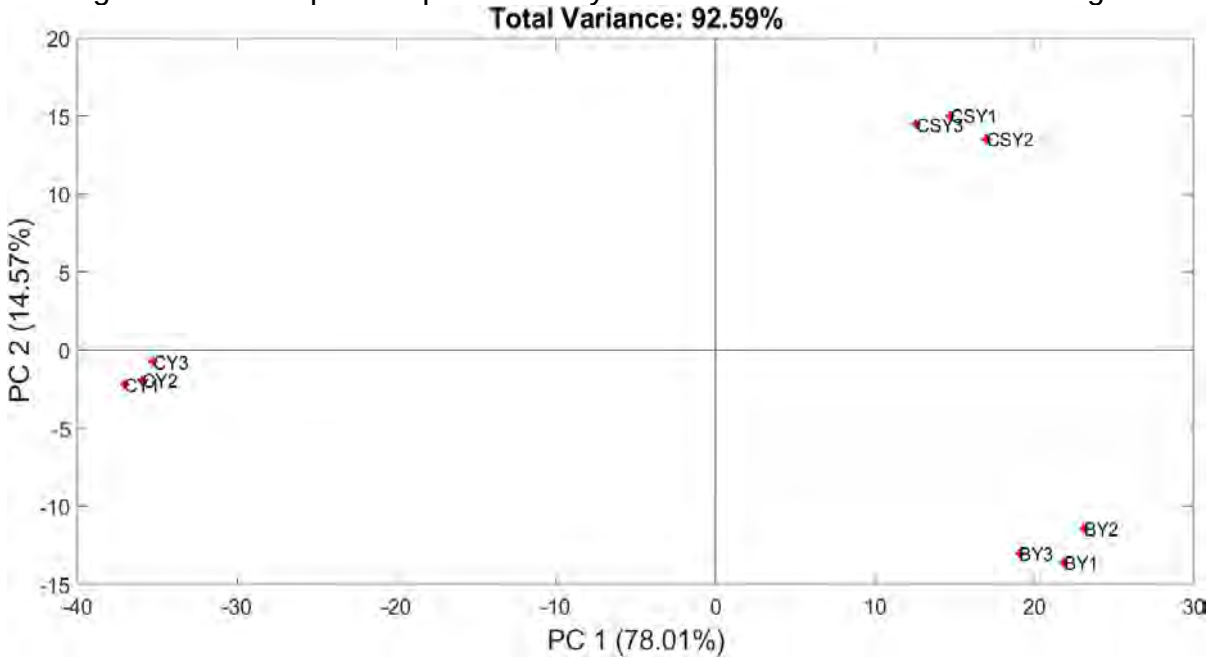
Phenolic compounds stability was evaluated in a period of 180 days and the results are shown in Table 4. The difference in phenolic compounds content between samples of enriched yeasts is consequence of the initial content on the obtained extracts after concentration – CSE presents a content of 3662.38±14.87 mgGAE L^{-1} whereas BE shows lower content of 2333.81±119.81 mgGAE L^{-1}. During 180 days, the stability of phenolic compounds did not follow a linear behavior, presenting increases and decreases in the content at every 30 days (data not shown). This could be related to oxidation reactions during storage time and, consequently, the degree of polymerization of the compounds, which may compromise the quantification by colorimetric methods (de Souza, Thomazini, Chaves, Ferro-Furtado, & Favaro-Trindade, 2020). After storage, 68.3% and 74.5% of the compounds of BY and CSY, respectively, were still retained in the biomass, resulting in half-life times of 326.77 and 423.29 days. It is important to mention that the loss of total phenolic compounds does not discard the protection effect of the yeasts over the core material, once spray-drying enables the coverage of the wall material with compounds, which is not inside the cell. Thus, this non-entrapped content is more propense to degradation and it is probable that the main loss is related to them. Further studies are necessary to investigate the encapsulation efficiency of grape pomaces compounds encapsulation into *S. cerevisiae*.

3.3.3 Principal component analysis (PCA) of MIR-ATR spectra

In this work, PCA was performed to cluster data based on spectra and to observe changes between yeasts before and after atomization of compounds, in an attempt to confirm there was an enrichment of the biomass with phenolic compounds. Figure 12 shows the principal component analysis scores and the plane

defined by two principal components – sufficient to explain almost 93% of the variability of the dataset. The variability of PC1 corresponds to 78,01%.

Figure 12 - Principal component analysis scores in the 900-1800 cm^{-1} region

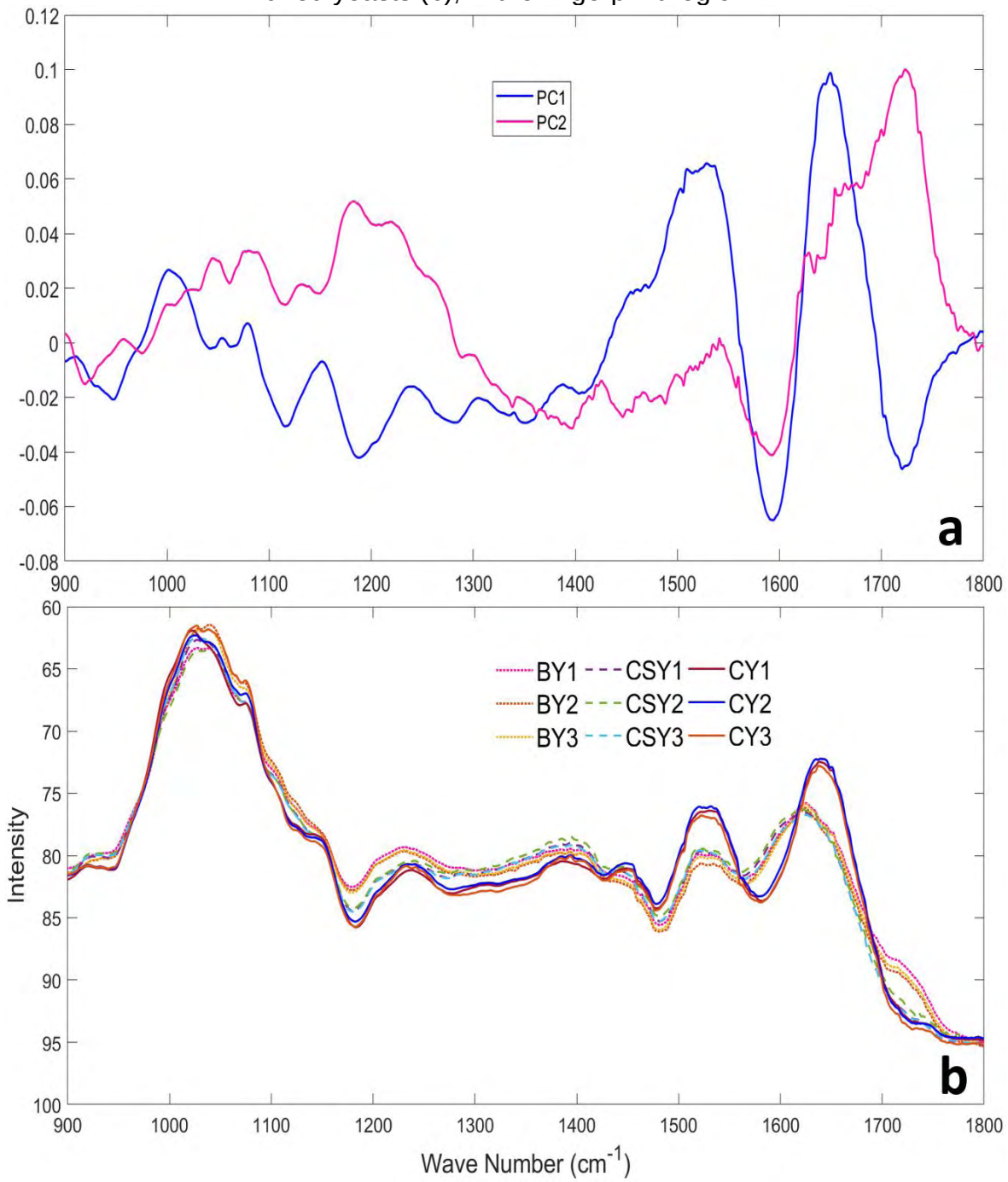


Where BY, CSY and CY represent yeasts after the atomization of phenolic compounds from Bordeaux and Cabernet Sauvignon extracts and control yeasts (yeasts before encapsulation), respectively. 1, 2 and 3 represent the three replicates for each sample.
Reference: Elaborated by the author

Based on the scores profile, samples can be clustered in three well defined groups. Control yeast functional groups are better explained by the negative sides of both PC1 and PC2, while yeasts enriched with phenolic compounds from CSE and from BE have their functional groups better explained by positive side of PC1 and positive and negative sides of PC2, respectively. These results imply that there is, indeed, a difference between all yeasts studied, control yeasts and yeasts atomized with CSE and BE. A suitable explanation for this result is that yeasts were loaded with phenolic compounds by the process of spray-drying and, also, bioactive compounds inherent of each extract have affinity for different chemical groups in yeasts structure.

Regarding to the loading profile, results will be discussed in parallel with the spectra of the samples, shown in Fig. 13 a and b, respectively.

Figure 13 - PC1 and PC2 loadings (a) and MIR-ATR spectra of control and spray-dried yeasts (b), in the fingerprint region



Where CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. 1, 2 and 3 represent the three replicates for each sample.
Reference: Elaborated by the author

The most important bands for control yeasts are found around 1590 cm^{-1}, 1280-1430 cm^{-1} and 900-960 cm^{-1}, where PC1 and PC2 are both negatives. In fact, correlating to spectra, is possible to observe that, in these regions, there are clear differences between CY and CSY and BY spectra. Yeasts enriched have overlapped

spectra comparing to CY, in all mentioned regions, pointing that phenolic compounds were probably bounded to chemical groups found in those regions. The first difference in absorbance peaks observed around 1590 cm^{-1} shows that amide II is an important group for the retention of phenolic compounds. The same behavior was reported by Rubio et al. (2018), where spectra in the region of amide II also presented higher intensity after the enrichment of yeasts with phenolic compounds from grape pomace. The region within 1280-1430 cm^{-1} comprises an important area where bands of phenolic compounds can be found, such as gallic and tannic acids and catechins (FRAGOSO et al., 2011). According to Galichet, Sockalingum, Belarbi, & Manfait (2001), mannans and glucans contents can appear in the spectral region of 790-1190 cm^{-1}, which comprehends a polysaccharide absorbing region. Thus, the slight change in the 900 to 960 cm^{-1} spectral area can be most probably related to the interaction of phenolic compounds with constituents of the surface of yeast cell through hydrogen-bonding or by insertion and bounding in the network of the cell wall. Similar result was observed by Paramera et al. (2011a), in which work the authors reported a strong interaction between curcumin and the cell wall constituents of *Saccharomyces cerevisiae*.

The main groups correlated to CSY samples, according to the loadings profile, are found in the absorbance areas of 1620-1695 cm^{-1}, 990-1040 cm^{-1}, and narrower peaks in 1050 and 1085 cm^{-1}. In the first area, a region where amide I appears (CHEN; WANG, 2016), obvious changes were observed after yeast enrichment, once the band is significantly less intense compared to control yeast spectra and moved to a lower frequency region, implying in chemical interaction between phenolic compounds from CSE and yeast cell. The other important areas for this sample reveal that organic acids and sugars found around 1050 and 1150 cm^{-1} (STAFUSSA et al., 2016) and polysaccharides found around 990-1040 cm^{-1} may have a significant bounding with phenolics from CS extract. At last, the main region responsible for explaining BY functional groups appear between 1430 and 1570 cm^{-1}, where amide III and amide II can be found and presented lower intensity compared to control yeasts.

3.3.4 Gastrointestinal digestion simulated *in vitro*

The changes on phenolic compounds content during the simulation of an *in vitro* digestion and bioaccessibility results are shown in Table 5.

Table 5 - Phenolic compounds from samples before and after the simulation of an in vitro digestion

Sample	Before *in vitro* digestion	After gastric phase	After intestinal phase	Bioaccessibility
	Phenolic compounds (mg GAE L^{-1})			%
BY	2288.1^{Da}±74.34	688.67^{Cc}±27.61	1105.76^{Cb}±28.28	$48,33^{A}$
CSY	3559.99^{Ca}±98.97	852.47^{Cc}±98.99	1253.38^{Cb}±44.45	$34,32^{B}$
FDBE	8829.24^{Ba}±75.24	2750.38^{Bc}±24.92	3162.24^{Bb}±17.51	$35,81^{B}$
FDCSE	13548.25^{Aa}±37.01	3257.05^{Ac}±65.32	4070.81^{Ab}±98.32	$30,04^{C}$

Capital letters in the same column and small letters in the same row within each sub-group do not differ statistically ($p > 0.05$). BY is the yeast after the atomization of phenolic compounds from Bordeaux extract, CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract and FDBE and FDCSE are the Bordeaux and Cabernet Sauvignon extracts after freeze drying, respectively.
Reference: Elaborated by the author

For all samples studied, the release of compounds occurred in majority in the gastric phase and the another part of the compounds was extracted and released in the intestinal phase. The gastric release corresponds to 62.3%, 68%, 87% and 80%, in relation to the bioaccessible fraction, for BY, CSY, FDBE and FDCSE, respectively. In fact, due to the low pH and the pepsin action, it is expected that phenolic compounds are released in the upper gastrointestinal tract, mainly those bound to carbohydrates (QIN et al., 2018). The stomach is the main site for extracting bioactives. Although, the transition from acid environment to an intestinal medium is critical for bioactive compounds since the combined effect of pH and enzymes activities may lead to changes in the bioavailability and mainly bioactivity of the ingested compounds (JILANI et al., 2016).

Regarding to the intestinal phase, there was an increase in phenolic compounds content for all samples studied, implying that compounds were still released even in alkaline conditions. Also, based on the obtained results, it can be seen that phenolic compounds from the bioaccessible fraction are just a part of the TPC retained in the biomass before digestion. The more probable explanation is that part of the compounds was retained in the surface of the capsule after encapsulation, thus, it is probable that these compounds were not bounded to yeast chemical groups and, then, they solubilize into gastrointestinal medium and they are more susceptible to degrade by enzymes action or mainly under pH conditions. Therefore, it is possible that the compounds found in the intestinal phase are released from

inside the capsule. Furthermore, it is also possible that compounds were not released integrally, that is, a percentage of them could be still retained inside the cells.

The results found in the present research are not consistent with previously reported data in literature involving the utilization of *Saccharomyces cerevisiae* as a material for the adsorption of phenolic compounds. These works showed there was an increase in the TPC content during gastric phase and a decrease after intestinal phase (de Oliveira et al., 2019; Ribeiro et al., 2019; Rubio et al., 2018). Authors explains that phenolic compounds are sensitive to alkaline environment, found in the small intestine, which may lead to interactions between chemical structures and the generation of different compounds with altered biological activities. Meanwhile, the present research shows that compounds encapsulated in yeasts by spray drying may be under a protective effect during both acid and alkaline conditions.

According to Czubinski et al. (2019), assessing bioaccessibility is the key step before concluding on any potential health-beneficial effects of phenolic compounds that are present in food. Although the initial content of phenolic compounds was higher in CSY, BY presented higher bioaccessibility after *in vitro* digestion. The digestive tract is a complex system and it cannot be assured that changes occur the same way with samples, mainly because of the different components inherent of each extract, which may behavior differently under pH conditions and enzymes activities.

Based on the bioaccessibility results found on Table 5, it is concluded that the percentage of bioaccessibility of compounds from BE and CSE encapsulated in yeasts is 35% and 14.25% higher than the respective free compounds bioaccessibility. The values found for microparticles justify microencapsulation, once yeasts are capable to protect and release compounds during gastrointestinal digestion and these bioactives are delivered into the intestine, where they might be absorbed for, then, exert their biological functions.

3.4 CONCLUSIONS

In this work, yeasts were proven to be a great wall material for encapsulation of bioactive compounds by spray-drying. It was possible to obtain powders with characteristics that enhance the shelf-life of the product.

Obtained powders present around one year of half-life times. The principal component analysis of MIR-ATR spectra showed that phenolics were successfully attached to the yeast surface. Amide I, amide II, amide III, mannans and glucans are cells important chemical groups for phenolic compounds bounding. Then, along with the evidences of yeast changes after atomization, showed by microscopy, it can be affirmed that yeasts were indeed loaded with compounds, thus, yeasts act as capsules and not only as a vehicle for compounds drying. Encapsulated compounds presented higher bioaccessibility compared to free compounds.

Both produced powders are of great interest for application, however, if the main purpose is the obtention of a pigment, the yeast enriched with phenolic compounds from Bordeaux grape pomace extract has to be further explored. This study may be helpful to promote the application of waste yeast as a vehicle for encapsulation of bioactive compounds as a novel food ingredient and may show some general interests for the stability of other natural substances.

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4 CHAPTER 3 - DEVELOPMENT OF NATURAL PIGMENTS MICROENCAPSULATED IN WASTE YEAST *SACCHAROMYCES CEREVISIAE* USING SPRAY DRYING TECHNOLOGY AND THEIR APPLICATION IN YOGURT[2]

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4 CHAPTER 3 – DEVELOPMENT OF NATURAL PIGMENTS MICROENCAPSULATED IN WASTE YEAST *SACCHAROMYCES CEREVISIAE* USING SPRAY DRYING TECHNOLOGY AND THEIR APPLICATION IN YOGURT

ABSTRACT

Although *Saccharomyces cerevisiae* has shown potential utilization as a bio-vehicle for encapsulation, there are no reports about the functionality of natural colorants encapsulated using yeast cells. The main objectives of this study were to produce natural food coloring by encapsulating extracts from grape pomace (GP) and jabuticaba byproducts (JB) into brewery waste yeast and evaluate the functionality of the pigments by their incorporation into yogurts. Particles produced by the encapsulation of extracts from GP and JB in *S. cerevisiae* using 5% of yeast had the highest encapsulation efficiencies for both anthocyanins (11.1 and 47.3%) and phenolic compounds (67.5 and 63.6%), highest concentration of both bioactives during storage and stable luminosity. Yogurts showed a pseudoplastic behavior and were considered weak gels. Colored yogurts had acceptance indexes between 73.9 and 81.4%. This work evidenced the utilization of enriched yeasts as coloring agents and interesting additives for the production of functional foods.

Keywords: grape pomace, jabuticaba, bio-vehicle, byproduct, functional food, natural dye.

4.1 INTRODUCTION

Over the years, industries have paid attention to changes in consumer behavior and expectances and, as a consequence, the searching for novel ingredients and additives has been of a great concern. Food appearance is highly related to the color and may be the most important factor to lead the consumer to select a product, since it is the first characteristic seen. According to Gebhardt et al. (2020), color positively influences on consumer's preference, purchase decision and eating desires. In this context, food colorants improve the attractiveness of foods to meet the color expectations of consumers (THALHAMER; BUCHBERGER, 2019) and, also, color can present marketing purposes when it is for flavor identification (LIN et al., 2018).

Although synthetic colorants are widely used in food industries due to their stability, strength and price (FEKETEA; TSABOURI, 2017), some pigments pose a potential risk to human health, especially in the case they are excessively consumed (AI et al., 2018), and the concern is exacerbated because artificially colored foods are often marketed to children (GUKOWSKY et al., 2018). There is recently a worldwide movement towards more use of natural colorants (AI et al., 2018). Among vegetable colorants, anthocyanins are considered the most important pigments of vascular plants (CASTAÑEDA-OVANDO et al., 2009) and represent a phenolic compounds class. Phenolic compounds can be found in low cost sources, such as bio-residues, and their valorization has been of a great interest for the sustainable production of value-added colorants (ALBUQUERQUE et al., 2020b).

Jabuticaba (also known as "Brazilian fruit") byproducts and grape pomace are amongst known sources rich in phenolic compounds. Grapes are a popular agricultural crop used in wine production. After their pressing for must preparation, the solid parts macerated are discarded as pomace, generating substantial quantities of wastes (TSALI; GOULA, 2018). On the other hand, jabuticaba is very appreciated for *in natura* consumption and for the production of jams, syrups and alcoholic beverages. However, the commercialization of this kind of fruit is difficult due to its high perishability and, with the main application of its pulp, residues represent about 50% of the total processed volume (ALBUQUERQUE et al., 2020a). Several works approach the antioxidant action of bioactive compounds extracted from these matrixes (BERES et al., 2019; CABRAL et al., 2018; FIDELIS et al., 2020;

MONTEIRO et al., 2021; SILVA et al., 2014; SOUZA et al., 2014) and their application as food colorants (ALBUQUERQUE et al., 2020b; BALDIN et al., 2016; DEMIRKOL; TARAKCI, 2018).

The application of natural pigments is limited yet by their poor stability, once most of them are sensitive to oxidation, pH changes and light, besides their inherent solubility which vary widely (LIN et al., 2018). To overcome these problems, encapsulation has been extensively studied for the protection of pigments, improvement of their stability and dispersibility in water. Among known techniques, spray-drying is scalable, relatively low-cost and the most common technique used to encapsulate food materials and active compounds (ABID et al., 2019). Furthermore, the short processing time is suitable for heat-sensitive compounds (ETZBACH et al., 2020).

Recent works have been studying the utilization of yeasts *Saccharomyces cerevisiae* to replace common carrier materials for encapsulation of anthocyanins (NGUYEN et al., 2018), flavor (SULTANA et al., 2017), curcumin (YOUNG; RAI; NITIN, 2020) and phenolic compounds from grape pomace (RUBIO et al., 2020) by spray-drying. Yeasts can be provided as residues from brewery industries after utilization in the fermentative process and, as a result, its low cost may be of great advantage compared to other carriers/vehicles. In addition, *S. cerevisiae* cells are generally recognized as safe (“GRAS”), they have high nutritional value and have been already consumed and incorporated into human nutrition. In a previous study of our research group, yeast cells were proven to be a great bio vehicle for incorporation of phenolic compounds, acting as a biocapsule, protecting phenolic compounds during gastric digestion and delivering them gradually until the intestine phase performed in an *in vitro* simulation (RUBIO et al., 2020). In addition, spray-dried powders presented interesting colors, but their potential as natural pigments and functionality still have to be further explored by their incorporation in food.

In this context, yogurt can be a suitable option to be studied as a food matrix to apply natural pigments because it is a food consumed by people of all ages with great acceptance and it is highly appreciated for its nutritional value and good digestibility (DE CAMPO et al., 2019; HELAL; TAGLIAZUCCHI, 2018). Yogurt matrix seems to be an excellent delivery vehicle for plant-derived phenolic compounds, once the low pH values increase the stability of phenolic compounds and the presence of proteins or large peptides and fat should maintain the integrity of phenolic compounds during digestion, increasing their bioaccessibility (HELAL;

TAGLIAZUCCHI, 2018). In addition, a yogurt colored with natural pigments may gain space in the market, enhancing attractiveness and the consumer interest. Therefore, the objective of this work was to encapsulate natural pigments from grape pomace and jabuticaba byproducts using yeasts *Saccharomyces cerevisiae* as vehicle and incorporate them in yogurt aiming its enrichment and coloring. To the best of our knowledge, this is the first work in literature that shows a food application for bio-based particles produced with yeasts.

4.2 MATERIAL AND METHODS

4.2.1 Materials and their preparation

For this work, three byproducts were used as raw material: residue from the Bordeaux grape vinification (*Vitis labrusca*), jabuticaba (*Myrciaria cauliflora*) pulping residues and waste brewery *Saccharomyces cerevisiae*. Grape pomace was donated by Vinícula Ferragut (Vinhedo, SP, Brazil) and the residue was dried in a forced air oven at 40 ºC for 36 hours (IORA et al., 2015). After drying, samples were milled in a knife mill. Jabuticaba fruits were purchased from local market and they were selected and washed with water, bleached for 3 minutes and then pulped on a pulping machine (EBERLE, 10C56), as proposed by Silva et al. (2014), in order to obtain peels and seeds as the raw material for subsequent steps. The obtained byproducts were dried in an oven with forced air circulation, at 60 ºC for 24 hours, and then samples were grounded in a blender (RODRIGUES et al., 2015). The biomass of *Saccharomyces cerevisiae* was kindly donated by Cervejaria Hausen Bier (Araras, SP, Brazil) after its utilization in Pilsen beer-type production. Yeasts were washed with distilled water consecutive times and the washing water was separated by decantation (RUBIO et al., 2018). After reaching the complete removal of the beer residue, the obtained biomass was placed in Petri dishes and frozen at - 20 ºC for 24 hours. After this time, yeasts were lyophilized in a Terroni freeze dryer (LC 1500, São Carlos, Brazil) for 48 hours at - 20 °C and pressure of 1–0.1 kPa. Afterward, grape pomace powder, grounded jabuticaba byproducts and dried yeasts were stored in dark environment at - 20 ºC in a freezer.

4.2.2 Preparation of extracts from Bordeaux grape pomace and jabuticaba byproducts

For the preparation of the grape pomace extract, 1 g of its powder was mixed with 20 mL of 40% (v/v) ethanol. Samples were shaken at 125 rpm (Orbital Shaker Marconi, MA420, Piracicaba, SP) and 25 °C for 3 hours, as described by Iora et al. (2015).

The extract from jabuticaba byproducts was prepared following the condition proposed by Rodrigues et al. (2015), using the ratio of 1:20 (w/v) of dry material and 46% (v/v) ethanol acidified to pH 2.0 with citric acid. Extraction was performed in an open rectangular ultrasound (Unique Model USC, 25 kHz, 150 W) for 10 minutes.

After extraction, samples from both materials were centrifuged at 4226 g for 10 min in a centrifuge (Eppendorf 543 0R). The resulting supernatants were evaporated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil), at 40 °C, until the extracts volumes were reduced to half of its initial volume. The obtained extracts were used for further analyses. Grape pomace and jabuticaba byproducts extracts were nominated by the acronyms GPE and JE, respectively.

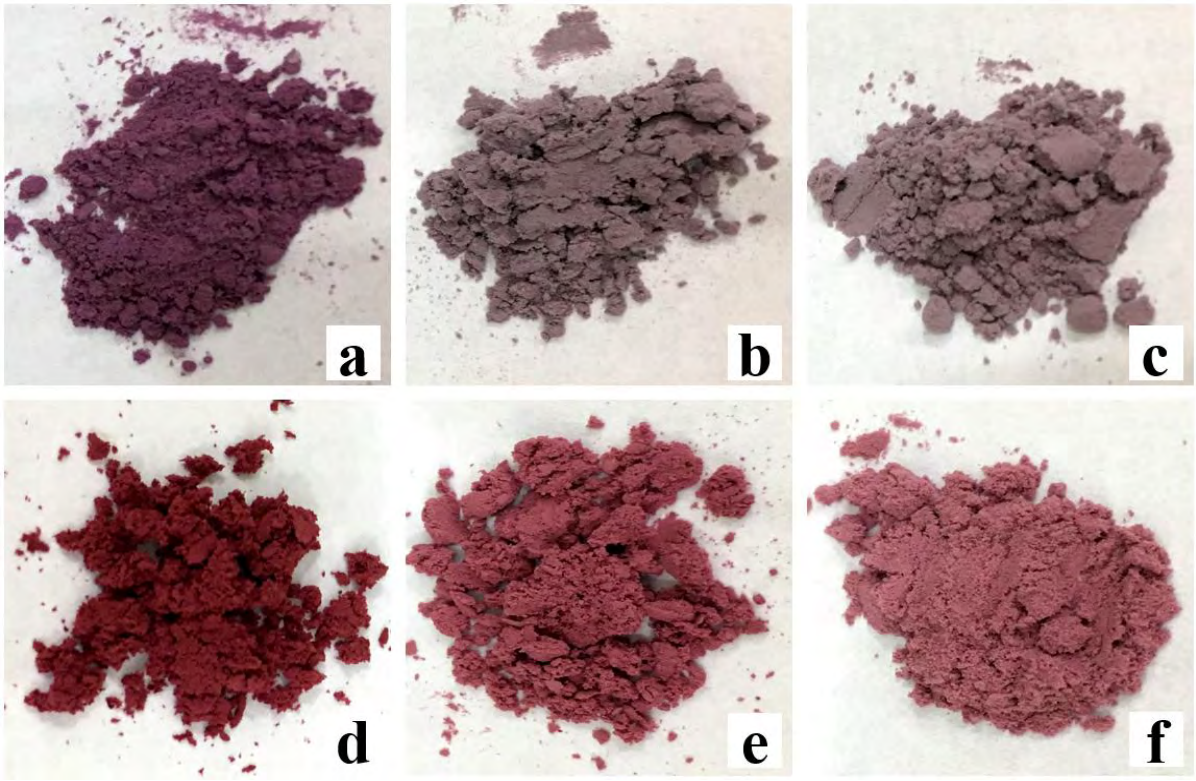
4.2.3 Spray drying operation conditions and powder stability evaluation

Mixtures of the extracts and yeasts were prior prepared by adding 5%, 10% and 15% (w/w) of dry *Saccharomyces cerevisiae* to the extracts. That is, 15 g of dry yeasts were mixed with 285, 135 and 85 g of extracts, respectively. The procedure was carried out according to Rubio et al. (2020). The suspensions were kept at room temperature and subjected to magnetic stirring in order to ensure that there was no phase separation. Then, samples were atomized in a bench spray dryer (model MSD 5.0, Labmaq do Brasil Ltda., Ribeirão Preto, Brazil), with a 2 mm nozzle, air flow of 65 L min^{-1} and feeding flow of 10.8 mL min^{-1}. The inlet temperature was 130 °C, the air compressor pressure was 0.2 MPa and the recorded outlet temperature was 80 ºC. The produced powders were collected in the cyclone compartment and they were separated in equal masses into penicillin flasks. After that, flasks were stored at 25 ºC in desiccators with $MgCl_2$ and controlled humidity at 33.3%. The stability of phenolic compounds and anthocyanins encapsulated in *Saccharomyces cerevisiae* yeast was evaluated on the day of production (day 0) and after 15, 30, 60 and 90

days of storage.

Powders produced with GPE and JE with 5, 10 and 15% of dried yeasts were named GP5, GP10, GP15 and J5, J10 and J15, respectively. Figure 14 shows the obtained powders.

Figure 14 - Powders produced by encapsulation of grape pomace extracts in *Saccharomyces cerevisiae* using proportions of 5, 10 and 15% of yeast (a, b and c, respectively) and by encapsulation of jabuticaba byproducts extracts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast (d, e and f, respectively)



Reference: Elaborated by the author

4.2.4 Determination of encapsulated compounds, encapsulation efficiency and bioactive retentions during storage

For determination, phenolic compounds and anthocyanins were extracted from the particles produced by adding to 0.05 g of powder into 1 ml of the reagent used in each extract production (40% ethanol for GPE and 46% acidified ethanol for JE). The suspension was sonicated in a rectangular ultrasound (Unique Model USC, 25 kHz, 150 W) for 5 minutes and then centrifuged for 5 minutes at 4226 g. This procedure was repeated until the obtained supernatant became colorless. The extract used for further analysis was the sum of supernatants obtained from each

extraction (RUBIO et al., 2020). For determination of surface compounds, 2 ml of the extraction solvent were added to 0.1 g of particles. The mixture was agitated and centrifuged for 1 minute at 3000 rpm. Finally, the supernatant was filtered through a 45 µm pore microfilter and the filtrate obtained was analyzed.

