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Tese de Doutorado

New insights about the beneficial properties of yerba mate for human health

Andreia Candal de Vasconcellos

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ANDREIA CANDAL DE VASCONCELLOS

TESE DE DOUTORADO

New insights about the beneficial properties of yerba mate for human health

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New insights about the beneficial properties of yerba mate for human health

Estudante de Pós-Graduação

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Dedicatória

Dedico este Título de Doutora ao meu Pai Verlaine, que se foi há exatos 2 anos (†17/03/2021), e que sempre foi meu melhor amigo, maior exemplo e incentivador. Tenho certeza que ele está orgulhoso e me dando forças lá de cima no dia de hoje. Obrigada por tudo, Pai. Eu nunca desisti por ti.

Abstract

Phenolic compounds have been extensively researched by hypothesis of their numerous health properties, the majority of which are related to their antioxidant capacity. Yerba Mate (YM), or *Ilex Paraguariensis*, is a South American native plant that seems to have antioxidant ability and stimulant properties, particularly due to the presence of bioactive components such as phenolic compounds (PCs) and methylxanthines. Several studies, on the other hand, reveal that the bioavailability and absorption of PCs from food products by the human body is very low, and that a variety of factors may influence their metabolism, many of which are currently little understood or unknown to the scientific community. The present work aims to study different methods of extraction of bioactive compounds from YM, in addition to identifying and quantifying PCs found in the extracts, in order to propose solutions to increase the amounts of extracted compounds and their bioavailability, optimizing their antioxidant capacity. When studying different extraction methods of bioactive compounds of YM, the optimum extraction conditions for phenolic and chlorophyll (Chl) components were in water at 80 ^{o}C and time of 60 minutes, obtaining values of 48.3 mg GAE g^{-1} and 2.0 g chlorophyll g^{-1}, respectively. It is noteworthy that all treatments were able to extract more *Chl b* than *Chl a*. In terms of antioxidant activity measured by DPPH and ABTS, the methanol solution extraction separated a greater number of compounds with higher activity; however, it was unable to separate compounds that protected deoxyribose against hydroxyl radicals; in this case, water extractions yielded higher values for HRSA. Our study demonstrates the separation of chemicals with beneficial characteristics at the temperature at which mate is typically consumed. Microwave Assisted Extraction (MAE) provided good conditions in terms of time and temperature for separation of phenolic and *Chls* compounds, but additional research is needed to determine the best separation parameters. In the HPLC analysis, a significant variety of PCs (29 total) were obtained as a result of each extraction method and around 16 to 18 compounds for extraction, mainly composed of caffeoylquinic acid and its dimers and a multitude of different hydroxycinnamic acids, such as ferulic acid (FA), p-coumaric acid (pCA) and caffeic acid (CA) and its derivatives. Caffeic acid, for example, was detected in all extracts. Flavonols, on the other hand, were obtained in the form of rutin, kaempferol, quercetin and also as combinations with carbohydrates such as Kaempferol rutinoside (329 - 340 nm, 29.2 - 29.4 min). When we analyze the total amounts of compounds found in the different extraction methods we can verify that

Aqueous Extraction (AE) 15 minutes and AE 60 minutes had no significant difference between the contents, with total amounts of 7020 mg L^{-1} (14,04 mg g^{-1}) and 6756 mg L^{-1} (13,51mg g^{-1}), respectively. AE 30 minutes showed a significant difference with a total amount of 12275 mg L^{-1} (24,55 mg g^{-1}). MAE 700 W and 1000 W showed total amounts of 5811 mg L^{-1} (11,62 mg g^{-1}) and 6793 mg L^{-1} (13,58 mg g-1) and Methanol Solvent Extraction (MSE) presented a significant different total amount of 25739 mg L^{-1} (51,47 mg $^{g-1}$), even though those values may not be replicable since consumers make their own extractions with water and never with methanol. Caffeoylquinic acids were the major constituents of the phenolic fraction of YM, representing more than 50% of all phenolics. In particular, 2-caffeoylquinic acid was the major compound in all extracts, followed by caffeoylquinic acid dimer and 4-caffeoylquinic acids.

Resumo

Os compostos fenólicos têm sido extensivamente pesquisados pela hipótese de suas inúmeras propriedades para a saúde, a maioria das quais relacionadas à sua capacidade antioxidante. A Erva Mate (EM), ou *Ilex Paraguariensis*, é uma planta nativa da América do Sul que parece ter capacidade antioxidante e propriedades estimulantes, principalmente devido à presença de componentes bioativos como compostos fenólicos (CFs) e metilxantinas. Vários estudos, por outro lado, revelam que a biodisponibilidade e a absorção de CFs de produtos alimentícios pelo corpo humano é muito baixa, e que uma variedade de fatores pode influenciar seu metabolismo, muitos dos quais são atualmente pouco compreendidos ou desconhecidos do comunidade científica. O presente trabalho visa estudar diferentes métodos de extração de compostos bioativos da EM, além de identificar e quantificar CFs encontrados nos extratos, a fim de propor soluções para aumentar as quantidades de compostos extraídos e sua biodisponibilidade, otimizando sua capacidade antioxidante. Ao estudar diferentes métodos de extração de compostos bioativos da EM, as condições ótimas de extração dos CFs e clorofilados foram em água a 80 ^{o}C e tempo de 60 minutos, obtendo valores de 48,3 mg GAE g^{-1} e 2,0 g clorofila g^{-1}, respectivamente. Vale ressaltar que todos os tratamentos foram capazes de extrair mais *Chl b* do que *Chl a*. Em termos de atividade antioxidante medida por DPPH e ABTS, a extração com solução de metanol separou um maior número de compostos com maior atividade; entretanto, não foi capaz de separar os compostos que protegiam a desoxirribose contra os radicais hidroxila; neste caso, as extrações com água forneceram

valores mais elevados para HRSA. Nosso estudo demonstra a separação de produtos químicos com características benéficas na temperatura em que o mate é normalmente consumido. A Extração Assistida por Micro-ondas (EAM) forneceu boas condições em termos de tempo e temperatura para separação de CFs e Chls, mas pesquisas adicionais são necessárias para determinar os melhores parâmetros de separação. Na análise de HPLC, uma variedade significativa de CFs (29 no total) foi obtida como resultado de cada método de extração e cerca de 16 a 18 compostos por extração, principalmente compostos de ácido cafeoilquínico e seus dímeros e uma infinidade de diferentes ácidos hidroxicinâmicos, como ácido ferúlico (AF), ácido p-cumárico (pCA) e ácido cafeico (CA) e seus derivados. O ácido cafeico, por exemplo, foi detectado em todos os extratos. Os flavonóis, por outro lado, foram obtidos na forma de rutina, kaempferol, quercetina e também como combinações com carboidratos como o Kaempferol rutinosídeo (329 - 340 nm, 29,2 - 29,4 min). Quando analisamos as quantidades totais de compostos encontrados nos diferentes métodos de extração podemos verificar que a Extração aquosa (EA) 15 minutos e EA 60 miutos não tiveram diferença significativa entre os teores, com quantidades totais de 7020 mg L^{-1} (14,04 mg g^{-1}) e 6756 mg L^{-1} (13,51mg g^{-1}), respectivamente. EA 30 minutos apresentou diferença significativa com uma quantidade total de 12275 mg L^{-1} (24,55 mg g^{-1}). EAM 700 W e 1000 W apresentaram quantidades totais de 5811 mg L^{-1} (11,62 mg g^{-1}) e 6793 mg L^{-1} (13,58 mg g^{-1}) e Extração por Solvente Metanol (ESM) apresentou uma quantidade total significativamente diferente de 25739 mg L^{-1} (51,47 mg g^{-1}), embora esses valores possam não ser replicáveis, pois os consumidores fazem suas próprias extrações com água e nunca com metanol. Os ácidos cafeoilquínicos foram os principais constituintes da fração fenólica do YM, representando mais de 50% de todos os fenólicos. Em particular, o ácido 2-cafeoilquínico foi o composto majoritário em todos os extratos, seguido pelo dímero do ácido cafeoilquínico e pelos ácidos 4-cafeoilquínicos.

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Sumário

1. Presentation of the Thesis

This Thesis Defense presentation will proceed through **5 chapters**. Each chapter presents a manuscript that was elaborated and written during the Doctorate Program.

The first two chapters include the demonstration of two review studies on Yerba Mate (YM), which will cover its bioactive compounds and antioxidant properties mainly associated with the phenolic fraction, as well as the relationship with the gut microbiota and Alzheimer Disease. The first study was recently published in "Plant Foods for Human Nutrition" Journal.

Subsequently, the **third chapter** will present a manuscript showing the identification and quantification of the phenolic compounds profiles of the three different extraction methods (Aqueous Extraction, Microwave-Assisted Extraction and Methanol Solvent Extraction) by high-performance liquid chromatography with electrospray ionization and mass spectrometry (LC-DAD-ESI-MS/MS), where a fifth manuscript has been developed.

The fourth chapter presents a manuscript showing analysis performed on different methods of extraction of phenolic compounds (Aqueous Extraction, Microwave-Assisted Extraction and Methanol Solvent Extraction), and compares each extraction method through the maintenance of YM proprieties, such as color, Total Phenolic Compounds (TPC), and antioxidant capacity (ABTS, DPPH and HRSA).

The **fifth chapter** brings in annex analysis performed in order to initialize the encapsulation of the extracts, testing dispersions with three encapsulating agents: partially hydrolyzed guar gum (GG), gum Arabic (GA) and polydextrose (PD). In the first moment, rheological tests were carried out in order to verify the behavior of these materials under the action of forces, such as viscosity, deformation, creep and recovery. In addition, zeta potential tests were performed to verify the dispersion system stability. The zeta potential analysis can be helpful in the determination of the net charge of the complexes present in the dispersions, indicating charge neutralization by electrostatic interactions. Further analysis are to be done.

The formatting of the texts that will follow are in accordance with the Journals to which they will be or have already been submitted. For this reason, templates may vary between texts.

2. Objectives

Main Objective

The present work aimed to analyze the latest studies on Yerba Mate to compose scientific reviews on the plant focusing on its phenolic compounds (PC), as well as to perform analysis to identify and quantify PC and evaluate the antioxidant capacity of yerba mate extracts obtained from different extraction methods, such as Microwave Assisted Extraction (MAE), Aqueous Extraction (AE) and Methanol Solvent Extraction (MSE).

Specific Objectives

1. Extraction of phenolic compounds from Yerba Mate by Aqueous Extraction (AE), Microwave-Assisted Extraction (MAE) and Methanol Solvent Extraction (MSE);
2. Evaluation of the antioxidant potential of YM extracts through *in vitro* tests - DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS*+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Total Phenolic Compounds (TPC) and Hydroxyl radical (OH•) scavenging capacity (HRSA);
3. Identification and quantification of phenolic compounds from the three extraction methods by LC-DAD-ESI-MS/MS.

3. Literature Review

Introduction

Food's purpose in modern life extends beyond fundamental nutritional requirements, such as relieving hunger and delivering nutrients to people; it is now focused on illness prevention and the enhancement of customers' physical and emotional well-being (Menrad, 2003; Roberfroid, 2000).

A diet rich in plant-based foods has been shown to be beneficial to human health (Slavin, 2012). The addition of functional ingredients to alter flavor, color, texture, or preservation capabilities is a common technique in the food business; moreover, bioactive substances with possible health advantages, such as antioxidants and probiotics, are becoming increasingly popular (Dordevic, 2015). These food items are high in or exclusive sources of a variety of phytochemicals that can aid in the promotion of several health benefits linked to plant foods and dietary patterns (Dauchet, 2009).

Yerba mate extracts (YM) have been proven in several studies to be an ally in preventing the development of diseases as well as defending against free radical reactivity. Several of these pharmacological activities of YM have recently been studied and documented in scientific articles, showing a wide variety of positive health effects, such as anti-obesity, anti-carcinogenic, and anti-inflammatory properties, prevention of pathologies such as cardiovascular disease, diabetes, and Alzheimer's, protection against DNA damage, improvement of glucosemia, promotion of greater bone mineral density and reduction of atherosclerosis (Heck, 2007; Bracesco, 2010; Bastos, 2007).

Researchers (Anesini, 2006; Bracesco, 2011; Heck, 2007; Schinella, 2005) have used chemical models and *in vivo* lipoprotein experiments to investigate the antioxidant effects of YM. They discovered that the phenolics in the extract are primarily responsible for its antioxidant action, once they delocalize electrons and establish intramolecular hydrogen bonds. As a result, chemicals in YM extracts have the power to prevent and treat illnesses by inhibiting chain reactions and repairing damage produced by reactive species (Bastos, 2007).

The addition of functional ingredients to suit flavor, color, texture, or preservation capabilities is common in modern industrial food manufacturing, and the current trend is the incorporation of bioactive substances with possible health advantages, such as antioxidants and probiotics. Bioactive compounds' stability (usually taken from natural

sources) is an important factor in their effective integration into diverse food systems. Vitamins, probiotics, minerals, phenolic compounds, omega-3 fatty acids, and phytosterols, for example, are health-promoting bioactive molecules that are sensitive to oxygen, light, heat, and water. Food items' shelf life and bioavailability are hampered by these circumstances (Champagne, 2007).

Furthermore, there is a financial interest in the production of functional foods that include a range of bioactive components with various, synergistic, and complementary actions that can provide several health advantages, once the stability and bioactivity of a mixture of bioactive components may be superior to a single one (Zhang *et al*., 2019).

Bioactive encapsulation is a new topic that offers a potential approach to the production of functionally active foods. Antioxidants, vitamins, essential oils or tastes, and antimicrobials can all be successfully encapsulated or co-encapsulated in an appropriate wall matrix and included into functional foodstuffs. In addition, co-encapsulation is particularly beneficial since it considers the synergistic impact of several bioactives in enhancing bioactivity in order to obtain specific health advantages. Co-encapsulation of many components is a well-established approach in the pharmaceutical industry, and it has been widely used by certain researchers to produce a novel strategy for curing specific diseases, offering pathogen resistance, or cancer therapy (Gursoy*et al*., 2004; Venturini *et al*., 2015; Doolaanea *et al*., 2016).

Recently, encapsulation has also been used in the food chain in order to protect the survival of probiotic bacteria (Iyer *et al*., 2005; Bakry *et al*., 2016). This is a new field of study that must be researched further in order to develop functional products that benefit numerous bioactive components. Synergistic effects between two or more bioactive chemicals, or between chemicals and prebiotics, resulting in an impact higher than the sum of all the individual components, have long been claimed, particularly in herbal medicine (Long *et al*., 2015).

In functional foods, the concept of synergistic effects has been established as a practical strategy for enhancing good health and preventing disease. The formulation of the bioactive mixture, with the selection of bioactives and their concentration in the mixture, is the key difficulty, as it exhibits a positive relationship with improving overall functioning. The success of the encapsulation is largely determined by the combination of bioactives chosen based on their synergistic effects, the wall materials used, and the microencapsulation method used, which determines the encapsulated sample's stability,

particle size distribution, release rate, system rheology, and functionality (Chawda *et al*., 2017).

On the other hand, the antagonistic effect on the stability of the bioactive components is one of the precautions that must be considered when formulating an encapsulated, and it is also necessary to investigate its impact on the interrelationship between the different molecules, such as solubility and chemical instability (Zhang *et al*., 2019).

Nanotechnology has been widely used to improve the bioavailability of bioactives, such as phenolic compounds, allowing them to reach their target tissues and cells without changing their physical structure, thereby avoiding metabolic biotransformation, which results in poor absorption (Rosas *et al*., 2017). However, because the physicochemical qualities and material composition of the nanoparticles might affect the bioavailability patterns of the pharmaceuticals, more clinical study is required to determine the method's efficiency.

Bioactive Compounds

Bioactive compounds have emerged as a promising therapeutic agent for health, paving the way for the creation of novel supplements and functional foods (Sharma, 2010). The development of new technologies, as well as increasing research efforts to determine the qualities and prospective uses of bioactive compounds, along with public interest and consumer needs, have all contributed to an increase in interest in these substances (Wildman, 2007).

As a result, novel bioactive compounds that can be employed as medications, functional food additives, or nutraceuticals are of interest to both the pharmaceutical and food industries (Sharma, 2010). Phenolic compounds, among all bioactive chemicals, have been intensively explored in recent years due to their bioactivity and influence on human health (Rodriguez-Mateos, 2014).