The phenolic content found in samples was estimated following the Folin-Ciocalteu colorimetric method proposed by Singleton & Rossi (1965). The absorbances were recorded at 765 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, Shanghai, China) and the results were found by using a prior standard calibration curve, prepared with concentrations between 45 and 500 mg of Gallic Acid per liter.

The anthocyanins quantification was based on the differential pH method, proposed by Giusti & Wrolstad (2001), technique based on spectrophotometric measurement (at 520 nm and 700 nm) of the absorbances of anthocyanin extract samples in two pH ranges, pH 1 and pH 4.5, using 0.025 mol L^{-1} potassium chloride (KCl) and 0.4 mol L^{-1} sodium acetate (CH_3COONa) solutions, respectively.

The encapsulation efficiency, in percentage, of phenolic compounds and anthocyanins was determined by the Equation 1, proposed by Tsali & Goula (2018). TC are the total compounds and SC are surface compounds.

$$EE\ (\%) = \frac{TC - SC}{TC} \times 100 \quad (1)$$

The compounds retention was determined by the Equation 2, where C_f is the compound content at the end of the storage period and C_i is the initial compound content.

$$CR(\%) = \frac{C_f}{C_i} \times 100 \quad (2)$$

4.2.5 Evaluation of powders instrumental color

Color was evaluated on the day of powders production and at the end of 90 days of storage (at 25 ºC and controlled humidity at 33.3%), using a HunterLab Mini Scan XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) to obtain the CIE L *, a * and b * color parameters. Chroma and hue angle were calculated according to the Equations 3 and 4 (TARONE et al., 2021), respectively.

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

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (4)$$

4.2.6 Microparticles morphology by confocal laser scanning microscopy

The analysis was carried out to observe differences between control and enriched cells, in attempt to confirm the presence of bioactive compounds inside yeast cells after atomization. Samples were prepared following the procedure described by Pham-Hoang et al. (2018) with slight modifications proposed by Rubio et al. (2020). Calcofluor White M2R and nile red solutions were prepared with distilled water and ethanol, respectively, at a concentration of 1 mg mL^{-1}. Cells were mixed with 1 µg mL^{-1} of calcofluor, washed three times with distilled water and stained with 1 µg mL^{-1} of nile red. Samples images were recorded using a Leica TCS SP5 Confocal Microscope (Leica Microsystems, Germany). For calcofluor, images were recorded in the excitation length at 405nm and emission wavelengths were collected between 430 and 480 nm. Nile red was excited at 488 nm and emission was recorded between 515 and 645 nm.

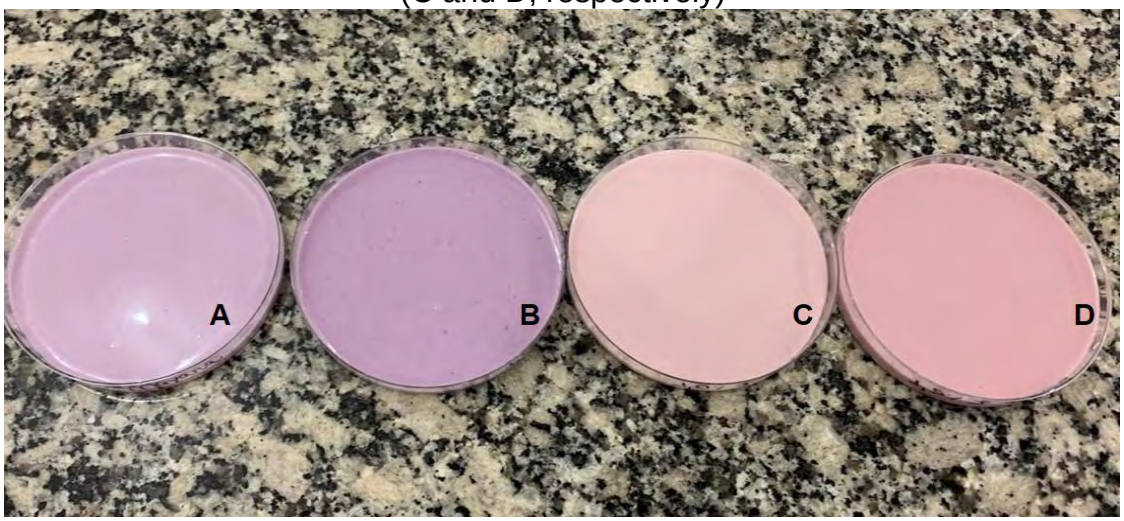
4.2.7 Enriched yogurts preparation

Yogurts were produced in the dairy plant of campus "Fernando Costa", at the University of São Paulo (Pirassununga, SP, Brazil). The procedures were followed as described by Comunian et al. (2017). Yogurt manufacturing involved the following the steps: milk heat-treatment at 90 ºC for 30 minutes; addition of sugar; cooling to 45 ºC; addition of the starter culture; incubation at 45 °C for 3 hours; cooling to 4 ºC and mixing of microcapsules in proportions of 1% and 1.5% (w/w). Grape aroma was added to the yogurt enriched with microparticles produced with grape pomace extract, in the concentration of 1.5 g L^{-1}. Jabuticaba aroma was added to the formulations with microcapsules produced with jabuticaba byproducts extract, in the concentration of 3 g L^{-1}. Yogurts were packed in 1000 mL plastic bottles and stored at 4 ºC for further characterization and stability analysis for 30 days.

The yogurts with 1% and 1.5% of microcapsules obtained with extracts from

grape pomace and 5% of yeast were named YGP1 and YGP15, respectively, and the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast were named YJ1 and YJ15. Figure 15 shows the yogurts with the incorporation of the above-mentioned pigments.

Figure 15 - Yogurts produced with 1 and 1.5% of particles obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5% of yeast (A and B, respectively) and with particles obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5% of yeast (C and D, respectively)



Reference: Elaborated by the author

4.2.8 Yogurts characterization

4.2.8.1 Determination of pH, titratable acidity and instrumental color

The pH of yogurts was measured using a previously calibrated digital pH meter (Mars MB-10, São Paulo, Brazil). The yogurt extracts for titratable acidity were obtained by diluting 10 g of original yogurt samples with 10 mL of deionized water. Thereafter, the mixture was titrated with 0.1 M NaOH to pH 8.3, controlled by a pH meter. The results were expressed as a percentage of lactic acid (IAL, 2008). Instrumental color was measured following the same procedure described in 2.5.

4.2.8.2 Rheology measurements

The rheological behavior of yogurts was determined using a rotational rheometer AR 2000, TA Instruments (New Castle, Delaware, USA), with parallel plate

geometry (60 mm, gap 500 µm). Tests were carried out following the procedure described by Comunian et al. (2017). Yogurts were pre-sheared for 30 s, at a shear rate of 500 s^{-1}, maintained in equilibrium for 30 s and then analyzed for 4 minutes. Samples were subjected to different rheological tests at 6 ºC. For steady-state tests, there was a gradual increase of shear rate from 0.1 to 100 s^{-1}, in which domain the evolution of shear stress and viscosity was monitored. The flow was modeled fitting data to the Herschel-Bulkley model (Equation 5).

$$\tau = \tau_0 + k_H.\gamma^{n_H} \quad (5)$$

where τ is the shear stress (Pa s), τ_0 is the yield stress (Pa), k_H is the consistency index (Pa s^n), γ is the shear rate (s^{-1}) and n_H is the flow behavior index (dimensionless). The type of fluid is determined by the parameter n_H: $n_H = 1$: Newtonian fluid; $n_H < 1$: pseudoplastic fluid; $n_H > 1$: dilating material.

For dynamic frequency sweep tests, an oscillatory stress of 0.2 Pa was set (region of linear viscoelasticity). The analysis was performed within the range of frequency of 0.1 and 10 Hz and storage modulus (G') and loss modulus (G'') were registered. Also, the complex modulus G* (Equation 6) was obtained at a frequency of 1 Hz.

$$G^* = [(G')^2 + (G'')^2]^{\frac{1}{2}} \quad (6)$$

4.2.8.3 Stability of yogurt phenolic compounds

For extraction and quantification of phenolic compounds and anthocyanins present in yogurts, 2 g of enriched yogurts were weighed in 15 mL centrifuge tubes following the addition of 4 mL of solvent (40% ethanol for YGP1 and YGP15 and 46% acidified ethanol for YJ1 and YJ15) and stirring for 3 minutes in vortex.

Mixtures were sonicated in and open rectangular ultrasound (Unique Model USC, 25 kHz, 150 W) for 5 minutes at 25 °C and, after that, samples were centrifugated for 5 minutes at 2935 g, obtaining the supernatant used for phenolic compounds and anthocyanins determination. This procedure was repeated at each seven days for one month. The half-life of the compounds in prepared yogurts was

calculated according to Equations 7 and 8, where C_o is the initial compound content, C_t is the compound content at the time t (days), $t_{\frac{1}{2}}$ is the half-life time and k is the reaction rate constant (SOUZA et al., 2014).

$$-\ln\frac{C_t}{C_o} = kt \tag{7}$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \tag{8}$$

4.2.8.4 Consumer acceptance test

A consumer acceptance test of enriched yogurts was performed to evaluate the effect of particles on sensory attributes, mainly color. The analysis with humans was previously approved by the Ethics in Research Committee of the University of São Paulo, SP, Brazil (protocol number 08111219.3.0000.5422, Attachment B). For the test, a panel consisting of 120 untrained panelists was employed. Panelists were recruited among students, professors and staff on the campus and they were selected on the basis of being regular consumers of yogurt. Samples were numbered with random three-digit codes and were served randomized, in plastic cups containing 25 mL, to consumers in individual cabins. Water and salty cracker biscuits were also served to rinse out the mouth and neutralize the flavors between samples. During analysis, samples were kept in a refrigerator, at 4 ºC, before serving. Before analysis, each panelist had to read and sign, if agreed, a Free and Informed Consent Form.

For the affective acceptance test, a 9–point hedonic scale was applied, varying from 1 = “extremely dislike” to 9 = “extremely like” to evaluate the product acceptance in relation to the attributes: appearance, color, aroma, flavor, texture and overall acceptability. The acceptance index (AI) was determined by obtaining the relation between the average score obtained for tested sample and the maximum score of the hedonic scale (9) multiplied by 100 (DUTCOSKY, 2007).

4.2.9 Statistical analysis

The analysis was carried out using STATISTICA 13.4.0.14 software

(StatSoft, Tulsa, USA). The comparison between means was performed by one-way analysis of variance (ANOVA) with post-hoc Tukey. A difference was considered statistically significant when $p \leq 0.05$. The software OriginPro 7.0 was used to perform the nonlinear regression for rheological data and the quality of the model applied was evaluated by the determination coefficient and chi-square value.

4.3 RESULTS AND DISCUSSION

4.3.1 Encapsulation efficiency and powders stability and retention of compounds

Table 6 shows the encapsulation efficiency obtained for all particles produced, the contents of phenolic compounds and anthocyanins in powders during the period of 90 days of storage and the retention of compounds after 90 days. Encapsulation efficiency ranged between 52.3 and 67.5% for phenolic compounds and from 4.2 to 47.4% for anthocyanins. Regarding to the effect of wall material concentration on encapsulation efficiency, in general, the increase in yeast concentration led to a decrease in encapsulated compounds. Thus, highest phenolic compounds and anthocyanins encapsulation efficiencies were obtained for GP5 and J5. The higher concentration of yeasts in relation to the extract may increase the viscosity of the feed solution, contributing to cells aggregation and, consequently, less contact area and active sites available for compounds bounding and entrance.

Encapsulation efficiencies of phenolic compounds were higher in comparison to anthocyanins, for both extracts GPE and JE. The complex nature of the extracts used in this work, presenting several different compounds in their composition, may lead to a competition to bind into the cell wall. In addition, as anthocyanins were retained mainly in the cell wall, it is possible that their molecule size could have impaired their passage through yeast pores.

Malvidin-3-glucoside is the major anthocyanin in Bordeaux grapes (DE SOUZA et al., 2015) and delphinidin-3-glucoside and cyanidin-3-glucoside are found in jabuticaba peels (BARROS et al., 2019). The chain length of these mentioned anthocyanins is higher than the chain length of gallic and ellagic acids, phenolic acids commonly found in Bordeaux grape pomace and jabuticaba peels (INADA et al., 2020; ROCKENBACH et al., 2011), respectively. With extended chains, the number of -CH_2- groups increases making

the apolar area longer and more difficult passage through the polar part in the cell structure. This polar region of the cell membrane plays an important role in the diffusion and the more polar the molecule is, the better its diffusion. Therefore, the polarity of molecules is an important factor able to influence the passage of molecules through yeast membrane and, consequently, the encapsulation achievement (PHAM-HOANG; VOILLEY; WACHÉ, 2016).

Table 6 - Phenolic compounds and anthocyanins stability in powders produced by encapsulation of extracts from grape pomace and jabuticaba byproducts in yeasts *Saccharomyces cerevisiae*, encapsulation efficiency (EE) and compound retention (CR) after storage

Sample	EE (%)	Day 0	Day 15	Day 30	Day 60	Day 90	CR (%)
	Phenolic compounds (mg EAG/g of particle)						
GP5	67.5^{A}	154.4^{Aa}±1.4	113.0^{Ab}±2.8	113.8^{Ab}±3.5	111.9^{Ab}±2.1	114.0^{Ab}±1.7	73.8^{C}
GP10	67.2^{A}	78.9^{Ba}±0.07	65.3^{Bb}±2.3	61.5^{Bb}±5.7	52.5^{Bc}±0.7	64.2^{Bb}±0.7	81.4^{B}
GP15	64.7^{B}	58.9^{Ca}±0.8	51.0^{Cc}±0.7	46.6^{Cd}±0.8	53.5^{Bbc}±0.7	54.7^{Cb}±2.4	92.9^{A}
J5	63.6^{A}	360.4^{Aa}±2.0	331.8^{Ab}±4.8	286.9^{Ac}±2.0	279.3^{Ac}±2.5	283.8^{Ac}±2.7	78.7^{B}
J10	56.1^{B}	222.8^{Ba}±1.5	214.6^{Ba}±16.7	209.3^{Ba}±2.5	212.8^{Ba}±2.5	212.8^{Ba}±8.7	95.5^{A}
J15	52.3^{C}	190.2^{Ca}±2.2	111.9^{Cc}±7.3	153.3^{Cb}±2.5	153.9^{Cb}±5.4	163.2^{Cb}±2.7	85.8^{AB}
	Anthocyanins (mg/g of particle)						
GP5	11.1^{A}	17.9^{Aa}±1.0	13.9^{Ab}±0.5	11.9^{Abcd}±2.1	10.7^{Acd}±0.6	9.9^{Ad}±0.8	55.3^{B}
GP10	7.4^{B}	11.1^{Ba}±0.3	5.9^{Bb}±0.3	3.5^{Bd}±0.2	5.4^{Bbc}±0.2	5.0^{Bc}±0.08	45.0^{C}
GP15	4.2^{C}	5.3^{Ca}±0.3	4.4^{Cb}±0.3	3.2^{Bc}±0.04	4.4^{Bb}±0.3	4.5^{Bab}±0.2	84.9^{A}
J5	47.4^{A}	14.8^{Aa}±0.5	14.1^{Aa}±0.5	11.2^{Ab}±0.8	11.9^{Ab}±0.2	9.2^{Ac}±0.1	62.2^{A}
J10	30.5^{B}	12.5^{ABa}±1.6	10.5^{Bab}±0.4	8.0^{Bbc}±0.2	7.3^{Ac}±1.7	7.0^{Bc}±0.4	56.0^{A}
J15	18.3^{C}	10.2^{Ba}±0.8	7.9^{Cb}±0.2	6.7^{Bb}±0.7	6.8^{Ab}±1.5	6.1^{Bb}±0.1	59.8^{A}

Where GP5. GP10 and GP15 are the powders obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeasts, respectively. J5, J10 and J15 are the powders obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast, respectively. Capital letters in the same column and small letters in the same row indicate there is no significant difference ($p > 0.05$) among samples, considering the same raw material.

Reference: Elaborated by the author

In comparison to other works performed using yeast cells as carrier materials, Nguyen et al. (2018) encapsulated anthocyanins from Hibiscus (*Hibiscus sabdariffa* L.) in yeast cells and obtained an encapsulation efficiency of around 27% under optimized conditions, using a concentration of 100 g L^{-1} of dry yeast for anthocyanin-rich hydroalcoholic extract. Medeiros et al. (2018) obtained encapsulation efficiencies of 33.1 and 49.5% for the internalization of curcumin and fisetin into *Saccharomyces cerevisiae* cells, respectively. From these results, it can be inferred that polarity is a really crucial factor that may have influence on the compounds entrapment. Although yeasts have affinity for both hydro and lipophilic compounds, due to the presence of phospholipids polar heads toward outside and nonpolar heads oriented to the center of the membrane (WANG; CHEN, 2009), it seems that is easier to incorporate hydrophilic compounds because of the more polar surface. That explains higher encapsulation efficiencies for compounds such as fisetin and other phenolic compounds and lower for curcumin.

Related to compounds stability, there was in general a decline in the content for both phenolic compounds and anthocyanins between time 0 and 90 days, as expected. For all times there was a significant difference among the three treatments, where GP5 and J5 presented the highest content of phenolics at all points analyzed. The retention of phenolics after 90 days of storage was higher with larger amounts of yeasts in the medium for samples prepared with grape pomace extract, with retentions of 73.8% for GP5 and 92.9% for GP15. This trend would allow to infer that the yeast in greater quantity in the sample would protect the phenolics, however, it was not so clear for the extract of jabuticaba, since the treatment with 10% had higher retention (95.5%) of phenolic compounds in comparison to GP5 and GP15. For anthocyanins, GP10 also showed an irregular behavior, presenting lower retention. Anthocyanin retentions for J5, J10 and J15 were not significantly different.

4.3.2 Changes in powders color parameters after storage

The parameters of color obtained for powders produced by encapsulation of extracts from grape pomace and jabuticaba extracts in *Saccharomyces cerevisiae* are shown in Table 7.

Table 7 - Color parameters L*, a*, b*, hue angle (h*) and chroma (C*) for powders produced by encapsulation of extracts from grape pomace and jabuticaba byproducts in yeasts *Saccharomyces cerevisiae*

Sample	**Day 0**					**Day 90**				
	L*	a*	b*	h*	C*	L*	a*	b*	h*	C*
GP5	42.5^{Ca}±0.04	15.1^{Aa}±0.1	-4.1^{Cb}±0.04	-15.3^{Cb}±0.1	15.6^{Aa}±0.1	41.8^{Bb}±0.2	14.0^{Ab}±0.2	-2.8^{Ca}±0.1	-11.2^{Ca}±0.6	$14,2^{Ab}$±0.2
GP10	56.7^{Ba}±0.006	7.0^{Bb}±0.1	-0.5^{Bb}±0.06	-4.9^{Bb}±0.5	7.0^{Bb}±0.06	53.6^{Ab}±1.2	7.1^{Ba}±0.07	0.4^{Ba}±0.08	3.6^{Ba}±0.6	$7,2^{Ba}$±0.07
GP15	59.0^{Aa}±0.03	7.0^{Ba}±0.02	-0.2^{Ab}±0.05	-1.6^{Ab}±0.4	7.0^{Ba}±0.02	53.2^{Ab}±0.1	6.9^{Cb}±0.04	1.1^{Aa}±0.04	10.3^{Aa}±0.4	$6,3^{Bb}$±0.04
J5	31.0^{Ca}±0.08	25.3^{Aa}±0.06	4.9^{Aa}±0.08	11^{Aa}±0.2	25.8^{Aa}±0.05	31.0^{Ca}±0.9	22.8^{Ab}±0.4	4.4^{Ab}±0.1	10.9^{Aa}±0.08	$23,3^{Ab}$±0.4
J10	41.8^{Ba}±0.05	23.2^{Ba}±0.06	3.1^{Bb}±0.04	7.6^{Bb}±0.09	23.4^{Ba}±0.06	37.5^{Bb}±6.2	21.8^{Bb}±0.6	3.5^{Ba}±0.7	8.4^{Ca}±0.04	$22,1^{Ab}$±0.7
J15	50.5^{Aa}±0.01	19.0^{Ca}±0.02	2.5^{Ca}±0.02	7.4^{Bb}±0.06	19.2^{Ca}±0.02	48.4^{Ab}±0.9	17.3^{Cb}±0.1	2.8^{Ca}±0.08	9.1^{Ba}±0.2	$17,5^{Bb}$±0.1

Where GP5. GP10 and GP15 are the powders obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeasts, respectively. J5, J10 and J15 are the powders obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast, respectively. Capital letters represent the comparison between different treatments in the same time, while small letters compare different times for the same treatment. Mean values followed by the same superscripts are not significantly different ($p > 0.05$).

Reference: Elaborated by the author

For samples obtained by the encapsulation of GPE and JE, the luminosity was lower in the powders with the addition of 5% of yeast biomass. The lower addition of yeast and higher proportion of extract in the mixture may have led to the greater dispersion of pigments and, in consequence, the improved darker color. In addition, it is possible to notice an increase in the luminosity with the increase in the material wall content used. Chroma values were inversely proportional to L values and confirmed that GP5 and J5 powders present more intense colors.

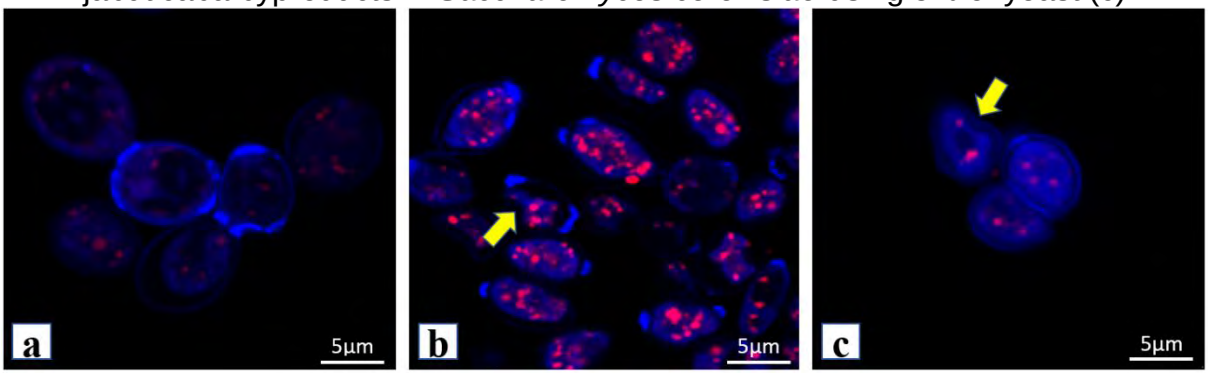
The powders GP5 and J5 presented higher intensity of a* parameter indicating greater intensity of red color. For b* parameter, while the powder GP5 presented the lowest value, of - 4.1, J5 presented the highest value comparing with the other treatments, of 4.9. These values are related to the blue color, which can be explained by the presence of different anthocyanins in extracts. Hue angles around 0º all over the storage period indicated that a red color was established, typically found in anthocyanins extracts of red berries in non-basic media (TARONE et al., 2021).

After 90 days, GP10, GP15, J10 and J15 had significant losses of luminosity bringing a change in the color profile. GP5 had a slight darkening passed 90 days (decrease in lightness and chroma), probably because of compounds oxidation, however, this change was lower comparing with the other treatments with GPE. J5 did not present difference in the parameter L* after storage and this result may be interesting from the point of view of the application, since it is interesting to apply darker pigments that maintain their intensity all over the storage. In general, for all particles, there was a decrease in the parameter a* and an increase in b*, indicating the degradation of anthocyanins and phenolic compounds over time.

4.3.3 Particles morphological analysis

For this analysis, GP5 and J5 were selected because of their highest content of phenolic compounds and anthocyanins entrapped. Confocal microscopy images represented by Figure 16 show cellular morphology before (a) and after enrichment with active compounds (b and d).

Figure 16 - Confocal scanning microscopy images of control yeasts (without encapsulation of bioactive compounds and spray-drying) (a), particles obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5% of yeast (b) and particles obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5% of yeast (c)



Yellow arrows indicate shrinkages and concavities in cells surface
Reference: Elaborated by the author

Enriched cells present slight changes in their outer surface, such as shrinkages and concavities, which are expected as a consequence of water evaporation through the spray-drying process. It cannot be observed any rupture in cells, the membrane seems to be intact even after the drying process. The use of confocal microscopy is very interesting due to the possibility of observing the intra-cellular area. According to Pham-Hoang et al. (2018), calcofluor is a cell-wall specific fluorochrome dye used to mark yeast cells (it binds to β-1,6-glucans and chitin in cell walls) and nile red has affinity for liposoluble structures, especially intracellular lipids.

Red bodies observed in both control and enriched cells were stained with nile red. In the particles without encapsulation of extracts (Figure 16a), this fluorochrome stained mainly the organelles of the yeast cell. However, for the particles obtained by encapsulation of extracts from grape pomace and jabuticaba byproducts extracts (Figures 16b and 16c, respectively), the red color was more intense, even using the same laser potency and stain methodology. Therefore, nile red probably stained active compounds bounded to the organelles as well as the lipid bodies within yeast cells (PHAM-HOANG; VOILLEY; WACHÉ, 2016). Furthermore, grape pomace extracts loaded particles presented the higher intensity of color, which could be attributed to a higher quantity of lower water-solubility compounds in this residue.

Under calcofluor excitation, control cells are stained mainly in their outer surface, whereas after enrichment with compounds, yeasts show a more pronounced intensity in their interior part, especially those enriched with extracts from jabuticaba byproducts. These observations indicate that there could be a homogeneous intra-

cellular distribution of the active compounds. The higher intensity for J5 (Figure 16c) might probably be related to the higher content of phenolic compounds in the jabuticaba extract encapsulated. With cells enrichment and the consequent fluorescence spread all over the cell structure, it can be assumed that compounds have perturbed significantly the cell organization and it is an indicative of phenolic compounds and anthocyanins incorporation inside the yeasts.

4.3.4 Yogurts characterization

GP5 and J5 obtained with 5% of yeast *Saccharomyces cerevisiae* and concentrated extracts from grape pomace and jabuticaba byproducts, were the powders chosen for application in yogurts, due to their highest encapsulation efficiency, content of phenolic compounds and anthocyanins and the maintenance of luminosity during storage. Table 8 shows the results for pH, titratable acidity and parameters of color for the yogurts produced with the chosen particles.

Table 8 - Values of pH, titratable acidity and parameters L*, a* and b* of color of the yogurts

Sample	pH	Titratable acidity	L*	a*	b*
YGP1	4.1	0.8	54.9±0.01	5.2±0.1	-2.9±0.05
YGP15	4.1	0.9	54.3±0.01	6.2±0.06	-3.8±0.03
YJ1	4.0	1.0	60.3±0.02	1.9±0.05	6.5±0.07
YJ15	4.0	1.1	62.0±0.03	3.3±0.03	6.4±0.04

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively.
Reference: Elaborated by the author

The pH values varied between 4.0 and 4.1, while values of titratable acidity were within the range of 0.8 and 1.1. Yogurts added with different proportions of J5 presented lower pH values maybe because of the extract used for encapsulation, which was acidified with citric acid. Jaster et al. (2018) recommend an acidity of milk products between 0.6 and 1,5% and Benedetti et al. (2016) reported that consumers prefer fermented products with pH on the range of 4.2 and 4.4.

Demirkol & Tarakci (2018) produced enriched yogurts applying grape pomace powders obtained from freeze drying or drying with forced air circulation at

different temperatures, and obtained pH and titratable acidity results from 3.99 to 4.25 and 0.74 to 0.93%, respectively. Karaaslan et al. (2011) enriched natural yogurts with extracts rich in phenolic compounds and anthocyanins from different species of grapes and obtained a range of pH and titratable acidity from 4.224 to 4.279 and 0.95 to 0.98%, respectively. Thus, the results obtained in the current work is in accordance with previous studies reported in literature.

In relation to the yogurt color, luminosity decreased with the addition of a higher percentage of particles and the intensities of red and blue were higher, as expected, as an obvious response to the enrichment with more particles.

4.3.5 Phenolic compounds and anthocyanins stability in yogurts

Based on Table 9, there was a significant difference in the content of phenolic compounds and anthocyanins in yogurt over time. Comparing samples with the same particles, but in different proportions, there was a significant difference between them in almost all points studied, with higher content of compounds for treatments using 1.5% of particles. This was expected once the proportion of pigment was higher and, consequently, phenolic compounds and anthocyanins contents were higher as well. Phenolic compounds in the sample YJ1 and anthocyanins in samples YGP15, YJ1 and YJ15 presented significant differences among day 0 and 7 and, after that, there was a stabilization in the compounds content during the other days of storage. The same trend was observed by Wallace & Giusti (2008) incorporating *Berberies boliviana* whole berry powder in yogurt. Anthocyanins can form complexes with the macromolecules of milk, protecting them from degradation. However, this interaction can make the compound less bioavailable, in addition to being able to precipitate (OLIVEIRA et al., 2015). This fact may explain the significant decline in anthocyanin levels after yogurt storage.

Table 9 - Phenolic compounds and anthocyanins stability in yogurts enriched with microcapsules produced by encapsulation of extracts from grape pomace and jabuticaba byproducts in yeasts *Saccharomyces cerevisiae* and half-life times

Sample	Day 0	Day 7	Day 15	Day 30	$T_{1/2}$ (days)
	Phenolic compounds (mg EAG/g of particle)				
YGP1	25.5^{Ba}±0.1	24.7^{Ba}±0.8	22.1^{Bb}±0.6	20.7^{Bc}±0.5	98.8
YGP15	33.0^{Aa}±1.8	33.1^{Aa}±0.4	29.2^{Ab}±1.4	27.1^{Ac}±0.4	105.0
YJ1	25.6^{Ba}±0.3	24.2^{Bb}±0.1	24.0^{Bbc}±0.9	23.0^{Bc}±0.8	193.5
YJ15	33.1^{Aa}±0.3	31.4^{Ab}±1.5	29.0^{Ac}±0.3	27.5^{Ad}±0.9	112.3
	Anthocyanins (mg/g of particle)				
YGP1	3.5^{Ba}±0.1	3.1^{Bb}±0.8	3.0^{Bb}±0.6	2.3^{Bc}±0.5	48.5
YGP15	5.3^{Aa}±1.8	5.0^{Aab}±0.4	4.8^{Aab}±1.4	5.6^{Ab}±0.4	101.1
YJ1	0.7^{Ba}±0.3	0.6^{Bab}±0.1	0.6^{Aab}±0.9	0.4^{Ab}±0.8	34.7
YJ15	1.1^{Aa}±0.3	0.9^{Aab}±1.5	0.8^{Aab}±0.3	0.6^{Ab}±0.9	39.5

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively. Capital letters in the same column and small letters in the same row indicate there is no significant difference ($p > 0.05$) among samples, considering the same raw material.
Reference: Elaborated by the author

Regarding to determined half-life times, phenolics half-lives were within the range of 98 and 194 days, while for anthocyanins, the variation was higher, between 34 and 102 days. Considering that the shelf life of yogurt is among 25 and 35 days (WALLACE; GIUSTI, 2008), the natural pigments evidenced in this work might be available during the yogurt shelf life and, in addition to the coloring function, compounds may offer a potential for health benefit.