In studies carried out in models using both animals and humans, the ingestion of foods rich in bioactive compounds has proved *in vivo* and *in vitro* antioxidant activity, as well as choleretic, hepatoprotective, antithrombotic, hypocholesterolemic, diuretic, anti-obesity, anti-rheumatic, anti-aging and anti-inflammatory (Bracesco, 2011; Dugo, 2009).

Both in animal models with physiological levels of isolated phenolic compounds (Del Rio, 2013; Peterson, 2012) as in humans eating foods rich in flavonoids (Hooper, 2012;

Hooper, 2008), foods high in phenolic compounds have been shown to have antioxidant, choleretic, hepatoprotective, antithrombotic, hypocholesterolemic, diuretic, antiobesity, antirheumatic, anti-aging, and anti-inflammatory properties *in vivo* and *in vitro* studies (Bracesco, 2011; Dugo, 2009). It has also demonstrated to influence several intermediate risk factors for cardiovascular disease, such as low-density lipoprotein (LDL), blood pressure (BP), and endothelial function, (Habauzit, 2012; Onakpoya, 2015) and inflamation (Gonzalez, 2011), in a favourable way.

The biggest challenge for science, in this context, is that phenolic compounds are unstable in food preparation and storage under a variety of circumstances, including the presence of oxidative enzymes, high temperatures, pH, humidity, light, and oxygen (Fang & Bhandari, 2011). **Figure 1** shows a summary on factors affecting the bioavailability of phenolics (Garavand *et al*., 2021). Recent techniques, such as encapsulation, are seen as alternatives increase the stability of vulnerable chemicals and protect them from harmful environmental conditions.

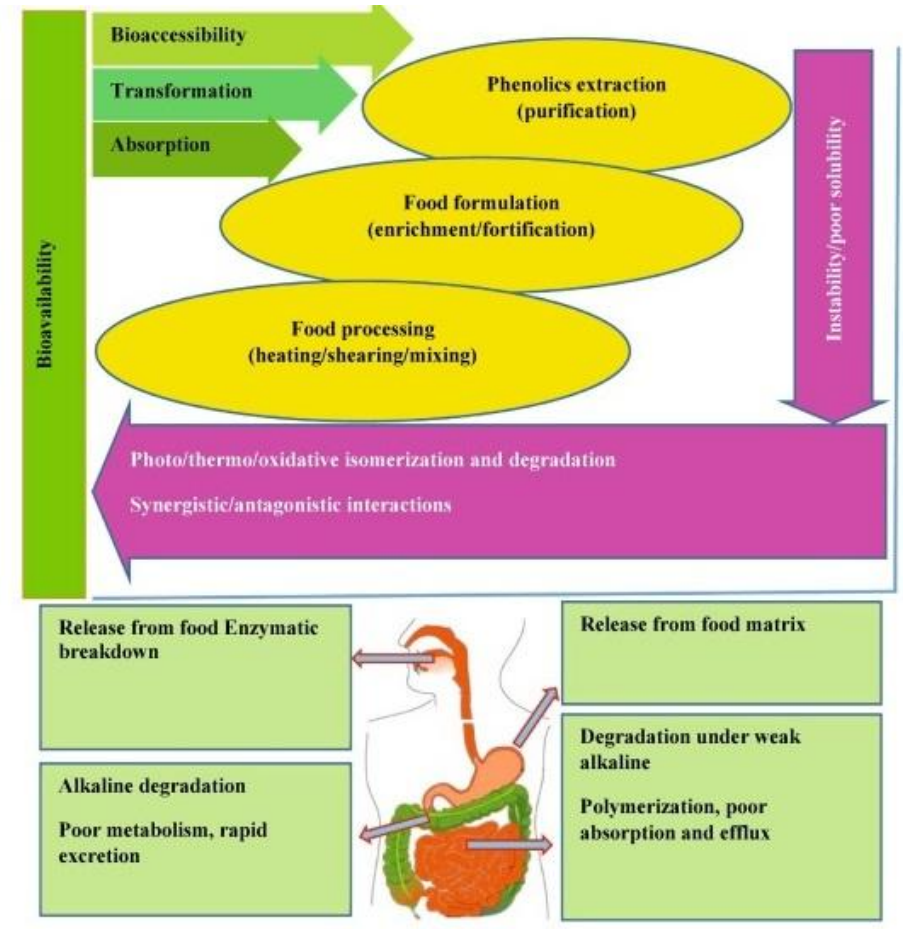


Figure 1: A summary on factors affecting the bioavailability of phenolics (Garavand *et al*., 2021).

Classification of phenols

Phenols are classified into two major groups: flavonoids and non-flavonoids. (Crozier, 2006; Fraga, 2009). The group of flavonoids is branched into several subclasses: flavonols, flavones, flavanones, flavan-3-ols, anthocyanins and isoflavones (**Figure 2**).

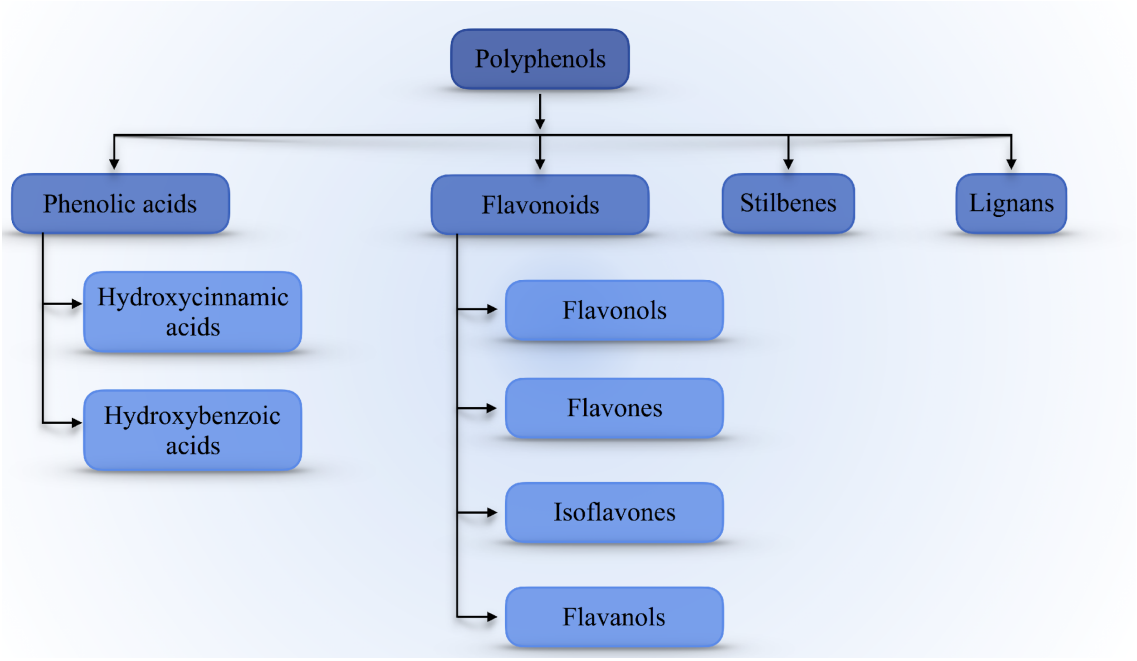


Figure 2: Classification of phenolic compounds (adapted from Crozier, 2006 and Fraga, 2009)

Flavonoids have 15 carbons in their structure, arranged in three rings: C6-C3-C6 (called A, B and C). The two six-carbon rings are aromatic (Fraga, 2009; Pietta, 2000). The flavonoid classes are differentiated from each other due to the level of oxidation and the pattern of C-ring substitution; the individual compounds within each of the classes differ from each other due to the substitution pattern of the A and B rings (Pietta, 2000). **Figure 3** illustrates the basic structure of flavonoids (Huber, 2008).

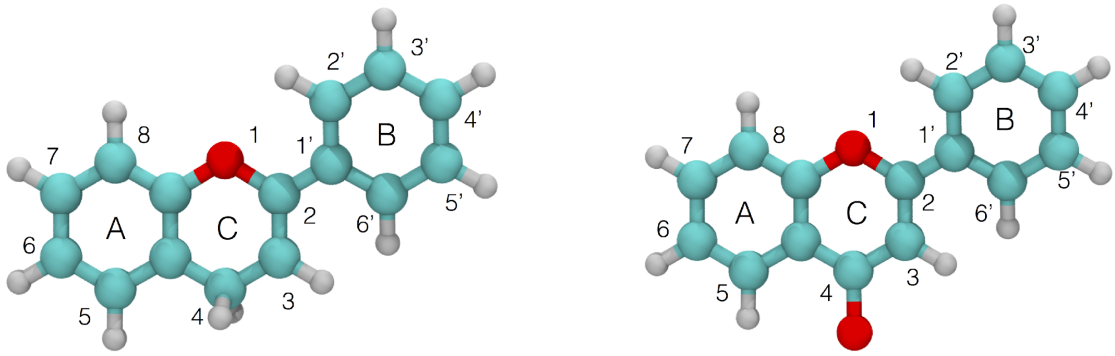


Figure 3: (A) Basic structure of flavonoids and (B) Basic structure of flavonoids with carbonyl group at C-4 (Huber, 2008).

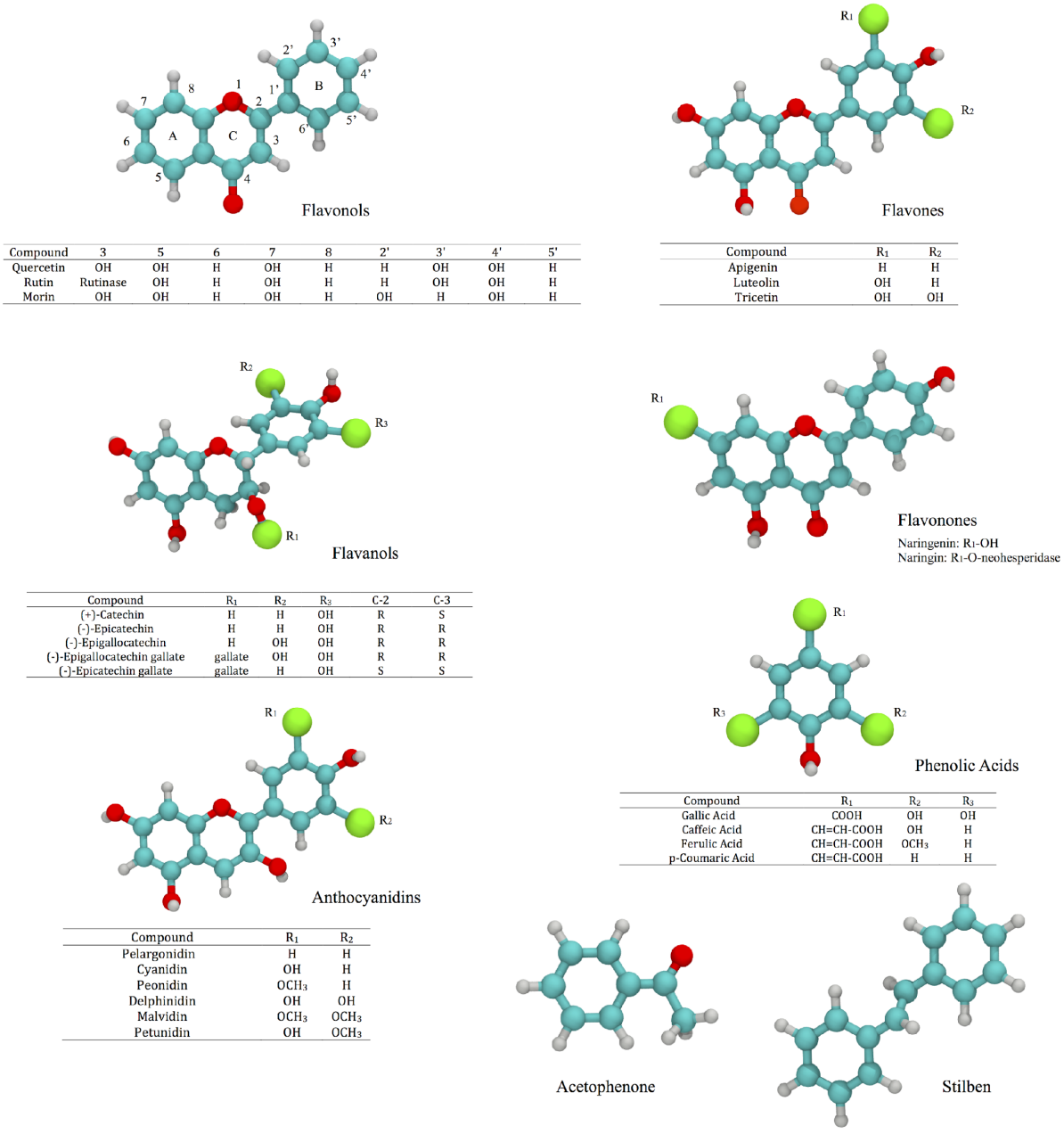
Flavonols are the most widespread flavonoids in foods, commonly found in the glycosylated form, ranging from white to yellow in color, and their main representatives are myricetin, isorhamnetin, kaempferol and quercetin (Crozier, 2006; Fraga, 2009). The later being the most ingested flavonol (Fraga, 2009). In contrast, flavones are not as widespread, and are found in some specific vegetables in their hydroxylated, methylated, alkylated or glycosylated forms. The main representatives are apigenin and luteolin (Crozier, 2006; Fraga, 2009).

Flavan-3-ols are the most complex group of flavonoids, ranging from simple monomers, such as catechin and its isomer epicatechin, to oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins (Crozier, 2006). Most proanthocyanidins in food are procyanidins, which are made up exclusively of (epi)catechin units (Balentine, 1997).

Not so common, proanthocyanidins, containing subunits of (epi)afzelequin and (epi)gallocatechin are called propelargonidines and prodelphinidins, respectively (Balentine, 1997). These compounds affect several food quality parameters, such as astringency, bitterness, acidity, sweetness, viscosity, aroma and color formation (Aron, 2007).

Flavanones are highly reactive and can undergo hydroxylation, glycosylation or methylation reactions (Crozier, 2006; Fraga, 2009). They are found exclusively in citrus fruits in their glycosidic forms, and their main representatives are naringenin, hesperetin, eriodictyol, in addition to other minor compounds (Fraga, 2009).

Isoflavones are found almost exclusively in leguminous plants, especially soybeans, and the presence of genistein, daidzein and glycitein is common (Crozier, 2006; Fraga, 2009). Isoflavones can also undergo modifications such as methylation, hydroxylation or polymerization (Fraga, 2009). **Figure 4** (Demirci *et al*., 2022) presents the chemical structures of main phenolic compounds.



Compound	3	5	6	7	8	2'	3'	4'	5'
Quercetin	OH	OH	H	OH	H	H	OH	OH	H
Rutin	Rutinase	OH	H	OH	H	H	OH	OH	H
Morin	OH	OH	H	OH	H	OH	H	OH	H

Compound	R_1	R_2
Apigenin	H	H
Luteolin	OH	H
Tricetin	OH	OH

Compound	R_1	R_2	R_3	C-2	C-3
(+)-Catechin	H	H	OH	R	S
(-)-Epicatechin	H	H	OH	R	R
(-)-Epigallocatechin	H	OH	OH	R	R
(-)-Epigallocatechin gallate	gallate	OH	OH	R	R
(-)-Epicatechin gallate	gallate	H	OH	S	S

Compound	R_1	R_2	R_3
Gallic Acid	COOH	OH	OH
Caffeic Acid	CH=CH-COOH	OH	H
Ferulic Acid	CH=CH-COOH	OCH_3	H
p-Coumaric Acid	CH=CH-COOH	H	H

Compound	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Petunidin	OH	OCH_3

Figure 4: Chemical structures of main phenolic compounds (adapted from Demirci *et al*., 2022).

The group of non-flavonoid phenols includes phenolic acids (C6-C1) (mainly gallic acid, precursor of tannins), hydroxycinnamates (C6-C3) and stilbenes (C6–C2–C6) (Crozier, 2006; Fraga, 2009). The main representative of phenolic acids is gallic acid, followed by p-hydroxybenzoic, protocatechuic, vanillic, syringic acid, among others (Fraga, 2009). Such acids are the main components responsible for astringency (Clifford, 2000) and are generally present in unbound form, composing complex structures such as lignins and hydrolysable tannins (ellagitannins and gallotannins) (Fraga, 2009).

The hydroxycinnamates, or hydroxycinnamic acids, are formed from cinnamic acid, through the phenylpropanoid pathway, and can therefore be called phenylpropanoids (Crozie, 2006). The most found hydroxycinnamates are p-coumaric, caffeic, ferulic and sinapic acids, which, in fruits and other vegetables, are often esterified to quinic acid, tartaric acid or carbohydrates and derivatives, forming chlorogenic acids (Crozier, 2006; Fraga, 2009; Oliveira, 2011).

Stilbenes are phytoalexins, which are compounds produced by plants in response to attack by fungi, bacteria and viruses, or other types of stress (Crozier, 2006; Fraga, 2009). Finally, resveratrol is the most common stilbene, and occurs as cis and trans isomers, being present in plant tissues mainly as trans-resveratrol-3-O-glycoside (Crozier, 2006).

Antioxidant Activity

The formation of reactive oxygen species (ROS) is a physiological process derived from aerobic metabolism. Still, excessive ROS production can cause a disproportion between the generation of reactive oxygen species and the antioxidant defense system, leading to a condition known as oxidative stress (Daiber *et al*., 2010; Halliwell *et al*., 2011).

In this context, when the antioxidant system is overloaded by excessive ROS formation, a series of events can occur leading to cell death or irreversible DNA damage, a process with a significant role in the etiology of numerous human diseases, such as diabetes and cancer, as well as certain neurodegenerative and cardiovascular diseases (Halliwell, 2011; Muller, 2007).

It is already proven that natural antioxidants can delay the oxidative damage caused by the unrestrained production of RNE (reactive nitrogen species) and ROS. Oxidation of cellular constituents can lead to structural changes and, consequently, cause

loss of cell biological activity (Bracesco, 2011). **Figure 5** illustrates the mechanisms of ROS production and antioxidant defense systems (Gandra, 2004).

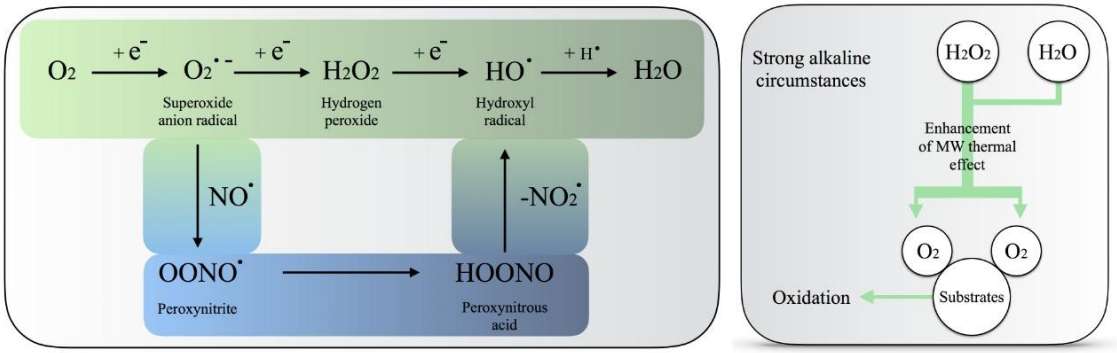


Figure 5: Mechanisms of ROS attack from monoelectronic O_2 reduction and antioxidant defense systems (adapted from Mwangi *et al*. 2022).

Antioxidant compounds can occur naturally in foods or be added in order to delay the onset of oxidation phenomena, maintaining sensory characteristics or bringing other technological advantages (Carocho, 2013). When present in a food in a balanced way, phenolic compounds can contribute to a lower incidence of chronic diseases such as cancer, atherosclerosis and diabetes, mainly due to their antioxidant effect, since the cellular components of the human body are not fully protected by endogenous antioxidants (Filip, 2000; Schuldt, 2005). In fact, several plant studies have demonstrated the relationship between free radical scavenging ability and polyphenolic content (Bixby, 2005; Vieira, 2009).

In addition to being related to odor, color, flavor and oxidative stability of foods, these compounds are essential in morphogenetic processes (fruiting and flowering, for example) and in the defense of numerous plants (Cansian, 2008). Therefore, antioxidant capacity is directly associated with the chemical structure of these compounds and could stabilize free radicals, inhibiting LDL (low-density lipoprotein) oxidation, preventing the development of atherosclerosis and performing anti-inflammatory, antiplatelet and vasodilatory actions (Da Silva, 2012).

In this context, antioxidants are commonly added to the food industry to minimize changes in aroma, nutritional value, flavor or color. Many synthetic antioxidants are commonly employed and are considered stable and effective; however, its use is limited in several countries as it can be harmful to human health (Schinella, 2000). On the other hand, natural antioxidants are significantly sensitive to conditions such as pH,

temperature and light variations and, to improve their effectiveness, they must be protected from the surrounding environment during food production using techniques such as encapsulation.

Yerba Mate (Ilex Paraguariensis)

Ilex paraguariensis A. St. Hil. (Mate, Aquifoliaceae) is a South American evergreen tree. When mate leaves and twigs are industrially processed, the major result is Yerba Mate (YM), which is sold to consumers in various countries across the world (Bastos, 2006).

It is typically used as a tea-like beverage in Brazil, Paraguay, Uruguay, and Argentina (Heck, 2007). YM stems and leaves powder is also used to produce a variety of stimulating cocktails. Mate is available in three flavors: *chimarrão* (hot water extract and dry green leaves), mate (hot water extract and toasted leaves), and tererê (cold water extract and dry green leaves) (Bracesco, 2010; Lima, 2014).

The association between beverage consumption and improved life quality in the elderly, as mentioned in the Guarani legend, is extremely important in understanding its functions in health. For many years, YM has been used in several cultures and has proven to be useful in the prevention and treatment of various diseases in tribal medicine (Heck, 2007).

In fact, several studies have linked YM to a variety of health benefits, including antioxidant properties (Bravo, 2007; Piovezan-Borges, 2016; Miranda, 2008), vasodilatory functions (Paganini, 2005; Yu, 2015; Stein, 2005), protection against DNA-induced damage (Miranda, 2008), hypoglycemic effects (Klein, 2011; Murakami, 2013), inhibition of glycation and atherosclerosis (Mosimann *et al*., 2006; Pomilio *et al*., 2002).

Also, minerals, vitamins, phenolic compounds, amino acids, enzymes, xanthines, polyphenols, saponins, lignin, cellulose, and organic acids have all been identified as chemical components responsible for the health benefits of yerba mate (Lunceford, 2005). Essentially, the relationship between its stimulating impact and potential antioxidant properties, among other health advantages, has resulted in a burgeoning YM industry with a global presence (Burris *et al*., 2011; Burris *et al*., 2012).

It has been suggested that mate consumption might be an efficient strategy to integrate strong antioxidants in the daily diet of the population, defending against the detrimental effects of free radicals and strengthening the human body's defensive system, based on

its chemical makeup (de Mejia, 2010). Many families of caffeoyl derivatives and flavonoids have been discovered in YM phytochemical research, including chlorogenic acid, caffeic acid, dicafoylquinic acids, rutin, quercetin, and kaempferol (Filip, 2001).

Due to their stimulant effect, the most active compounds detected in YM are the purine alkaloids (methylxanthines) which basically consist of caffeine (0.8 to 1.9%), theobromine (0.3 to 0.9%) and traces of theophylline (Filip, 1998; Vazquez, 1986), although most of the positive health effects of YM are attributed to its phenolic fraction, by virtue of its antioxidant, anti-inflammatory, and anticancer effects, as well as its ability to regulate gene expression (Bravo, 2007; Filip, 2000, Heck , 2007). The main phenolic compounds present in mate are caffeoyl derivatives, such as chlorogenic, 3,5-dicafoylquinic, 4,5-dicafoylquinic, 3,4-dicafoylquinic and caffeic acid (De Souza, 2011; Filip, 2001; Reginatto, 1999; Schenkel, 1996).

The phenolic portion accounts for 7-10% of the dry weight of YM leaves, with antioxidant capabilities equivalent to red wine and tea when tested *in vitro* by ABTS and FRAP methods. Hydroxycinnamas (a series of esters generated between quinic acid and one or more hydroxycinnamic acids, such as caffeic acid, ferulic acid, and p-coumaric acid), also known as chlorogenic acids, make up to 95 percent of the phenolic component in YM, with flavonoids comprising for just 5% (Bravo, 2007). It has also been shown that chlorogenic acid and its isomers represent around 3% of the weight of YM leaves, or 1–10% of the chemicals in the aqueous extract (Streit, 2007).

According to Gnoatto *et al*. 2005; Heck *et al*. 2007; Coelho *et al*. 2010 and Murakami *et al*. 2013, mate-based drinks also contain triterpene saponins formed with ursolic and oleanolic aglycones, in addition to a small amount of flavonoids. These compounds are responsible for foaming and the bitter taste, typical of mate-based drinks.

Silveira *et al*. (2016) discovered lutein for the first time in mate extract, indicating that YM might be a source of this carotenoid. Other glycosylated flavonoids, including kaempferol and quercetin, are found in the phenolic fraction of YM, although in minor levels compared to the other phenolic components (Bravo, 2007; Baeza, 2016). However, because kaempferol is entirely insoluble in water, its identification is still questionable (Heck *et al*., 2007; Gnoatto *et al*., 2005).

Chlorophyll is another key component found in YM that deserves more exploration. The presence of this pigment gives yerba mate its green hue, and the strength of the green is proportional to the concentration of this pigment (Morawicki *et al*., 1999). Chlorophyll

breakdown may be caused by a variety of variables, including changes in pH, enzymes, oxygen, temperature, and light, and the pace of reaction seems to be determined by the food's water activity (Lajolo *et al*., 1971; Schwartz *et al*., 1971; Schwartz *et al*. al., 1990; Bohn *et al*., 2004).

The most likely reactions of chlorophyll degradation in dehydrated vegetables, such as YM, are phaeophytinization and oxidation, which produce derivatives with spectral characteristics different from the parent pigment (Lajolo *et al*., 1982; King *et al*., 2001; Santos *et al*., 2004), changing the color of the product. The magnesium in the core of the chlorophyll molecule is replaced by hydrogen in the phaeophytinization process, changing the brilliant green color of chlorophyll to the olive brown hue of pheophytin. This reaction is considered the most significant mechanism of chlorophyll destruction in processed foods due to its greater degradation rate than other chlorophyll degradation pathways (Streit, 2005).

Other findings on chemicals with thermogenic capacity, and therefore a powerful ally to weight reduction, led to weight loss study on YM (Riachi, 2017). In the last 20 years, only a few clinical studies have been conducted to investigate the plant's potential to increase energy expenditure, increase satiety, reduce appetite, and improve body fat composition (Alkhatib *et al*., 2014; Andersen *et al*., 2014; Andersen *et al*., 2014; Andersen *et al*. al., 2001; Jung *et al*., 2016; Martinet *et al*., 1999; Kim *et al*., 2012; Oliveira *et al*., 2016; Kim *et al*., 2015). Martinet *et al*. examined the thermogenic impact of YM in humans for the first time in double-blind placebo-controlled research in 1999.

Further research is needed to confirm and assess the potential of YM as a useful functional product in the fight against obesity. Similarly, numerous methodologies for future study have been proposed with the goal of establishing the impacts of mate on phytochemical content and health consequences (Cardozo Junior *et al*., 2016).

Microwave Assisted Extraction (MAE)

Microwave Assisted Extraction (MAE) is a promising alternative to conventional extraction methods (Eskilsson *et al*., 2000; Heleno *et al*., 2016; Sinha *et al*., 2012). Several authors have reported the advantages of MAE over conventional extraction, such as reduced processing time, lower solvent and energy demand, and higher yield (Cardoso-Ugarte, 2014; Chen, 2008; Hemwimon, 2007; Proestos, 2008).

Microwaves are electromagnetic waves with both electric and magnetic field components. Microwave irradiation works in the frequency range from 0.3 to 300 GHz and wavelength (λ) ranging from 1 mm to 1 m (Larhed *et al*., 2002). Microwaves can penetrate certain materials and interact with polar components to generate heat. The heating generated by microwaves acts directly on the molecules by ionic conduction and dipole rotation and therefore only selective and targeted materials can be heated based on their dielectric constant (Chan, *et al*., 2011; Eskilsson *et al*., 2000).

The interaction of the electric field component with the matrix is called the dipole rotation mechanism. It is necessary for the molecule to have a dipole moment for alignment with the electric field to be applied. When the electric field is removed, the molecules return to their disordered state and the energy that was absorbed during orientation in the dipoles is dissipated in the form of heat, heating the medium (De Souza, 2011; Tsukui, 2014).

During microwave irradiation, cells become thermally disordered. As a result, the temperature and pressure within the cell increase to levels that result in the breakdown of the cell walls of the plant material, releasing the intracellular compounds of interest (Pap *et al*., 2013). Cell disruption facilitates the entry of the extraction solvent to solubilize the compounds, which leads to a faster extraction, preventing the degradation of the compounds of interest (Mandal *et al*., 2008). Most plant extracts obtained through microwave extraction are polyphenols, including flavonoids (flavones, flavonols, isoflavones, catechins, flavanones, flavonones and cinnamic acid derivatives), anthocyanins and carotenoids (Li *et al*., 2013).

The efficiency of microwave heating is the main advantage of MAE. The use of closed containers allows the use of higher temperatures, accelerating the extraction process of the compound of interest. The volume of solvent usually used in this method is about 10 times smaller than the volume used in conventional extraction techniques, with a usual increase in yield and process reproducibility (Tsukui *et al*., 2014).

Encapsulation

Microencapsulation is a process in which small amounts of solid, liquid or gaseous matter are coated by a thin polymeric layer, forming small-sized capsules, which protect the encapsulated compound from adverse environmental conditions. The encapsulated

material is called the core, internal phase or filling, while the material that forms the capsule is called the encapsulant, covering material, wall material or membrane (Dubey *et al*., 2009; Poshadri and Kuna, 2010; Sri *et al*., 2012; Umer *et al*., 2012).

Microparticles can be classified into microcapsules or microspheres, according to their structure. In microspheres there is a continuous phase of one or more polymers, where the encapsulated particles are dispersed, however part of the encapsulated material particles remains exposed on the surface, that is, there is no well-defined wall. In microcapsules, which is called true encapsulation, the core is clearly concentrated in the central region, and surrounded by a defined and continuous film formed by the wall material (Azeredo *et al*., 2005; Depypere *et al*., 2003; Mathiowitz *et al*., 2003; Mathiowitz *et al*., 2003; Mathiowitz *et al*. al., 1999; Suave *et al*., 2006).

The term encapsulation has been used in its broadest sense, encompassing both the formation of microcapsules and microspheres (Azeredo, 2005). According to Shahidi *et al*. (1993), there are six main reasons for the application of the microencapsulation technique in the food industry:

1. Reduce the reactivity of the nucleus towards the external environment (eg. light, oxygen and water).
2. To decrease the rate of evaporation or transfer of material from the core to the external environment.
3. Promote easier handling of core material to: a) Avoid agglomerates; b) positioning the core material more evenly through a mixture, giving it a size and outer surface like the rest of the materials in the mixture; c) Converting a liquid into a solid form; d) Promote easy mixing of the core material.
4. To control the release of core material in order to achieve the proper delay to the right stimulus.
5. To mask the core flavor.
6. Dilute the core material when it is used only in very small amounts, but achieve uniform dispersion in the host material.

Although most of the studies focused on the atomization method (spray drying or spray cooling), several other methods can be used in the encapsulation of compounds of interest in the food industry, such as lyophilization, emulsification, coacervation,

fluidized bed, extrusion, molecular inclusion and liposomes, co-crystallization and polymerization (Azeredo, 2005; Desai *et al*., 2005; Dubey *et al*., 2009; Favaro-Trindade *et al*., 2008; Poshadri *et al*., 2010; de Vos *et al*., 2010).