Although there was a decrease in phenolic compounds and anthocyanins content over the storage period, it is probable that yeasts are not releasing entrapped compounds and those which are retained in the yeast surface are spread in yogurt and, as a consequence, they have less protection and greater tendency to degradation. To confirm whether *Saccharomyces cerevisiae* is able to protect entrapped compounds in a medium with proteins, fat and sugar, further studies are necessary to evaluate compounds release in yogurt and their bioaccessibility after digestion simulations.

4.3.6 Rheological characterization of yogurts

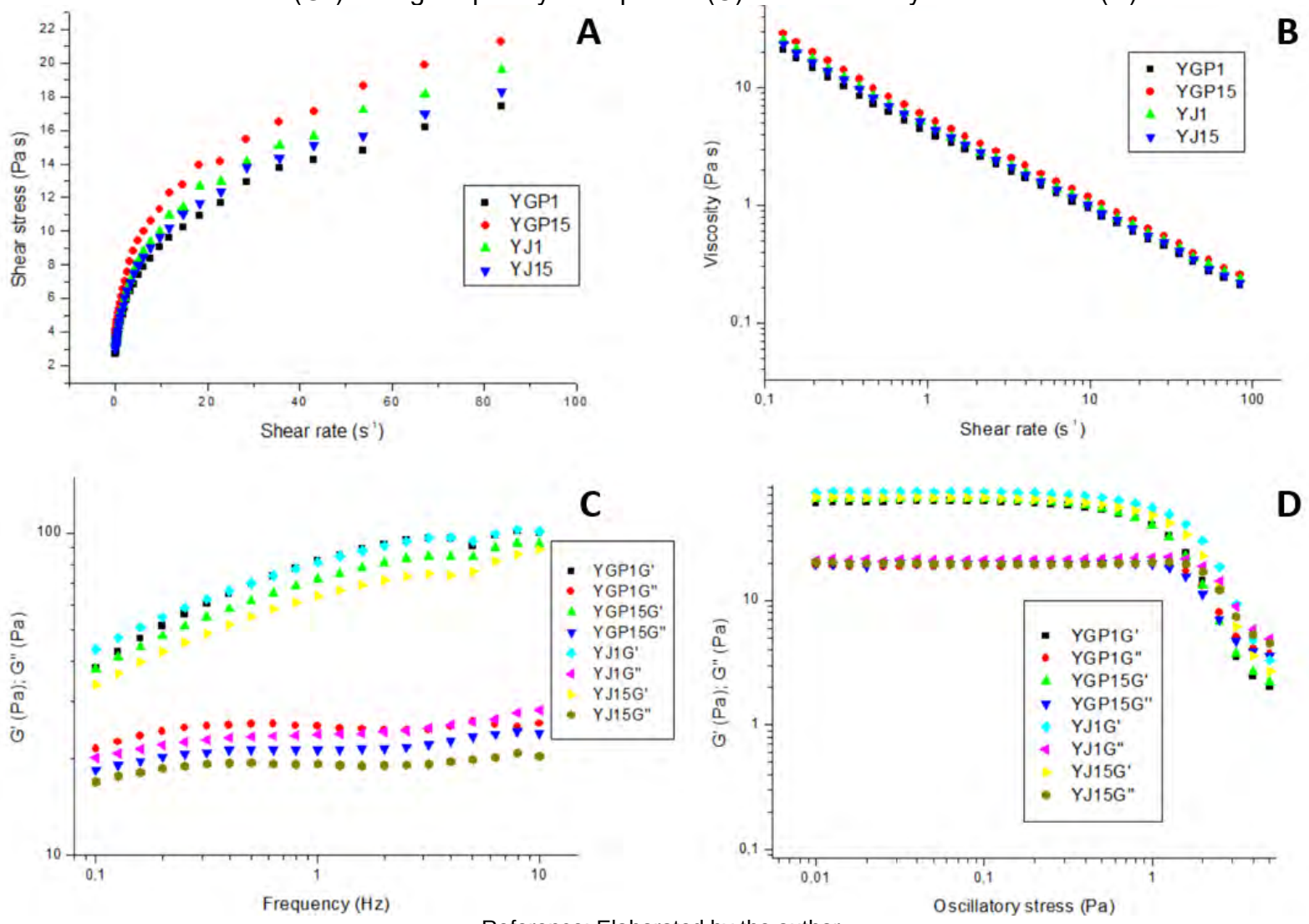
The flow behavior of yogurts colored with different proportions of GP5 and J5 was properly explained by the Herschel Bulkley model, with determination coefficients higher than 0.997 and χ^2 lower than 0.09. Table 10 lists the parameters obtained by the model. The flow index (n_H) was lower than 1 ($n < 1$), showing a shear thinning behavior for all yogurts and confirming that samples are non-Newtonian materials (Figure 17A).

Table 10 - Rheological parameters for data fitted by Herschel-Bulkley model and storage modulus (G'), loss modulus (G'') and complex modulus (G*) at a frequency of 1 Hz

Sample	τ_0 (Pa)	k_H (Pa s^n)	n_H (-)	χ^2	R^2	G' (Pa)	G'' (Pa)	G* (Pa)
YGP1	0.2^A	4.4^A	0.3^A	0.07	0.997	81.9^A	25.2^A	85.9^A
YGP15	0.6^A	5.2^A	0.3^A	0.09	0.997	64.9^B	19.8^A	67.9^B
YJ1	0.7^A	4.4^A	0.3^A	0.08	0.997	81.2^A	23.7^A	84.6^A
YJ15	0.3^A	4.7^A	0.3^A	0.06	0.998	63.9^B	19.9^A	66.7^B

Where τ_0 is the yield stress (Pa), k_H is the consistency index (Pa s^n), n_H is the flow behavior index (dimensionless), χ^2 is chi-square value and R^2 is the determination coefficient. YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively. Mean values in the same row followed by the same superscripts are not significantly different ($p > 0.05$).
Reference: Elaborated by the author

Figure 17 - Steady-state flow curves (A), viscosity as a function of the shear rate (B), and evolution of storage modulus (G') and loss modulus (G'') during frequency sweep tests (C) and oscillatory stress at 1 Hz (D)



Reference: Elaborated by the author

The pseudoplastic behavior can also be visualized in the Figure 17B, once the viscosity decreases with the increasing shear rate. When shear forces are applied in a fluid, weak bonds are destroyed and there is a reduction in the hydrophobic interaction between molecules and in the electrostatic repulsion. The disruption is greater at the beginning of shearing and, after that, there is an alignment of the particles with the flow and it reduces the viscosity (JASTER et al., 2018). Also based on Figure 17A, the shear stress curve does not begin at the origin of the plot and it is concave downwards. The existence of a yield stress in the flow curve of a material indicates that there is a cross-linked or another interactive structure that must be broken before flow can occur at an appropriate rate (PEREIRA; DE RESENDE; GIAROLA, 2014).

Storage modulus was higher than loss modulus (G’ > G’’) in all cases (Figure 17C and Table 10), which allowed characterizing the behavior of all samples of yogurts as predominantly elastic. In addition, all yogurts could be considered weak gels (G’/G’’ < 10) (AUGUSTO; CRISTIANINI; IBARZ, 2012), regardless of the addition of particles. The addition of a higher proportion of microparticles resulted in lower complex modulus (G*) values at frequency of 1 Hz, suggesting decreased resistance to deformation (GHEONEA (DIMA) et al., 2020). A possible explanation is that added microparticles are somehow interacting with the milk proteins, reducing aggregation of the casein network and reducing the resistance of yogurt to flow (JASTER et al., 2018). In Figure 17D, at lower values of oscillatory stress, the storage modulus was also higher than loss modulus, assuming the gel-like nature of all yogurts. Above the cross point (oscillatory stress at which G’ and G’’ assume the same value), the loss modulus is slightly higher than the storage modulus, which is a characteristic of a liquid. This implies the sol-gel transition of the material (PASQUI; DE CAGNA; BARBUCCI, 2012).

4.3.7 Sensory analysis – Acceptance test

A consumer acceptance test was applied to evaluate whether the addition of particles had positive influence on the sensory parameters of yogurts, mainly the color. According to the Table 11, in general, the difference was not significant between the samples for appearance, color, aroma and overall average.

Table 11 - Results of yogurts sensory evaluation and acceptance index

Parameters	YGP1	YGP15	YJ1	YJ15
Appearance	7.5^{a}±1.3	7.5^{a}±1.4	6.1^{a}±1.8	6.3^{a}±1.6
Color	7.7^{a}±1.3	7.8^{a}±1.3	5.9^{a}±1.8	6.3^{a}±1.7
Aroma	7.5^{a}±1.3	7.4^{a}±1.4	7.0^{a}±1.6	7.2^{a}±1.6
Flavor	6.7^{a}±1.9	6.1^{b}±2.0	6.8^{a}±2.0	6.5^{a}±2.1
Texture	7.4^{a}±1.5	7.2^{a}±1.5	7.5^{a}±1.3	7.0^{b}±1.7
Overall acceptability	7.2^{a}±1.5	6.7^{b}±1.6	6.8^{a}±1.6	6.5^{a}±1.7
Overall average	7.3^{a}±1.1	7.1^{a}±1.2	6.7^{a}±1.3	6.7^{a}±1.4
Acceptance index (%)	81.4	79.1	74	73.9

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively. Mean values in the same row followed by the same superscripts are not significantly different ($p > 0.05$).
Reference: Elaborated by the author

Yogurts produced with 1,5% of grape pomace particles had lower values of flavor and overall acceptability, while panelists evaluated yogurts produced with 1,5% of jabuticaba byproducts with lower scores for texture. This result might be interestingly correlated to rheological measurements, which showed yogurts had higher tendency to flow with the increasing proportion of added particles. The acceptance index (AI) varied within the range of 73 and 82%. According to Dutcosky (2015), samples are considered well accepted when they have an AI (%) greater than 70%, thus, when observing this criterion, it is noted that all the samples of yogurt produced were well accepted.

According to the free comments from the panelists, although the average of scores did not differ significantly, most of the panelists found the colors of the yogurts quite attractive, highlighting 1.5% YGP and 1.5% YJ treatments, a fact that coincides with the instrumental color analysis previously discussed, since these treatments presented higher values of the color parameter chroma. In addition, few panelists commented about a slightly bitter taste they felt in formulations. The more probable explanations for this result are: first, the source of the waste yeast is the production of beer, thus, the biomass still present a bitter residual taste; second, once this yeast was used as a carrier material, its taste was not fully masked, despite the presence of extracts and flavorings. Other panelists pointed out the presence of some agglomerated powder in the product. This fact can be explained by the possible incomplete disintegration of the particle agglomerates when incorporating them into

yogurt samples and also by the yeast sedimentation, already expected, since it is not a soluble material. Then, it is recommended to shake the yogurt before consuming. To overcome the sedimentation, a more viscous or creamy yogurt could have been used. However, although it was possible to see sedimented yeasts at the bottom of the yogurt bottles, yeast is not expected to be sensorially sensed, and even when yeasts are agglomerated, they do not confer a grainy texture to the product.

Also based on the sensory evaluation, 70 panelists said they would buy YGP1 yogurt and 45 panelists would buy YGP15 yogurt. Regarding to the yogurts enriched with powders from the byproducts of jabuticaba, 69 panelists said they would buy YJ1 yogurt and 49 panelists would buy YJ15 yogurt.

CONCLUSIONS

In this work, waste yeast biomass was used as wall material for encapsulation of natural pigments. Yeasts are much more complex than other materials used for encapsulation, because of their biological nature. Thus, it is important to understand how entrapped compounds behave after their incorporation and what are the effects of the enriched yeast application into a food matrix.

Results showed that pigments produced with lower proportion of yeast (5%) had more intense colors, highest encapsulation efficiencies for both phenolic compounds and anthocyanins, higher compounds content all over the storage period and luminosity maintained after 90 days. Thus, these pigments were chosen for application in yogurt. All yogurt samples were well accepted by panelists, mainly the formulations prepared using 1% of pigments produced with grape pomace and jabuticaba byproducts extracts, with acceptance indexes of 81.4 and 74%, respectively.

From the point of view of the production cost, it is really interesting to use lower amounts of raw material (yeasts) for obtaining pigments and lower proportions of pigments for coloring a food matrix. In addition to the reuse of bioproducts from food industries as sources of bioactive compounds, this research is responsible for pointing a novel application for waste yeasts *Saccharomyces cerevisiae* as carrier material and pigments for the production of novel functional products.

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5 CHAPTER 4 – INVESTIGATION OF BREWER'S SPENT YEAST AS A BIO-VEHICLE FOR ENCAPSULATION OF CAROTENOIDS FROM PUMPKIN PEELS (*CUCURBITA MOSCHATA*)

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ABSTRACT

Brewer's spent yeast (BSY) *Saccharomyces cerevisiae* has been currently explored as a bio-vehicle for encapsulation of bioactive compounds and as a delivery system. The main objectives of this work were encapsulating carotenoids from pumpkin peels extract using BSY as encapsulant agent and evaluating the influence of an ultrasound treatment on carotenoids incorporation, stability and release. Microcapsules produced by encapsulation of carotenoids in BSY presented physical and microbiological stability during storage, presenting low values of water activity (< 0.406), moisture content (< 7.0%) and hygroscopicity (< 6.8 g/100g), characteristics of greatest importance for powder formulations. The best retention of carotenoid (273.3 µg g^{-1} of particle) was obtained applying the ultrasound treatment before atomization, which probably led to an adsorption of carotenoids into yeasts. Ultrasound also showed a positive effect on the color protection of powders during storage and on the protection of compounds under simulated gastrointestinal digestion. BSY released the compounds gradually during the digestion and, fortunately, the higher carotenoid release occurred in the intestinal phase (IP), around the double content found in the beginning of the IP. Yeasts are a suitable carrier material and shows promising characteristics for technological application in foodstuff.

Keywords: Ultrasound, byproducts, spray-drying, natural colorants, simulated digestion, *Saccharomyces cerevisiae*.

5.1 INTRODUCTION

Annually, agricultural processing industries generate significant amounts of waste, mainly composed by solid parts of processed foods that are not used in the manufacture of the product, considered a problem from an economic and environmental point of view (VAN DYK et al., 2013). Despite the greater use for animal feed or as organic fertilizer, the disposal from the food industry constitutes a rich source, but still underexplored, of compounds that can be applied as new ingredients, additives or supplements (GENEVOIS; FLORES; DE ESCALADA PLA, 2016). A wide range of bioactive compounds can be found in different fractions of the waste generated (BANERJEE et al., 2016).

In the pumpkin processing, the most used part in the elaboration of products is the pulp, and about 18 to 21% of the fruit is discarded as a byproduct, mainly the peels (GENEVOIS; FLORES; DE ESCALADA PLA, 2016). While bioactive compounds are widely distributed in fruits, carotenoids are also present, and even more concentrated, on the surface of tissues and seeds, parts that are normally rejected by consumers and food processing (MARTINS; FERREIRA, 2017). When compared to pulp, fruit peels are generally richer in carotenoids (SAINI; NILE; PARK, 2015). Carotenoids are natural pigments usually found in fruits and vegetables of intense yellow or orange color, capable of interacting with free radicals, whose presence in the body results in cell damage and the resulting oxidative stress. Therefore, they play an antioxidant function and reduce the risk of several disorders resulting from the imbalance of the antioxidant defense system (KONOPACKA et al., 2010; MONEGO; DA ROSA; DO NASCIMENTO, 2017; SAMANTA; CHAUDHURI; DUTTA, 2016).

However, the amount of carotenoid present in a food does not directly reflect its functional property, once the biological function depends on the fraction of the compound that will be able to reach its biological target (CARRILLO et al., 2017). That is, for carotenoids to be able to perform their function in the human organism, they must be absorbed and enter the bloodstream, becoming bioavailable (SAINI; NILE; PARK, 2015). The limiting factor in the use of natural bioactives for industrial application is their low resistance to changes in pH, heating, presence of oxygen and exposure to light (JANISZEWSKA-TURAK, 2017). Considering that during digestion

carotenoids are constantly exposed to the action of temperature, pH and enzymes, the bioaccessibility of this important phytochemical can be reduced and its bioactivity modified (MOSELE et al., 2016). In this regard, it is essential to apply techniques capable of protecting the bioactivity of the carotenoids of interest.

Spray drying is a well-known process and one of the most popular techniques for encapsulation, because of being reasonably priced and simple. Its low processing time is appropriate for protecting ingredients sensitive to heat (GERANPOUR; ASSADPOUR; JAFARI, 2020). Recent studies have shown the encapsulation of phenolic compounds (RUBIO et al., 2020) and flavors (SULTANA et al., 2017, 2018) by spray drying using yeasts *Saccharomyces cerevisiae* as a wall material. Particularly, in the previous work carried out by our research group (RUBIO et al., 2020), besides being a promising novel carrier for encapsulation of bioactive compounds, yeasts were able to protect compounds during storage and deliver them to the intestine phase under simulated gastrointestinal digestion. Also, compared with free extract, encapsulated compounds showed a higher bioaccessibility.

The yeast cell membrane has a continuous bilayer formed by phospholipids (WANG; CHEN, 2009) which are able to interact with both hydrophobic and hydrophilic molecules (PARAMERA; KONTELES; KARATHANOS, 2011a). The ability to not decompose easily with temperature also makes yeast an attractive wall material with excellent potential for industrial application (PARAMERA; KONTELES; KARATHANOS, 2011b). Beer yeast is already used in human nutrition, is considered safe for consumption (GRAS), is low cost and source of B vitamins, amino acids and proteins (FERREIRA et al., 2010). In addition, yeast cell wall polysaccharides, particularly β-glucans, have antibacterial, antioxidant, antimutagenic and potent wound healing actions (MOKHTARI; JAFARI; KHOMEIRI, 2017).

In this context, the brewer’s spent yeast, an underutilized brewing byproduct, represent a promising biological material for incorporation and protection of bioactives. In addition to proposing the use of two industrial residues generated on large scale - yeasts and pumpkin peels - this work proposes the enrichment of the yeast biomass with carotenoids evaluating the effect of an ultrasound treatment on powders stability, carotenoids retention and their release during a gastrointestinal digestion.

5.2 MATERIAL AND METHODS

5.2.1 Material

Absolute ethanol used in the extract preparation and for determination of carotenoids retention was acquired from Êxodo Científica (Sumaré, Brazil). Methanol and t-butyl methyl ether used for HPLC were acquired from Synth (Diadema, Brazil) and α and β-carotene standards were purchased from Sigma-Aldrich (St. Louis, USA). Magnesium chloride used in hygroscopicity and stability analyses was purchased from Synth (Diadema, Brazil). Nile red and calcofluor used to stain cells were purchased from Sigma-Aldrich (St. Louis, USA). Potassium chloride, potassium phosphate monobasic, sodium bicarbonate, sodium chloride, magnesium chloride hexahydrate and ammonium carbonate were used in the production of the simulated fluids for *in vitro* digestion and were acquired from Synth (Diadema, Brazil). Sodium hydroxide and hydrochloric acid were used for pH adjustment and were purchased from Synth (Diadema, Brazil) and Êxodo Científica (Sumaré, Brazil), respectively. Calcium chloride dihydrate was acquired from Synth (Diadema, Brazil). Pepsin from porcine gastric mucosa, bile salts and pancreatin from porcine pancreas were purchased from Sigma-Aldrich (St. Louis, USA). Petroleum ether, used in the carotenoid determination after *in vitro* digestion, was purchased from Synth (Diadema, Brazil). All reagents were of analytical grade.

5.2.1.1 Byproducts and their preparation

In this work, two byproducts were used: pumpkin peels and brewer's spent yeast. Pumpkin peels from the species "jerimum de leite" (*Cucurbita moschata*) were donated by "Doces Frutas de Minas" as a byproduct from the compotes production. After sanitizing, peels were crushed in a domestic processor and placed in trays for drying in an oven with air circulation (Marconi, MA035/1152), at 40 °C for 20 hours. Subsequently, dried peels were ground in a knife mill (Marconi, MA340) and submitted to sieving in an 80-mesh sieve (Figure 18).

Brewer's spent yeast (BSY), as source of *Saccharomyces cerevisiae*, were kindly provided by "Hausen Bier" brewery, after their utilization in beer production. In order to remove beer residues, the first step in preparing residual yeast biomass

consisted of repeated washing of the material with distilled water. At each washing step, yeasts sedimented and the waste water was removed, until the remaining water was clear. After this procedure, yeasts were dried in a bench atomizer (model MSD 5.0, Labmaq do Brasil Ltda., Ribeirão Preto, Brazil).

Pumpkin peel flour and dried yeasts were manually packed in polypropylene packages and kept at - 20 ºC, protected from light.

Figure 18 - Crushed pumpkin peels before and after drying (a and b, respectively) and pumpkin peel flour (c)

Reference: Elaborated by the author

5.2.2 Preparation and characterization of extracts

The extracts rich in carotenoids were prepared following a Central Rotational Composite Design (DCCR), with three independent variables: extraction time, extraction temperature and quantity of solute. The optimal extraction conditions were previously defined in response to the higher concentration of carotenoids present in the extracts. Therefore, the mixture of 0.92 g of pumpkin peel flour and 10 mL of absolute ethanol were stirred in a thermostatic shaker (Shaker - Marconi, MA420) at a temperature of 40 ºC, at 200 rpm and for 38.4 minutes. Afterward, samples were centrifuged (Eppendorf, 5810R) at 4850 g for 10 minutes. The collected supernatant was concentrated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 40 ºC until the extract was reduced to half of its volume. The concentrated extract was used for carotenoids quantification and incorporation in yeasts.

Identification and quantification of α and β-carotene in the extract was carried out by high-performance liquid chromatography (HPLC) (Model Prominence, Shimadzu, Kyoto, Japan) with photodiode array detector, following the method described by de Carvalho et al. (2012) with minor modifications. A C30 column (YCM

carotenoid, 5 µm, 4.6 mm x 250 mm) was used. The mobile phase consisted of 5% of t-butyl methyl ether in methanol (v/v), the flow rate was 0.8 mL/min and 40 µL of the extract was injected. The analysis temperature was 35 ºC with total analysis time of 65 min. The scan was carried out at 450 nm using the LC solution software.

5.2.3 Atomization process and powders stability

The first step prior atomization was the preparation of a 30% (w/w) yeast dispersion in distilled water and subsequent formulation of four different treatments, changing the proportion of extract and yeast dispersion and the application or not of ultrasound: 1) mixture of extract and yeast dispersion in the proportion of 1:1 (T1:1); 2) mixture of extract and yeast dispersion in the proportion of 1:1 with application of ultrasound (U1:1); 3) mixture of extract and yeast dispersion in the proportion of 1:2 (T1:2); 4) mixture of extract and yeast dispersion in the proportion of 1:2 with application of ultrasound (U1:2). In the application of ultrasound (Sonifier SFX550, BRANSON, Danbury-USA, with 550 W of power and 20 kHz of frequency), 30 minutes of operation were dedicated: 1 minute of operation and 15 seconds of rest, totalizing 24 work cycles (LIU et al., 2013). During the procedure, samples were immersed in an ice bath, to avoid superheating.

For complete homogenization of all treatments before atomization, ultra turrax (model IKA T25 digital ULTRA TURRAX) was used for 5 minutes at 10,000 rpm. Finally, samples were kept under magnetic stirring to avoid yeast sedimentation during atomization in a bench spray dryer (model MSD 1.0, Labmaq do Brasil Ltda, Ribeirão Preto, Brazil) at 160 ºC, and air flow rate of 60 mL min^{-1}. Produced powders were collected in the cyclone compartment. Figure 19 shows the produced powders.

Samples were equally divided into penicillin flasks and stored in desiccators with $MgCl_2$, under controlled humidity of 33.3% and at the temperature of 25 ºC. To assess the stability of the powders, carotenoids content, water activity, moisture content and instrumental color were evaluated starting on the day of powders production and every 15 days, during 75 days of storage.

Figure 19 – BSY (control yeast) and powders produced by enriching yeasts with carotenoids from pumpkin peel extract

Where T1:1 and T1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, without application of ultrasound; U1:1 and U1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, with application of ultrasound.
Reference: Elaborated by the author

5.2.4 Carotenoids retention

For the determination of carotenoids retention, compounds were extracted from BSY through successive washing steps using ethanol. An amount of 50 mg of the samples was mixed with 1 ml of ethanol in an Eppendorf micro tube. The suspension was vortexed for 1 min. followed by extraction in a water bath ultrasound (Unique, USC-1400, Indaiatuba - SP, Brazil) for 5 minutes, at 40 kHz and 135 W. Afterward, the suspension was centrifuged at 4226 g for 5 minutes. Washing and centrifugation steps were performed until a colorless supernatant was obtained, indicating no longer extraction of carotenoids from the yeasts. The extract used for quantification of carotenoids was the mixture of all the supernatants obtained from the sequential extractions.

5.2.5 Total carotenoids quantification

The concentration of total carotenoids present in ethanolic extracts was determined by spectrophotometry in the visible ultraviolet region, using the Equation 1 (DE CARVALHO et al., 2012).

$$C = \frac{A \times V \times 10^4}{A_{1cm}^{1\%} \times P} \quad (1)$$

where C is the carotenoid content (μg g^{-1}); A is the absorbance measured at a wavelength of 450 nm; V is the volume of extract (mL); $A_{1cm}^{1\%}$ is the absorption coefficient of β-carotene in ethanol, which value is 2620, according to Rodriguez-Amaya (2001); and P (g) is the mass of sample used for reading.

5.2.6 Powders characterization

5.2.6.1 Water Activity, moisture content and instrumental color

The water activity was measured at a temperature of 25 ºC using an Aqualab Pre Water Activity Analyzer (Decagon Devices Inc., USA). For the moisture content determination, 1 g of each powder was measured in a moisture analyzer (MB35 Halogen, Ohaus, Switzerland). The moisture was expressed in terms of the percentage in dry basis. The color of the powders was measured using a colorimeter (Mini Scan XE, HunterLab, Reston, USA) and the CIELAB color space was used to obtain the color coordinates L* (variation from lightness to darkness), a* (variation between green and red) and b* (variation between blue and yellow). The color parameters hue angle, chroma and total color difference were calculated according to the Equations 2, 3 (TARONE et al., 2021) and 4 (RIVAS; CABRAL; ROCHA-LEÃO, 2019), respectively.

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

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

$$\Delta E = \left[\left(L_i^* - L_f^*\right)^2 + \left(a_i^* - a_f^*\right)^2 + \left(b_i^* - b_f^*\right)^2\right]^{\frac{1}{2}} \quad (4)$$

5.2.6.2 Hygroscopicity

The hygroscopicity was determined as proposed by Souza et al. (2014). Triplicates of 0.5 g of each sample were placed in desiccators with a $MgCl_2$ saturated solution and relative humidity of 32.8%. Desiccators were stored in incubators at 25 ºC. After 7 days of storage, samples were weighed and hygroscopicity was expressed as g of adsorbed water per 100 g of dry solids.

5.2.6.3 Scanning electron microscopy (SEM)

For this analysis, samples were prior slightly milled using a mortar and pistil to facilitate agglomerates separation and for better visualization of cells under microscope zoom. Afterward, samples were mounted on aluminum stubs using carbon adhesive tapes. Control yeasts (BSY without carotenoids encapsulation) and yeasts enriched with carotenoids were analyzed using a scanning electron microscope (Tabletop Microscope, Hitachi TM300) and a voltage of 15 kV (RUBIO et al., 2018).

5.2.6.4 Confocal laser scanning microscopy

Confocal images of the obtained microparticles were acquired with a Leica TCS SP5 Confocal Microscope (Leica Microsystems, Germany). Control cells and cells with carotenoids incorporated were analyzed with and without staining. For staining, nile red and calcofluor were prepared with ethanol and water, respectively, both in the concentration of 1 µg mL^{-1} (PHAM-HOANG et al., 2018). Cells were firstly immersed in the calcofluor solution, rinsed three times in distilled water and finally immersed in the nile red solution, with each step following a centrifugation procedure. Calcofluor and nile red excitation was carried out at 405 nm and 488 nm, respectively. Emission wavelengths were recorded by a band-pass filter at 430-480 nm for calcofluor and at 515-645 nm, for nile red (RUBIO et al., 2020).

5.2.7 Release of carotenoids from particles during simulated gastrointestinal digestion

Samples used were produced one day before this analysis, to avoid carotenoids degradation before digestion. Carotenoid release was analyzed after an oral phase and at the beginning and the end of gastric and intestinal phases, following a standardized *in vitro* digestion procedure, proposed by Minekus et al., (2014). A minor adjustment was made in the quantity of sample used, as it is a powdered sample, in order to obtain the thin paste-like consistency suggested by the authors. For this purpose, a proportion of 1:2 (w/v) of particles and simulated salivary fluid was used. To start the simulation evaluating the influence of the oral phase on carotenoids release, 1 g of particles were weighed and 1.05 mL of simulated salivary fluid (SSF), 7.5 µL of 0.3 mol L^{-1} $CaCl_2$ and 0.9425 mL of distilled water were added, resulting in a final concentration of 0.5 g mL^{-1}. The mixture was vortexed in order to obtain a viscous sample. Afterward, samples were incubated at 37 ºC for 2 min., under stirring. Then, 2.25 mL of simulated gastric fluid (SGF) were added, following the addition of 1.5 µL of 0.3 mol L^{-1} $CaCl_2$ and the homogenization in vortex. The pH of samples was adjusted to 3 using HCl 6 mol L^{-1}, the total volume was completed to 6 mL using distilled water and, finally, 0.48 mL of a pepsin solution (25000 U mL^{-1}) was added. Samples were kept under stirring at 37 ºC for 2 hours in a thermostatic bath (TE-053, Tecnal, Piracicaba, Brazil). To perform the intestinal phase, 3.3 mL of simulated intestinal fluid (SIF) were added and the samples were homogenized immediately to increase the pH. Afterward, 0.75 mL of a 160 mmol L^{-1} bile solution was added, following the addition of 12 µL of 0.3 mol L^{-1} $CaCl_2$ and pH correction to 7, using 5 mol L^{-1} NaOH. Then, the final volume was completed to 12 mL with distilled water and 1.5 mL of pancreatin (800 U mL^{-1}) were added. Samples were incubated at 37 ºC for 2 hours. Samples taken before or after each phase were centrifuged at 6169 × g for 10 min. and supernatants were collected and stored at – 20 ºC.