Encapsulation of Phenolic Compounds

The number of phenolic compounds used in the food and pharmaceutical industries is increasing annually (Spigoni *et al*., 2017). Oral administration of phenolic compounds has limitations in solubility, permeability and absorbance, leading to reduced bioavailability (Boonchu *et al*., 2015). In addition, the processing of phenolic compounds, storage time and gastrointestinal instability also influence their bioactivity. We already know that natural phenolics are sensitive and easily oxidized by exposure to sunlight and other environmental factors, which lead to changes in their biological, chemical and physical properties before in vivo administration, limiting their activities (Kalusevic *et al*., 2017).

Microencapsulation technology for phenolic compounds attracts many research interests, as the medicinal properties of the compounds are considerably less bioavailable if consumed directly (Rosas *et al*., 2017). Applications of phenolic compounds encapsulation technology are already underway in some pharmaceutical and food industries.

However, since these compounds are quite unstable under different processing conditions, many studies have tried to overcome this problem. There are multiple microencapsulation methods for trapping active ingredients and compounds, which are dependent on the desired criteria and the encapsulated material. Various formulations of the microcapsule coating material are available for all encapsulation methods (Costamagna *et al*., 2017). Kalusevic *et al*. (2017) employed various methods of microencapsulation of anthocyanins extracted from grape skins, which showed highly beneficial effects.

The type of encapsulation plays an important role in the efficiency of the retained phenolic compounds. Natural fiber polymers such as inulin, sodium alginate, HPMC and β-cyclodextrin have been tested and successful in forming microcapsules for phenolic compounds with an effective and beneficial targeted delivery method for human health (Waterhouse *et al*., 2013). According to recent research, the spray drying method is more

suitable for the encapsulation of phenolics obtained from plant source, with a higher retention rate and preserved bioactivity (Rigon *et al*., 2016; Kalusevic *et al*., 2017).

In the co-encapsulated system, it is known that a variety of factors influence the stability of the microcapsules, and that the delivery of the active ingredient needs to be tailored depending on the application requirements of the food system. Bioaccessibility and bioavailability are important parameters that are essential to establish the potential of coencapsulation in the food industry.

Nevertheless, simultaneous administration of multiple bioactive components can produce synergistic effects and offer multiple health benefits. This motivates the development of co-delivery systems that can simultaneously encapsulate a plurality of bioactive components (Zhang *et al*, 2019).

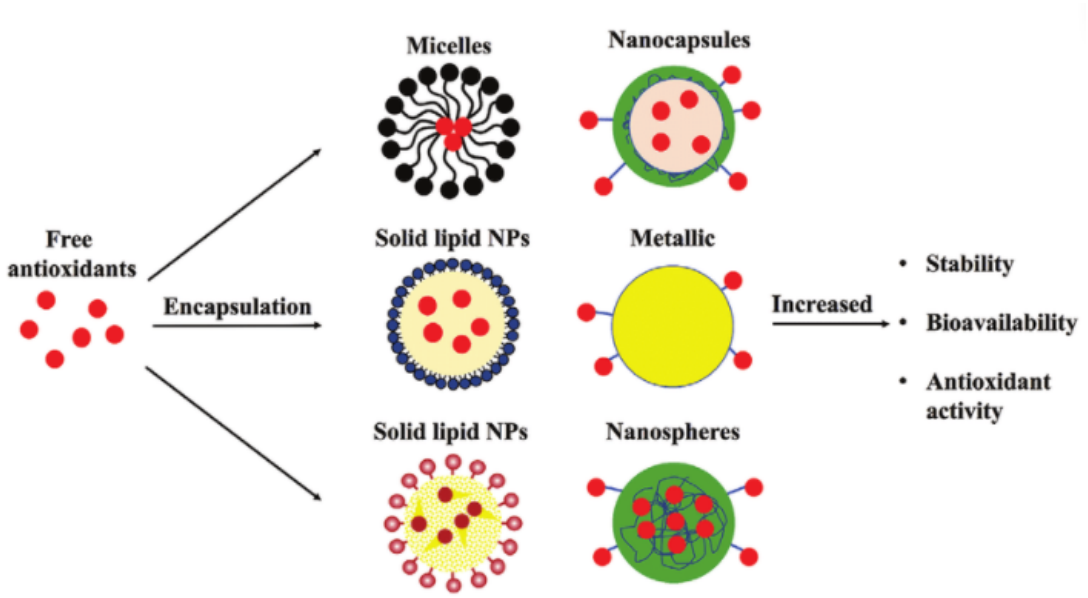


Figure 6: Encapsulation of antioxidants in different nanocarriers (Eleftheriadou *et al*., 2020).

Encapsulation Methods

Atomization (Spray Drying)

The atomization or spray drying technique represents a unique and continuous process that allows the conversion of liquid solutions, emulsions, suspensions or pastes into dry powders with controlled size and morphological aspects (Oliveira *et al*., 2013).

This method has a short drying time and is economically viable, being used in several food industries (Oliveira *et al*., 2013). Atomization is used to encapsulate a wide range of food ingredients, such as flavors, vitamins, minerals, colors, fats and oils, in order to protect them from their surrounding environment and prolong their stability during storage (Pillai *et al*., 2012).

The principle of the method is the dissolution of the core, meaning that the compound will be encapsulated in a wall material forming a dispersion, which will then be atomized in heated air, promoting the rapid removal of the solvent and resulting in particles in the form of powder that will be separated from the drying air (Gibbs *et al*., 1999; Sri *et al*., 2012; Taylor *et al*. 1983; Park *et al*., 2008; de Vos *et al*., 2010).

Two disadvantages observed in this method are the formation of structures such as those of microspheres, where part of the bioactive components can be exposed, and the high temperatures required for application of the technique (de Vos *et al*., 2010). Despite the need for high temperatures, the negative consequences are not so serious because the high surface area/volume ratio of the particles promotes the rapid evaporation of water and, therefore, the exposure time to these high temperatures is short, so that the core does not exceed temperatures close to 100 °C (Azeredo, 2005).

Lyophilization (Freeze Drying)

Lyophilization is a drying technology that consists of removing water from a frozen sample by sublimation (Aydin *et al*., 2017). The process is carried out under conditions of temperature and pressure below the triple point, to allow the sublimation of ice, being suitable for the drying of thermolabile compounds (Nireesha *et al*., 2013). However, it is a very expensive unit operation and often economically unfeasible (Sane *et al*., 2017). It consists of three steps: pre-freezing (solidification), primary drying (low pressure ice sublimation) and secondary drying (desorption of unfrozen water) (Abdelwahed *et al*., 2006; Nireesha *et al*., 2013).

In the pre-freezing step, the liquid sample is cooled to form pure crystalline ice from part of the liquid and the remaining liquid is frozen in a glassy state where the viscosity is too high to allow further crystallization. In primary drying, ice formed during freezing is removed by sublimation under vacuum (pressures from 10-4 to 10-5 atmospheres) at low temperatures (-45 to -20 °C), leaving a highly porous structure in the remaining amorphous solute. In secondary drying, most of the remaining water is

removed from the product as the temperature is gradually increased (Nireesha *et al*., 2013). This drying method allows the formation of nanocapsules, without changing the structure and shape of the compounds present in the composition (Ezhilarasi *et al*., 2013).

Wall Materials

Coating materials, which are basically film-forming materials, can be selected from a wide variety of natural and synthetic polymers, according to the desired characteristics for the microcapsules (Poshadri and Kuna, 2010). Although several types of encapsulating agents can be used, some characteristics must be observed, including film forming ability, biodegradability, resistance to the gastrointestinal tract, solids content, hygroscopicity, cost (Silva *et al*., 2013), good rheological properties at high concentrations, ability to disperse or emulsify the active material and stabilize the emulsion produced, as well as do not react with the material to be encapsulated both during the process and during storage (Desai *et al*., 2005).

Dietary fibers, which are carbohydrate polymers with a degree of polymerization greater than 3, are one type of wall material used for encapsulation of compounds. Because of their degree of polymerization, they are neither digested nor absorbed in the small intestine, meaning that they resist hydrolysis by food enzymes present in the human body (Phillips *et al*., 2008; Phillips and Williams, 2008). Dietary fibers generally have the following properties: reduce intestinal transit time and increase stool volume; being fermentable by the colon microflora; reduce LDL cholesterol levels; reduce postprandial blood glucose and/or insulin levels (Phillips *et al*., 2008).

According to Gibson and Roberfroid (1995), dietary fibers can be classified as prebiotics if they are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of a limited number of colonic bacteria, thereby improving host's health. In this way, they act to promote beneficial physiological effects, including laxative, attenuation of blood cholesterol levels, attenuation of blood glucose levels, with an indication that they may also have a potential effect on specific diseases such as constipation, infections, inflammation and colon diseases (Phillips *et al*., 2008).

However, it is worth noting that dietary fiber and prebiotics cannot be considered automatically synonymous, as all prebiotics must not be hydrolyzed or digested in the

upper gastrointestinal tract, thus evidencing nutritional properties and stimulating the growth of healthy bacteria in the colon. All prebiotics, therefore, are dietary fiber, but not all dietary fiber is prebiotic (Phillips *et al*., 2008).

Following, we briefly describe the three dietary fibers that were used as wall materials in this research: guar gum, polydextrose and gum arabic.

Guar Gum

Guar gum is a galactomannan isolated from the endosperm of guar seed, used as a soluble fiber supplement (Slavin, 2003). It is effective in decreasing postprandial hyperglycemia and cholesterol concentrations, both in experimental studies (Stanogias *et al*., 1985) and in healthy subjects and in diabetics (Kishida *et al*., 2002).

The mechanism of action of guar gum and other soluble fibers is based on their action to sequester bile acids in the duodenum. As a result, fecal excretion of bile acids increases in the feces, decreasing the amount that reaches the liver via the enterohepatic route. This increased excretion leads to a greater conversion of hepatic cholesterol to bile acids, reducing intrahepatic cholesterol concentration. Other mechanisms also studied are the increase in intestinal viscosity, directly inhibiting the absorption of cholesterol and its production, by bacterial fermentation, of short-chain fatty acids (such as propionate), which would inhibit hepatic cholesterogenesis. Although with proven physiological effects, this high viscosity limits the use of guar gum or its incorporation in foods (Farmer *et al*., 1995).

Partially hydrolyzed guar gum (PHGG) has its viscosity significantly reduced, making this fiber easily added to foods and accepted by consumers. Its use as a modulator of intestinal transit, improving both diarrhea and constipation, has been proven by several studies. However, it is not clear that after loss of viscosity, all the effects of guar gum (including hypoglycaemic and hypoglycaemic effects) are maintained (Alam *et al*., 2000; Spapen *et al*., 2001).

Polydextrose

Polydextrose is a low molecular weight glucose polysaccharide obtained by vacuum polycondensation of glucose with a small amount of sorbitol and food grade acids as a catalyst (Flood *et al*. 2004). This biopolymer has a low caloric content and is considered a dietary fiber with probiotic activity, due to its low digestibility in the small intestine, passing directly to the colon where it is gradually fermented by the endogenous microbiota (Craig *et al*., 1999).

Polydextrose is used in the food and cosmetic industry as a stabilizing, thickening and gelling agent, as well as in the formulation of edible and biodegradable films, adhesives, paper substitutes, plasticizers, materials for biomedical implantation and has many positive effects on human health, including reducing serum cholesterol and glucose levels, inducing apoptosis of prostate cancer cells, and stimulating immune responses (Jackson *et al*., 2007; Mohnen *et al*., 2008).

Gum Arabic

Gum Arabic is a natural polysaccharide with a neutral or slightly acidic chain composed of D-galactopyranose units joined by ® (1→4) glycosidic bonds, while the side chains are composed of two to five D-galactopyranose units linked to the chain mainly by ® (1→4) bonds, also presenting in its molecule a mixed saline complex of calcium, magnesium and potassium of arabic acid (Whistler *et al*., 1993; Ali *et al*., 2009; Shi *et al*., 2017).

It is a dietary fiber with antioxidant properties, effects against liver, kidney and heart toxicity and is used in the treatment of kidney diseases and diabetes (Ali *et al*., 2009). This gum is used as a wall material in several encapsulation processes, largely due to its low cost, non-toxicity, biocompatibility, biodegradability, good ability to act as a stabilizer, emulsifier and protection against oxidation processes (Fernandes *et al*., 2016; Eratte *et al*., 2014; Garcia-Saldaña *et al*., 2016; Kuck and Noreña, 2016; Premi and Sharma, 2017).

Although a variety of encapsulating agents can be used, certain properties must be considered, such as film forming ability, biodegradability, gastrointestinal tract resistance, viscosity, solids content, hygroscopicity, and cost (Silva *et al*, 2013). Despite its outstanding qualities, an encapsulating agent is unlikely to possess all the required attributes. As a result, using wall material mixtures as a way to improve the encapsulation process' efficiency is an option.

1. REFERENCES

ABDELWAHED, W., DEGOBERT, G., STAINMESSE, S., FESSI, H., (2006). Freeze-drying of nanoparticles: formulation, process and storage considerations. Advanced drug delivery reviews 58(15), 1688-1713.

ALAM, N.H, Meier R, Schneider H, Sarker SA, Bardhan PK, Mahalanabis D, et al. Partially hydrolyzed guar gum-supplemented oral rehydration solution in the treatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000; 31(5):503-7.

ALI, B. H.; Ziada, A.; Blunden, G. Biological effects of gum arabic. A review of some recent research. Food and Chemical Toxicology, v.47, n.1, p. 1-8, 2009.

ALKHATIB, A. (2014). Yerba Maté (Illex Paraguariensis) ingestion augments fat oxidation and energy expenditure during exercise at various submaximal intensities. Nutrition & Metabolism, 11(1), 42

ANDERSEN, T., & FOGH, J. (2001). Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. Journal of Human Nutrition and Dietetics, 14, 243–250.

ANESINI, C., FERRARO, G., & FILIP, R. (2006). Peroxidase-like activity of Ilex paraguariensis. Food Chemistry, 97, 459-464.

ARÇARI, D. P., BARTCHEWSKY, W., DOS SANTOS, T. W., OLIVEIRA, K. A., FUNCK, A., PEDRAZZOLI, J., DE SOUZA, M. F., SAAD, M. J., BASTOS, D. H., GAMBERO, A., CARVALHO, P. O., & RIBEIRO, M. L. (2009). Antiobesity effects of yerba maté extract (Ilex paraguariensis) in high-fat diet-induced obese mice. Obesity, 12, 2127–2133.

ARON, P. M.; KENNEDY, J. A. Compositional investigation of phenolic polymers isolated from Vitis vinifera L. cv. Pinot Noir during fermentation. Journal of Agricultural and Food Chemistry, v.55, p.5670–5680, 2007.

AYDIN, E.S., YUCEL, O., SADIKOGLU, H., (2017). Modelling and simulation of a moving interface problem: freeze drying of black tea extract. Heat and Mass Transfer, 1-12.

AZEREDO, H. M. C. Encapsulação: aplicação à tecnologia de alimentos. Alimentos e Nutrição, v.16, n.1, p.89-97, 2005.

BAEZA, G., SARRIA, B., MATEOS, R., & BRAVO, L. (2016). Dihydrocaffeic acid, a major microbial metabolite of chlorogenic acids, shows similar protective effect than a

yerba mate phenolic extract against oxidative stress in HepG2 cells. Food Research International, 87, 25-33.

BAKRY, A. M., FANG, Z., NI, Y., CHENG, H., CHEN, Y. Q., LIANG, L. (2016). Stability of tuna oil and tuna oil/peppermint oil blend microencapsulated using whey protein isolate in combination with carboxymethyl cellulose or pullulan. Food Hydrocolloids, 60: 559–571.

BALENTINE, D. A.; WISEMAN, S. A.; BOUWNES, L. C. The chemistry of tea flavonoids. Critical Review in Food and Science Nutrition, v.37, p.693–704, 1997.

BASTOS, D. H. M., FORNARI, A. C., QUEIROZ, Y. S., & TORRES, E. A. F. S. (2006). Bioactive compounds content of Chimarrao infusions related to the moisture of yerba mate (Ilex paraguariensis) leaves. Brazilian Archives of Biology and Technology, 49, 399–404.

BASTOS, D. H. M., OLIVEIRA, D. M., MATSUMOTO, R. L. T., CARVALHO, P. O., & RIBEIRO, M. L. (2007). Yerba maté: Pharmacological properties, research and biotechnology. Medicinal and Aromatic Plant Science and Biotechnology, 1(1), 37–46.

BIXBY, M., SPIELER, L., MENINI, T., & GUGLIUCCI, A. (2005). Ilex paraguariensis extracts are potent inhibitors of nitrosative stress: A comparative study with green tea and wines using a protein nitration model and mammalian cell cytotoxicity. Life Sciences, 77(3), 345–358.

BOHN, T.; WALCZYK, T. Determination of chlorophyll in plant samples by liquid chromatography using zinc-phthalocyanine as an internal standard. Journal of Chromatography, v.1024, n.1-2, p.123-128, 2004.

BOONCHU, T. UTAMA-ANG, N., 2015. Optimization of extraction and microencapsulation of bioactive compounds from red grape (Vitis vinifera L.) Pomace. J. Food Sci. Technol. 52, 783-792.

BRACESCO, A.G. SANCHEZ, V. CONTRERAS, T. MENINI, A. GUGLIUCCI. Recent advances on Ilex paraguariensis research: minireview. J. Ethnopharmacol. 136 (2010) 378-384.

BRACESCO, N., SANCHEZ, A. G., CONTRERAS, V., MENINI, T., & GUGLIUCCI, A. (2011). Recent advances on Ilex paraguariensis research: Mini review. Journal of Ethnopharmacology, 136(3), 378–384.

BRAVO, L., L. GOYA, E. LECUMBERRI. LC/MS characterization of phenolic constituents of mate (Ilex paraguariensis A. St.-Hill) and its antioxidant activity compared to commonly consumed beverages. Food Res. Int. 40 (2007), 303-405.

BURRIS, K.P., DAVIDSON, P.M., STEWART JR, C.N., HARTE, F.M., 2011. Antimicrobial activity of yerba mate (Ilex paraguariensis) aqueous extracts against Escherichia coli O157: H7 and Staphylococcus aureus. J. Food Sci. 76, M456–M462.

BURRIS, K.P., HARTE, F.M., DAVIDSON, P.M., STEWART JR, C.N., ZIVANOVIC, S., 2012. Composition and bioactive properties of yerba mate (Ilex paraguariensis A. St.-Hil.): a review. Chil. J. Agric. Res. 72, 268–274.

CANSIAN, R. L., MOSSI, A. J., MAZUTTI, M., OLIVEIRA, J. V., PAROUL, N., DARIVA, C., & ECHEVERRIGARAY, S. (2008). Semi-volatile compounds variation among Brazilian populations of Ilex paraguariensis St. Hil. Brazilian Archives of Biology and Technology, 51, 175–181.

CARDOSO-UGARTE, G.; SOSA-MORALES, M. E.; BALLARD, T.; LICEAGA, A.; SAN MARTÍN-GONZALEZ, M. F. Microwave-assisted extraction of betalains from red beet (Beta vulgaris). LWT - Food Science and Technology 59, 276-282, 2014.

CARDOZO JUNIOR, E., MORAND, C. Interest of mate (Ilex paraguariensis A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review. Journal of Functional Foods 21(2016) 440–454.

CAROCHO, M., FERREIRA, I.C.F.R., 2013. A review on antioxidants, pro-oxidant sand related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. 51, 15-25.

CHAMPAGNE, C.P; FUSTIER, P. (2007) Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol 18:184–190.

CHAN, C.; YUSOFF, R.; NGOH, G.; KUNG, F. W. Microwave-assisted extractions of active ingredients from plants. Journal of Chromatography A, 1218, 6213-6225, 2011.

CHAWDA, P.J et al. Co-encapsulation of bioactives for food applications. Food Quality and Safety, 1.4 (2017): 302-309.

CHEN, L.; JIN, H.; DING, L.; ZHANG, H.; LI, J.; QU, C.; ZHANG, H. Dynamic microwave assisted extraction of flavonoids from Herba Epimedii. Separation and Purification Technology, 59 (1), 50-57, 2008.

CLIFFORD, M. N. Anthocyanins: Nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture, v.80, p.1063–1072, 2000.

COELHO, G. C., GNOATTO, S. B., BASSANI, V. L., & SCHENKEL, E. P. (2010). Quantification of saponins in extractive solution of mate leaves (Ilex paraguariensis A. St. Hil). Journal of Medicinal Food, 13(2), 439–443.

COSTAMAGNA, M.S., MASCARAQUE, L.G.G., ZAMPINI, I.C., ALBERTO, M.R., PEREZ, J., RUBIO, A.L., ISLA, M.I., 2017. Microencapsulated chanar phenolics: A potential ingredient for functional foods development. J. Funct. Foods. 37, 523-530.

CRAIG, S. et al. Polydextrose as soluble fiber and complex carbohydrate. Food Science and Technology, New York, Marcel Dekker, p. 229-248, 1999.

CROZIER, A.; CLIFFORD, M. N.; ASHIHARA, H. Plant secondary metabolites. Ocurrence, structure and role in the human diet. Blackwell Publishing Ltd., Oxford, UK, 2006. 384p.

DA SILVA, R.C., CRESTANI, S., DE SOUZA, P., BOLIGON, A.A., ATHAYDE, M.L., SANTO, A.R., MARQUES, M.C., KASSUYA, C.A., DA SILVA, S.J.E., 2012. Endothelium-dependent andindependent vasorelaxation induced by an n-butanolic fraction of bark of Scutiabuxifolia Reiss (Rhamanaceae). J. Ethnopharmacol. 141,997–1004.

DA SILVEIRA, T. F. F., MEINHART, A. D., COUTINHO, J. P., DE SOUZA, T. C. L., CUNHA, E. C. E., & DE MORAES, M. R. (2016). Content of lutein in aqueous extracts of yerba mate (Ilex paraguariensis St. Hil). Food Research International, 82, 165–171.

DAIBER, A., 2010. Redox signaling (crosstalk) from and to mitochondria involvesmitochondrial pores and reactive oxygen species. Biochim. Biophys. Acta 1797,897–906.

DAUCHET, L., AMOUYEL, P., & DALLONGEVILLE, J. (2009). Fruits, vegetables and coronary heart disease. Nature Reviews Cardiology, 6, 599–608.

DE MEJIA, E. G., SONG, Y. S., HECK, C. I., & RAMIREZ-MARES, M. (2010). Yerba mate tea (Ilex paraguariensis): Phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. Journal of Functional Foods, 2, 23-34.

DEMIRCI-Çekiç, Sema, et al. "Biomarkers of Oxidative Stress and Antioxidant Defense." Journal of pharmaceutical and biomedical analysis 209 (2022): 114477.

DE SOUZA, R. O. M. A.; MIRANDA, L. S. M. Irradiação de micro-ondas aplicada à síntese orgânica: uma história de sucesso no Brasil. Química Nova, 24, 497, 2011.

DE VOS, P. et al. Encapsulation for preservation of functionality and targeted delivery os bioactive food components. International dairy journal 20.4 (2010): 292-302.

DEL RIO, D., RODRIGUEZ-MATEOS, A., SPENCER, J. P. E., TOGNOLINI, M., BORGES, G., & CROZIER, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxidants and Redox Signaling, 18, 1818–1892.

DEPYPERE, F., DEWETTINCK, K., RONSSE, F., PIETERS, J.G. (2003). Food powder microencapsulation: principles, problems and opportunities. Applied Biotechnology Food Science and Policy, 1 (2), pp 75-94.

DESAI, H.; PARK, H. Recent developments in microencapsulation of food ingredients. Drying Technology, 23, 1361-1394, 2005.

DESAI, K.G.H AND JIN, PARK H. (2005) Recent Developments in Microencapsulation of Food Ingredients. Drying Technology, 23, 1361-1394.

DOOLAANEA, A. A., MANSOR, N. ‘., MOHD NOR, N. H., MOHAMED, F. (2016). Coencapsulation of nigella sativa oil and plasmid dna for enhanced gene therapy of alzheimer’s disease. Journal of Microencapsulation, 33: 114–126.

DORDEVIC, V.; BALANC, B.; BELSCAK-CVITANOVIC, A.; LEVIC, S.; TRIFKOVIC, K.; KALUSEVIC, A.; KOSTIC, I.; KOMES, D.; BUGARSKI, B.; NEDOVIC, V. Trends in encapsulation technologies for delivery of food bioactive compounds. Food Engineering Reviews, v. 7, n .4, p. 452–490, 2015.

DUBEY, R.; SHAMI, T. C.; BHASKER RAO, K. U. Microencapsulation technology and applications. Defence Science Journal, 59, 1, 82-95, 2009.

DUFRESNE, A. (1997). Mechanical Behavior of Films Prepared from Sugar Beet Cellulose Microfibrils, J. Appl. Poly. Sci. 64(6): 1185–119.

DUGO, P., CACCIOLA, F., DONATO, P., JACQUES, R. A., CARAMAO, E. B., & MONDELLO, L. (2009). High efficiency liquid chromatography techniques coupled to mass spectrometry for the characterization of mate extracts. Journal of Chromatography A, 1216(43), 7213-7221.

ERATTE, D. et al. Complex Coacervation with whey protein isolate and gum Arabic for the microencapsulation of mega-3 rich tuna oil. Food & Functions, v. 5, n. 11, p. 2743-2750.

ELEFTHERIADOU, Despoina, et al. "Redox-responsive nanobiomaterials-based therapeutics for neurodegenerative diseases." Small 16.43 (2020): 1907308.

ESKILSSON, C.; BJORKLUND, E. Analytical-scale microwave assisted extraction. Journal of Chromatography A, 902 (1), 227-250, 2000.

EZHILARASI, P., KARTHIK, P., CHHANWAL, N., ANANDHARAMAKRISHNAN, C., (2013). Nanoencapsulation techniques for food bioactive components: a review. Food and Bioprocess Technology 6 (3), 628-647.

FANG, Z., & BHANDARI, B. (2011). Effect of spray drying and storage on the stability of bayberry polyphenols. Food Chemistry, 129, 1139–1147.

FARMER, J.A, Gotto AM, Jr. Currently available hypolipidaemic drugs and future therapeutic developments. Baillieres Clin Endocrinol Metab. 1995; 9(4):825-47.

FAVARO-TRINDADE, C. S.; PINHO, S. C.; ROCHA, G. A. Revisão: Microencapsulação de ingredientes alimentícios. Brazilian Journal of Food Technology, Campinas, v. 11, n. 2, p. 103-112, 2008.

FERNANDES, R.V.D.B. et al. Study of ultrasound-assisted emulsions on microencapsulation of ginger essential oil by spray drying. Industrial Crops and Products. 94 (2016): 413-423.

FILIP R, LOTITO SB, FERRARO G, FRAGA CG 2000. Antioxidant activity of Ilex paraguariensis and related species. Nutr Res 20: 1437-1446.

FILIP, R., LÓPEZ, P., GIBERTI, G., COUSSIO, J., FERRARO, G., 2001. Phenolic compounds in seven South American Ilex species. Fitoterapia 72, 774–778.

FILIP, R., P. LÓPEZ, J. COUSSIO, G. FERRARO. Mate substitutes or adulterants: Study of Xanthine Content. Phytotherapy Research, 12 (1998), pp. 129-131.

FLOOD, M.T.; Auerbach, M.H.; Craig, S.A.S. A review of the clinical toleration studies of polydextrosein food. Food and Chemical Toxicology, v. 42, n.9, p.1531-1542, 2004.

FRAGA, C. G. Plant phenolics and human health: biochemistry, nutrition and pharmacology. New Jersey: Wiley & Sons, 2009. 593p.

GANDRA, P.G, et al. “Determinação eletroquímica da capacidade antioxidante para avaliação do exercício físico.” Química Nova (2004).

GARAVAND, Farhad, et al. "Encapsulation of phenolic compounds within nano/microemulsion systems: A Review." Food Chemistry (2021): 130376.

GARCÍA-SALDAÑA, J. S. et al., Microencapsulation of sulforaphane from broccoli seed extracts by gelatin/gum Arabic ang gelatin/pectin complexes. Food Chemistry, v.201, p. 94-100, 2016.

GIBBS, F., Kermasha, S., Alli, I., Mulligan, C.N., Bernard. "Encapsulation in the food industry: a review." International journal of food sciences and nutrition 50.3 (1999): 213-224.

GIBSON, G. R.; ROBERFROID, M. B. Dietary modulation of the human colonie microbiota: introducting the concept of prebiotics. Journal of Nutrition, v.125, n.6, p.1401-1412, 1995.

GNOATTO, S. C. B., SCHENKEL, E. P., & BASSANI, V. L. (2005). HPLC method to assay total saponins in Ilex paraguariensis aqueous extract. Journal of the Brazilian Chemical Society, 16(4), 723–726.

GONZALEZ, R., BALLESTER, I., LOPEZ-POSADAS, R., SUAREZ, M. D., ZARZUELO, A., MARTINEZ-AUGUSTIN, O., & SANCHES DE MEDINA, F. (2011). Effects of flavonoids and other polyphenols on inflammation. Critical Reviews in Food Science and Nutrition, 51, 331–362.

GÜRSOY, Ayla, Ece Kut, and Sumru Özkırımlı. "Co-encapsulation of isoniazid and rifampicin in liposomes and characterization of liposomes by derivative spectroscopy." International journal of pharmaceutics 271.1-2 (2004): 115-123.

HABAUZIT, V., MORAND, C. (2012). Evidence for the cardioprotective benefits of polyphenols-containing foods: Na update for clinicians. Therapeutic Advances in Chronic Disease, 3, 87–106.

HALLIWELL, B., 2011. Free radicals and antioxidants—quo vadis? Trends Pharmacol. Sci. 32, 125–130.

HECK, C. I., MEJIA, E. G. (2007). Yerba mate tea (Ilex paraguariensis): A comprehensive review on chemistry, health implications, and technological considerations. Journal of Food Science, 72, 138–151.

HELENO, S. A.; PRIETO, M. A.; BARROS, L.; RODRIGUES, A.; BARREIRO, M. F. Optimization of microwave-assisted extraction ofergosterol from Agaricus bisporus L. by-products using response surface methodology. Food and Bioproducts Processing, 100, 25-35, 2016.

HEMWIMON, S.; PAVASANT, P.; SHOTIPRUK, A. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Separation and Purification Technology, 54 (1), 44-50, 2007.

HOOPER, L., KAY, C., ABDELHAMID, A., KROON, P. A., COHN, J. S., RIMM, E. B., CASSIDY, A. (2012). Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: A systematic review and meta-analysis of randomized trials. The American Journal of Clinical Nutrition, 95, 740–751.

HOOPER, L., KROON, P. A., RIMM, E. B., COHN, J. S., HARVEY, I., LE CORNU, K. A., RYDER, J. J., HALL, W. L., CASSIDY, A. (2008). Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition, 88, 38–50.

HUBER, L.S., RODRIGUES-AMAYA, D.B. “Flavonóis e flavonas: fontes brasileiras e fatores que influenciam a composição em alimentos.” Câncer 21.42 (2008):55.

IYER, C., KAILASAPATHY, K. (2005). Effect of co-encapsulation of probiotics with prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt conditions and in yogurt. Journal of Food Science, 70: M18–M23.

JACKSON, C. L. et al. Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. Glycobiology, v. 17, n.8, p. 805-819, 2007.

JUNG, J.-H., HUR, Y.-I. (2016). The Effect of Maté Extract on Body Weight and Fat Reduction in Obese Women: A Randomized Placebo-controlled Clinical Trial. The Korean Journal of Obesity, 25(4), 197–206.

KALUSEVIC, A.M., LEVIC, S.M., CALIJA, B.R., MILIC, J.R., PAVLOVIC, V.B., BUGARSKI, B.M., NEDOVIC, V.A., 2017. Effects of different carrier materials on

physicochemical properties of microencapsulated grape skin extract. J. Food Sci. Technol. 54, 3411-3420

KIM, H. J., KO, J., STORNI, C., SONG, H. J., CHO, Y. G. (2012). Effect of green mate in overweight volunteers: A randomized placebo controlled human study. Journal of Functional Foods, 4, 287–293.

KIM, S.-Y., OH, M.-R., KIM, M.-G., CHAE, H.-J., CHAE, S.-W. (2015). Anti-obesity effects of Yerba Mate (Ilex Paraguariensis): a randomized, double-blind, placebo-controlled clinical trial. BMC Complementary and Alternative Medicine, 15(1), 338.