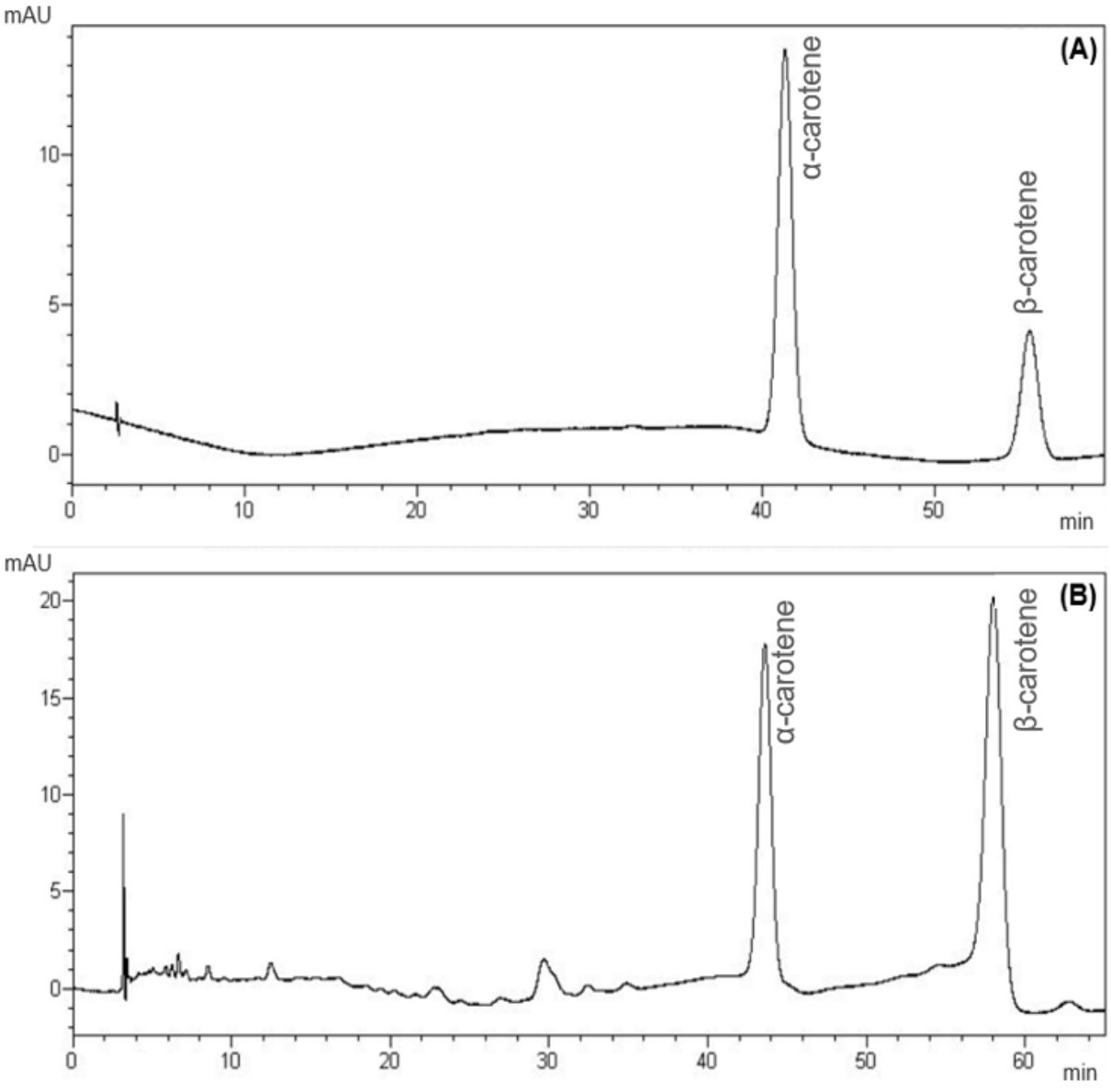
To determine the carotenoid content in each sample, supernatants were mixed with ethanol and petroleum ether (1:1:4, respectively), vortexed for 1 min. and centrifuged at 6169 × g for 10 min. The extraction was performed twice. Then, the phase containing carotenoids and petroleum ether was separated and the absorbance of the solution was measured by spectrophotometer (Genesys 10s, Thermo Scientific, Waltham, USA), at 450 nm.

5.3 RESULTS AND DISCUSSION

5.3.1 Extract characterization

The total carotenoid content in the concentrated extract was 866.08 µg/g of extract and the chromatography characterization showed β and α-carotene as the main carotenoids found in the pumpkin peel extract, with concentrations of 398±5.8 and 176±1.7 µg/g of extract, respectively. Figure 20 shows the chromatograms obtained for standards and extract from pumpkin peels.

Figure 20 - Chromatogram of β and α-carotene standards (A) and β and α-carotene in the extract from pumpkin peels (B)



Reference: Elaborated by the author

5.3.2 Characterization of the produced particles

5.3.2.1 Moisture content, water activity and hygroscopicity of particles

Table 12 shows the moisture content and water activity results for all samples during the storage period of 75 days. For both moisture content and water activity, in general, there was no significant difference between same proportions and different treatments. It suggests that the utilization of ultrasound did not have influence on these parameters. At the day 0, moisture varied between 4.5 and 4.9 for treatments prepared in the proportion of 1:1 and ranged between 4.6 and 5.1 for the proportion 1:2 of extract from pumpkin peels and yeast suspension. After 15 days of storage, moisture content presented an increase, but no significant difference was noticed over the rest of the period of storage, which indicates that moisture content reached an equilibrium. The same behavior was observed for water activity. There was no significant difference between samples of the same proportion at the day 0 and after 15 days of storage there was an increase in water activity values and posterior equilibrium during the rest of the storage.

Despite the increase in water activity and moisture content during storage, values are within the range considered appropriate for powder physical and microbiological stability. When water activity is near 0.3, samples are stable against microorganism development and enzymatic activities and, if any spoilage occurs, it is more probably induced by chemical reactions rather than by microorganisms (ÁLVAREZ-HENAO et al., 2018). In addition, low values of moisture content and water activity are desirable for powders to prevent agglomeration and caking, which can result in wet powders and hindrance of the flowability and dispersion (RAMAKRISHNAN et al., 2018).

Table 12 - Moisture content and water activity of each treatment during 75 days of storage

Sample	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75
			Moisture (%)			
T1:1	4.9^{Ba}±0.07	6.9^{Aa}±0.3	6.6^{Aa}±0.1	6.7^{Aa}±0.01	6.4^{Aa}±0.2	6.9^{Aa}±0.04
U1:1	4.5^{Ba}±0.1	6.8^{Aa}±0.4	6.7^{Aa}±0.1	6.5^{Aa}±0.3	6.3^{Aa}±0.1	7.0^{Aa}±0.03
T1:2	4.6^{Ba}±0.6	6.3^{Aa}±0.07	6.3^{Aa}±0.1	6.3^{Aa}±0.03	6.3^{Aa}±0.3	6.9^{Aa}±0.02
U1:2	5.1^{Ba}±0.3	6.8^{Aa}±0.3	6.3^{ABa}±0.1	6.6^{ABa}±0.3	6.2^{ABa}±0.07	6.6^{ABa}±0.4
			Water Activity			
T1:1	0.245^{Ba}±0.03	0.380^{Aa}±0.01	0.377^{Aa}±0.005	0.360^{Aa}±0.001	0.357^{Aa}±0.002	0.407^{Aa}±0.007
U1:1	0.317^{Aa}±0.07	0.392^{Aa}±0.004	0.380^{Aa}±0.001	0.406^{Ab}±0.001	0.378^{Ab}±0.004	0.407^{Aa}±0.001
T1:2	0.188^{Ba}±0.05	0.379^{Aa}±0.01	0.389^{Aa}±0.008	0.398^{Aa}±0.004	0.368^{Aa}±0.008	0.419^{Aa}±0.001
U1:2	0.263^{Ba}±0.02	0.386^{Aa}±0.002	0.400^{Aa}±0.002	0.404^{Aa}±0.006	0.369^{Aa}±0.001	0.396^{Ab}±0.003

Where T1:1 and T1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, without application of ultrasound; U1:1 and U1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, with application of ultrasound. Different capital letters indicate significant differences ($p \leq 0.05$) for the same sample at different times and different small letters show a significant difference between samples on the same day.

Reference: Elaborated by the author

Regarding to the hygroscopicity (Table 13), values ranged between 6.1 and 6.8 g/100 g for the proportion 1:1 and 5.4 and 6.0 g/100 g for the proportion of 1:2 of extract from pumpkin peels and yeast suspension, without significant differences between same proportion. Low hygroscopicity values are also important to maintain the powder stability, suggesting a minimal water absorption. In a previous work, Rubio et al. (2020) incorporated phenolic compounds into yeasts *S. cerevisiae* and reported higher hygroscopicity values, around 13.7. Alves et al. (2017) encapsulated carotenoids from pequi extracts using a matrix composed of maltodextrin and gum arabic and obtained hygroscopicity values ranging 10.43-11.19 g/100g. In another work, values ranging 9.1 and 14% were reported by Souza et al. (2018) for microparticles produced by encapsulation of a lycopene-rich tomato concentrate with different composition of encapsulating agents. The lower values found in the current research are the result of the combination of yeasts, which are not a hygroscopic material, and the extract from pumpkin peels, that is expected to have few or no low molecular sugars and acids.

Table 13 - Hygroscopicity (g/100 g) and color parameters chroma, hue angle and total difference of colors for yeasts enriched with carotenoids from pumpkin peels extract

Sample	Hygroscopicity	$h^{o}t_0$	$h^{o}t_{75}$	$C^{*}t_0$	$C^{*}t_{75}$	ΔE
T1:1	6.8^{a}±0.7	80.9^{Aa}±0.4	82.1^{Aa}±0.1	33.0^{Aa}±0.3	28.3^{Bb}±0.4	5.3^{a}±0.1
U1:1	6.1^{a}±0.7	80.6^{Ba}±0.2	81.6^{Aa}±0.2	33.7^{Aa}±1.3	31.6^{Aa}±0.4	2.8^{b}±0.1
T1:2	6.0^{a}±0.5	82.9^{Aa}±0.2	84.3^{Aa}±0.6	25.4^{Aa}±0.1	20.8^{Bb}±0.3	5.3^{a}±0.2
U1:2	5.4^{a}±0.4	81.9^{Bb}±0.1	83.7^{Aa}±0.3	26.1^{Aa}±0.6	24.3^{Aa}±0.2	3.6^{b}±0.2

Where T1:1 and T1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, without application of ultrasound; U1:1 and U1:2 are particles produced by mixing extract and yeast dispersion in the proportion of 1:1 and 1:2, respectively, with application of ultrasound. t_0 and t_{75} represent the time zero and after 75 days, respectively. h^{o} and C^{*} are the hue angle and chroma. Different capital letters indicate significant differences ($p \leq 0.05$) for the same sample at different times and different small letters show a significant difference between samples on the same day.
Reference: Elaborated by the author

5.3.2.2 Color differences after storage

The color parameters for produced powders are shown in Table 13. The data for colorimetry analyses were located in the first quadrant of the CIELab color chart. At day 0 and after 75 days, hue angles ranged from 80.6 to 82.9 and 81.6 to 84.3, respectively, confirming the yellow color of the powders (qualitatively visualized in Figure 19), which was expected once the extract was rich in α and β-carotene.

Similar results were found by Pal & Bhattacharjee (2018), who reported a hue angle of 89.63 for encapsulated lutein extracted from African marigold flowers. Rivas et al. (2019) encapsulated carotenoids from mango and passion fruit and reported hue angles ranging between 79.97 and 79.34 after 90 days of storage.

For T1:1 and T1:2 treatments, chroma values had a significant decrease, indicating that those powders presented less intense color after 75 days of storage. For U1:1 and U1:2, chroma did not present a significant change, suggesting that these powders maintained their saturation. The values of total difference of colors (ΔE) higher than 2.8 indicate that there was a visual loss of color after storage and the shade has begun to change (RIVAS; CABRAL; ROCHA-LEÃO, 2019; TUPUNA-YEROVI et al., 2020). However, particles produced using ultrasound treatment had lower values of ΔE. These results may suggest that the ultrasound treatment led to a better protection of β and α-carotene, main responsible for the extract color. The loss or change of color in carotenoids gives an indication of degradation or structural modification, as a consequence of isomerization and oxidation to which carotenoids are susceptible (RODRIGUEZ-AMAYA, 2001).

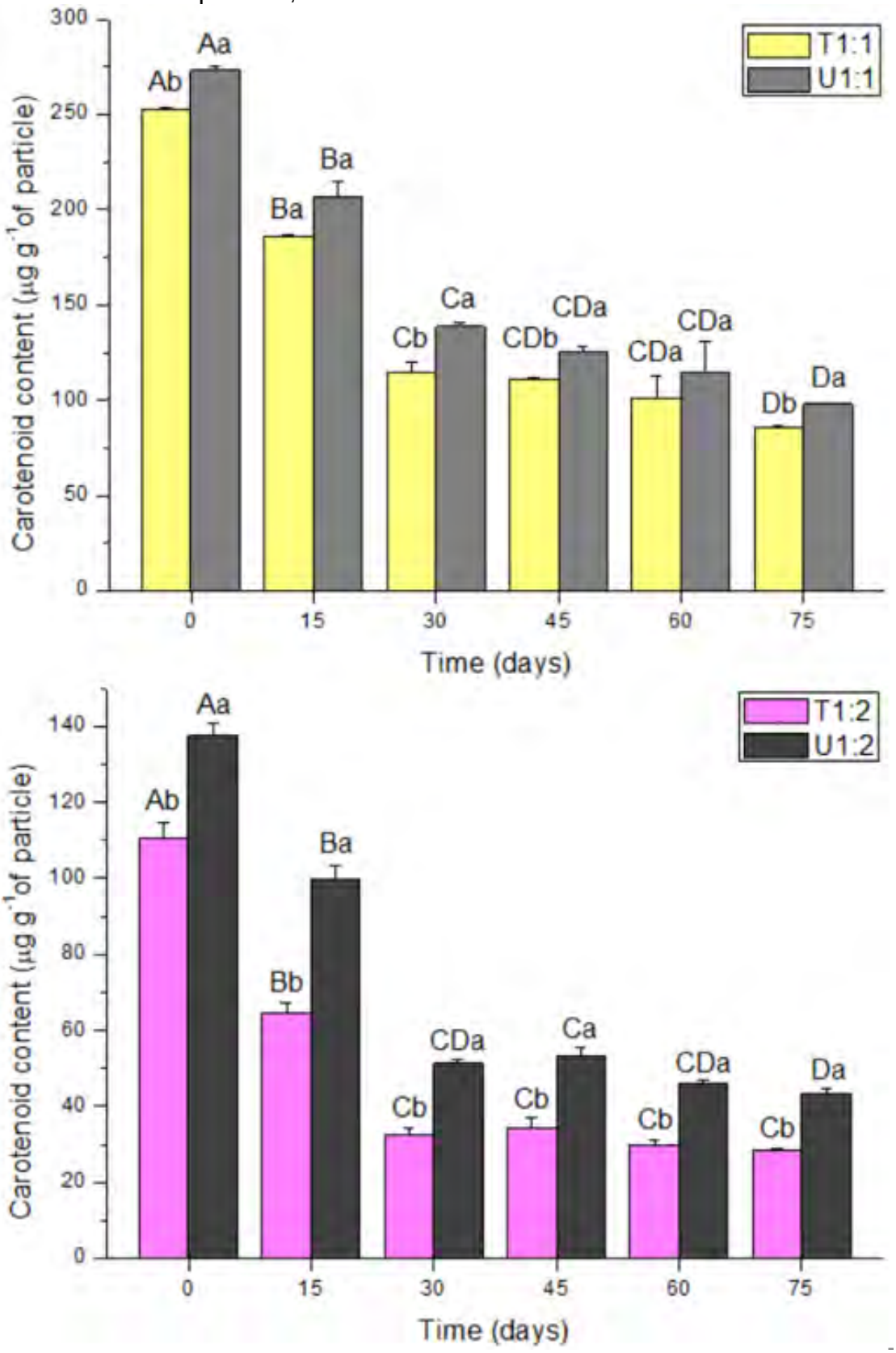
5.3.2.3 Storage stability and retention of carotenoids

Figure 21 shows the stability data of carotenoids encapsulated in BSY. The content of carotenoids was significantly higher on day 0, with carotenoid contents of 252.6 and 273.3 μg g^{-1} of particle for T1:1 and U1:1 and 110.4 and 134.8 μg g^{-1} of particle for T1:2 and U1:2. In most stability points, samples treated with ultrasound had significant higher carotenoid retention. In the case of samples treated with ultrasound, is possible that a biosorption of carotenoids have occurred during the treatment, before the step of spray drying. Due to the presence of functional groups on yeasts surface, such as carboxyl, hydroxyl sulfhydryl, phosphoryl and amino groups, *S. cerevisiae* is able to adsorb compounds present in solution (JILANI et al., 2016) and the simple contact between carotenoids and yeasts may result in the adsorption of the compounds by the biological material. In fact, the cavitation and microscopic turbulence generated by the ultrasound treatment can enhance the adsorption process by hitting the yeast surface, creating pores and exposing more binding sites and facilitating the transfer of the compounds across the liquid film surrounding yeasts (TAO et al., 2019). Furthermore, ultrasound technique is

commonly used to enhance cell membrane permeability by inducing transient holes in the membrane, allowing the transfer of molecules inside the cells (PHAM-HOANG et al., 2018). Thus, the ultrasound treatment may have enhanced the carotenoid content both in the surface and interior of the yeast cells. This explains the higher content of carotenoids in samples treated with ultrasound.

Similar results were found by Pham-Hoang et al. (2018), who related an increase in the β-carotene loading in yeasts *Yarrowia lipolytica* using an ultrasound-mediated encapsulation process. Moreover, Tao et al. (2019) evaluated the influence of increasing ultrasound acoustic energy on biosorption of phenolic compounds in yeasts *S. cerevisiae* and the researchers also showed a positive effect of ultrasound on yeast biosorption capacity.

Figure 21 - Total carotenoids content during the storage of the powders produced in the proportion of 1:1 (A) and in the proportion of 1:2 (B) of extract and yeast suspension, with and without ultrasound treatment



Different capital letters indicate significant differences ($p \leq 0.05$) for the same sample at different times and different small letters show a significant difference between samples on the same day.
Reference: Elaborated by the author

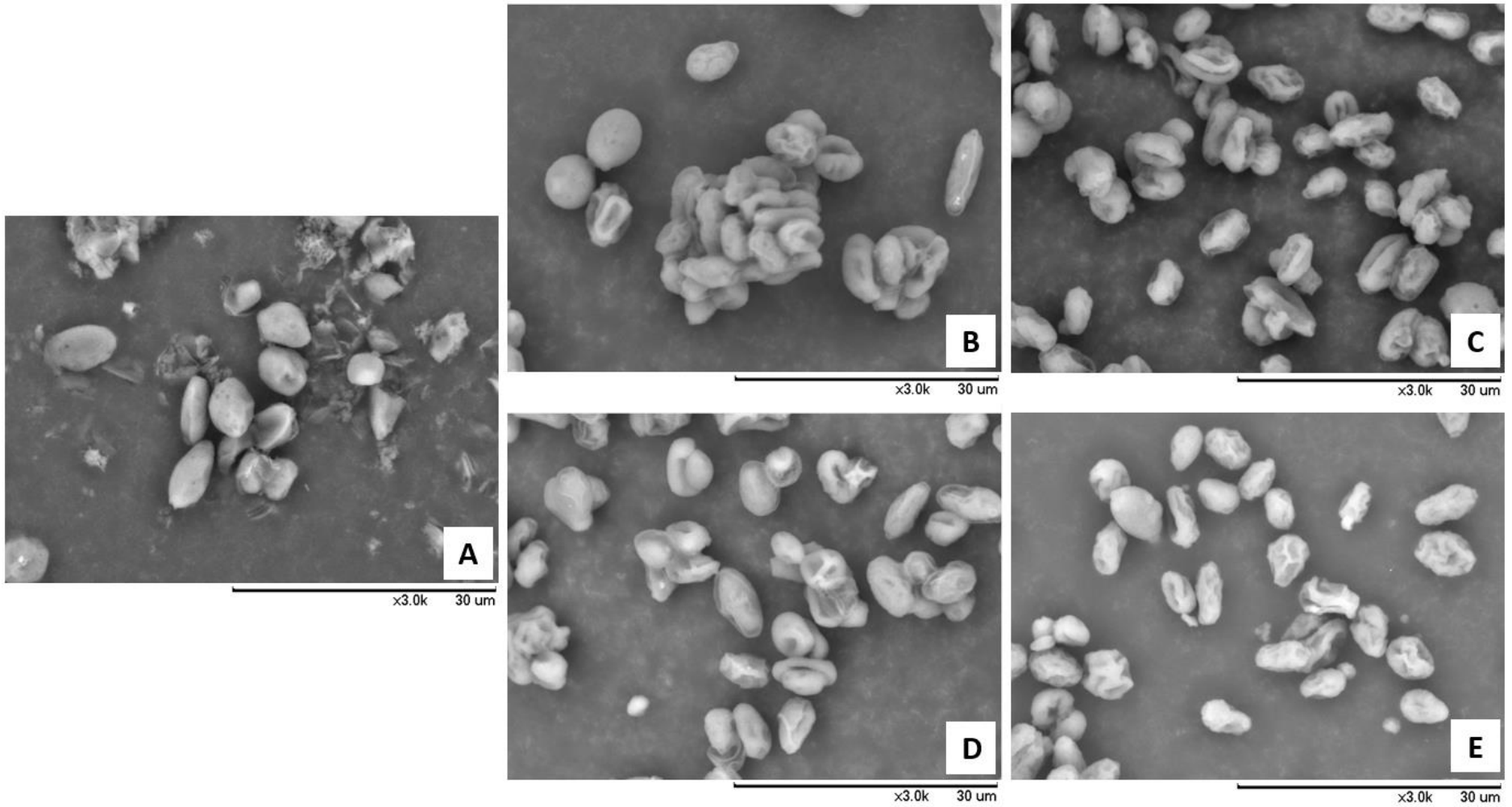
Regarding to the period of storage, the first 30 days were the most critical, where a significant decrease in carotenoids content is evident at days 15 and 30. An explanation for the significant decline of carotenoids content in this initial period is that possibly these first degraded carotenoids were those mainly retained on the yeasts surface (probably by adsorption and binding with the surface chemical groups) which were, consequently, more exposed. In addition, this decline can also be a consequence of increased moisture and mainly the water activity in the first 15 days of storage, which might increase the oxidation rate by enhancing the mobility of reactants and bringing catalysts into solution (LAVELLI; ZANONI; ZANIBONI, 2007).

After stabilization of moisture and water activity, there was also a stabilization in the content of carotenoids up to the day 75, where a significative decrease was evidenced for samples T1:1, U1:1 and U1:2. Lavelli et al. (2007) reported a similar behavior. The authors dehydrated carrots and evaluated the effect of the water activity on β and α-carotene stability and authors reported that carotenoids showed a maximum stability at water activity ranging 0.341-0.537, which are in agreement with the range of water activity found in this present research and the carotenoid retention stabilization.

5.3.2.4 Morphological characterization of yeasts enriched with carotenoids

Figure 22 shows the micrographs of control yeasts (A) and yeasts enriched with carotenoids from pumpkin peels (B, C, D and E). Control yeasts present smooth surface and oval shape. After enrichment, there is a roughness on the surface, yeasts are shrinked and present several concavities, caused by evaporation of water from the interior of the cells during atomization. The same morphology was reported in a previous work published by our research group (RUBIO et al., 2020). Even after the enrichment, cells seem intact and there are no evidences of cells rupture. Although the aggregation of cells may be a consequence of the atomization process, the presence of β-glucans in cells walls is probably the main reason for agglomeration, because of their binding properties (SULTANA et al., 2017).

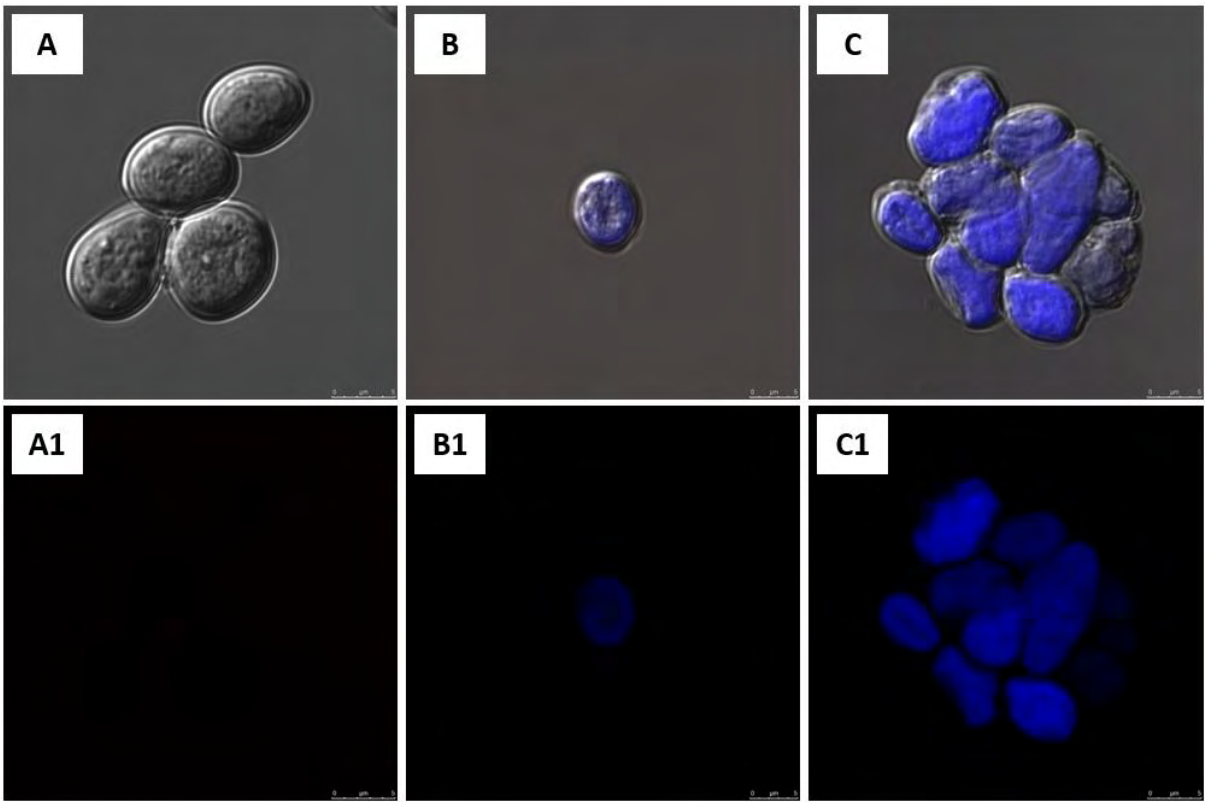
Figure 22 - Scanning electron microscopy micrographs of control cells (A), cells enriched with carotenoids without ultrasound treatment T1:1 and T1:2 (B and C, respectively) and cells enriched with carotenoids with ultrasound treatment U1:1 and U1:2 (D and E, respectively)



Reference: Elaborated by the author

Confocal micrographs of stained yeasts before and after enrichment with carotenoids are shown in Figure 23. This technique may be useful for exploring how carotenoids are distributed in the cell and whether they are incorporated only on the surface or inside the cells. Control cells (A and A1) show no fluorescence under excitation. After yeasts loading with carotenoids, cells show fluorescence, which indicates the probable incorporation of carotenoids inside the cells. The fluorescence intensity is higher for cells that passed through ultrasound treatment (C and C1). This result corroborates with the carotenoid retention in yeasts, which, in general, was higher after ultrasound treatment, as a consequence of cavitation. Based on the dispersion of fluorescence, carotenoids are spread all over the yeast cells and no precipitation or nucleation can be noticed.

Figure 23 - Confocal laser scanning microscopy of unstained control and enriched cells

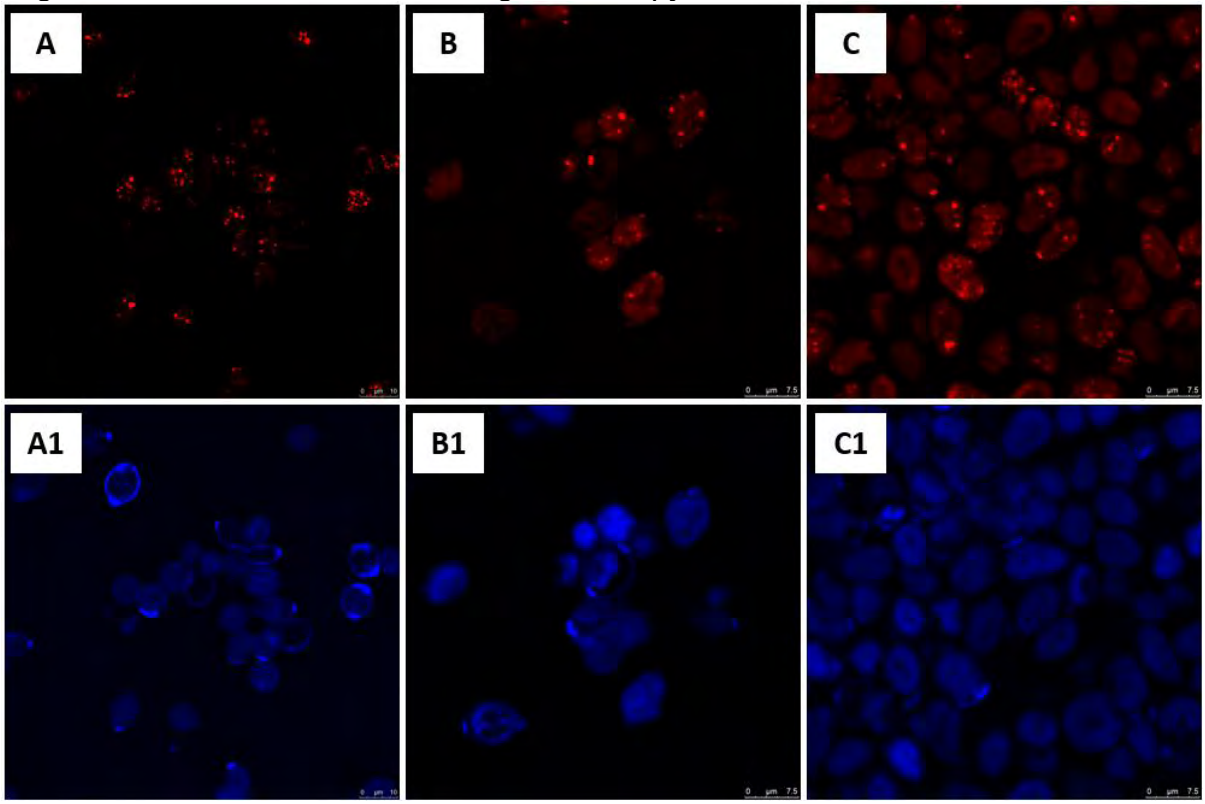


Where A and A1 represent control yeasts; B and B1 represent cells enriched with carotenoids without using ultrasound treatment; and C and C1 represent yeasts enriched with carotenoids with ultrasound treatment. A, B and C represent images in white light and A1, B1 and C1, without white light.
Reference: Elaborated by the author

In addition, Figure 24 shows that nile red stains only organelles in control yeasts (A) and, in enriched cells (B and C), stains organelles and the whole interior of the cells, also proving the presence of carotenoids inside yeast cells. The same

occurs under excitation of calcofluor, which stains mainly the membrane of control cells (A1) and stains the whole cell of enriched yeasts with higher intensity (B1 and C1). Comparing cells with and without ultrasound treatment, staining is more pronounced for cells treated with ultrasound, confirming cells enrichment.