KING, V.A.E. et al. Chlorophyll stability in spinach dehydrated by freeze-drying and controlled low-temperature vacuum dehydration. Food Research International, v.34, n.2-3, p.167-175, 2001.

KISHIDA T, Nogami H, Ogawa H, Ebihara K. The hypocholesterolemic effect of high amylose cornstarch in rats is mediated by an enlarged bile acid pool and increased fecal bile acid excretion, not by cecal fermented products. J Nutr. 2002; 132(9):2519-24.

KLEIN, G.A., A. STEFANUTO, B.C. BOAVENTURA, E.C. MORAIS L.S. CAVALCANTE, F. ANDRADE, E. WAZLAWIK, P.F. DI PIETRO, M. MARASCHIN, E.L. Silva. Mate tea (Ilex paraguariensis) improves glycemic and lipid profiles of type 2 diabetes and pre-diabetes individuals: a pilot study. J. Am. Coll. Nutr. 30 (2011) 320-32.

KUCK, L.S.; Noreña, C.P.Z. 2016. Microencapsulation of grape (Vitis labrusca var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. Food Chemistry 194: 569-576.

LAJOLO, F.M. et al. Reaction at limited water concentration. 2. Chlorophyll degradation. Journal of Food Science, v.36, p.850-853, 1971.

LAJOLO, F.M.; LANFER MARQUEZ, U.M. Chlorophyll degradation in a spinach system at low and intermediate water activities. Journal of Food Science, v.47, p.1995-1998, 1982.

LARHED, M.; MOBERG, C.; HALLBERG, A. Microwave-accelerated homogeneous catalysis in organic chemistry. Accounts of Chemical Research, 35, 717, 2002.

LI, Y.; FABIANO-TIXIER, A.; ABERT-VIAN, M.; CHEMAT, F. Microwave-assisted extraction of antioxidants and food colors. Microwave-assisted extraction for bioactive compounds. Food Engineering Series, 4, 103-125, 2013.

LIMA, N.S., E. OLIVEIRA, A.P. SILVA, L.A. MAIA, E.G. MOURA, P.C. LISBOA. Effects of Ilex paraguariensis (yerba mate) treatment on leptin resistance and inflammatory parameters in obese rats primed by early weaning. Life Sci. 12 (2014) 115, 29-35.

LONG, F., YANG, H., XU, Y., HAO, H., LI, P. (2015). a strategy for the identification of combinatorial bioactive compounds contributing to the holistic effect of herbal medicines. Scientific Reports, 5: 12361.

LUNCEFORD, N.L., A. GUGLIUCCI. Ilex paraguariensis extracts inhibit AGE formation more efficiently than green tea. Fitoterapia, 76 (2005) 419-427.

MANDAL, V.; MOHAN, Y.; HEMALATHA, S. Microwave assisted extraction of curcumin by sample-solvent dual heating mechanism using Taguchi L9 orthogonal design. Journal of Pharmaceutical and Biomedical Analysis, 46, 322-327, 2008.

MARTINET, A., HOSTETTMANN, K., & SCHUTZ, Y. (1999). Thermogenic effects of commercially available plant preparations aimed at treating human obesity. Phytomedicine, 6(4), 231–238.

MATHIOWITZ, E.; KREITZ, M. Microencapsulation. In: MATHIOWITZ, E. (ed). Encyclopedia of Controlled Drug Delivery, 2 Volume Set. 1 edition. Wiley, 1999. 1061p.

MENRAD, K. (2003) Market and marketing of functional food in Europe. J Food Eng 56:181–188.

MIRANDA, D.D, ARCARI DP, PEDRAZZOLI JR J, CARVALHO PDE O, CERUTTI SM, BASTOS DH, et al. Protective effects of mate tea (Ilex paraguariensis) on H2O2-induced DNA damage and DNA repair in mice. Mutagenesis 2008; 23:261–5.

MOHNEN, D. Pectin Structure and biosynthesis. Current opinion in plant biology, v.11, n.3, p. 266-277, 2008.

MORAWICKI, R.O. et al. Chlorophyll stability in yerba maté leaves in controlled atmospheres. Brazilian Archives of Biology and Technology, v.42, n.1, p.85-90, 1999.

MOSIMANN, A.L., WILHELM-FILHO, D., DA SILVA, E.L. Aqueous extract of Ilex paraguariensis attenuates the progression of atherosclerosis in cholesterol-fed rabbits. Biofactors 2006; 26:59–70.

MULLER, F.L., LUSTGARTEN, M.S., JANG, Y., RICHARDSON, A., VAN REMMEN, H., 2007.Trends in oxidative aging theories. Free Radic. Biol. Med. 43, 477–503.

MURAKAMI, A.N., AMBONI, R.D., PRUDÊNCIO, E.S., AMANTE, E.R., FRITZEN-FREIRE, C.B., BOAVENTURA, B.C., MUÑOZ, I.B., BRANCO, C.S., SALVADOR, M., MARASCHIN, M. Concentration of biologically active compounds extracted from Ilex paraguariensis St Hil. By nanofiltration. Food Chem. 141 (2013) 60-65.

MWANGI, Ruth W., et al. "The antioxidant potential of different edible and medicinal mushrooms." Biomedicine & Pharmacotherapy 147 (2022): 112621.

NIREESHA, G., DIVYA, L., SOWMYA, C., VENKATESHAN, N., BABU, M.N., LAVAKUMAR, V., (2013). Lyophilization/freeze drying—an review. International journal of novel trends in pharmaceutical sciences 3(4), 87-98.

OLIVEIRA, A. et al. Nano spray drying as an innovative technology for encapsulating hydrophilic active pharmaceutical ingredients (API). Journal of Nanomedicine and Nanotechnology, v. 4, n. 186, p. 2, 2013.

OLIVEIRA, D. M.; BASTOS, D. H. M. Biodisponibilidade de ácidos fenólicos. Química Nova, v.34, n.6, 2011.

OLIVEIRA, E. P; TOREZAN, G. A., GONÇALVES, L. DES., CORRENTE, J. E., MCLELLAN, K. C. P., BURINI, R. C. (2016). O consumo agudo de erva mate aumenta o gasto energético de homens jovens saudáveis: um estudo piloto. Revista Brasileira de Obesidade, Nutrição E Emagrecimento, 10(59), 242–249.

ONAKPOYA, I. J., SPENCER, E. A., THOMPSON, M. J., & HENEGHAN, C. J. (2015). The effect of chlorogenic acid on blood pressure: A systematic review and meta-analysis of randomized clinical trials. Journal of Human Hypertension, 29, 77–81.

PAGANINI STEIN, S.F.L., B. SCHMIDT, E.B. FURLONG, L.A. SOUZA-SOARES, M.C. SOARES, M.R. VAZ, A.L. MUCCILLO BAISCH. Vascular responses to extractable fractions of Ilex paraguariensis in rats fed standard and high-cholesterol diets. Biol. Res. Nurs. 7 (2005) 146-156.

PAP, N.; BESZÉDES, S., PONGRÁCZ, E., MYLLYKOSKI, L., GÁBOR, M., GYIMES, E.; HODÚR, C.; KEISKI, R. Microwave-assisted extraction of anthocyanins from black currant marc. Food and Bioprocess Technology, 6: 2666-2674, 2013.

PARK, M., Bae, J., Lee, D.S., (2008). Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytotherapy Research 22(11), 1446-1449.

PETERSON, J. J., DWYER, T. J., JACQUES, P. F., & MCCULLOUGH, M. L. (2012). Associations between flavonoids and cardiovascular disease incidence or mortality in European and US populations. Nutrition Reviews, 70, 491–508.

PHILLIPS, G. O.; OGASAWARA, T.; USHIDA, K. The regulatory and scientific approach to defining gum arabic (Acacia senegal and Acacia seyal) as a dietary fibre. Food Hydrocolloids, v. 22, p. 24-35, 2008.

PHILLIPS, G. O.; WILLIAM, P. A. Handbook of Hydrocolloids, 2nd edition, CRC Press, Boca Raton, FL, USA, 2000. 901p.

PIETTA, P. G. Flavonoids as antioxidants. Journal of Natural Products, v.63, p.1035-1042, 2000.

PILLAI, Dipin S., et al. "Microencapsulation of Garcinia cowa fruit extract and effect of its use on pasta process and quality." International Journal of Food Properties 15.3 (2012): 590-604.

PIMENTEL, G.D., F.S. LIRA, J.C. ROSA, A.V. CARIS, F. PINHEIRO, E.B. RIBEIRO, C.M. Oller do Nascimento, L.M. Oyama. Yerba mate extract (Ilex paraguariensis) attenuates both central and peripheral inflammatory effects of diet-induced obesity in rats. J. Nut. Biochem. 24 (2013) 809-818.

PIOVEZAN-BORGES, A.C.P., C. Valério-Júnior, I.L. Gonçalves, A.A. Mielniczki-Pereira, A.T. Valduga. Antioxidant potential of yerba mate (Ilex paraguariensis St. Hil.) extracts in Saccharomyces cerevisae deficient in oxidant defense genes. Braz. J. Biol. 76 (2016) 539-544.

POMILIO, A.B, Trajtemberg S, Vitale AA. High-performance capillary electrophoresis analysis of mate infusions prepared from stems and leaves of Ilex paraguariensis using automated micellar electrokinetic capillary chromatography. Phytochem Anal 2002; 13:235–41.

POSHADRI, A.; KUNA, A. Microencapsulation technology: a review. Journal of Research ANGRAU, v. 38, n. 1, p. 86-102, 2010.

PREMI, M.; Sharma, H. Effect of different combinations of maltodextrin, gum Arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick.

PROESTOS, C.; KOMAITIS, M. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT- Food Science and Technology, 41 (4), 652-659, 2008.

REGINATTO, F.H., ATHAYDE, M.L., GOSMANN, G., SCHENKEL, E.P., 1999. Methylxanthinesaccumulation in Ilex species – caffeine and theobromine in erva-mate (Ilexparaguariensis) and other Ilex species. J. Braz. Chem. Soc. 10, 443–446.

RIACHI, L., DE MARIA, C. Yerba mate: An overview of physiological effects in humans. Journal of Functional Foods 38 (2017) 308–320.

RIGON, R.T., NORENA, C.P.Z., 2016. Microencapsulation by spray-drying of bioactive compounds extracted from Blackberry (Rubus fruticosus). Journal of Food Science and Technology. 53(3): 1515-1524.

ROBERFROID, M. B. (2000). Chicory fructooligossacharides and the gastrointestinal tract. Nutrition, 16, 677-679.

RODRIGUEZ-MATEOS, A., VAUZOUR, D., KRUEGER, C. G., SHANMUGANAYAGAM, D., REED, J., CALANI, L., MENA, P., DEL RIO, D., & CROZIER, A. (2014). Bioavailability, bioactivity and impact on health of dietary

flavonoids and related compounds: An update. Archives of Toxicology, 88(10), 1803–1853.

ROSAS, J.C., GROSSO, C.R.F., ALDAPA, C.A.G., VARGAS, E.R., MARIN, M.L.R., ORTIZ, F.A.G., CORTEZ, R.N.F., 2017. Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food - A review. Food Res, Intern.102, 575-587.

SANE, P., VARMA, N., GANGULY, A., PIKAL, M., ALEXEENKO, A., BOGNER, R.H., (2017). Spatial variation of pressure in the lyophilization product chamber part 2: experimental measurements and implications for scale-up and batch uniformity. AAPS PharmSciTech 18(2), 369-380.

SANTOS, K.A. Estabilidade da erva-mate (*Ilex paraguariensis* St. Hill.) em embalagens plásticas. 2004. 109f. Dissertação de Mestrado em Tecnologia de Alimentos - Setor de Tecnologia, Universidade Federal do Paraná, Curitiba, PR.SCHENKEL, E.P.,

MONTANHA, J.A., GOSMANN, G., 1996. Triterpene saponins from mate, Ilex paraguariensis. Saponins Used in Foodand Agriculture, pp. 47–56.

SCHINELLA, G. R., TROIANI, G., DÁVILA, V., BUSCHIAZZO, P. M., &

TOURNIER, H. A. (2000). Antioxidant effects of an aqueous extract of Ilex paraguariensis. Biochemical and Biophysical Research Communications, 269, 357–360.

SCHINELLA, G., FANTINELLI, J. C., & MOSCA, S. M. (2005). Cardioprotective effects of Ilex paraguariensis extract: evidence for a nitric oxide-dependent mechanism. Clinical Nutrition, 24, 360e366.

SCHULDT, E. Z., BET, A. C., HORT, M. A., IANSSEN, C., MARASCHIN, M.,

CKLESS, K., & RIBEIRODO- VALLE, R. M. (2005). An ethyl acetate fraction obtained from a southern Brazilian red wine relaxes rat mesenteric arterial bed through hyperpolarization and NO-cGMP pathway. **Vascular Pharmacology**, 43, 62–68.

SCHWARTZ, S.J.; LORENZO, T.V. Chlorophylls in foods. Critical Reviews in Food Science and Nutrition, v.29, n.1, p.1-17, 1990.

SHAHIDI, FEREIDOON, AND XIAO-QING HAN. "Encapsulation of food ingredients." Critical Reviews in Food Science & Nutrition 33.6 (1993): 501-547.

SHARMA, R; SINGH, R.B (2010) Bioactive foods and nutraceutical supplementation criteria in cardiovascular protection. Open Nutraceuticals J 3:141–153.

SHI, Y *et al*. Characterization and emulsifying properties of octenyl succinate anhydride modified Acacia seyal gum (gum arabic). Food Hydrocolloids, v. 65, p. 10-16, 2007.

SILVA, P. I., STRINGHETA, P. C., TEÓFILO, R. F., & OLIVEIRA, I. R. N. (2013). Parameter optimization for spray-drying microencapsulation of jaboticaba (Myrciaria jaboticaba) peel extracts using simultaneous analysis of responses. Journal of Food Engineering, 117, 538–544.

SINHA, K.; SARA, P. D.; DATTA, S. Response surface optimization and artificial neural network modeling of microwave assisted natural dye extraction from pomegranate rind. Industrial Crops and Products. 37, 408-414, 2012.

SLAVIN, J. L., LLOYD, B. (2012). Health benefits of fruits and vegetables. Advances in Nutrition: An International Review Journal, 3, 506–516.

SLAVIN, J. L.; GREENBER, N. A. Partially hydrolyzed guar gum: clinical nutrition uses. Nutrition, v. 19, p. 549-552, 2003.

SPIGONI, V., MENA, P., FANTUZZI, F., TASSOTTI, M., BRIGHENTI, F., BONADONNA, R.C., RIO, D.D., CAS, A.D., 2017. Bioavailability of Bergamot (*Citrus bergamia*) flavanones and biological activity of their circulating metabolites in human pro-angiogenic cells. Nutrients. 9, 1328.

SRI, J. S.; SEETHADEVI, A.; PRABHA, K. S.; MUTHUPRASANNA, P.; PAVITRA, P. Microencapsulation: a review. International Journal of Pharma and Bio Sciences, v.3, n.1, p.509-531, 2012.

STANOGIAS G, Pearce GR. The digestion of fibre by pigs. 3. Effects of the amount and type of fibre on physical characteristics of segments of the gastrointestinal tract. Br J Nutr. 1985; 53(3): 537-48.

STEIN, F.L.P., SCHIMIDT, B., FURLONG, E.B., 2005. Vascular responses to extractable fractions of Ilex paraguariensis in rats fed standard and high-cholesterol diets. Biol. Res. Nurs. 7, 146–156.

STREIT, N. A. *et al*. Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (Ilex paraguariensis). Elsevier, v. 102, p. 560-564, 2007.

STREIT, N.M. *et al*. As clorofilas. Ciência Rural, v.35, n.3, p.748-755, 2005.

SUAVE, J.; DALL'AGNOL, E. C.; PEZZIN, A. P. T.; SILVA, D. A. K.; MEIER, M. M.; SOLDI, V. Microencapsulação: Inovação em diferentes áreas. Revista saúde e ambiente, v.7, n.2, p.12-20, 2006.

TAYLOR, A. H., and TAYLOR AH. "Encapsulation systems and their applications in the flavour industry." (1983).

TSUKUI, A.; REZENDE, C. M. Extração Assistida por Micro-ondas e Química Verde. Revista Virtual de Química, 6 (6), 1713-1725, 2014.

UMER, H.; NIGAM, H.; TOMBOLI, A. M.; NAINAR, M. S. M. Microencapsulation: Process, Techniques and Applications. International Journal of Research in Pharmaceutical and Biomedical Sciences, v.2, n.2, p.474-481, 2011.

VAZQUEZ, A., MOYNA, P. Studies on mate drinking. Journal of Ethnopharmacology. 18 (1986), 267-272.

VENTURINI, Cristina G., et al. "Co-encapsulation of imiquimod and copaiba oil in novel nanostructured systems: promising formulations against skin carcinoma." European Journal of Pharmaceutical Sciences 79 (2015): 36-43.

VIEIRA, M. A. (2009). Análise de hidrocarbonetos policíclicos aromáticos (HPAs) nas etapas do processamento da erva-mate (Ilex paraguariensis) e caracterização química dos resíduos da trituração para o desenvolvimento de produto. Tese de Doutorado, Federal University of Santa Catarina, Florianópolis, Brazil.

WATERHOUSE, D.S., WADHWA, S.S., WATERHOUSE, G.I.N., 2013. Spray-drying microencapsulation of polyphenol bioactives: A comparative study using different natural fibre polymers as encapsulants. Food Biopr. Technol. 6, 2376-2388.

WHISTLER, R. L. Exudate gums. In: (Ed). Industrial gums: Elsevier, 1993. p.309-339.

WILDMAN, R.E.C; KELLEY, M. (2007) Nutraceuticals and Functional Foods. In: Wildman RCE (ed) Handbook of nutraceuticals and functional foods, 2nd edn. Taylor and Francis, Boca Raton, pp 1–22

YU, S.I., S.W. YUE, Z. LIU, T. ZHANG, N. XIANG, H. FU. Yerba mate (Ilex paraguariensis) improves microcirculation of volunteers with high blood viscosity: a randomized, double-blind, placebo-controlled trial. Exp. Gerontol. 62 (2015) 14-22.

ZHANG, F., Khan, M.A., Cheng, H., Liang, L., et al. Co-encapsulation of α-tocopherol and resveratrol within zein nanoparticles: Impact on antioxidant activity and stability. Journal of Food Engineering. 247(2019): 9-18.

4. CHAPTER I

2. Article 1

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REVIEW ARTICLE

Phenolic Compounds Present in Yerba Mate Potentially Increase Human Health: A Critical Review

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Abstract

Yerba Mate (YM) is a food product derived from *Ilex paraguariensis* whose constituents obtained from its extract, mainly the phenolic fraction, have been linked to numerous health benefits, such as cardiovascular protection, weight reduction, glucose control, and gene modulation. However, evidences linking phenolic compounds (PC) intake and human health are still limited and often contentious. Several researches have shown that key PC elements are poorly absorbed in humans and exist predominantly as conjugates, which may not be bioactive but may play a crucial role when interacting with the gut microbiota (GM). As the intestine is the largest microorganism-populated organ in the human body, GM has been regarded as a "microbial organ", acting as a second genome for modulating the host's health phenotype. For this reason, the study of intestinal microbiota has received considerable attention in recent years. Its impact on the development of nutrition-related diseases must motivate broader researches on the interaction between YM's PC and GM regarding the production of metabolites that may influence human health. This review aimed to gather and assess the available information about how PC from YM may impact host metabolism and the immune system and GM.

Keywords Tea · Polyphenol · Antioxidant · Intestine · Stimulant

Introduction

The aging of the world's population is placing emphasis on the development of health-care policies and research methodologies to enhance nutrition and human health relationships. Bioactive compounds (BAC) of natural origin, which are secondary metabolites derived from seeds, food, and fermentation-based metabolic products, are currently on researchers focus [1]. Several factors, including food matrix, molecule size, environmental factors, and association with gastrointestinal material, can inhibit BAC bioavailability and absorption in host cell systems and target sites. As a result, the isolation of such natural BAC can result in promising multifunctional extracts that can be used in food applications to aid in health-promoting effects in host cell systems [2].

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For instance, the most common plant-based BAC from food are phenolic compounds (PC). Numerous health benefits associated to them have made the interest and demand for phenolic-rich foods, identified as preventive diets, increase. In addition, because of their antioxidant properties and mechanisms such as enzymatic activity modulation, cellular signaling, and gene expression, foods high in phenolic compounds have been linked to the prevention of several chronic diseases [3].

Nevertheless, the same emerging interest has been observed regarding yerba mate (YM) as a food product derived from *Ilex paraguariensis A. St. Hil.* (mate) whose constituents obtained from its extract, mainly its phenolic fraction, have been linked to numerous health benefits. In Brazil, Paraguay, Uruguay, and Argentina, it is usually consumed as a tea-like beverage [4].

It is estimated that among the countries with the largest consumption of YM, Uruguay has the greatest per capita (8–10 kg/hab/year); Argentina´s consumption is around 6.5 kg/hab/year and in southern Brazil it is ingested 3–5 kg/ hab/year [5]. Nowadays, YM products are also consumed in different countries, including Germany, Syria, and the United States for the production of energy drinks and teas.

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More recently, the consumption of YM products has been amplified to other countries such as Italy, France, Spain, Japan, Australia, Russia, and Korea since its taste and stimulant properties are very attractive [6]. In addition, the use of mate has already surpassed the tradition of infusion, starting to be used in the manufacture of cosmetics and in the pharmaceutical industry [7].

This plant is a rich source of several bioactive chemicals that apparently have their health effects influenced in a synergistic or complementary manner. Beyond that, it seems clear that the several benefits may not be exclusively related to a specific nutrient, but rather to the inter- action between them, the human body and the GM [8]. The interaction between GM and PC has been extensively addressed by a plethora of studies using animal models or *in-vitro* colonic models. Although the findings reveal that dietary PC increases the number of beneficial bacteria and antimicrobial activity against pathogenic bacteria on GM, the main elements of PC are poorly absorbed by animals, and are mostly present as non-active conjugates when in the bloodstream [9].

Therefore, in order to take advantage of the nutritional effects of BAC, improvements in the absorption rate of these components should be studied, at the same time as potential food sources rich in PC must be known in better details so that we can safely introduce them into our diet. GM modulation through dietary changes has proven to be a key to improvements on PC absorption by animals. Several aspects present in GM modulation, such as eating habits seem to be particularly important in defining its characteristics. Long-term diet may not only have a crucial effect on the human GM but also, slight diet changes may affect the species composition [10]. For instance, diets rich in PC are reported to change the nature of GM, which in turn, may metabolize phenolics into bioactive compounds, improving their regulatory bioavailability [11].

Despite several researches displaying interaction between GM and PC have already been published there are almost no studies on the effect of YM and its PC on the human GM. This is certainly a relevant topic that should be better investigated once YM is a potential source of PC even when compared with most beverages and food products already studied [12].

For this reason, to know better the impact of PC from YM on gut microbiota (GM) and human health, this review has gathered and assessed relevant papers selected from Science Direct, Scopus, Web of Sciences, PubMed, Scielo, and Google Scholar databases resulting in a total of 74 publications were selected, considering the novelty and the impact in the area of this review.

An Overview on Yerba Mate's Health Benefits

Over the last two decades, clinical trials have explored the use of YM in the prevention and treatment of a variety of health conditions [13]. Figure S1, of the supplementary material, shows the processing ow for YM products and details of the production processes in different countries.

Several authors have linked YM to a wide range of health benefits, including antioxidant capabilities [8, 14], vasodilator functions [15], gene modulation and DNA damage defense [16], hypoglycemic effects [17], anti-obesity and weight loss properties [13, 18], cardioprotective effects [19], cholesterol improvement [20] and thermogenic effects [21].

Heck and Mejia [4] described that YM extracts are especially rich in CGAs (ester formed by quinic acid, QA, and caffeic acid, CA). The hydrolytic products, QA and CA are significant as chemicals of high interest and present great commercial values. For instance, CA has demonstrated antioxidant capacity, with several mechanisms concerning metal ion chelation, inhibitory effects to some specific enzymes involved in free-radical generation and free-radical scavenging [22].

Studies *in vitro and in vivo* have presented a wide variety of biological activities of mono and di-caffeoylquinic acids, also found on YM extracts. Caffeoylquinic acids derivatives exhibited antioxidant capacity and anti-in ammatory activity [23], apoptosis-mediated cytotoxicity and α-glucosidase inhibitory effects [24], hypoglycaemic properties [25], anti- obesity effects, and lipid metabolism improvement [26]. Table S1, of the supplementary material, presents a compilation of studies suggesting some of YM benefficial health effects.

Different chemical components responsible for YM's health benefits have already been identified, such as organic acids, minerals, enzymes, vitamins, amino acids, xanthines, saponins, lignin, lutein, cellulose, and especially PC [27]. For instance, methylxanthines, the main stimulant com- pounds present in YM, have presented several biological properties, including peripheral vasoconstriction, central nervous system and myocardial stimulation, smooth muscle relaxation, neuroprotective, hypoglycemic, anti-inflammatory, diuretic, and cardioprotective effects, among other benefits [28].

Some studies have also linked the effects of YM health benefits regarding its antioxidant capacity and more recent global health outbreak. In general, the antioxidant capacity of food products is already known to be related to free radical neutralization by PC even though, their potential within the human body is still arguable [29]. However, the main antioxidant effect in YM seems to be primarily due to the PC in the extract, delocalizing electrons and forming intra- molecular hydrogen bonds [4, 19].

De Lima et al. [8] investigated the ability of YM to protect rat brain from chemically induced reactive oxygen species (ROS), glutathione balance disturbance, mitochondrial dysfunction, and lipid peroxidation. Glutathione depletion and mitochondrial dysfunction were both prevented by YM, and both benefits were associated to its ability to decrease ROS formation. Their results have also suggested that the preventive properties of YM may be due to a coordinated action amongst the numerous components in the extract, rather than just the phenolic fraction.

Augusti et al. [30] have very recently published a review on the utilization of dietary bioactive substances, such as PC, as a potential supplement to decrease COVID-19 symptoms. The synthesis of PC-derived postbiotics has been hypothesized to boost host's antioxidant and immune response against SARS-CoV-2 infection, along with GM remodeling.

Phenolic Compounds in Yerba Mate

In YM leaves, the PC fraction represents 7–10% of the dry weight. Its main PC fraction is composed by hydroxycinnamates, a family of esters formed mostly by QA and a plethora of distinct hydroxycinnamic acids, such as ferulic acid, *p*-coumaric acid, and CA accounting for up to 95% of the phenolic content. The remaining 5% of the PC fraction is composed by flavonols [31]. Among flavonoids found in Yerba Mate are rutin, quercetin 3-rhamnoside and 3-gluco- side, kaempferol 3-rhamnoside and 3-glucoside, and luteolin diglycoside [5].

CA is considered an important biosynthetic precursor representing the main hydroxycinnamic moiety, forming mono- and dicaffeoylquinic acid isomers and counting over 90% of the total PC, with 5-caffeoylquinic acid being the main hydroxycinnamate in YM [15, 31].

In detailed research, Mateos et al. [32] found 58 PC in YM, such as four isomers of ca eoyl-2,7-anhydro-3-deoxy- 2-octulopyranosonic acid, two isomers of tri methoxy cinnamoyl shikimic acid, di- and tri-methoxy cinnamoyl quinic acids and 4-sinapoyl quinic acid. In addition, 2-methylxanthines and 46 PC were also found. Alike the aforementioned ratio, in their study, hydroxy cinnamic acid derivatives and avonols represented 90 and 10% of YM's PC, respectively. Along with rutin (7.1–7.8%), 5-caffeoylquinic (21.1–22.4%), 4-caffeoylquinic (12.6–14.2%), 3-caffeoylquinic (26.8–28.8%), and 3,5-dicaffeoylquinic acids (9.5–11.3%) were the most abundant phenols, and caffeine was the major methylxanthine (90%) [33].

These phenolic compounds can also be obtained from numerous vegetable sources, although in different compositions and amounts when compared to those found in YM.

In particular, Meinhart et al. [12] analyzed the existence of CGAs in 89 plants infusions. They found these com- pounds in 93% of infusions, however, YM presented the greatest content of CGAs (52.6 mg in 100 mL), being an important source of this nutrient compared to other beverages and food products.

Likewise, according to Duarte and Farah [34], 100 mL of *chimarrão* has double the quantity of 3,4-dicaffeoylquinic acid, 15 times more 3,5-dicaffeoylquinic acid, and six times more 4,5-dicaffeoylquinic acid than the same volume of coffee. The values of 5- caffeoyilquinic acid in 100 ml of *chimarrão* are on average 100, 60, and 20 times higher than in the same amount of white, green, and black tea, respectively [35]. Similar results were obtained for *tererê* extract, which presented amounts 300, 100, and 50 times greater than white, green, and black tea infusions, respectively [35].

In addition, YM-based drinks produced 120 times more 5-caffeyolquinic acid than mountain tea and 15 times more than chamomile tea when aqueous extracts of YM and Mediterranean herbs were compared [36]. Infusions commonly consumed in South America, such as those prepared from macela (*Achyrocline satureioides*) and carqueja (*Baccharis trimera*) leaves, showed concentrations of dicaffeyolquinic acids isomers 100 times lower than those in *chimarrão* and *tererê* extracts [35]. Therefore, *chimarrão* and *tererê* are great alternative sources of CGAs.

Interaction Between PC and the GM

The human body provides a nutrient-rich environment for intestinal bacteria, and the microbiota, in its turn, per- forms essential functions not exerted by humans, such as the production of valuable nutrients, modulation of bile acid metabolism, intestinal cell barrier, and immune system regulation. The balance of gut bacteria has been associated with immunological fortification, prevention of autoimmune dis- orders and immune inflammation, and preservation of the integrity of the intestinal epithelium (which avoids permeation of pathogens and immune-triggering compounds into the blood circulation) [37–39]. Polyphenols might indirectly regulate these functions by modulating the composition and activity of this microbiota [38]. In addition, some polyphenols are involved with the immune system, mainly with immunoglobulin A [40].

It also plays an important role in breaking down original complex PC into phenolic metabolites that are absorbed in the small intestine region [41].

In its turn, the bioavailability and bioactive impact of PC and their metabolites seem to influence GM composition. For instance, dietary PC is able to increase the number of beneficial bacteria and antimicrobial activity against pathogenic bacteria, although most researches have been performed in animal models or *in-vitro* colonic models [42]. In fact, there is also a strong relationship between PC activity modifying GM and, consequently, impacting *Bacteroides/Firmicutes* balance. Several studies have shown the importance of this ratio since decreased values indicate lower index of insulin resistance, adiposity, and obesity [39]. This ratio changes across the lifetime. It is lower in the first years of life (0.4), rises in adulthood (10.9), and diminished during elderliness (0.6) [43].

Phenolic compounds are poorly absorbed by the stomach and the small intestine, being that the small intestine absorbs from 5 to 10% of the total phenolic intake [38, 39, 44–46]. The low absorption is due to molecular structure complex and polymerization of polyphenols, while free aglycones can be absorbed efficiently [44, 46]. Next, unabsorbed poly- phenols are carried to the large intestine, where are metabolized and biotransformed by the gut microbiota [38, 44, 45, 47]. Enzymes from gut microbiota degrade polyphenols into bioactive phenolic metabolites which might regulate the metabolic functions and composition of gut microbiota [38–40, 44]. Polyphenols are metabolized via dihydroxylation, glucosidase, esterase, demethylation, and decarboxylation, resulting in more simple phenolic structures by cleavage, hydrolysis, and reduction reactions [37, 46, 48]. Some of these metabolites produced have higher bioactivity and bioavailability than their precursors, such as simple phenolic acids and lactones [44–46, 49]. This way, the inter- action between polyphenols and gut microbiota promotes the production of active phenolic metabolites, which its turn results in the modulation of gut microbiota composition [38, 50]. Phenolic metabolites prompt the swap of the gut micro- biota population, usually favoring the growth of beneficial

over pathogenic gut microbiota [39, 47]. For this reason, phenolic compounds act as prebiotics [38, 40, 44, 47]. For instance, caffeic and ferulic acids act selectively, reducing the rates of growth of pathogens without disturbing benefficial microorganisms [39, 44]. In addition, caffeic and chlorogenic acids might reduce the fermicutes-bacteroidetes ratio of the gut microbiota [51]. Polyphenols are also related to the prevention of gut dysbiosis, caused by the imbalance of gut microbiota [38, 46]. In addition, gut bacteria produce short-chain fatty acids by fermentation of dietary fibers and resistant starch, which have several health benefits, such as supplying energy to intestinal epithelial cells, reducing inflammation, absorbing minerals, and maintaining the gut and immune homeostasis [45, 47].