Figure 24 - Confocal laser scanning microscopy of stained control and enriched cells



Where A and A1 represent control yeasts; B and B1 represent cells enriched with carotenoids without ultrasound treatment; and C and C1 represent yeasts enriched with carotenoids with ultrasound treatment. In A, B and C, cells were under excitation of nile red and in A1, B1 and C1, the same cells were under excitation of calcofluor.
Reference: Elaborated by the author

5.3.2.5 Principal component analysis (PCA) of the MIR-ATR spectra

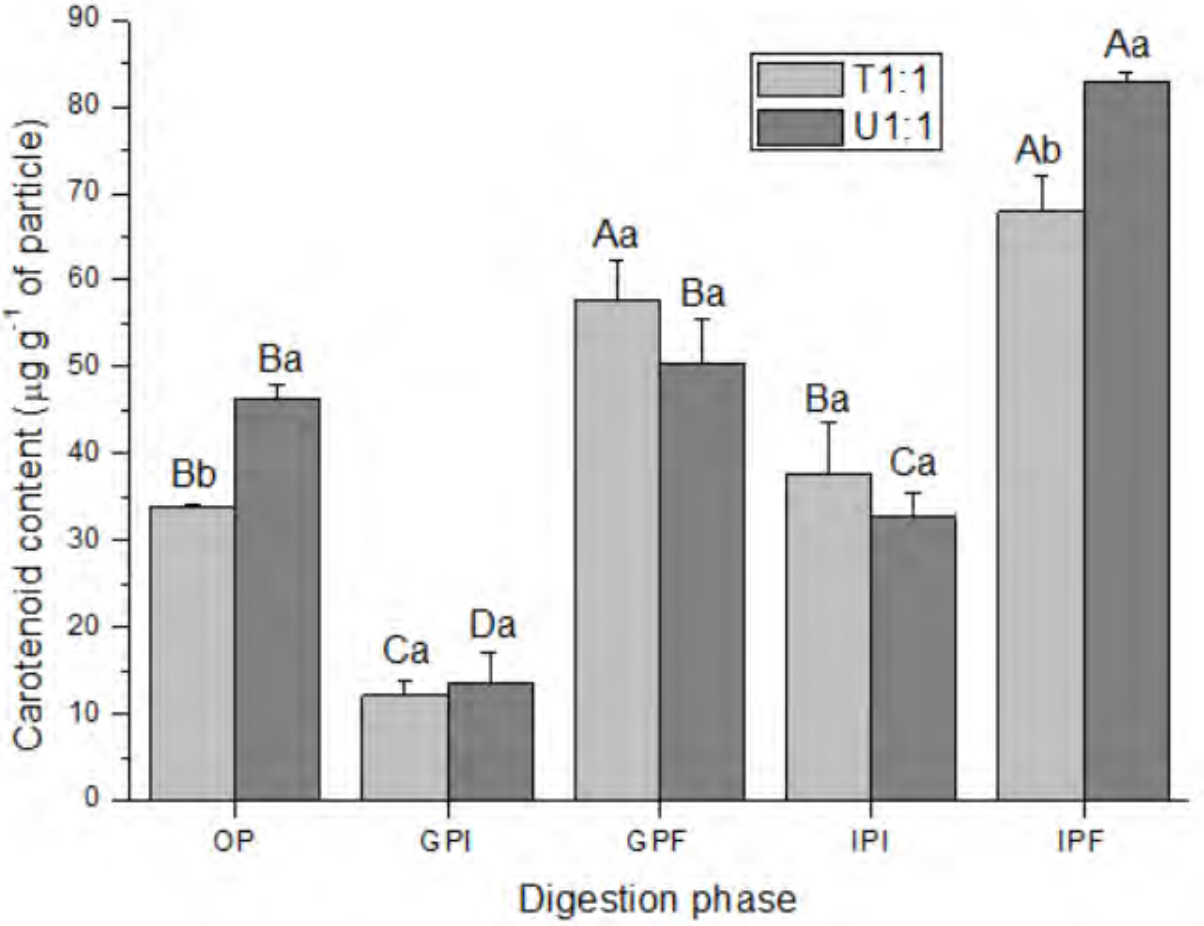
To better understand the main chemical differences between samples, a principal component analysis was carried out. Figure 5 shows the scores profile and the two main principal components identified (PC1 and PC2) explained 100% of the total variance. Samples were separated in different quadrants and this result implies that control yeasts, T1:1 and U1:1 are significantly different. The functional groups of control yeasts are better explained by negative sides of both PC1 and PC2. For U1:1, chemical groups are better explained by the positive sides of both PC1 and PC2 and for T1:1, by positive side of PC1 and negative side of PC2.

Figure 6 shows the PCA loadings plot and the spectra for the three samples. The PC1 high intensities at around 1640, 1620, 1550 and 1040 cm^{-1} indicate that the functional groups found in these regions are responsible for best explaining the differences between samples. The most important bands for U1:1 are found around 1645-1675 cm^{-1} and 1535-1600 cm^{-1}, where amide I and amide II – signals of protein – can be found, respectively (VULIĆ et al., 2019). In these both regions, especially in the region of amide I, the bands for enriched yeasts T1:1 and U1:1 are significantly less intense and dislocated to a lower frequency region, which may indicate that there was a chemical interaction between carotenoids and amides from the yeast cells. The main bands correlated with control yeasts are around 1600-1630 cm^{-1} – where new peaks appear for U1:1 and T1:1 – and 1280-1485 cm^{-1}, representing C-H scissoring and bending, typical for carotenoids (SUN et al., 2018). In this last region, it is evident that enriched yeasts have overlapped spectra over control cells, probably related to the carotenoid incorporation in yeasts. Specifically, the peak at approximately 1400 cm^{-1} denoted the stretching of carboxyl groups in amide III (TAO et al., 2019). T1:1 is best explained by the regions in 1500-1520 cm^{-1} and mainly 1239 cm^{-1}. According to Paramera et al. (2011a), this last peak is indicative of DNA/RNA, molecules that are exclusively localized in the cytoplasm. The lower intensity of this peak after enrichment of yeasts might indicate that carotenoids interfered somehow in the interior of the cells. The region comprehending peaks within the range of 900 and 1100 cm^{-1} is characteristic of polysaccharides, where mannans absorption band appears around 1057 cm^{-1} (DADKHODAZADE et al., 2018) and β-glucans may appear around 1026 cm^{-1} (KARAMAN, 2020). Shifted bands for U1:1 and T1:1 in this spectra area may suggest that there is an interaction between carotenoids from pumpkin extracts and cell wall components, confirming that part of the carotenoids was retained in the cell wall. A higher intensity for U1:1 is observed at 930 and 980 cm^{-1} and might be explained by the ultrasound treatment and consequent biosorption and retention in cell wall. The results suggest that there was a physical and chemical incorporation of carotenoids in yeast cells and that amides and polysaccharides show important role in the encapsulation of these compounds.

5.3.3 Release of carotenoids from microparticles

The microparticles produced with a higher proportion of extract (samples T1:1 and U1:1) were chosen for carrying out the simulated gastrointestinal digestion due to their higher retention of carotenoids. Figure 25 shows the non-cumulative release profile of carotenoids from BSY. For both treatments, the release occurred in similar behavior, showing that yeasts released carotenoids gradually and there was a degradation of carotenoids in the beginning of the gastric and intestinal phases.

Figure 25 - Release profile of carotenoids from microparticles in oral, gastric and intestinal phases



OP is the oral phase; GPI and GPF represent the beginning and the end of the gastric phase, respectively; IPI and IPF correspond to the beginning and the end of the intestinal phase, respectively. Different capital letters indicate significant differences ($p \leq 0.05$) for the same sample at different times and different small letters show a significant difference between samples on the same day.
Reference: Elaborated by the author

In the oral phase, there was an initial carotenoid release, probably caused by the dispersion of carotenoids present on BSY surface in the oral fluid. The explanation for the higher release for U1:1 is that the biosorption promoted during the

ultrasound treatment possibly led to the binding of a higher content of carotenoids in the yeast surface and, as a consequence, these compounds were more exposed. At the beginning of the gastric phase, a carotenoid degradation is evidenced, but at the end of the gastric phase, the content of carotenoid was significantly higher, showing that a further release of the bioactive occurred. The presence of pepsin may induce changes in the cell wall, causing a protein denaturation, increasing cell permeability (YOUNG; RAI; NITIN, 2020) and influencing the compound release.

When the intestinal phase initiated, there was also a degradation of carotenoids but, during the process, carotenoids were released in almost the double content found in IPI for T1:1 and more than double for U1:1. The most interesting result was obtained for U1:1, because of the significant higher release of carotenoids at the end of the intestinal phase compared to T1:1. A suitable explanation for this result is that the ultrasound treatment may have also enhanced the content of carotenoids retained in an inner part of the cell, in the most apolar part of the membrane, for example, and these compounds were not easily released without the presence of facilitators that increase micellization. In this context, the action of pancreatin and bile salts in the intestine phase showed important role on carotenoids release, probably facilitating the micellization of carotenoids. In addition, the ultrasound may modify the protein structure and consequently exposes the hydrophobic groups, facilitating the carotenoid release. The content of the bioactive compound that reaches the end of the intestinal phase is of greatest importance, once carotenoids have to be firstly bioaccessible to be taken up by the epithelial cells in the small intestine, where they can be absorbed by the organism and exert the health-promoting biological activities (RODRIGUES; MARIUTTI; MERCADANTE, 2016).

The total release of carotenoids was 43.4% for T1:1 and 48.7% for U1:1. The bioaccessible fraction of T1:1 and U1:1 represents 26.9% and 30.3% of the initial carotenoid content, respectively. The low release of total carotenoids during the simulation of gastrointestinal digestion can be explained by the low affinity of carotenoids with the aqueous phase and also because the yeasts are not disrupted or solubilized during the process, in addition to the carotenoid degradation throughout the experiment. Furthermore, there is an inverse relationship between carotenoid hydrophobicity and micelle incorporation. Carotenes, such as β and α-carotene, are generally less bioaccessible than xanthophylls, because of their more

nonpolar character that makes difficult the transfer to mixed micelles (RODRIGUES; MARIUTTI; MERCADANTE, 2016), which also explains their low release at the end of the gastrointestinal digestion. An alternative to increase the release of carotenoids during digestion is to apply the microparticles into a food that contains lipids or in another lipidic matrix for which carotenoids could have more affinity, facilitating their release into the medium.

An important point to discuss and consider is the effect of the kind of particle on the compound release during a gastrointestinal digestion. Atomization using other carrier materials generally results in a microparticle of the matrix type, in which carotenoids are dispersed throughout the particle, including the surface. For this kind of particle, the release can occur significantly both in oral and gastric fluids. On contrary, particles of reservoir type or coated matrix particles can present a reduced premature release (SANTOS et al., 2021b). Once yeasts are pre-formed capsules, they are able to be loaded with compounds acting as a reservoir, where carotenoids are surrounded by the membrane and represent the nucleus of the particle. At the same time, yeast can have carotenoids dispersed and linked on its surface. Because of this yeast characteristic, the release of bioactives starts on oral phase, continues on gastric phase and a further release on intestine conditions is expected, considering that this fraction corresponds mainly to the compounds entrapped inside the cells. Exemplifying, the same release profile was noticed for yeasts *S. cerevisiae* enriched with phenolic compounds (RUBIO et al., 2020). On the other side, carotenoids from tucumã oil encapsulated with gum arabic showed a premature release in the oral and gastric phases and a lower release in intestinal fluids (SANTOS et al., 2021a). Therefore, yeasts may be advantageous over other types of encapsulating materials in protecting and releasing compounds.

5.4 CONCLUSIONS

This work evidenced the possibility of reusing two industrial byproducts for obtainment of a novel product, associating the nutritional value of yeasts and bioactive compounds. Brewer's spent yeast (BSY) was proven to be a suitable material for incorporation of carotenoids from pumpkin peels extract, showed a gradual release of compounds under simulated gastrointestinal digestion and its pre-capsule morphology may be of advantage over other materials for encapsulation of

bioactive compounds. Furthermore, the results of this work showed that ultrasound is able to increase the carotenoid incorporation in BSY. Considering further researches, it would be interesting to evaluate the application of yeasts enriched with carotenoids into a food product, mainly a lipidic matrix, to better understand the role of lipids in carotenoids stability and release.

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6 GENERAL CONCLUSIONS

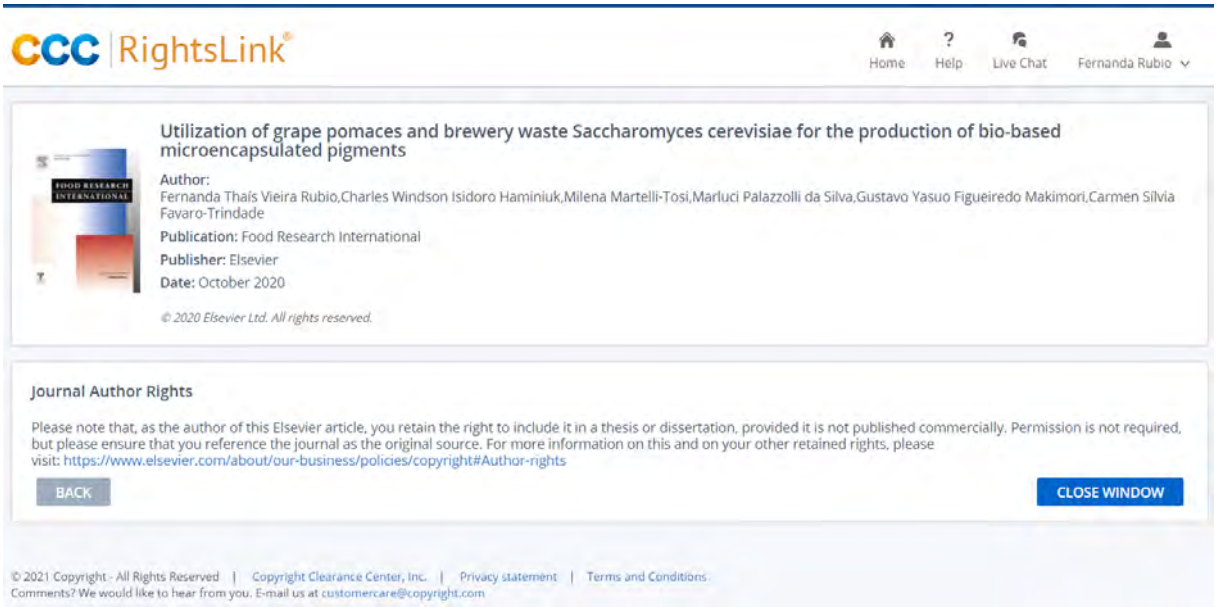
Considering the current great attempt to reuse industrial wastes and byproducts and the search for new options for the industry, the encapsulation of bioactive extracts in a biological wall material, both coming from industrial byproducts, may suggest an interesting solution. The utilization of extracts containing different bioactive compounds, such as phenolic compounds, anthocyanins and carotenoids was helpful to show that yeast indeed have affinity by both hydrophobic and hydrophilic compounds. Although, in this work, it was noticed that the polarity of the compounds can contribute to their incorporation and it is easier to incorporate hydrophilic compounds in yeasts, because of the exteriorized polar part of the yeast membrane.

Brewer's spent yeast (BSY), as a source of *Saccharomyces cerevisiae*, was evidenced as a suitable and low-cost option to replace other carrier materials widely used for encapsulation. The microparticles obtained in this work are of low cost and simple obtention, which may present an advantage for a large-scale manufacturing. Microparticles produced using BSY as carrier material have good physical and microbiological stability, which can enable their future application. When added to a food product, the microparticles enrich the product not only with bioactive compounds, but also with the yeast and its health benefits. Furthermore, BSY enriched with natural pigments can confer color to a product, acting as a natural and innovative food colorant, and do not negatively interfere in the consumer acceptance.

In the current work, the intact cells of yeast were used. Although, for future works, it would be interesting to explore plasmolyzed cells or their fragments for encapsulation of compounds. For example, comparing intact and plasmolyzed cells in order to discover the role of the internal cell content in the incorporation and stability of the compounds. Furthermore, the phenomenon of the compounds incorporation could be better understood studying a less complex extract or a pure compound.

This research may be helpful to promote the application of BSY as a vehicle for encapsulation of compounds from any source as novel products with added value and may show some general interests for the application of yeast-based materials.

ATTACHMENT A – PAPER PUBLISHED IN FOOD RESEARCH INTERNATIONAL



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Utilization of grape pomaces and brewery waste *Saccharomyces cerevisiae* for the production of bio-based microencapsulated pigments

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ABSTRACT

This research approaches the utilization of brewery waste yeast *Saccharomyces cerevisiae* as a vehicle for the encapsulation and protection of phenolic compounds from Cabernet Sauvignon and Bordeaux grape pomace extracts. The main purpose of this research was to enrich the biomass of yeast to investigate its potential as a novel vehicle for further application as pigment or functional ingredient. The obtained powders presented characteristics appropriated for storage, such as low water activity (< 0.289), hygroscopicity (< 13.71 g/100 g) and moisture (< 7.10%) and particle sizes lower than the sensory perceptible (< 11.45 μm). This work proved that yeasts were loaded after spray-drying, thus, they might be considered as biocapsules. Furthermore, the bioaccessibility of encapsulated phenolic compounds from Bordeaux and Cabernet Sauvignon extracts was 34.96% and 14.25% higher compared to their respective free extracts, proving that yeasts are not only biocapsules of easy application, but also a biological material capable of protecting and delivering the compounds during gastrointestinal digestion.

1. Introduction

The increasing interest in the valorization of food and agricultural industries byproducts is a recurrent subject in the scientific literature and the importance of studies involving natural sources of bioactive compounds, such as grape pomace, is well established. Several studies highlight this winery byproduct as an attractive source of phenolic compounds (Goula, Thymiatis, & Kaderides, 2016; Nayak, Bhushan, Rosales, Turienzo, & Cortina, 2018; Peixoto et al., 2018; Rubio et al., 2018), which exert antiproliferative properties against colon cancer cells (Jara-Palacios et al., 2015), antioxidant (Farhadi, Esmaeilzadeh, Hatami, Forough, & Molaie, 2016; Iora et al., 2015; Peixoto et al., 2018), antibacterial (Peixoto et al., 2018; Xu, Burton, Kim, & Sismour, 2016), cardioprotective (Rodriguez-Rodriguez et al., 2012) and skin anti-aging (Wittenauer, Mäckle, Sußmann, Schweiggert-Weisz, & Carle, 2015) activities.

Although the benefits and promising applications, phenolic compounds stability is a crucial aspect to consider their utilization as antioxidants and colorants in foods (Bakowska-Barczak & Kolodziejczyk, 2011; De Souza, Thomazini, Balieiro, & Fávaro-Trindade, 2015; De Souza et al., 2014). Phenolic compounds can be affected by pH variation, presence of metal ions, light, temperature, oxygen and enzymatic activities (Aizpurua-olaizola, Navarro, Vallejo, Olivares, & Etxebarria, 2016) and, in addition to the poor long-term stability, the bioavailability and bioactivity of the potential bioactive compounds can be also altered during the exposure to different chemical, physical and biochemical conditions under gastrointestinal digestion (Mosele, Maciá, Romero, & Motilva, 2016). In this context, it is essential to apply some kind of technology to overcome these problems.

Microencapsulation is a technique widely used by food industry to protect functional food ingredients due to the low cost and flexibility (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Shamaei, Seiiedlou, Aghbashlo, Tsotsas, & Kharaghani, 2017). Among microencapsulation techniques, spray drying is a cost-effective method that can often result in the formation of stable and free-flowing powders (Flores, Singh, Kerr, Pegg, & Kong, 2014) with high quality, low water activity and good storage capability (Shamaei et al., 2017). The quick evaporation of the solvent employed in the mixture keeps the

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Fig. 1. Control yeast *Saccharomyces cerevisiae* (a) and powders BY and CSY obtained after encapsulation of extracts from Bordeaux and from Cabernet Sauvignon grape pomaces in yeast, respectively (b and c).

temperature of the formed particles low and, therefore, enables the drying of heat-sensitive products without affecting their quality in a significant way (De Souza et al., 2015).

Recently, there is an increasing interest in the use of yeast cells as a carrier material for encapsulation (Pham-Hoang, Romero-Guido, Phan-Thi, & Waché, 2018; Sultana et al., 2017; Young & Nitin, 2019; Young, Rai, & Nitin, 2020) because of its structure and nutritional benefits. The cell wall of *Saccharomyces cerevisiae*, for instance, consists of β-glucans, mannoproteins and small amounts of chitin which are permeable for both hydrophilic and hydrophobic compounds (Sultana et al., 2017). Yeasts semi-permeable membrane has a proven efficacy in protecting intracellular components from undesirable effects, such as light and oxygen, and the structure of yeast capsules is resistant to temperatures higher than 265 °C (Paramera, Konteles, & Karathanos, 2011a, 2011b). Furthermore, the presence of *Saccharomyces cerevisiae* in human nutrition is not a novelty, once this eukaryotic structure is recognized as GRAS (generally recognized as safe), natural (Mokhtari, Jafari, & Khomeiri, 2016) and represents a great source of B vitamins, proteins, nucleic acids and minerals (Ferreira, Pinho, Vieira, & Tavarela, 2010). *S. cerevisiae* is well known for its fermentative action in wine and beer production (Capece et al., 2018; Shi, Wang, Chen, & Zhang, 2019; Yan, Zhang, Joseph, & Waterhouse, 2020). After their utilization, yeasts are usually discarded as liquid effluent or used as animal feed and they lost their commercial value, what makes them a great low-cost material for reutilization.

Recent studies have proven that *Saccharomyces cerevisiae* acts like a delivery system for phenolic compounds, capable to protect them from *in vitro* gastrointestinal digestion and increase their bioaccessibility (Jilani, Cilla, Barberá, & Hamdi, 2015, 2016; Ribeiro et al., 2019; Rubio et al., 2018). Thus, the objective of this study was to enrich the biomass of waste *Saccharomyces cerevisiae* with phenolic compounds from grape pomace using the spray drying technique in order to produce a bio-based pigment or colorant with health benefits and increased stability under *in vitro* gastrointestinal digestion.

2. Material and methods

2.1. Residual materials

In this work, two varieties of industrial residue of grape pressing for vinification were used: grape pomaces from Cabernet Sauvignon (*Vitis vinifera*) and Bordeaux (*Vitis labrusca*) grapes, from the regions of Toledo and Marialva (PR, Brazil), respectively. Grapes were harvested in 2016 and after pressing for winemaking, their grape pomaces were kindly provided by cooperatives. The drying conditions of both pomaces are described by Rubio et al. (2018), in which work the same material was used. Samples were stored at 4 °C and protected from light.

The yeast biomass, *Saccharomyces cerevisiae* (S-33, Fermentis Safbrew), was kindly provided by Cervejaria Campanária (Pirassununga, SP, Brazil), after its utilization twice in the Pilsen brewing. The biological material was washed with distilled water several times and it was separated from the wash water by decantation (Rubio et al., 2018). After complete removal of beer residue, the yeast was placed in Petri dishes and frozen at −20 °C for 24 h. Then, the biomass was lyophilized in a Terroni freeze-dryer (model LC 1500, São Carlos, Brazil) for 48 h, at −20 °C and pressure of 1–0.1 kPa. Biomass was stored in polyethylene plastic bags, at −20 °C, for 6 months prior its utilization.

2.2. Preparation of extracts

For the Bordeaux and Cabernet Sauvignon extracts preparation, 1 g of grape pomace was dissolved in 20 mL of 40% ethanol, according to Iora et al. (2015). After shaking (125 rpm) (Orbital Shaker Marconi, MA420, Piracicaba, SP) at 25 °C for 180 min, samples were centrifuged at 6000 rpm for 10 min in an Eppendorf 543 0R centrifuge. The final volume of extracts was concentrated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 40 °C, until the extracts were reduced to half of the initial volume. In this work, Bordeaux and Cabernet Sauvignon extracts will be called by the acronyms BE and CSE, respectively.

2.3. Microparticles preparation by spray drying

The mixtures of yeast-extract were prepared by suspending 5% (w/w) of dry yeast in each extract. The flask containing the suspension was kept under magnetic stirring and room temperature (23–25 °C) during the drying procedure in order to not separate the liquid and solid phases. The solutions were atomized in a pilot scale spray dryer (model MSD 1.0, Labmaq do Brasil Ltda, Ribeirão Preto, Brazil), using a 2 mm nozzle and air flow of 65 L min^{-1}. The inlet temperature and air compressor pressure used were 130 °C and 0.2 MPa, respectively. The outlet temperature was recorded around 80 °C. Powders were collected at the bottom of the dryer's cyclone and were stored in bottles hermetically sealed, in absence of light, at −20 °C. Fig. 1 shows the yeasts before encapsulation and the produced microparticles. The biomass

2.4. Particle characterization

The particle characterization was carried out with yeasts without incorporation of extracts (named control yeasts) and with microparticles of yeasts containing the extracts from BE and CSE (called by the acronyms BY and CSY, respectively), in order to observe yeast changes after atomization and incorporation of extracts.

2.4.1. Moisture content

The moisture content of microparticles was measured in a moisture

analyzer (MB35 Halogen, Ohaus, Switzerland) using infrared radiation and a halogen-heating lamp.

2.4.2. Water activity

The water activity (a_w) of the powders was measured using an Aqualab Pre Water Activity Analyzer (Decagon Devices Inc., USA) at 25 °C after stabilization of the samples at this temperature.

2.4.3. Hygroscopicity

Hygroscopicity measurements were carried out as described by Cai and Corke (2000), with slight modifications. Triplicates of 0.5 g of microparticles were placed in Petri dishes in a desiccator containing NaCl saturated solution (relative humidity of 79.6%). After one week of storage, samples were weighed and hygroscopicity was expressed as g of adsorbed water per 100 g of dry solids.

2.4.4. Particle sizing

Analysis was carried out as described by Silva et al. (2018), with modifications. Small samples of microparticles were slightly macerated with a pistil and were suspended in distilled water. The samples were immersed in an ultrasound bath and subjected to ultrasound treatment at constant frequency of 25 kHz (150 W) for 2 min, in an attempt to disrupt agglomerates of particles. Then, the mixture was placed in a proper cuvette, the particle size was measured using a laser diffraction particle analyzer (Shimadzu SALD-201 V, Kyoto - Japan) and results were expressed as D[4,3], the mean diameter over the volume distribution.

2.4.5. Scanning electron microscopy (SEM)

Samples were slightly macerated, in order to separate agglomerates of yeasts and facilitate the microscope zoom, as well as being mounted on aluminum stubs using carbon adhesive tapes. The microstructure of control and encapsulated yeasts was analyzed using a scanning electron microscope (Tabletop Microscope, Hitachi TM300) and a 15 kV voltage was applied (Rubio et al., 2018).

2.4.6. Confocal laser scanning microscopy

Samples preparation followed the procedure described by Pham-Hoang et al. (2018), with some modifications. The analysis was carried out with yeasts before and after atomization, in an attempt to observe changes in yeasts outer and inner morphology. Samples were immersed in 1 μg mL^{-1} Calcofluor White M2R solution, rinsed three times in distilled water and, then, immersed in Nile Red solution (1 μg mL^{-1}) in order to stain cell walls and lipid bodies, respectively. Yeast cells were observed using a Leica TCS SP5 Confocal Microscope (Leica Microsystems, Germany). Calcofluor was excited at 405 nm and light emitted was recorded between 430 and 480 nm by a band-pass filter. Nile Red was excited at 488 nm and emission wavelengths were recorded between 515 and 645 nm.

2.4.7. Color

Powders were filled into a glass cell against the light source and color was measured in a colorimeter (Mini Scan XE, HunterLab, Reston, USA). The instrument was calibrated with black and white calibration plates before color measurement and the results were expressed according to the CIELAB color system (L*, a* and b*), where the L* value indicates the measure of lightness, the parameter a* is a measure of redness or greenness and b* value is a measure of the amount of yellowness or blueness (Zhang, Li, & Fan, 2019).

2.5. Stability test

Microencapsulated yeasts were stored at −20 °C and total phenolic compounds (TPC) analysis was carried out every 30 days. During a period of 180 days, the extraction of encapsulated compounds was performed and TPC content was assessed in order to determine the stability of the compounds under storage conditions. Three replicates per sample were tested throughout the experiment. The half-life time ($t_{1/2}$) of encapsulated compounds was obtained by Eqs. (1) and (2) (De Souza et al., 2014), where k is the reaction rate constant, C_o is the initial TPC content and C_t is the TPC content at the reaction time t (days).

$$-\ln\frac{C_t}{C_o} = kt \tag{1}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{2}$$

2.6. Extraction of encapsulated compounds and retention

To determine phenolic compounds retention into the yeast cells, the TPC of the extracts before drying and TPC content in the microparticles have been determined. Encapsulated phenolic compounds were extracted from yeast cells by washing the microcapsules with the solvent used to prepare the extracts. For this purpose, 50 mg of each sample were mixed with 1 mL of 40% ethanol in Eppendorf micro tubes and samples were kept for 5 min. in an ultrasonic water bath (Unique, USC-1400, Indaiatuba – SP, Brazil) with 40 kHz of frequency and power of 135 W. After washing, samples were centrifuged at 6000 rpm for 5 min. The washing and centrifugation steps were repeated for sufficient times until the resultant supernatant became colorless. The extracts used for phenolic compounds quantification were the mixture of all obtained supernatants from each extraction. Retention was determined by Eq. (3).

$$Retention = \frac{TPC\ \ content\ \ in\ \ microparticles}{TPC\ \ content\ \ in\ \ extracts} \times 100 \tag{3}$$

2.7. Mid-infrared attenuated total reflectance (MIR-ATR)

Samples of biomasses, before and after the atomization, were characterized by Fourier transform infrared spectroscopy, in the mid-infrared region. Spectra were recorded on a Perkin Elmer spectrometer (Spectrum ONE FT-IR, Universal ATR Sampling Accessory) over the range of 4000–650 cm^{-1}, with 32 scans and 4 cm^{-1} resolution (Rubio et al., 2018).

2.8. In vitro simulated digestion

2.8.1. Samples preparation

The effect of a gastrointestinal digestion on free and encapsulated TPC was evaluated using the method described by Koehnlein et al. (2016) with modifications proposed by Rubio et al. (2018). After rotaevaporation, crude Bordeaux and Cabernet Sauvignon extracts were dried by lyophilization on a freeze-dryer (Terroni, model LC 1500, São Carlos, Brazil) for 24 h. Six different samples were used: yeasts containing the extracts from BE and CSE (BY and CSY), freeze-dried extracts from BE and CSE (FDBE and FDCSE), gastric and intestinal blanks. Each sample was prepared in duplicate for gastric and intestinal phases. For BY, CSY, FDBE and FDCSE, samples were prepared suspending 1 g of the respective powder with 10 mL of distilled water.

2.8.2. Simulated gastrointestinal procedure

The first step was to adjust the pH of the samples to 1.2 by the addition of 5 mol L^{-1} HCl. Then, 30 mL of simulated gastric fluid (prepared with 3.2 g L^{-1} of pepsin in 0.03 mol L^{-1} NaCl solution previously adjusted to pH 1.2) was added. Samples were shaken in an orbital shaker (Marconi, MA420, Piracicaba, SP) at 150 rpm for 120 min and 37 °C in the protection of light. Afterward, samples were kept on ice for 10 min in order to stop the pepsin activity. Then, half of the samples and gastric blanks were centrifuged at 6000 rpm for 10 min, the supernatants were collected and stored at −20 °C. The pH

of the other half of the samples and intestinal blanks was adjusted to 6.0 with 1 mol L^{-1} $NaHCO_3$. After the addition of 5 mL of 120 mmol L^{-1} NaCl and 5 mL of 5 mmol L^{-1} KCl, 30 mL of freshly prepared intestinal fluid (prepared by dissolving 0.05 g of pancreatin and 0.3 g of bile salts for each 35 mL of 0.1 mol L^{-1} $NaHCO_3$ solution) was added. The mixtures were shaken for 180 min under the same incubation conditions and, then, the digests were also kept on ice for 10 min, following centrifugation and supernatants storage at −20 °C.

2.8.3. Total phenolic compounds content

The TPC analysis was carried out following the Folin-Ciocalteu colorimetric method proposed by Singleton and Rossi (1965), with a previously determined standard calibration curve (45–500 mg of Gallic Acid/L). Absorbances were recorded at 765 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV–Vis, Shanghai, China) and the results of TPC content were expressed as milligrams of Gallic Acid Equivalents (GAE) per liter of extract. The bioaccessibility was calculated following Eq. (4) (Vu et al., 2019).

$$Bioaccessibility\ (\%) = \frac{PC_{SF}}{PC_{fi}} \times 100 \quad (4)$$

where PC_{SF} is the phenolic compounds content in the soluble fractions after *in vitro* digestion and PC_{fi} is the phenolic content in the samples before digestion.

2.9. Statistical analysis

All analyses were performed in triplicate and results are presented in terms of mean and respective standard deviation. One-way analysis of variance (ANOVA) with post-hoc Tukey was used for comparison between means. The analysis was performed using STATISTICA 13 software (StatSoft, Tulsa, OK, USA).

MIR-ATR spectra were preprocessed through the isolation of the fingerprint area followed by a multiplicative scatter correction (MSC) algorithm. Then, a principal component analysis (PCA) was performed in the treated data, using MATLAB R2008 (The MathWorks Inc., Natick, USA).

3. Results and discussion

3.1. Powders characterization

3.1.1. Morphology and confocal imaging

Scanning electron microscopy was applied to reveal changes in the yeasts surface and morphology after phenolic compounds encapsulation. The micrographs obtained are shown in Fig. 2. In the Fig. 2a and b (before atomization), yeast cells presented a typical ellipsoid shape with smooth surface. The same aspect was also reported by Zhang, Liu, Zhang, Wang, and Zhao (2011) and Qin, Feng, Dai, and Chang (2017). However, waste yeast cells can present damages in their structure caused by the previous utilization in the brewery fermentation and by the washing and drying processes that they were submitted before the atomization (Rubio et al., 2018). After spray-drying, in general, cells presented a more irregular surface, observed in Fig. 2c–f. Some cells showed a shrinked shape and concavities, which can be related to the evaporation of liquid droplets during the drying process, characteristic of atomized products, as cited by Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, and Netto (2010). Also, yeasts seem to be larger after atomization and mostly swollen cells can be observed, fact that can be explained by the loading of compounds inside the cell structure. The aggregation of microparticles can also be observed after spray-drying, in Fig. 2c, d and mainly in e and f, which is a common characteristic of spray-dried materials. The same behavior was evidenced in works using yeasts (Sultana et al., 2017) and other carrier materials (Paini et al., 2015; Shamaei et al., 2017).

By confocal microscopy observations, the cell outer morphology was almost the same, regardless the treatment used. Under calcofluor excitation, yeast cells exhibited some fluorescence, which can be observed in blue (Fig. 3). *Saccharomyces cerevisiae* cell wall is composed mainly by a high-order complexes of mannoproteins, β-1,3- and β-1,6-glucan and some amount of chitin (Orlean, 2012) and calcofluor is responsible for staining β-1,6-glucans and chitin. Control cells were more homogeneous, with no cell deformation and the cell wall was clearly stained by calcofluor. On the other hand, after spray-drying, the cell wall fluorescence was faint and the internal part of the cell was stained with higher intensity. Some authors evidenced the intense fluorescence at excitation 405 nm and emission 530–600 due to the presence of polyphenols inside the yeast cell (Mekoue Nguela, Vernhet, Julien-Ortiz, Sieczkowski, & Mouret, 2019). In addition, it can be observed that the cell organization was slightly perturbed after atomization of phenolic compounds. While the inner material is basically homogeneously distributed inside control cells, cells after spray-drying present some shrinkage in the inner material, as if it was unstuck from the cell membrane. The red structures that appear under Nile Red excitation are possibly lipid bodies, such as organelles. Nile Red stain fluoresces when in contact with hydrophobic components, according to Pham-Hoang et al. (2018). In control yeasts, organelles are spread all over the cell structure, whereas in cells after atomization, they appear more agglomerated. Once there was the impregnation with hydrophilic compounds, it is possible that organelles were repulsed by phenolic compounds and attracted by each other, what explains the more irregular format that they have after spray-drying and the inner liposoluble area extended, showing a more intense red area under excitation. In conclusion, all differences observed between control cells and cells after atomization might indicate the presence of phenolic compounds inside the cells.

3.1.2. Particle size, water activity, moisture and hygroscopicity

Independent of the extract dried using *Saccharomyces cerevisiae*, both obtained powders presented similar characteristics, interesting for application as a novel ingredient, such as a pigment or a food supplement. The average powder diameter was found to be within the range of 9.42–11.45 μm (Table 1), which is in accordance with the diameter showed by the micrographs. There was no significant difference between treatments. Sultana et al. (2017) encapsulated flavors (d-limonene, ethyl hexanoate, citral and ethyl propionate) in yeast cells and reported lower particle sizes, ranging between 4.8 and 9.1 μm. The authors highlight the role of the equipment nozzle structure and drying conditions on particles sizes. Lower particle sizes obtained by the authors can be mainly related to the higher inlet temperature used in their work, of 200 °C. There are evidences that when the inlet temperature is higher, because of the faster evaporation of droplets of water, particle structure tends to be more porous (De Souza et al., 2015) and the lower diameter is probably related to particle shrinkage. An issue in measuring of particle size of yeasts using laser diffraction is that cells are generally agglomerated and the base of the size distribution may be large. According to Sultana et al. (2017), the aggregation between cells might occur because of the binding properties of β-glucans, naturally present in the thick yeast cell. Although, sizes obtained in this work were within the range of 5–150 μm, expected for microparticles produced by common spray dryers (Favaro-Trindade et al., 2010) and, in addition, particles with diameters below 100 μm can be incorporated into food without interfering negatively with the texture of the final product and consumers perception (Comunian et al., 2017).

Table 1 also shows the values obtained for the water activity of control, varying between 0.166 and 0.289. Microparticles obtained after spray-drying presented higher water activity in comparison to control yeasts. The difference of a_w between yeasts before and after spray drying may be related mainly to the rehydration of yeasts with the extracts and posterior drying process applied. Values of water activity lower than 0.6 are within the recommended limit to assure the proper microbiological stability of the powders and capacity for a long

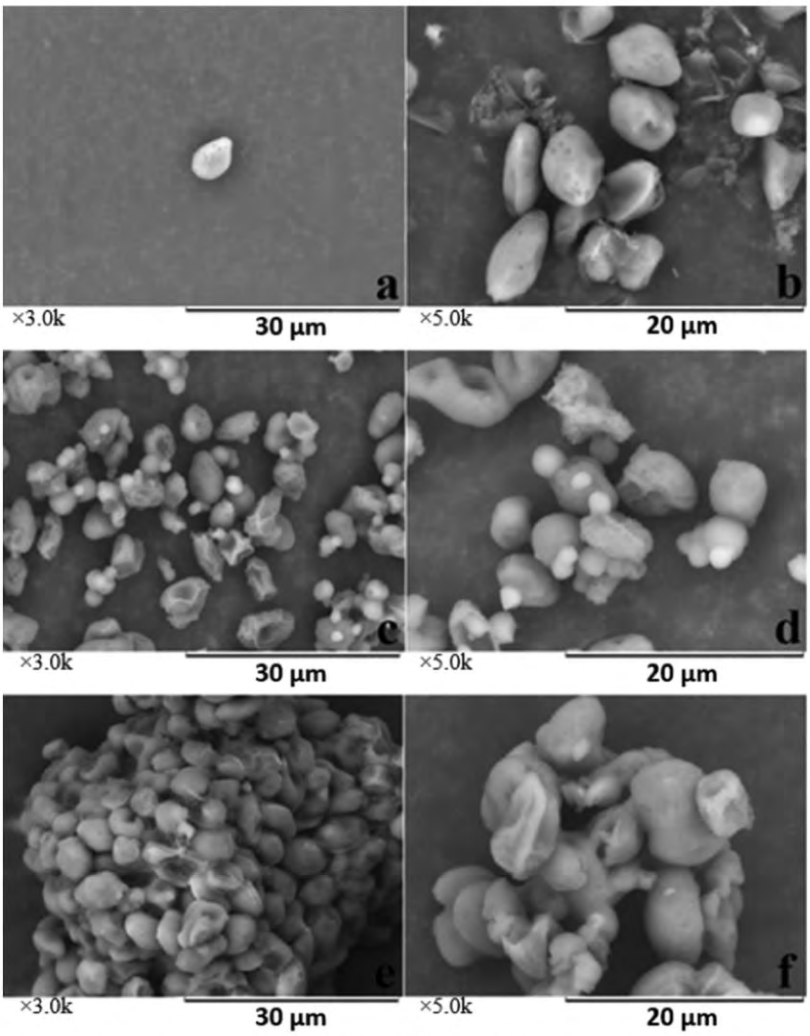

Fig. 2. Scanning electron microscopy micrographs of control yeast *Saccharomyces cerevisiae* (a and b); microparticles obtained after atomization of BE in yeasts (c and d); and microparticles obtained after atomization of CSE in yeasts (e and f).

time storage in suitable packaging and storage conditions (Righi da Rosa et al., 2019; Sarabandi, Sadeghi Mahoonak, Hamishekar, Ghorbani, & Jafari, 2018).

In relation to the moisture content (Table 1), values were within the range of 5.6% and 7.1%, with significant differences between samples and lower value for control yeast. Similar values were found by Sultana et al. (2017), with values of moisture varying from 5.1% to 9.12% when using yeasts *S. cerevisiae* for the encapsulation of flavors. Low values of moisture are desirable for powders, because the increase of moisture content is frequently related to the instability of encapsulated compounds. Moisture can promote the release or diffusion of encapsulated bioactive through the encapsulation matrix to the capsule surface, where the compound is more vulnerable to oxidation or the solubility of the compound can be increased and, then, microcapsules present higher losses of the bioactive material (Paramera, Konteles, & Karathanos, 2011b; Zheng, Ding, Zhang, & Sun, 2011).

Control yeasts present hygroscopicity of 8.98 g of water/100 g of dry matter, while yeasts after encapsulation by spray-drying show higher values of 13.70 and 13.71 g of water/100 g of dry matter. Notwithstanding using different wall material, De Souza et al. (2015) reported similar values for hygroscopicity of pigments extracted from grape byproducts and encapsulated by spray-drying with maltodextrin. The authors found values between 12.44 and 16.90 g of water/100 g of dry matter. Also, Rezende, Nogueira, and Narain (2018) reported hygroscopicity values of powders produced by freeze and spray-drying of extracts from acerola pulp and residue into maltodextrin and gum arabic ranging from 9.24 to 12.46 g of water/100 g of dry matter. Lower hygroscopicity of powders facilitates their conservation and

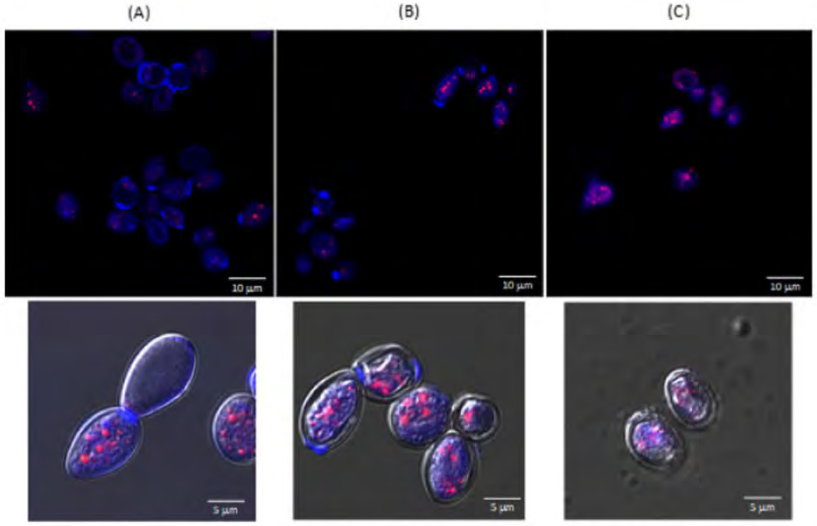

Fig. 3. Confocal laser scanning microscope images of control *Saccharomyces cerevisiae*, without extract (A); microparticles obtained after atomization of BE in yeasts (B); and microparticles obtained after atomization of CSY in yeasts (C).

preservation of color and bioactive compounds (Rezende et al., 2018).

3.1.3. Color changes after encapsulation and the influence of storage on powder color

Based on Fig. 1, visually analyzing, while the powder obtained after encapsulation of phenolic compounds from Cabernet Sauvignon have a lighter color, the particles obtained after encapsulation of Bordeaux extracts present a more intense purple color, which may be more interesting from the point of view of an industry whose goal is to add it to a product as a natural pigment.

Table 2 shows the color parameters for control and spray-dried yeasts. Control yeast parameters of L*, a* and b* differ from those obtained for yeasts after spray-drying, indicating that the color of yeast was altered by the enrichment with phenolic compounds from grape pomaces. After encapsulation of the extracts in yeasts, lower L* and b* values were observed, while a* values increased. According to Bernardes et al. (2019), normally, when the carrier material is white or light colored, the color of the added extract tends to predominate and the obtained sample will be darker, presenting lower luminosity (L*). The higher a* values reveal a deeper red and lower b* values indicate an increase in the blue color after enrichment with phenolic compounds, probably related to the incorporation of pigments such as anthocyanins, one class of the phenolic compounds and the most important pigments of vascular plants (De Souza et al., 2015). The main anthocyanins found in both Cabernet Sauvignon and Bordeaux grape pomaces are peonidin-3-O-acetylglucoside, peonidin-3-O-glucoside, malvidin-3-O-acetylglucoside, malvidin-3-O-*p*-coumaroylglucoside and malvidin-3-O-glucoside, as reported by Ribeiro et al. (2015).

In relation to the color of the powders after 180 days of storage (Table 2), both BY and CSY presented a decrease in the parameter of luminosity (L*), implying in lower levels of lightness. According to Taofiq et al. (2018), the decrease in lightness may be derived from the oxidation of incorporated compounds. For BY samples, there was an increase in b* parameter from −3.17 to −2.44 and no significant difference was observed for a* values. The evolution from negative to less negative b* values can be related to the loss of copigmentation effects accompanied by the formation of anthocyanin-derived red-or-angish pigments, such as pyranoanthocyanins (Tsali & Goula, 2018). On the contrary, there was an increase in a* values for CSY samples, from 7.71 to 8.58, and no significant difference was found for b* parameters. The formation of pigments derived from anthocyanins that stabilize the flavylium red-colored form can explain this behavior (Lago-Vanzela et al., 2014). Likewise, Moser et al. (2017) also reported an increase in the red color component after 150 days of storage of grape juice encapsulated with a mixture of whey protein and maltodextrin.

3.2. Phenolic compounds retention and stability of compounds

The retention of phenolic compounds from Cabernet Sauvignon and Bordeaux grape pomaces extracts encapsulated into *Saccharomyces*

Table 1
Properties of control and spray-dried yeasts.

	Properties			
Sample	Particle size (µm)	Moisture (%)	Water activity	Hygroscopicity (g/100 g)
CY	$11.45^{a} \pm 0.01$	$5.60^{c} \pm 0.106$	$0.166^{b} \pm 0.005$	$8.98^{b} \pm 0.314$
BY	$9.69^{a} \pm 0.30$	$6.40^{b} \pm 0.145$	$0.289^{a} \pm 0.007$	$13.70^{a} \pm 0.001$
CSY	$9.42^{a} \pm 1.70$	$7.10^{a} \pm 0.247$	$0.286^{a} \pm 0.011$	$13.71^{a} \pm 0.692$

Mean values in the same column followed by the same superscripts are not significantly different ($p > 0.05$). CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract.

Table 2
Color parameters L* (luminosity), a* (difference between red and green) and b* (difference between blue and yellow) for control and spray-dried yeasts.

	Samples				
Parameter	CY	BYt_0	$CSYt_0$	BYt_{180}	$CSYt_{180}$
L^*	$55.31^{a} \pm 0.04$	$35.49^{d} \pm 0.81$	$49.44^{b} \pm 1.18$	$30.54^{e} \pm 0.07$	$44.18^{c} \pm 0.03$
a^*	$6.02^{d} \pm 0.19$	$17.57^{a} \pm 0.12$	$7.71^{c} \pm 0.02$	$17.61^{a} \pm 0.04$	$8.58^{b} \pm 0.04$
b^*	$21.76^{a} \pm 0.40$	$-3.17^{d} \pm 0.11$	$12.44^{b} \pm 0.03$	$-2.44^{c} \pm 0.12$	$12.84^{b} \pm 0.10$

Where t_0 and t_{180} represent the time zero and after 180 days, respectively. CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. Mean values in the same line followed by the same superscripts are not significantly different ($p > 0.05$).

Table 3
Percentage of retention, stability and half-life time of total phenolic compounds.

Sample	Retention (%)	$TPCt_0$ (mgGAE L^{-1})	$TPCt_{180}$ (mgGAE L^{-1})	$t_{1/2}$ (days)
BY	95.22	$2288.09^{A} \pm 74.34$	$1561.9^{B} \pm 50.51$	326.77
CSY	97.20	$3560^{A} \pm 98.97$	$2651.19^{B} \pm 75.76$	423.29

Where $TPCt_0$ and $TPCt_{180}$ represent the total phenolic compounds in the time zero and after 180 days of storage, respectively. $t_{1/2}$ is compounds half-life time, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. Mean values in the same line followed by the same superscripts are not significantly different ($p > 0.05$).

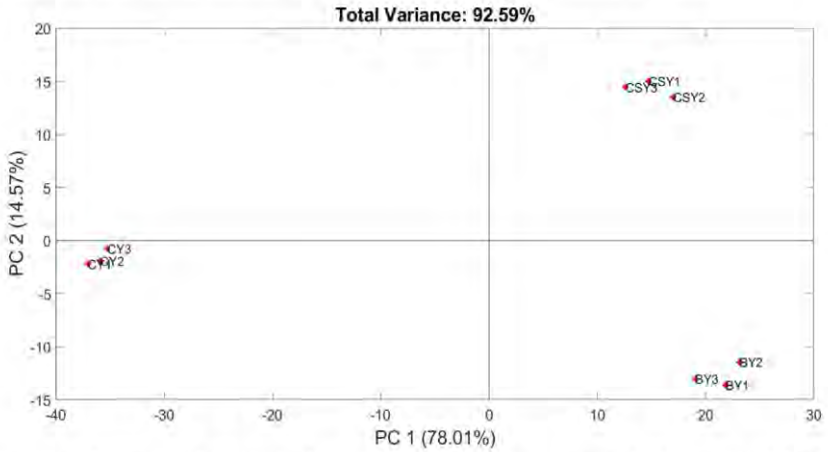

Fig. 4. Principal component analysis scores in the 900–1800 cm^{-1} region. Where BY, CSY and CY represent yeasts after the atomization of phenolic compounds from Bordeaux and Cabernet Sauvignon extracts and control yeasts (yeasts before encapsulation), respectively. 1, 2 and 3 represent the three replicates for each sample.

cerevisiae is shown in Table 3. The obtained values were within the range from 95.22 to 97.2%. Retentions close to 100% suggests that phenolic compounds were not affected by the temperature of 130 °C used in the atomization process. Yet, it is known that the structure of yeast capsules can resist high temperatures up to 265 °C (Paramera et al., 2011a), so, if phenolic compounds have affinity for wall components and they are able to bound into cell wall, it is possible that yeasts can also protect them from high temperatures applied in spray-drying. Due to the amphiphilic character of its membrane structure, formed with a continuous bilayer of lipids oriented with the polar lipid heads toward outside and the nonpolar heads toward the center of the membrane (Wang & Chen, 2009), yeast presents a great affinity for both hydrophobic and hydrophilic compounds, such as phenolic compounds. This affinity can increase the accessibility of phenolic extract to the diffusion process. Moreover, as yeasts are probable to agglomerate in consequence of β-glucans presence, bioactives can be entrapped in the interlacing formed between cells.

Phenolic compounds stability was evaluated in a period of 180 days and the results are shown in Table 3. The difference in phenolic compounds content between samples of enriched yeasts is consequence of the initial content on the obtained extracts after concentration – CSE presents a content of 3662.38 ± 14.87 mgGAE L^{-1} whereas BE shows lower content of 2333.81 ± 119.81 mgGAE L^{-1}. During 180 days, the stability of phenolic compounds did not follow a linear behavior, presenting increases and decreases in the content at every 30 days (data not shown). This could be related to oxidation reactions during storage time and, consequently, the degree of polymerization of the compounds, which may compromise the quantification by colorimetric methods (de Souza, Thomazini, Chaves, Ferro-Furtado, & Favaro-Trindade, 2020). After storage, 68.3% and 74.5% of the compounds of BY and CSY, respectively, were still retained in the biomass, resulting in half-life times of 326.77 and 423.29 days. It is important to mention that the loss of total phenolic compounds does not discard the protection effect of the yeasts over the core material, once spray-drying enables the coverage of the wall material with compounds, which is not inside the cell. Thus, this non-entrapped content is more propense to

degradation and it is probable that the main loss is related to them. Further studies are necessary to investigate the encapsulation efficiency of grape pomaces compounds encapsulation into *S. cerevisiae*.

3.3. Principal Component Analysis (PCA) of MIR-ATR spectra

In this work, PCA was performed to cluster data based on spectra and to observe changes between yeasts before and after atomization of compounds, in an attempt to confirm there was an enrichment of the biomass with phenolic compounds. Fig. 4 shows the principal component analysis scores and the plane defined by two principal components – sufficient to explain almost 93% of the variability of the dataset. The variability of PC1 corresponds to 78.01%.

Based on the scores profile, samples can be clustered in three well defined groups. Control yeast functional groups are better explained by the negative sides of both PC1 and PC2, while yeasts enriched with phenolic compounds from CSE and from BE have their functional groups better explained by positive side of PC1 and positive and negative sides of PC2, respectively. These results imply that there is, indeed, a difference between all yeasts studied, control yeasts and yeasts atomized with CSE and BE. A suitable explanation for this result is that yeasts were loaded with phenolic compounds by the process of spray-drying and, also, bioactive compounds inherent of each extract have affinity for different chemical groups in yeasts structure.

Regarding to the loading profile, results will be discussed in parallel with the spectra of the samples, shown in Fig. 5 a and b, respectively. The most important bands for control yeasts are found around 1590 cm^{-1}, 1280–1430 cm^{-1} and 900–960 cm^{-1}, where PC1 and PC2 are both negatives. In fact, correlating to spectra, is possible to observe that, in these regions, there are clear differences between CY and CSY and BY spectra. Yeasts enriched have overlapped spectra comparing to CY, in all mentioned regions, pointing that phenolic compounds were probably bounded to chemical groups found in those regions. The first difference in absorbance peaks observed around 1590 cm^{-1} shows that amide II is an important group for the retention of phenolic compounds. The same behavior was reported by Rubio et al. (2018), where spectra in the region of amide II also presented higher intensity after the enrichment of yeasts with phenolic compounds from grape pomace. The region within 1280–1430 cm^{-1} comprises an important area where bands of phenolic compounds can be found, such as gallic and tannic acids and catechins (Fragoso, Aceña, Guasch, Busto, & Mestres, 2011). According to Galichet, Sockalingum, Belarbi, and Manfait (2001), mannans and glucans contents can appear in the spectral region of 790–1190 cm^{-1}, which comprehends a polysaccharide absorbing region. Thus, the slight change in the 900–960 cm^{-1} spectral area can be most probably related to the interaction of phenolic compounds with constituents of the surface of yeast cell through hydrogen-bonding or by insertion and bounding in the network of the cell wall. Similar result was observed by Paramera et al. (2011a), in which work the authors reported a strong interaction between curcumin and the cell wall constituents of *Saccharomyces cerevisiae*.

The main groups correlated to CSY samples, according to the loadings profile, are found in the absorbance areas of 1620–1695 cm^{-1}, 990–1040 cm^{-1}, and narrower peaks in 1050 and 1085 cm^{-1}. In the first area, a region where amide I appears (Chen & Wang, 2016), obvious changes were observed after yeast enrichment, once the band is significantly less intense compared to control yeast spectra and moved to a lower frequency region, implying in chemical interaction between phenolic compounds from CSE and yeast cell. The other important areas for this sample reveal that organic acids and sugars found around 1050 and 1150 cm^{-1} (Stafussa et al., 2016) and polysaccharides found around 990–1040 cm^{-1} may have a significant bounding with phenolics from CS extract. At last, the main region responsible for explaining BY functional groups appear between 1430 and 1570 cm^{-1}, where amide III and amide II can be found and presented lower intensity compared to control yeasts.

3.4. Gastrointestinal digestion simulated in vitro

The changes on phenolic compounds content during the simulation of an *in vitro* digestion and bioaccessibility results are shown in Table 4. For all samples studied, the release of compounds occurred in majority in the gastric phase and the other part of the compounds was extracted and released in the intestinal phase. The gastric release corresponds to 62.3%, 68%, 87% and 80%, in relation to the bioaccessible fraction, for BY, CSY, FDBE and FDCSE, respectively. In fact, due to the low pH and the pepsin action, it is expected that phenolic compounds are released in the upper gastrointestinal tract, mainly those bound to carbohydrates (Qin et al., 2018). The stomach is the main site for extracting bioactives. Although, the transition from acid environment to an intestinal medium is critical for bioactive compounds since the combined effect of pH and enzymes activities may lead to changes in the bioavailability and mainly bioactivity of the ingested compounds (Jilani, Cilla, Barberá, & Hamdi, 2016).

Regarding to the intestinal phase, there was an increase in phenolic compounds content for all samples studied, implying that compounds were still released even in alkaline conditions. Also, based on the obtained results, it can be seen that phenolic compounds from the bioaccessible fraction are just a part of the TPC retained in the biomass before digestion. The more probable explanation is that part of the compounds was retained in the surface of the capsule after encapsulation, thus, it is probable that these compounds were not bounded to yeast chemical groups and, then, they solubilize into gastrointestinal medium and they are more susceptible to degrade by enzymes action or mainly under pH conditions. Therefore, it is possible that the compounds found in the intestinal phase are released from inside the capsule. Furthermore, it is also possible that compounds were not released integrally, that is, a percentage of them could be still retained inside the cells.

The results found in the present research are not consistent with previously reported data in literature involving the utilization of *Saccharomyces cerevisiae* as a material for the adsorption of phenolic compounds. These works showed there was an increase in the TPC content during gastric phase and a decrease after intestinal phase (de Oliveira et al., 2019; Ribeiro et al., 2019; Rubio et al., 2018). Authors explains that phenolic compounds are sensitive to alkaline environment, found in the small intestine, which may lead to interactions between chemical structures and the generation of different compounds with altered biological activities. Meanwhile, the present research shows that compounds encapsulated in yeasts by spray drying may be under a protective effect during both acid and alkaline conditions.

According to Czubinski et al. (2019), assessing bioaccessibility is the key step before concluding on any potential health-beneficial effects of phenolic compounds that are present in food. Although the initial content of phenolic compounds was higher in CSY, BY presented higher bioaccessibility after *in vitro* digestion. The digestive tract is a complex system and it cannot be assured that changes occur the same way with samples, mainly because of the different components inherent of each extract, which may behavior differently under pH conditions and enzymes activities.

Based on the bioaccessibility results found on Table 4, it is concluded that the percentage of bioaccessibility of compounds from BE and CSE encapsulated in yeasts is 35% and 14.25% higher than the respective free compounds bioaccessibility. The values found for microparticles justify microencapsulation, once yeasts are capable to protect and release compounds during gastrointestinal digestion and these bioactives are delivered into the intestine, where they might be absorbed for, then, exert their biological functions.

4. Conclusions

In this work, yeasts were proven to be a great wall material for encapsulation of bioactive compounds by spray-drying. It was possible

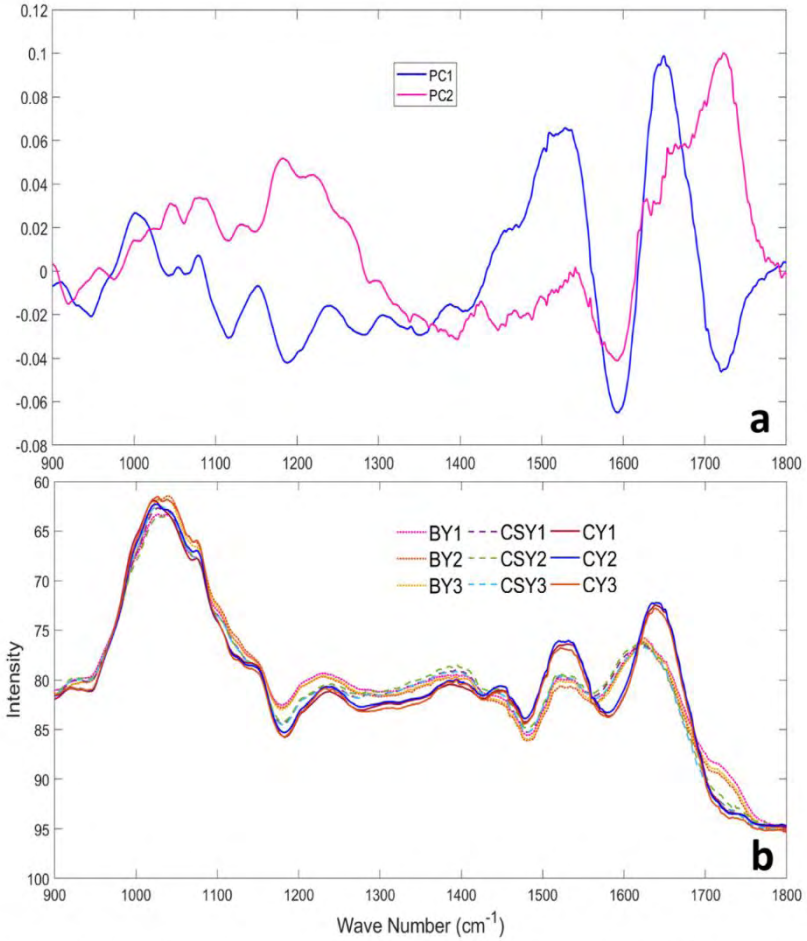

Fig. 5. PC1 and PC2 loadings (a) and MIR-ATR spectra of control and spray-dried yeasts (b), in the fingerprint region. Where CY is the control yeast of *S. cerevisiae*, BY is the yeast after the encapsulation of phenolic compounds from Bordeaux extract and CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract. 1, 2 and 3 represent the three replicates for each sample.

Table 4
Phenolic compounds from samples before and after the simulation of an *in vitro* digestion.

Sample	Before *in vitro* digestion	After gastric phase	After intestinal phase	Bioaccessi-bility
	Phenolic compounds (mg GAE L^{-1})			%
BY	$2288.1^{Da} \pm 74.34$	$688.67^{Cc} \pm 27.61$	$1105.76^{Cb} \pm 28.28$	48.33^{A}
CSY	$3559.99^{Ca} \pm 98.97$	$852.47^{Cc} \pm 98.99$	$1253.38^{Cb} \pm 44.45$	34.32^{B}
FDBE	$8829.24^{Ba} \pm 75.24$	$2750.38^{Bc} \pm 24.92$	$3162.24^{Bb} \pm 17.51$	35.81^{B}
FDCSE	$13548.25^{Aa} \pm 37.01$	$3257.05^{Ac} \pm 65.32$	$4070.81^{Ab} \pm 98.32$	30.04^{C}

Capital letters in the same column and small letters in the same row within each sub-group do not differ statistically ($p > 0.05$). BY is the yeast after the atomization of phenolic compounds from Bordeaux extract, CSY is the yeast after the encapsulation of phenolic compounds from Cabernet Sauvignon extract and FDBE and FDCSE are the Bordeaux and Cabernet Sauvignon extracts after freeze drying, respectively.

to obtain powders with characteristics that enhance the shelf-life of the product.

Obtained powders present around one year of half-life times. The principal component analysis of MIR-ATR spectra showed that phenolics were successfully attached to the yeast surface. Amide I, amide II, amide III, mannans and glucans are cells important chemical groups for phenolic compounds bounding. Then, along with the evidences of yeast changes after atomization, showed by microscopy, it can be affirmed that yeasts were indeed loaded with compounds, thus, yeasts act as capsules and not only as a vehicle for compounds drying. Encapsulated compounds presented higher bioaccessibility compared to free compounds.

Both produced powders are of great interest for application, however, if the main purpose is the obtention of a pigment, the yeast enriched with phenolic compounds from Bordeaux grape pomace extract has to be further explored. This study may be helpful to promote the application of waste yeast as a vehicle for encapsulation of bioactive compounds as a novel food ingredient and may show some general interests for the stability of other natural substances.

CRediT authorship contribution statement

Fernanda Thaís Vieira Rubio: Conceptualization, Investigation, Methodology, Formal analysis, Project administration, Data curation, Writing - original draft. **Charles Windson Isidoro Haminiuk:** Conceptualization, Writing - review & editing. **Milena Martelli-Tosi:** Methodology, Data curation. **Marluci Palazzolli da Silva:** Methodology, Investigation, Data curation. **Gustavo Yasuo Figueiredo Makimori:** Methodology, Software. **Carmen Sílvia Favaro-Trindade:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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ATTACHMENT B – ETHICS COMMITTEE ACCEPTANCE

USP - FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS DA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação sensorial de iogurte enriquecido com compostos bioativos extraídos do bagaço de uva Bordeaux (Vitis Labrusca) e dos resíduos de Jabuticaba (Myrciaria cauliflora) e encapsulados em levedura Saccharomyces cerevisiae

Pesquisador: FERNANDA THAIS VIEIRA RUBIO

Área Temática:

Versão: 4

CAAE: 08111219.3.0000.5422

Instituição Proponente: UNIVERSIDADE DE SAO PAULO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.338.771

Apresentação do Projeto:

O projeto de pesquisa se refere a pesquisa que será realizada pela pesquisadora responsável Fernanda Thaís Vieira Rubio que realizada doutorado sob orientação da Profa. Dra. Carmen Sílvia Fávaro-Trindade, ambas vinculadas a FZEA. Trata-se de projeto de pesquisa que envolve a participação de seres humanos para avaliação sensorial de iogurtes enriquecidos com compostos bioativos com capacidade antioxidante. Deste modo a avaliação e aprovação por Comitê de Ética em Pesquisa é necessária para execução da pesquisa.

Objetivo da Pesquisa:

O objetivo deste projeto é colorir e enriquecer iogurte por meio da adição de compostos bioativos extraídos dos resíduos do processamento de vinho e do resíduo da jabuticaba, nas formas livre e encapsulados em células de Saccharomyces cerevisiae.

Avaliação dos Riscos e Benefícios:

Sobre os riscos, a equipe responsável observa que todos os procedimentos serão realizados dentro das mais absolutas normas de higiene e segurança e os provadores serão informados sobre os ingredientes do produto. As pesquisadoras indicam que se responsabilizarão caso haja qualquer dano ao provador decorrente da participação na pesquisa.

Endereço: Avenida Duque de Caxias Norte, 225
Bairro: Campus Universitário da USP **CEP:** 13.635-900
UF: SP **Município:** PIRASSUNUNGA
Telefone: (19)3565-4299 **Fax:** (19)3565-4114 **E-mail:** cepfzea@usp.br

Sobre os benefícios as pesquisadoras informam que não haverá qualquer benefício ao provador ao participar da pesquisa.

Comentários e Considerações sobre a Pesquisa:

A pesquisa trata de tema relevante, propondo o desenvolvimento de um produto à base de iogurte com a adição de compostos (compostos fenólicos encapsulados em levedura) que tragam benefícios a saúde do consumidor.

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatórios estão adequados.

Recomendações:

Aprovação da pesquisa.

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências ou inadequações.

Considerações Finais a critério do CEP:

O CEPH FZEA aprova o desenvolvimento da pesquisa a partir desta data.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1285409.pdf	14/05/2019 20:36:11		Aceito
Projeto Detalhado / Brochura Investigador	Projetosensoriaiogurte.doc	14/05/2019 20:35:54	FERNANDA THAIS VIEIRA RUBIO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tclecorrigido.docx	14/05/2019 20:35:32	FERNANDA THAIS VIEIRA RUBIO	Aceito
Folha de Rosto	DocPlataforma.pdf	31/01/2019 18:27:57	FERNANDA THAIS VIEIRA RUBIO	Aceito

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Aprovado

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ATTACHMENT C – PAPER PUBLISHED IN FOOD & FUNCTION

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		Expected presentation date	2021-09-15
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Food & Function

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Development of natural pigments microencapsulated in waste yeast *Saccharomyces cerevisiae* using spray drying technology and their application in yogurt

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Although *Saccharomyces cerevisiae* has shown potential utilization as a bio-vehicle for encapsulation, there are no reports about the functionality of natural colorants encapsulated using yeast cells. The main objectives of this study were to produce natural food coloring by encapsulating extracts from grape pomace (GP) and jabuticaba byproducts (JB) in brewery waste yeast and evaluate the functionality of the pigments by their incorporation into yogurts. Particles produced by the encapsulation of extracts from GP and JB in *S. cerevisiae* using 5% of yeast had the highest encapsulation efficiencies for both anthocyanins (11.1 and 47.3%) and phenolic compounds (67.5 and 63.6%), the highest concentration of both bioactives during storage and stable luminosity. Yogurts showed a pseudoplastic behavior and were considered weak gels. Colored yogurts had acceptance indexes between 73.9 and 81.4%. This work evidenced the utilization of enriched yeasts as coloring agents and interesting additives for the production of functional foods.

1 Introduction

Over the years, industries have paid attention to changes in consumer behavior and expectations and, as a consequence, the search for novel ingredients and additives has been of great concern. Food appearance is highly related to color and may be the most important factor to lead the consumer to select a product since it is the first characteristic seen. According to Gebhardt *et al.* (2020),[1] color positively influences a consumer's preference, purchase decision and eating desires. In this context, food colorants improve the attractiveness of foods to meet the color expectations of consumers[2] and, also, color can present marketing purposes when it is for flavor identification.[3]

Although synthetic colorants are widely used in food industries due to their stability, strength and price,[4] some pigments pose a potential risk to human health, especially in the case when they are excessively consumed,[5] and the concern is exacerbated because artificially colored foods are often marketed to children.[6] Recently, there has been a worldwide movement towards more use of natural colorants.[5] Among vegetable colorants, anthocyanins are considered the most important pigments of vascular plants[7] and represent a phenolic compound class. Phenolic compounds can be found in low cost sources, such as bio-residues, and their valorization has been of great interest for the sustainable production of value-added colorants.[8]

Jabuticaba (also known as "Brazilian fruit") byproducts and grape pomace are amongst known sources rich in phenolic compounds. Grapes are a popular agricultural crop used in wine production. After their pressing for must preparation, the solid parts macerated are discarded as pomace, generating substantial quantities of wastes.[9] On the other hand, jabuticaba is very appreciated for *in natura* consumption and for the production of jams, syrups and alcoholic beverages. However, the commercialization of this kind of fruit is difficult due to its high perishability and, with the main application of its pulp, residues represent about 50% of the total processed volume.[10] Several works have reported the antioxidant action of bioactive compounds extracted from these matrixes[11–16] and their application as food colorants.[8,17,18]

The application of natural pigments is limited yet by their poor stability as most of them are sensitive to oxidation, pH changes and light, besides their inherent solubility which

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varies widely.[3] To overcome these problems, encapsulation has been extensively studied for the protection of pigments, improvement of their stability and dispersibility in water. Among known techniques, spray-drying is a scalable, relatively low-cost and the most common technique used to encapsulate food materials and active compounds.[19] Furthermore, the short processing time is suitable for heat-sensitive compounds.[20]

Recent works have been studying the utilization of yeast *Saccharomyces cerevisiae* to replace common carrier materials for encapsulation of anthocyanins,[21] flavors,[22] curcumin[23] and phenolic compounds from grape pomace[24] by spray-drying. Yeasts can be obtained as residues from brewery industries after utilization in the fermentative process and, as a result, its low cost may be of great advantage compared to other carriers/vehicles. In addition, *S. cerevisiae* cells are generally recognized as safe ("GRAS"), have high nutritional value and have been already consumed and incorporated into human nutrition. In a previous study of our research group, yeast cells were proven to be a great bio-vehicle for the incorporation of phenolic compounds, acting as a biocapsule, protecting phenolic compounds during gastric digestion and delivering them gradually until the intestine phase performed in an *in vitro* simulation.[24] In addition, spray-dried powders presented interesting colors, but their potential as natural pigments and functionality still has to be further explored by their incorporation in food.

In this context, yogurt can be a suitable option to be studied as a food matrix to apply natural pigments because it is a food consumed by people of all ages with great acceptance and it is highly appreciated for its nutritional value and good digestibility.[25,26] The yogurt matrix seems to be an excellent delivery vehicle for plant-derived phenolic compounds, as the low pH values increase the stability of phenolic compounds and the presence of proteins or large peptides and fat maintains the integrity of phenolic compounds during digestion, thus increasing their bioaccessibility.[26] In addition, a yogurt colored with natural pigments may gain space in the market by enhancing attractiveness and consumer interest. Therefore, the objective of this work was to encapsulate natural pigments from grape pomace and jabuticaba byproducts using yeast *Saccharomyces cerevisiae* as a vehicle and incorporate them in yogurt aiming at its enrichment and coloring. To the best of our knowledge, this is the first work in the literature that shows a food application for bio-based particles produced with yeasts.

2 Experimental

2.1 Materials and their preparation

For this work, three byproducts were used as raw materials: residue from the Bordeaux grape vinification (*Vitis labrusca*), jabuticaba (*Myrciaria cauliflora*) pulping residues and waste brewery *Saccharomyces cerevisiae*. Grape pomace was donated by Vinícula Ferragut (Vinhedo, SP, Brazil) and the residue was dried in a forced air oven at 40 °C for 36 hours.[27] After drying, the samples were milled in a knife mill. Jabuticaba fruits were purchased from a local market and they were selected and washed with water, bleached for 3 minutes and then pulped using a pulping machine (EBERLE, 10C56), as proposed by Silva *et al.* (2014),[16] in order to obtain peels and seeds as the raw material for the subsequent steps. The obtained byproducts were dried in an oven with forced air circulation at 60 °C for 24 hours and then the samples were ground in a blender.[28] The biomass of *Saccharomyces cerevisiae* was kindly donated by Cervejaria Hausen Bier (Araras, SP, Brazil) after its utilization in Pilsen beer-type production. Yeasts were washed with distilled water consecutive times and the washing water was separated by decantation.[29] After reaching the complete removal of the beer residue, the obtained biomass was placed in Petri dishes and frozen at −20 °C for 24 hours. After this time, yeasts were lyophilized in a Terroni freeze dryer (LC 1500, São Carlos, Brazil) for 48 hours at −20 °C and a pressure of 1–0.1 kPa. Afterward, grape pomace powder, ground jabuticaba byproducts and dried yeasts were stored in a dark environment at −20 °C in a freezer.

2.2 Preparation of extracts from Bordeaux grape pomace and jabuticaba byproducts

For the preparation of the grape pomace extract, 1 g of its powder was mixed with 20 mL of 40% (v/v) ethanol. Samples were shaken at 125 rpm (Orbital Shaker Marconi, MA420, Piracicaba, SP) and 25 °C for 3 hours, as described by Iora *et al.* (2015).[27]

The extract from jabuticaba byproducts was prepared following the condition proposed by Rodrigues *et al.* (2015),[28] using the ratio of 1 : 20 (w/v) of dry material and 46% (v/v) ethanol acidified to pH 2.0 with citric acid. Extraction was performed in an open rectangular ultrasound instrument (Unique Model USC, 25 kHz, 150 W) for 10 minutes.

After extraction, samples from both materials were centrifuged at 4226*g* for 10 min in a centrifuge (Eppendorf 543 0R). The resulting supernatants were evaporated using a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 40 °C until the extract volumes were reduced to half of their initial volume. The obtained extracts were used for further analyses. Grape pomace and jabuticaba byproduct extracts were nominated by the acronyms GPE and JE, respectively.

2.3 Spray drying operation conditions and powder stability evaluation

Mixtures of the extracts and yeasts were prior prepared by adding 5%, 10% and 15% (w/w) of dry *Saccharomyces cerevisiae* to the extracts. That is, 15 g of dry yeasts was mixed with 285, 135 and 85 g of extracts, respectively. The procedure was carried out according to Rubio *et al.* (2020).[24] The suspensions were kept at room temperature and subjected to magnetic stirring in order to ensure that there was no phase separation. Then, the samples were atomized using a bench spray dryer (model MSD 5.0, Labmaq do Brasil Ltda., Ribeirão Preto, Brazil) with a 2 mm nozzle at an air flow of 65 L min^{-1} and a feeding flow of 10.8 mL min^{-1}. The inlet temperature was

130 °C, the air compressor pressure was 0.2 MPa and the recorded outlet temperature was 80 °C. The produced powders were collected in the cyclone compartment and separated in equal masses into penicillin flasks. Then the flasks were stored at 25 °C in desiccators with $MgCl_2$ and a controlled humidity of 33.3%. The stability of phenolic compounds and anthocyanins encapsulated in *Saccharomyces cerevisiae* yeast was evaluated on the day of production (day 0) and after 15, 30, 60 and 90 days of storage. Powders produced with GPE and JE with 5, 10 and 15% of dried yeasts were named GP5, GP10, GP15 and J5, J10 and J15, respectively. Fig. 1 shows the obtained powders.

2.4 Determination of encapsulated compounds, encapsulation efficiency and bioactive retentions during storage

For determination, phenolic compounds and anthocyanins were extracted from the particles produced by adding 0.05 g of the powder into 1 ml of the reagent used in each extract production (40% ethanol for GPE and 46% acidified ethanol for JE). The suspension was sonicated using a rectangular ultrasound equipment (Unique Model USC, 25 kHz, 150 W) for 5 minutes and then centrifuged for 5 minutes at 4226 g. This procedure was repeated until the obtained supernatant became colorless. The extract used for further analysis was the sum of supernatants obtained from each extraction.[24] For the determination of surface compounds, 2 ml of the extraction solvent was added to 0.1 g of the particles. The mixture was agitated and centrifuged for 1 minute at 3000 rpm. Finally, the supernatant was filtered through a 45 μm pore microfilter and the filtrate obtained was analyzed.

The phenolic content found in the samples was estimated following the Folin–Ciocalteu colorimetric method proposed by Singleton & Rossi (1965).[30] The absorbances were recorded at 765 nm using a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, Shanghai, China) and the results were found by using a prior standard calibration curve, prepared with concentrations between 45 and 500 mg of gallic acid per liter.

The anthocyanin quantification was based on the differential pH method, proposed by Giusti & Wrolstad (2001),[31] a technique based on the spectrophotometric measurement (at 520 nm and 700 nm) of the absorbances of anthocyanin extract samples in two pH ranges, pH 1 and pH 4.5, using 0.025 mol L^{-1} potassium chloride (KCl) and 0.4 mol L^{-1} sodium acetate (CH_3COONa) solutions, respectively.

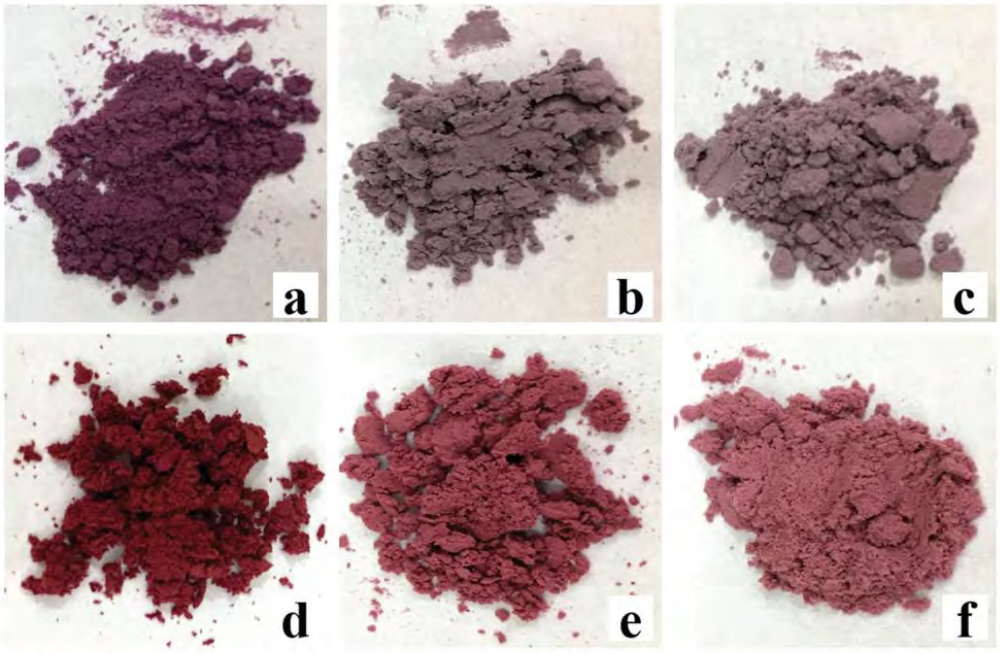

Fig. 1 Powders produced by the encapsulation of grape pomace extracts in *Saccharomyces cerevisiae* using proportions of 5, 10 and 15% of yeast (a, b and c, respectively) and by the encapsulation of jabuticaba byproduct extracts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast (d, e and f, respectively).

The encapsulation efficiencies, in percentage, of the phenolic compounds and anthocyanins were determined by eqn (1) proposed by Tsali & Goula (2018).[9] TC are the total compounds and SC are surface compounds.

$$\mathrm{EE}(\%) = \frac{\mathrm{TC} - \mathrm{SC}}{\mathrm{TC}} \times 100 \quad (1)$$

The compound retention time was determined by eqn (2), where C_f is the compound content at the end of the storage period and C_i is the initial compound content.

$$\mathrm{CR}(\%) = \frac{C_f}{C_i} \times 100 \quad (2)$$

2.5 Evaluation of powder instrumental color

Instrumental color parameters were evaluated on the day of powder production and at the end of 90 days of storage (at 25 °C and controlled humidity of 33.3%) using a HunterLab Mini Scan XE colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) to obtain the CIE L^*, a^* and b^* color parameters. Chroma and hue angle were calculated according to eqn (3) and (4),[32] respectively.

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

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (4)$$

2.6 Microparticle morphology by confocal laser scanning microscopy

Analyses were carried out to observe the differences between control and enriched cells in an attempt to confirm the presence of bioactive compounds inside yeast cells after atomization. Samples were prepared following the procedure described by Pham-Hoang *et al.* (2018)[33] with slight modifications proposed by Rubio *et al.* (2020).[24] Calcofluor White M2R and Nile red solutions were prepared with distilled water and ethanol, respectively, at a concentration of 1 mg mL^{-1}. The cells were mixed with 1 μg mL^{-1} of calcofluor, washed three times with distilled water and stained with 1 μg mL^{-1} of Nile red. The sample images were obtained using a Leica TCS SP5 Confocal Microscope (Leica Microsystems, Germany). For calcofluor, the images were recorded at an excitation wavelength of 405 nm and the emission wavelengths were recorded between 430 and 480 nm. Nile red was excited at 488 nm and the emission was recorded between 515 and 645 nm.

2.7 Enriched yogurt preparation

Yogurts were produced in the dairy plant of campus "Fernando Costa", at the University of São Paulo (Pirassununga, SP, Brazil). The procedures were followed as described by Comunian *et al.* (2017).[34] Yogurt manufacturing involved the following the steps: milk heat-treatment at 90 °C for 30 minutes; addition of sugar; cooling to 45 °C; addition of the starter culture; incubation at 45 °C for 3 hours; cooling to 4 °C and mixing of microcapsules in proportions of 1% and 1.5% (w/w). Grape aroma was added to the yogurt enriched with microparticles produced with grape pomace extract at a concentration of 1.5 g L^{-1}. Jabuticaba aroma was added to the formulations with microcapsules produced with jabuticaba byproduct extract at a concentration of 3 g L^{-1}. Yogurts were packed in 1000 mL plastic bottles and stored at 4 °C for further characterization and stability analyses for 30 days.

The yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast were named YGP1 and YGP15, respectively, and the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast were named YJ1 and YJ15. Fig. 2

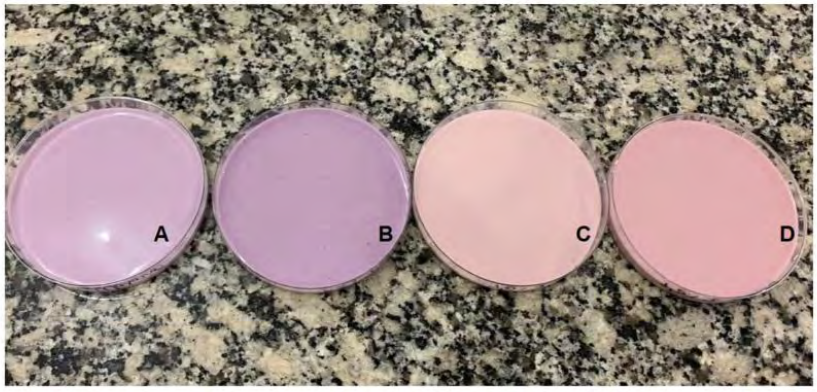

Fig. 2 Yogurts produced with 1 and 1.5% of particles obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5% of yeast (A and B, respectively) and with particles obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5% of yeast (C and D, respectively).

shows the yogurts incorporated with the abovementioned pigments.

2.8 Yogurt characterization

2.8.1 Determination of pH, titratable acidity and instrumental color. The pH of yogurts was measured using a previously calibrated digital pH meter (Mars MB-10, São Paulo, Brazil). The titratable acidity of yogurt extracts was obtained by diluting 10 g of original yogurt samples with 10 mL of deionized water. Thereafter, the mixture was titrated with 0.1 M NaOH to pH 8.3, controlled by a pH meter. The results were expressed as a percentage of lactic acid.[35] Instrumental color was measured by following the same procedure described in section 2.5.

2.8.2 Rheology measurements. The rheological behavior of yogurts was determined using an AR 2000 rotational rheometer, TA Instruments (New Castle, Delaware, USA), with parallel plate geometry (60 mm, gap 500 μm). The tests were carried out following the procedure described by Comunian *et al.* (2017).[34] Yogurts were pre-sheared for 30 s at a shear rate of 500 s^{-1}, maintained in equilibrium for 30 s and then analyzed for 4 minutes. The samples were subjected to different rheological tests at 6 °C. For steady-state tests, there was a gradual increase of shear rate from 0.1 to 100 s^{-1}, in which domain the evolution of shear stress and viscosity was monitored. The flow was modeled by fitting the data to the Herschel–Bulkley (eqn (5)) model.

$$\tau = \tau_0 + k_H \cdot \gamma^{n_H} \quad (5)$$

where τ is the shear stress (Pa s), τ_0 is the yield stress (Pa), k_H is the consistency index (Pa s^n), γ is the shear rate (s^{-1}) and n_H is the flow behavior index (dimensionless). The type of fluid is determined by the parameter n_H: $n_H = 1$: Newtonian fluid; $n_H < 1$: pseudoplastic fluid; $n_H > 1$: dilating material.

For dynamic frequency sweep tests, oscillatory stress of 0.2 Pa was set (region of linear viscoelasticity). The analysis was performed within the frequency range of 0.1 and 10 Hz and the storage modulus (G') and loss modulus (G'') were registered. In addition, the complex modulus G^* (eqn (6)) was obtained at a frequency of 1 Hz.

$$G^* = [(G')^2 + (G'')^2]^{\frac{1}{2}} \quad (6)$$

2.8.3 Stability of yogurt phenolic compounds. For extraction and quantification of phenolic compounds and anthocyanins present in yogurts, 2 g of enriched yogurts were weighed in 15 mL centrifuge tubes following the addition of 4 mL of solvents (40% ethanol for YGP1 and YGP15 and 46% acidified ethanol for YJ1 and YJ15) and stirring for 3 minutes in a vortex.

The mixtures were sonicated in an open rectangular ultrasound instrument (Unique Model USC, 25 kHz, 150 W) for 5 minutes at 25 °C and then the samples were centrifugated for 5 minutes at 2935*g*, obtaining the supernatant used for phenolic compound and anthocyanin determination. This procedure was repeated seven days once for one month. The half-lives of the compounds in the prepared yogurts were calculated according to eqn (7) and (8), where C_0 is the initial compound content, C_t is the compound content at the time t (days), $t_{\frac{1}{2}}$ is the half-life time and k is the reaction rate constant.[36]

$$-\ln\frac{C_t}{C_0} = kt \quad (7)$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \quad (8)$$

2.8.4 Consumer acceptance test. A consumer acceptance test of enriched yogurts was performed to evaluate the effect of the particles on sensory attributes, mainly color. The analysis with humans was previously approved by the Ethics in Research Committee of the University of São Paulo, SP, Brazil (protocol number 08111219.3.0000.5422). For the test, a panel consisting of 120 untrained panelists was employed. The panelists were recruited among students, professors and staff on the campus and they were selected on the basis of being regular consumers of yogurt. The samples were numbered with random three-digit codes and were served randomly, in 25 mL plastic cups, to consumers in individual cabins. Water and salty cracker biscuits were also served to rinse out the mouth and neutralize the flavors between samples. During analysis, the samples were kept in a refrigerator, at 4 °C, before serving. Before analysis, each panelist had to read and sign, if agreed, a Free and Informed Consent Form.

For an effective acceptance test, a 9-point hedonic scale was applied, varying from 1 = "extremely dislike" to 9 = "extremely like" to evaluate product acceptance in relation to the attributes: appearance, color, aroma, flavor, texture and overall acceptability. The acceptance index (AI) was determined by obtaining the relationship between the average score obtained for the tested sample and the maximum score of the hedonic scale (9) multiplied by 100.[37]

2.9 Statistical analysis

The analysis was carried out using the STATISTICA 13.4.0.14 software (StatSoft, Tulsa, USA). The comparison between means was performed by one-way analysis of variance (ANOVA) with *post-hoc* Tukey. A difference was considered statistically significant when $p \leq 0.05$. The software OriginPro 7.0 was used to perform the nonlinear regression of rheological data and the quality of the model applied was evaluated by the determination coefficient and chi-square value.

3 Results and discussion

3.1 Encapsulation efficiency, powder stability and retention of compounds

Table 1 shows the encapsulation efficiency obtained for all particles produced, the contents of phenolic compounds and anthocyanins in powders during the period of 90 days of storage and the retention of compounds after 90 days. The encapsulation efficiency ranged from 52.3 to 67.5% for pheno-

Table 1 Phenolic compound and anthocyanin stability in powders produced by encapsulation of extracts from grape pomace and jabuticaba byproducts in yeast *Saccharomyces cerevisiae*, encapsulation efficiency (EE) and compound retention (CR) after storage

Sample	EE (%)	Day 0	Day 15	Day 30	Day 60	Day 90	CR (%)
	Phenolic compounds (mg EAG per g of particle)						
GP5	67.5^{A}	$154.4^{Aa} \pm 1.4$	$113.0^{Ab} \pm 2.8$	$113.8^{Ab} \pm 3.5$	$111.9^{Ab} \pm 2.1$	$114.0^{Ab} \pm 1.7$	73.8^{C}
GP10	67.2^{A}	$78.9^{Ba} \pm 0.07$	$65.3^{Bb} \pm 2.3$	$61.5^{Bb} \pm 5.7$	$52.5^{Bc} \pm 0.7$	$64.2^{Bb} \pm 0.7$	81.4^{B}
GP15	64.7^{B}	$58.9^{Ca} \pm 0.8$	$51.0^{Cc} \pm 0.7$	$46.6^{Cd} \pm 0.8$	$53.5^{Bbe} \pm 0.7$	$54.7^{Cb} \pm 2.4$	92.9^{A}
J5	63.6^{A}	$360.4^{Aa} \pm 2.0$	$331.8^{Ab} \pm 4.8$	$286.9^{Ac} \pm 2.0$	$279.3^{Ac} \pm 2.5$	$283.8^{Ac} \pm 2.7$	78.7^{B}
J10	56.1^{B}	$222.8^{Ba} \pm 1.5$	$214.6^{Ba} \pm 16.7$	$209.3^{Ba} \pm 2.5$	$212.8^{Ba} \pm 2.5$	$212.8^{Ba} \pm 8.7$	95.5^{A}
J15	52.3^{C}	$190.2^{Ca} \pm 2.2$	$111.9^{Cc} \pm 7.3$	$153.3^{Cb} \pm 2.5$	$153.9^{Cb} \pm 5.4$	$163.2^{Cb} \pm 2.7$	85.8^{AB}
	Anthocyanins (mg per g of particle)						
GP5	11.1^{A}	$17.9^{Aa} \pm 1.0$	$13.9^{Ab} \pm 0.5$	$11.9^{Abcd} \pm 2.1$	$10.7^{Acd} \pm 0.6$	$9.9^{Ad} \pm 0.8$	55.3^{B}
GP10	7.4^{B}	$11.1^{Ba} \pm 0.3$	$5.9^{Bb} \pm 0.3$	$3.5^{Bd} \pm 0.2$	$5.4^{Bbc} \pm 0.2$	$5.0^{Bc} \pm 0.08$	45.0^{C}
GP15	4.2^{C}	$5.3^{Ca} \pm 0.3$	$4.4^{Cb} \pm 0.3$	$3.2^{Bc} \pm 0.04$	$4.4^{Bb} \pm 0.3$	$4.5^{Bab} \pm 0.2$	84.9^{A}
J5	47.4^{A}	$14.8^{Aa} \pm 0.5$	$14.1^{Aa} \pm 0.5$	$11.2^{Ab} \pm 0.8$	$11.9^{Ab} \pm 0.2$	$9.2^{Ac} \pm 0.1$	62.2^{A}
J10	30.5^{B}	$12.5^{ABa} \pm 1.6$	$10.5^{Bab} \pm 0.4$	$8.0^{Bbc} \pm 0.2$	$7.3^{Ac} \pm 1.7$	$7.0^{Bc} \pm 0.4$	56.0^{A}
J15	18.3^{C}	$10.2^{Ba} \pm 0.8$	$7.9^{Cb} \pm 0.2$	$6.7^{Bb} \pm 0.7$	$6.8^{Ab} \pm 1.5$	$6.1^{Bb} \pm 0.1$	59.8^{A}

Where GP5, GP10 and GP15 are the powders obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeasts, respectively. J5, J10 and J15 are the powders obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast, respectively. Capital letters in the same column and small letters in the same row indicate there is no significant difference ($p > 0.05$) among samples, considering the same raw material.

lic compounds and from 4.2 to 47.4% for anthocyanins. Regarding the effect of wall material concentration on encapsulation efficiency, in general, the increase in yeast concentration led to a decrease in encapsulated compounds. Thus, the highest phenolic compound and anthocyanin encapsulation efficiencies were obtained for GP5 and J5. The higher concentration of yeasts in relation to the extract may increase the viscosity of the feed solution, contributing to cell aggregation and, consequently, less contact area and active sites available for compound bounding and entrance.

The encapsulation efficiencies of phenolic compounds were higher in comparison to anthocyanins, for both GPE and JE extracts. The complex nature of the extracts used in this work, presenting several different compounds in their composition, may lead to a competition to bind to the cell wall. In addition, as anthocyanins were retained mainly in the cell wall, it is possible that their molecule size could have impaired their passage through yeast pores.

Malvidin-3-glucoside is the major anthocyanin in Bordeaux grapes[38] and delphinidin-3-glucoside and cyanidin-3-glucoside are found in jabuticaba peels.[39] The chain length of these mentioned anthocyanins is higher than the chain length of gallic and ellagic acids, phenolic acids commonly found in Bordeaux grape pomace and jabuticaba peels,[40,41] respectively. With extended chains, the number of $-CH_2-$ groups increases making the apolar area longer and more difficult the passage through the polar part in the cell structure. This polar region of the cell membrane plays an important role in diffusion and the more polar the molecule is, the better is its diffusion. Therefore, the polarity of molecules is an important factor that is able to influence the passage of molecules through yeast membrane and, consequently, the encapsulation achievement.[42]

In comparison to other works performed using yeast cells as carrier materials, Nguyen *et al.* (2018)[21] encapsulated anthocyanins from Hibiscus (*Hibiscus sabdariffa* L.) in yeast cells and obtained an encapsulation efficiency of around 27% under optimized conditions, using a concentration of 100 g L^{-1} of dry yeast for anthocyanin-rich hydroalcoholic extract. Medeiros *et al.* (2018)[43] obtained encapsulation efficiencies of 33.1 and 49.5% for the internalization of curcumin and fisetin into *Saccharomyces cerevisiae* cells, respectively. From these results, it can be inferred that polarity is a crucial factor that may have influence compound entrapment. Although yeasts have an affinity for both hydro and lipophilic compounds, due to the presence of phospholipid polar heads oriented toward the outside and nonpolar heads oriented to the center of the membrane,[44] it seems that it is easier to incorporate hydrophilic compounds because of the more polar surface. This explains the higher encapsulation efficiencies for compounds such as fisetin and other phenolic compounds and the lower encapsulation efficiency for curcumin.

With regard to compound stability, in general, there was a decline in the content of both phenolic compounds and anthocyanins between time 0 and 90 days, as expected. At all times, there was a significant difference among the three treatments, where GP5 and J5 presented the highest content of phenolics at all points analyzed. The retention of phenolics after 90 days of storage was higher with larger amounts of yeast in the medium for samples prepared with grape pomace extract, with retentions of 73.8% for GP5 and 92.9% for GP15. This trend would allow us to infer that the yeast in greater quantity in the sample would protect the phenolics; however, it was not so clear for the extract of jabuticaba as the treatment with 10% had higher retention (95.5%) of phenolic compounds in comparison to GP5 and GP15. For anthocyanins, GP10 also showed an irregular behavior, presenting lower retention. Anthocyanin retentions for J5, J10 and J15 were not significantly different.

3.2 Changes in powder color parameters after storage

The parameters of color obtained for powders produced by encapsulation of extracts from grape pomace and jabuticaba extracts in *Saccharomyces cerevisiae* are shown in Table 2. For samples obtained by the encapsulation of GPE and JE, luminosity was lower in the powders with the addition of 5% of yeast biomass. The addition of lower amount of yeast and a higher proportion of extract in the mixture might have led to the greater dispersion of pigments and, in consequence, the improved darker color. In addition, it is possible to notice an increase in the luminosity with the increase in the material wall content used. Chroma values were inversely proportional to L values and confirmed that the GP5 and J5 powders present more intense colors.

The GP5 and J5 powders presented higher intensity of the a^* parameter indicating a greater intensity of red color. For the b^* parameter, while the GP5 powder presented the lowest value of −4.1, J5 presented the highest value of 4.9 compared to the other treatments. These values are related to the blue color, which can be explained by the presence of different anthocyanins in the extracts. Hue angles around 0° over the storage period indicated that a red color was established, typically found in the anthocyanin extracts of red berries in non-basic media.[32]

After 90 days, GP10, GP15, J10 and J15 exhibited significant loss of luminosity bringing a change in the color profile. GP5 had a slight darkening after 90 days (decrease in lightness and chroma), probably because of compound oxidation; however, this change was lower compared to the other treatments with GPE. J5 did not present any difference in the parameter L^* after storage and this result may be interesting from the point of view of the application, since it is interesting to apply darker pigments that maintain their intensity all over the storage. In general, for all particles, there was a decrease in the parameter a^* and an increase in b^*, indicating the degradation of anthocyanins and phenolic compounds over time.

3.3 Particle morphological analysis

For this analysis, GP5 and J5 were selected because of their highest content of entrapped phenolic compounds and anthocyanins. Confocal microscopy images in Fig. 3 show the cellular morphology before (a) and after enrichment with active compounds (b and d). Enriched cells present slight changes in their outer surface, such as shrinkages and concavities, which are expected as a consequence of water evaporation through the spray-drying process. No rupture was observed in cells and the membrane seems to be intact even after the drying process. The use of confocal microscopy is very interesting due to the possibility of observing the intra-cellular area. According to Pham-Hoang *et al.* (2018),[33] calcofluor is a cell-wall specific fluorochrome dye used to mark yeast cells (it binds to β-1,6-glucans and chitin in cell walls) and Nile red has an affinity for liposoluble structures, especially intracellular lipids.

Red bodies observed in both control and enriched cells were stained with Nile red. In the particles without encapsula-

Table 2 Color parameters L^*, a^*, b^*, hue angle (h^*) and chroma (C^*) for powders produced by the encapsulation of extracts from grape pomace and jabuticaba byproducts in yeast *Saccharomyces cerevisiae*

	Day 0					Day 90				
Sample	L^*	a^*	b^*	h^*	C^*	L^*	a^*	b^*	h^*	C^*
GP5	$42.5^{Ca} \pm 0.04$	$15.1^{Aa} \pm 0.1$	$-4.1^{Cb} \pm 0.04$	$-15.3^{Cb} \pm 0.1$	$15.6^{Aa} \pm 0.1$	$41.8^{Bb} \pm 0.2$	$14.0^{Ab} \pm 0.2$	$-2.8^{Ca} \pm 0.1$	$-11.2^{Ca} \pm 0.6$	$14.2^{Ab} \pm 0.2$
GP10	$56.2^{Ba} \pm 0.006$	$7.0^{Bb} \pm 0.1$	$-0.5^{Bb} \pm 0.06$	$-4.9^{Bb} \pm 0.5$	$7.0^{Bb} \pm 0.06$	$53.6^{Ab} \pm 1.2$	$7.1^{Ba} \pm 0.07$	$0.4^{Ba} \pm 0.08$	$3.6^{Ba} \pm 0.6$	$7.2^{Ba} \pm 0.07$
GP15	$59.0^{Aa} \pm 0.03$	$7.0^{Ba} \pm 0.02$	$-0.2^{Ab} \pm 0.05$	$-1.6^{Ab} \pm 0.4$	$7.0^{Ba} \pm 0.02$	$53.2^{Ab} \pm 0.1$	$6.9^{Cb} \pm 0.04$	$1.1^{Aa} \pm 0.04$	$10.3^{Aa} \pm 0.4$	$6.3^{Bb} \pm 0.04$
J5	$31.0^{Ca} \pm 0.08$	$25.3^{Aa} \pm 0.06$	$4.9^{Aa} \pm 0.08$	$11^{Aa} \pm 0.2$	$25.8^{Aa} \pm 0.05$	$31.0^{Ca} \pm 0.9$	$22.8^{Ab} \pm 0.4$	$4.4^{Ab} \pm 0.1$	$10.9^{Aa} \pm 0.08$	$23.3^{Ab} \pm 0.4$
J10	$41.8^{Ba} \pm 0.05$	$23.2^{Ba} \pm 0.06$	$3.1^{Ba} \pm 0.04$	$7.6^{Bb} \pm 0.09$	$23.4^{Ba} \pm 0.06$	$37.5^{Bb} \pm 6.2$	$21.8^{Ba} \pm 0.6$	$3.5^{Ba} \pm 0.7$	$8.4^{Ca} \pm 0.04$	$22.1^{Ab} \pm 0.7$
J15	$50.5^{Aa} \pm 0.01$	$19.0^{Ca} \pm 0.02$	$2.5^{Ca} \pm 0.02$	$7.4^{Bb} \pm 0.06$	$19.2^{Ca} \pm 0.02$	$48.4^{Ab} \pm 0.9$	$17.3^{Cb} \pm 0.1$	$2.8^{Ca} \pm 0.08$	$9.1^{Ba} \pm 0.2$	$17.5^{Ab} \pm 0.1$

Where GP5, GP10 and GP15 are the powders obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeasts, respectively. J5, J10 and J15 are the powders obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5, 10 and 15% of yeast, respectively. Capital letters represent the comparison between different treatments in the same time, while small letters compare different times for the same treatment. Mean values followed by the same superscripts are not significantly different ($p > 0.05$).

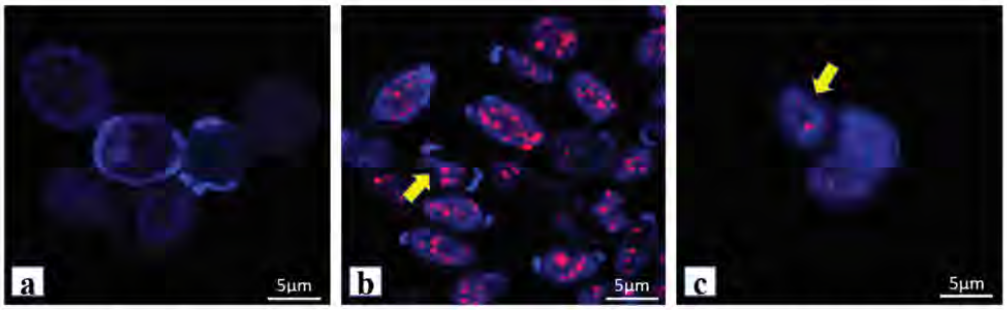

Fig. 3 Confocal scanning microscopy images of control yeasts (without encapsulation of bioactive compounds and spray-drying) (a), particles obtained by the encapsulation of extracts from grape pomace in *Saccharomyces cerevisiae* using 5% of yeast (b) and particles obtained by the encapsulation of extracts from jabuticaba byproducts in *Saccharomyces cerevisiae* using 5% of yeast (c). Yellow arrows indicate shrinkages and concavities in cell surface.

tion of extracts (Fig. 3a), this fluorochrome stained mainly the organelles of the yeast cell. However, for the particles obtained by the encapsulation of extracts from grape pomace and jabuticaba byproducts (Fig. 3b and c, respectively), the red color was more intense, even upon using the same laser potency and stain methodology. Therefore, Nile red probably stained active compounds bound to the organelles as well as the lipid bodies within yeast cells.[42] Furthermore, grape pomace extract loaded particles presented a higher color intensity, which could be attributed to a higher quantity of lower water-solubility compounds in the residue.

Under calcofluor excitation, control cells are stained mainly in their outer surface, whereas after enrichment with compounds, yeasts show a more pronounced intensity in their interior part, especially those enriched with extracts from jabuticaba byproducts. These observations indicate that there could be a homogeneous intra-cellular distribution of the active compounds. The higher intensity for J5 (Fig. 3c) might probably be related to the higher content of phenolic compounds in the jabuticaba extract encapsulated. With cell enrichment and the consequent fluorescence spread all over the cell structure, it can be assumed that compounds have perturbed significantly the cell organization and it is indicative of phenolic compounds and anthocyanins incorporation inside the yeasts.

3.4 Yogurt characterization

GP5 and J5 obtained with 5% of yeast *Saccharomyces cerevisiae* and concentrated extracts from grape pomace and jabuticaba byproducts were the powders chosen for application in yogurts due to their highest encapsulation efficiency, content of phenolic compounds and anthocyanins and the maintenance of luminosity during storage.

Table 3 shows the results for pH, titratable acidity and parameters of color for the yogurts produced with the chosen particles. The pH values varied between 3.9 and 4.1, while the titratable acidity values were within the range of 0.9 and 1.1. Yogurts with different proportions of J5 presented lower pH values may be because of the extract used for encapsulation, which was acidified with citric acid. Jaster *et al.* (2018)[45] recommend acidity of milk products between 0.6 and 1.5% and Benedetti *et al.* (2016)[46] reported that consumers prefer fermented products with pH in the range of 4.2 and 4.4.

Table 3 Values of pH, titratable acidity and parameters L^*, a^* and b^* of the color of the yogurts

Sample	pH	Titratable acidity	L^*	a^*	b^*
YGP1	4.1	0.8	54.9 ± 0.01	5.2 ± 0.1	−2.9 ± 0.05
YGP15	4.1	0.9	54.3 ± 0.01	6.2 ± 0.06	−3.8 ± 0.03
YJ1	4.0	1.0	60.3 ± 0.02	1.9 ± 0.05	6.5 ± 0.07
YJ15	4.0	1.1	62.0 ± 0.03	3.3 ± 0.03	6.4 ± 0.04

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively.

Demirkol & Tarakci (2018)[17] produced enriched yogurts by applying grape pomace powders obtained from freeze drying or drying with forced air circulation at different temperatures and obtained pH and titratable acidity results from 3.99 to 4.25 and 0.74 to 0.93%, respectively. Karaaslan *et al.* (2011)[47] enriched natural yogurts with extracts rich in phenolic compounds and anthocyanins from different species of grapes and obtained a range of pH and titratable acidity from 4.224 to 4.279 and 0.95 to 0.98%, respectively. Thus, the results obtained in the current work are in accordance with previous studies reported in the literature.

Regarding yogurt color, luminosity decreased with the addition of a higher percentage of particles and the intensities of red and blue were higher, as expected, as an obvious response to the enrichment with more particles.

3.5 Phenolic compounds and anthocyanins stability in yogurts

Based on Table 4, there was a significant difference in the content of phenolic compounds and anthocyanins in yogurt over time. Upon comparing samples with the same particles, but in different proportions, there was a significant difference between them in almost all points studied, with higher content of compounds for treatments using 1.5% of particles. This was expected as the proportion of pigment was higher and, consequently, the phenolic compound and anthocyanin contents were higher as well. Phenolic compounds in the sample YJ1 and anthocyanins in the samples YGP15, YJ1 and YJ15 presented significant differences among day 0 and day 7; the compound content was stable during the other days of storage. The same trend was observed by Wallace & Giusti (2008)[48] upon incorporating *Berberis boliviana* whole berry powder in yogurt. Anthocyanins can form complexes with the macromolecules of milk, protecting them from degradation. However, this interaction can make the compound less bioavailable, in addition to being able to precipitate.[49] This fact may explain the significant decline in anthocyanin levels after yogurt storage.

Regarding the determination of half-life times, phenolic half-lives range from 98 to 194 days, while for anthocyanins, the variation was higher, between 34 and 102 days. Considering that the shelf life of yogurt is between 25 and 35 days,[48] the natural pigments evidenced in this work might be available during the yogurt shelf life and, in addition to the coloring function, the compounds may offer a potential for health benefit.

Although there was a decrease in the phenolic compound and anthocyanin contents over the storage period, it is probable that yeasts are not releasing entrapped compounds and those which are retained in the yeast surface are spread in yogurt and, as a consequence, they have less protection and a greater tendency to degradation. To confirm whether *Saccharomyces cerevisiae* is able to protect entrapped compounds in a medium with proteins, fat and sugar, further studies are necessary to evaluate compound release in yogurt and their bioaccessibility after digestion simulations.

3.6 Rheological characterization of yogurts

The flow behavior of yogurts colored with different proportions of GP5 and J5 was properly explained by the Herschel–Bulkley model, with determination coefficients higher than 0.997 and χ^2 lower than 0.09. Table 5 lists the parameters obtained by the model. The flow index (n_H) was lower than 1 ($n < 1$), showing a shear thinning behavior for all yogurts and confirming that

Table 4 Phenolic compound and anthocyanin stability in yogurts enriched with microcapsules produced by the encapsulation of extracts from grape pomace and jabuticaba byproducts in yeast *Saccharomyces cerevisiae* and half-life times

Sample	Day 0	Day 7	Day 15	Day 30	$T_{1/2}$ (days)
	Phenolic compounds (mg EAG per g of particle)				
YGP1	$25.5^{Ba} \pm 0.1$	$24.7^{Ba} \pm 0.8$	$22.1^{Bb} \pm 0.6$	$20.7^{Bc} \pm 0.5$	98.8
YGP15	$33.0^{Aa} \pm 1.8$	$33.1^{Aa} \pm 0.4$	$29.2^{Ab} \pm 1.4$	$27.1^{Ac} \pm 0.4$	105.0
YJ1	$25.6^{Ba} \pm 0.3$	$24.2^{Bb} \pm 0.1$	$24.0^{Bbc} \pm 0.9$	$23.0^{Bc} \pm 0.8$	193.5
YJ15	$33.1^{Aa} \pm 0.3$	$31.4^{Ab} \pm 1.5$	$29.0^{Ac} \pm 0.3$	$27.5^{Ad} \pm 0.9$	112.3
	Anthocyanins (mg per g of particle)				
YGP1	$3.5^{Ba} \pm 0.1$	$3.1^{Bb} \pm 0.8$	$3.0^{Bb} \pm 0.6$	$2.3^{Bc} \pm 0.5$	48.5
YGP15	$5.3^{Aa} \pm 1.8$	$5.0^{Aab} \pm 0.4$	$4.8^{Aab} \pm 1.4$	$5.6^{Ab} \pm 0.4$	101.1
YJ1	$0.7^{Ba} \pm 0.3$	$0.6^{Bab} \pm 0.1$	$0.6^{Aab} \pm 0.9$	$0.4^{Ab} \pm 0.8$	34.7
YJ15	$1.1^{Aa} \pm 0.3$	$0.9^{Aab} \pm 1.5$	$0.8^{Aab} \pm 0.3$	$0.6^{Ab} \pm 0.9$	39.5

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively. Capital letters in the same column and small letters in the same row indicate there is no significant difference ($p > 0.05$) among samples, considering the same raw material.

Table 5 Rheological parameters for data fit by the Herschel–Bulkley model and the storage modulus (G'), loss modulus (G'') and complex modulus (G^*) at a frequency of 1 Hz

Sample	τ_0 (Pa)	k_H (Pa s^n)	n_H (—)	χ^2	R^2	G' (Pa)	G'' (Pa)	G^* (Pa)
YGP1	0.2^A	4.4^A	0.3^A	0.07	0.997	81.9^A	25.2^A	85.9^A
YGP15	0.6^A	5.2^A	0.3^A	0.09	0.997	64.9^B	19.8^A	67.9^B
YJ1	0.7^A	4.4^A	0.3^A	0.08	0.997	81.2^A	23.7^A	84.6^A
YJ15	0.3^A	4.7^A	0.3^A	0.06	0.998	63.9^B	19.9^A	66.7^B

Where τ_0 is the yield stress (Pa), k_H is the consistency index (Pa s^n), n_H is the flow behavior index (dimensionless), χ^2 is the chi-square value and R^2 is the determination coefficient. YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from the jabuticaba byproducts and 5% of yeast, respectively. Mean values in the same row followed by the same superscripts are not significantly different ($p > 0.05$)

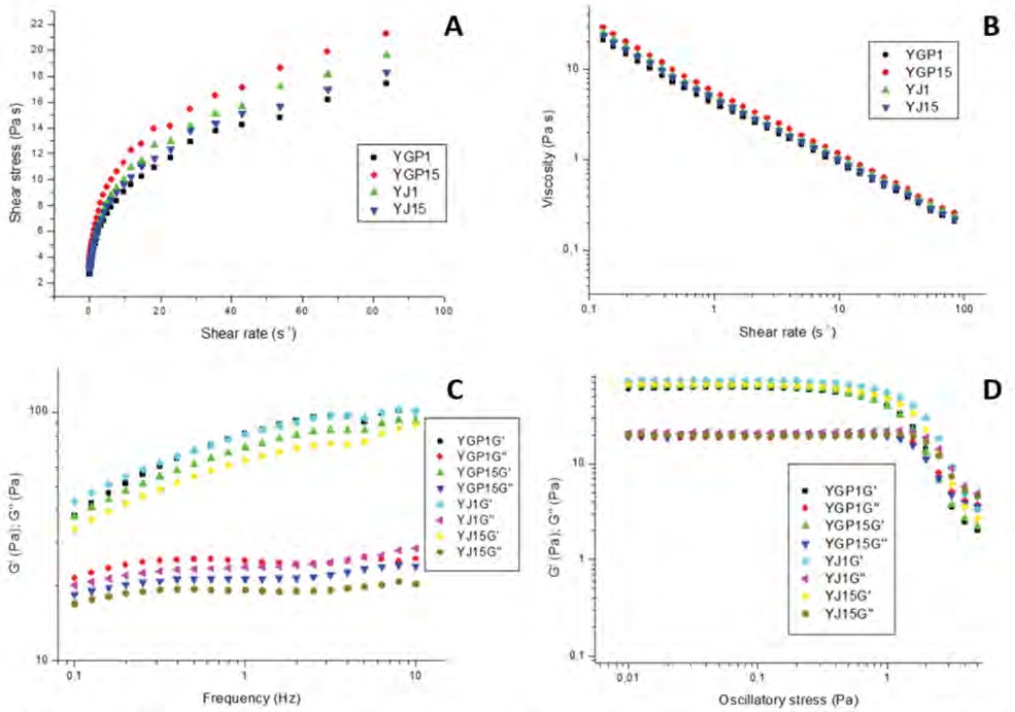

Fig. 4 Steady-state flow curves (A), viscosity as a function of the shear rate (B), and evolution of storage modulus (G') and loss modulus (G'') during frequency sweep tests (C) and oscillatory stress at 1 Hz (D).

the samples are non-Newtonian materials (Fig. 4A). The pseudoplastic behavior can also be visualized as shown in Fig. 4B, once the viscosity decreases with the increasing shear rate. When shear forces are applied in a fluid, weak bonds are destroyed and there is a reduction in the hydrophobic interaction between molecules and in the electrostatic repulsion. The disruption is greater at the beginning of shearing and, after that, there is an alignment of the particles with the flow which reduces the viscosity.[45] In addition, based on Fig. 4A, the shear stress curve does not begin at the origin of the plot and it is concave downwards. The existence of yield stress in the flow curve of a material indicates that there is a cross-linked or another interactive structure that must be broken before the flow can occur at an appropriate rate.[50]

The storage modulus was higher than the loss modulus ($G' > G''$) in all the cases (Fig. 4C and Table 5), which allowed characterizing the behavior of all samples of yogurts as predominantly elastic. In addition, all yogurts could be considered weak gels ($G'/G'' < 10$),[51] regardless of the addition of particles. The addition of a higher proportion of microparticles resulted in lower complex modulus (G^*) values at a frequency of 1 Hz, suggesting decreased resistance to deformation.[52] A possible explanation is that the added microparticles are somehow interacting with the milk proteins and thus reduce the aggregation of the casein network and the resistance of yogurt to flow.[45] As shown in Fig. 4D, at lower values of oscillatory stress, the storage modulus was also higher than the loss modulus, assuming the gel-like nature of all yogurts. Above the cross point (oscillatory stress at which G' and G'' assume the same value), the loss modulus is slightly higher than the storage modulus, which is a characteristic of a liquid. This implies the sol–gel transition of the material.[53]

3.7 Sensory analysis – acceptance test

A consumer acceptance test was applied to evaluate whether the addition of particles had a positive influence on the sensory parameters of yogurts, mainly the color. According to Table 6, in general, the difference was not significant between the samples in terms of appearance, color, aroma and overall average. Yogurts produced with 1.5% of grape pomace particles had lower values of flavor and overall acceptability, while

Table 6 Results of yogurt sensory evaluation and acceptance index

Parameters	YGP1	YGP15	YJ1	YJ15
Appearance	$7.5^{a} \pm 1.3$	$7.5^{a} \pm 1.4$	$6.1^{a} \pm 1.8$	$6.3^{a} \pm 1.6$
Color	$7.7^{a} \pm 1.3$	$7.8^{a} \pm 1.3$	$5.9^{a} \pm 1.8$	$6.3^{a} \pm 1.7$
Aroma	$7.5^{a} \pm 1.3$	$7.4^{a} \pm 1.4$	$7.0^{a} \pm 1.6$	$7.2^{a} \pm 1.6$
Flavor	$6.7^{a} \pm 1.9$	$6.1^{b} \pm 2.0$	$6.8^{a} \pm 2.0$	$6.5^{a} \pm 2.1$
Texture	$7.4^{a} \pm 1.5$	$7.2^{a} \pm 1.5$	$7.5^{a} \pm 1.3$	$7.0^{b} \pm 1.7$
Overall acceptability	$7.2^{a} \pm 1.5$	$6.7^{b} \pm 1.6$	$6.8^{a} \pm 1.6$	$6.5^{a} \pm 1.7$
Overall average	$7.3^{a} \pm 1.1$	$7.1^{a} \pm 1.2$	$6.7^{a} \pm 1.3$	$6.7^{a} \pm 1.4$
Acceptance index (%)	81.4	79.1	74	73.9

Where YGP1 and YGP15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from grape pomace and 5% of yeast, respectively; YJ1 and YJ15 are the yogurts with 1% and 1.5% of microcapsules obtained with extracts from jabuticaba byproducts and 5% of yeast, respectively. Mean values in the same row followed by the same superscripts are not significantly different ($p > 0.05$).

the panelists evaluated yogurts produced with 1.5% of jabuticaba byproducts with lower scores for texture. This result might be interestingly correlated to the rheological measurements that showed yogurts had a higher tendency to flow with the increasing proportion of the added particles. The acceptance index (AI) varied within the range of 73 and 82%. According to Dutcosky (2015),[54] the samples are considered well accepted when they have an AI (%) greater than 70%; thus, when observing this criterion, it is noted that all the samples of yogurt produced were well accepted.

According to the free comments from the panelists, although the average of scores did not differ significantly, most of the panelists found the colors of the yogurts quite attractive, highlighting the 1.5% YGP and 1.5% YJ treatments, a fact that coincides with the instrumental color analysis previously discussed as these treatments presented higher values of the color parameter chroma. In addition, few panelists commented on a slightly bitter taste they felt in the formulations. The more probable explanation for this result is that, once the yeast was used as a carrier material, its taste was not fully masked, despite the presence of extracts and flavorings. Other panelists pointed out the presence of some agglomerated powder in the product. This fact can be explained by the possible incomplete disintegration of the particle agglomerates when incorporating them into yogurt samples and also by yeast sedimentation, as already expected, as it is not a soluble material. Therefore, it is recommended to shake the yogurt before consumption. To overcome the sedimentation, a more viscous or creamy yogurt could have been used. However, although it was possible to see sedimented yeasts at the bottom of the yogurt bottles, yeast is not expected to be sensorially sensed, and even when yeasts are agglomerated, they do not confer a grainy texture to the product.

In addition, based on the sensory evaluation, 70 panelists said they would buy YGP1 yogurt and 45 panelists would buy YGP15 yogurt. Regarding the yogurts enriched with powders from the byproducts of jabuticaba, 69 panelists said they would buy YJ1 yogurt and 49 panelists would buy YJ15 yogurt.

4. Conclusions

In this work, waste yeast biomass was used as a wall material for encapsulation of natural pigments. Yeasts are much more complex than other materials used for encapsulation because of their biological nature. Thus, it is important to understand how entrapped compounds behave after their incorporation and what are the effects of enriched yeast application in a food matrix.

The results showed that the pigments produced with a lower proportion of yeast (5%) had more intense colors, the highest encapsulation efficiencies for both phenolic compounds and anthocyanins, the higher compound content over the storage period and luminosity maintained after 90 days. Thus, these pigments were chosen for application in yogurt. All yogurt samples were well accepted by the panelists, mainly the formulations prepared using 1% of pigments produced with grape pomace and jabuticaba byproduct extracts, with acceptance indexes of 81.4 and 74%, respectively.

From the point of view of the production cost, it is really interesting to use lower amounts of raw material (yeasts) for obtaining pigments and lower proportions of pigments for coloring a food matrix. In addition to the reuse of bioproducts from food industries as sources of bioactive compounds, this research is responsible for pointing out a novel application for waste yeast *Saccharomyces cerevisiae* as a carrier material and pigments for the production of novel functional products.

Conflicts of interest

There are no conflicts to declare.

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