According to Loo et al. [44], quercetin suppresses the development of *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, and Lactobacillus rhamnosus* at minimum inhibitory concentrations (MIC) ranging from 62.5 to 250 g mL^{-1}, however, it seems to suppress the

growth of *Bacteroides galacturonicus, Enterococus caccae, Lactobacillus* spp., *Ruminococcus gauvreauii, Bi dobacte rium catenulatum, and E. coli* at doses ranging from 4 to 50 g mL^{-1}.

Other studies have reported MIC of hydroxycinnamic acids (HCA) ranging from 125 to 1,000 μg mL^{-1} to strains of *S. aureus, E. coli, S. typhimurium, and L. rhamnosus* [44]. HCA on GM has also been reported to increase the lactic acid bacteria growth in the human intestine as a con- sequence of a high dose of CGAs. At the same time, a positive effect on the adhesion of probiotic bacteria such as *L. acidophilus* has been proved to be caused by CA presence [39].

As it can be seen, numerous studies have shown that PC modulate the microbial intestinal community through pre- biotic or antimicrobial action against pathogenic intestinal bacteria [52].

As a consequence, there has been an increase in the number of studies associating the antioxidant, anti-inflammatory, antiadipogenic, antidiabetic, cardioprotective, neuroprotective, and anticarcinogenic effects of phenolic-rich substances through interaction with GM, in recent years [53]. However, there are almost no published studies on the effect of YM and its PC on the human GM, and this is certainly a relevant topic that should be better investigated. On the other hand, different sources of PC have already been assessed regarding their beneficial impact on human GM.

For instance, Gil-Sánchez et al. [54] researched grape pomace, a wine product rich in fibers and PC, two elements of foods in which bioaccessibility involves the microbiota. In this study, the *in vitro* colonic digestion of grape pomace extracts was analyzed for the first time. From the release of the main bioaccessible phenolic metabolites of grape pomace extract, various benzoic, phenylacetic, and phenyl propionic acids have been identified. It was observed a significant increase in the amounts of acetic, propionic, and butyric acids posteriorly to enhanced feeding indicating microbial fermentative activity [54]. Moreover, most classes of bacteria increased during chronic feeding, with the highest increases for groups of Lactobacillus and Bacteroids.

Nash et al. [55] published an overview of recent studies in humans concerning the impact of PC from grape and red wine on GM. All studies confirmed the regulation of those ingested PC performed by the intestinal microbiota, through the increase in the number of phenolic metabolites found in blood, urine, ileal fluid, and fecal fluids. According to the authors, the consumption of grape and red wine-derived PC may modulate GM and lead to beneficial microbial ecology improving human health. In addition, GM has demonstrated to modulate grape and red wine PC, suggesting an important two-way relationship [55].

Ramírez-Pérez et al. [56] also demonstrated a two-way interaction where host metabolism may be affected by both microbial modifications of bile acids, either by altering bile acid receptors signals as well as the microbiota composition. It becomes increasingly clear that the GM of individuals may determine the health effects of PC and several other bioactive compounds.

Despite all these demonstrated bene ts for the regulation of GM, the observed limitation in animal absorption of key PC elements requires researches aimed at improving the bioaccessibility of bioactive chemicals derived from plant sources [9].

Approaches to Enhance the Bioavailability of Phenolic Compounds

Researches in the digestion processes of YM and other plants revealed a modification in the number of bioactive compounds after it passes through the several compartments of the gastrointestinal tract (GIT) due to enzymatic actions, metabolic activity of the GM and pH alterations [57]. Temperature and length of digestion may also influence the final qualitative and quantitative outcome. For instance, only one-third of all CGAs amount is absorbed in the small intestine while two-thirds reach the colon where they may be metabolized by the microbiota [58].

In accordance with this finding, Gómez-Juaristi et al. [59] evaluated the bioavailability of YM's PC in healthy humans. They found that aside from unmetabolized caffeoyl-, feruloyl-, and *p*-coumaroilquinic acids, more than 34 metabolites with quick onset and clearance in plasma have been discovered, implying small intestine absorption. These chemicals accounted for 13.1% of the metabolites found in urine. In addition to feruloylglycine, delayed absorption of dihydroca eic, dihydroferulic, and dihydrocoumaric acids and their phase II metabolites, accounting for 81.0% of excreted metabolites, revealed bacterial origin and intestinal absorption, suggesting that YM's PC are highly metabolized, mainly by the microbiota.

Furthermore, GM not only appears to be responsible for most of the PC metabolism, but it can also be modied through specific interventions in order to favorably affect human metabolism. Notwithstanding, GM must be first maintained so as to exert its main function properly. In this regard, pre-and probiotics may play an important role.

Prebiotics such as inulin, fructooligosaccharides, and galactooligosaccharides have demonstrated to improve intestinal permeability, decrease inflammation, and improve insulin control *in vivo* [60].

Probiotics as *Lactobacillus* spp. and *Bi dobacterium* spp. are equally beneficial to human health even when used

isolated. However, combinations of pre- and probiotics have suggested a better potential in the GM and host health than isolated consumption, since the combination of both components stimulate bacteria growth and survival in the gastrointestinal tract [61].

In addition, isolated nutrients are rarely consumed and for this reason, in recent years, science has been evaluating the ability of dietary and nutritional patterns to adjust GM in pathological conditions. It seems that a long-term adherence to a high- fiber, phenolic compounds-enriched and vegetable-protein-based diet may provide benefits to the GM com- position, as well as improvement in obesity and metabolic syndrome symptoms [41].

Diet has been shown to be a primary predictor of GM composition. Different degrees of *in vivo* scientific evidence support nutrition as a crucial component in GM modulation since certain foods, bioactive chemicals, and dietary pat- terns may impact health outcomes via their effect on the GM. In this context, it is crucial to understand how specific nutrients, such as PC may act in the modulation of the GM in order to clarify their action and effect in the human body. The discovery that food may have a significant impact on host-microbe interactions suggests that future treatment techniques to change the GM and reduce dysbiosis caused by nutrition-related disorders should be pursued [41].

Nowadays, dietary polyphenols have been used as an emerging therapy in the prevention of several diseases. For instance, the association between gut microbiota and polyphenols was related to the improvement of the signs of depression, mitigation of cognitive dysfunction, improvement in blood ow and vasodilation in cerebrovascular circulation, and acting as a neuronal protector due to diminishing neuroinflammation [62, 63], with immunomodulatory effect [40]. In addition, dietary polyphenols can prevent inflammatory processes, cardiovascular diseases, obesity, cancer, and type 2 diabetes [37, 46]. These properties were also reported for yerba mate, being yerba mate tea is recommended as dietary therapy [5, 33].

It is salient to promote investigations focusing on metagenomic, transcriptomic, and proteomic that help to comprehend the interaction between dietary polyphenols and gut microbiota, for this way, to know the genes and microorganisms that participate in the metabolism of these polyphenols, and thus, elucidate

how the dose and polyphenol compounds from yerba mate extract impact on the gut microbiome and the immune system [37].

Aside from the maintenance of GM, the extraction method used to obtain PC elements from plant sources has to be efficient and provide a high quantity of compounds in order to improve PC absorption by human GIT.

It is already known that different extraction conditions such as time, temperature, type of solvent, and concentration may affect PC composition. Conventional methods for bioactive compounds extraction can be an alternative to increase their bioavailability and include solvent maceration, direct boiling, distillation, compression, etc. [64], although, such processes are time-consuming and can lead to the degradation of thermolabile compounds. Traditional methods such as *Soxhlet* and maceration present numerous disadvantages, including the employment of large amounts of organic solvents that may be toxic and also harmful to the environment, in addition to their high consumption of energy and time [65].

The stability of bioactive compounds derived from natural sources is a crucial factor for their effective integration into various food systems. In this context, methods such as microwave-assisted extraction emerged as an alternative to reduce the exposure times of bioactive compounds to high temperatures, energy costs, and environmental degradation [65]. Ultrasound-assisted extraction represents another option for the acquisition of bioactive compounds, using acoustic energy to improve the release and diffusion of target compounds from several matrices [66].

Subsequently, as natural antioxidants are significantly sensitive to the environment, in order to have its efficacy improved, they may also be protected from the surrounding medium through several methods. Recent techniques such as encapsulation may be valuable options for this purpose. The encapsulation process packs particles with the assistance of an encapsulating material in order to protect internal compounds and their functionalities. Protective delivery vehicles may also permit targeted release in tissues such as the small intestine, in addition to encasing, protecting, and conveying the desired bioactive molecules into the circulatory system [67].

In particular, in the pharmaceutical and nutritional domains, the use of encapsulated micro and nanoparticles for efficient oral administration of biomolecules has been a growing trend. Modern bioactive carriers, which mainly apply natural dietary macromolecules as functional materials, are meant to increase bioactive component absorption, physicochemical stability, and bioavailability through several routes while not presenting safety or health risks [68]. Successful application of these bioengineered vehicles of food compounds can bring benefits beyond basic nutrition to human health.

Encapsulation may also represent an alternative to trans- form some of the product's characteristics, enhancing its appearance or avoiding unpleasant interactions with the carrier food matrix [67]. Phenolics having greater water solubility may be more readily liberated from the food matrix, dissolved in digestive juice, and absorbed by the small intestine mucosa during digestion. Hydrophobic molecules, on the other hand, are more likely to interact with other

food components like fibers and lipids, delaying or reducing absorption [69]. Several wall materials can be used for food encapsulation, such as fibers, proteins, and gums. However, depending on the structure and features of each encapsulating agent, the employment of multiple agents may result in varied physical attributes [70].

Several biocompatible and biodegradable polysaccharides have been designed into micro or nanoparticles in an attempt to address the absorption issues of PC. Cyclodextrins, cyclic oligosaccharides presenting a hydrophilic outer surface and a lipophilic inner chamber, are a viable choice. Likewise, chitosan is another type of polysaccharide positively charged often employed for entrapping hydrophilic molecules. Because of interactions with the negatively charged mucus layer, the chitosan-based particles promote absorption by facilitating the passage through the tight junctions [71].

Furthermore, dietary proteins such β-lactoglobulin, β-casein, gelatin, and isolated soy protein are appealing as macronutrients and functional components, making them suitable materials for carriers to efficiently

transport nutraceuticals. As can be seen, by the use of electrostatic inter- actions, proteins and polysaccharides may be designed to produce self-assembled particles [68].

In fact, encapsulated PC compounds have already demonstrated higher bioavailability and stability [72]. According to Berté et al. [73], spray-dried YM extract presented greater amounts of phenolic acids compared to the leaves. Becker et al. [74] assessed the antioxidant capacity and clinical effects of spray-dried YM extract capsules in healthy individuals. The ingestion of the capsules increased the antioxidant biomarkers while decreasing lipid peroxidation both in the short and long term.

Concluding Remarks

Ilex paraguariensis has been shown to have several health advantages. Although many of these bene ts are not yet fully established, multiple studies have shown that the plant has the potential to be a promising functional food product, mostly due to its phenolic component. Because the relationship with the GM is essential for PC metabolism, the final fraction of YM compounds ingested as well as their mode of action in the human body should be better understood.

In this context, authors should exercise caution in light of the abundance of repetitive and misleading information, since multiple poor research can dilute solid works. Inter- individual variation, on the other hand, emerges within rigorous research, such as varied responses to PC utilization depending on the individual. Before precise findings can be drawn, it is necessary to determine the probable interplaying elements surrounding the PC and GM interaction in human health. Also, because PC bioavailability and effects are frequently contested, it is critical to qualify the various YM products in terms of PC, as well as understand how different extractions and modes of consumption impact the degree of phenolic migration to water as well as absorption by the human body.

Subsequently, as natural antioxidants are significantly sensible, in order to have its efficacy improved, they may also be protected from the surrounding medium through several methods. Recent techniques such as encapsulation could be valuable options for this purpose. Protective or encapsulated delivery vehicles may also permit targeted release in tissues such as the small intestine, in addition to encasing, protecting, and conveying the desired bioactive molecules into the circulatory system [67].

Abbreviations

BAC Bioactive compounds
CGAs ester formed by quinic acid (QA) and caffeic

acid (CA)
DNA Deoxyribonucleic acid

GIT the gastrointestinal tract GM the gut microbiota HCA Hydroxycinnamic acids PC phenolic compounds ROS reactive oxygen species YM Yerba Mate

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Declarations

Conflict of Interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

1. Shetty K, Sarkar D (2019) Introduction: Metabolic-driven eco- logical rationale to advance biotechnological approaches for functional foods. In: Shetty K, Sarkar D (eds) Functional Foods

and Biotechnology, 1st edn. CRC Press, Florida, pp 1–4. https://

doi.org/10.1201/9781003003830

2. Banwo K, Olojede AO, Adesulu-Dahunsic AT, Verma DK, Thakur M, Tripathy S, Singh S, Patelh AR, Gupta AK, Agui- lar CN, Utama GL (2021) Functional importance of bioactive compounds of foods with potential health bene ts: A review on recent trends. Food Biosci 43:101320. https://doi.org/10.1016/j. fbio.2021.101320

3. Dragan M, Deval C, Dubray C, Mazur A, Morand C (2011) Hesperidin displays relevant role in the nutrigenomic e ect of orange juice on blood leukocytes in human volunteers: a random- ized controlled cross-over study. PLoS ONE e26669. https://doi. org/10.1371/journal.pone.0026669

4. Heck CI, Mejia EG (2007) Yerba mate tea (Ilex paraguariensis): A comprehensive review on chemistry, health implications, and technological considerations. J Food Sci 72:138–151. https://doi. org/10.1111/j.1750-3841.2007.00535.x

5. Gawron-Gzella A, Chanaj-Kaczmarek J, Cielecka-Piontek J (2021) Yerba Mate -A long but current history. Nutrients 13:3706. **https**://**doi**.**org**/**10**.**3390**/**nu13113706**

6. Meinhart AD, Bizzotto CS, Ballus CA, Rybka ACP, Sobrinho MR, Cerro-Quintana RS, Teixeira-Filho J, Godoy HT (2010) Methylxanthines and phenolics content extracted during the con- sumption of mate (Ilex paraguariensis St. Hil) beverages. J Agric Food Chem 58:2188–2193. https://doi.org/10.1021/jf903781w

7. Reichert CL, Friedrich JC, Cassol GH, Pensin CF, Mitsui ML, Donaduzzi CM, Cardozo-Junior EL (2013) Chemical stability and dissolution study of mate (Ilex paraguariensis St. Hil.) extract and some formulations. Int J Pharm Sci Technol 8:33–49

8. de Lima ME, Colpo AC, Maya-López M, Rosa H, Túnez I, Galván-Arzate S, Santamaría A, Folmer V (2017) Protective e ect of Yerba Mate (Ilex paraguariensis St. Hill.) against oxida- tive damage in vitro in rat brain synaptosomal/mitocondrial P2 fractions. J Funct Foods 34:447–452. https://doi.org/10.1016/j. j .2017.05.026

9. Cli ord MN, Hooft JJ, Crozier A (2013) Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. Am J Clin Nutr 98:1619S–1630S. https://doi. org/10.3945/ajcn.113.058958

10. Wu GD, Chen J, Ho mann C et al (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334:105– 108. https://www.science.org/doi/https://doi.org/10.1126/ science.1208344

11. Anhê FF, Choi BSY, Dyck JRB, Schertzer JD, Marette A (2019) Host-microbe interplay in the cardiometabolic bene ts of dietary polyphenols. Trends Endocrinol Metab 30:384–395. https://doi. org/10.1016/j.tem.2019.04.002

12. Meinhart AD, Caldeirão L, Damin FM, Teixeira Filho J, Godoy HT (2018) Analysis of chlorogenic acids isomers and ca eic acid in 89 herbal infusions (tea). J Food Compos Anal 73:76–82. https://doi.org/10.1016/j.jfca.2018.08.001

13. Kim SY, Oh MR, Kim MG, Chae HJ, Chae SW (2015) Anti-obe- sity e ects of Yerba Mate (Ilex Paraguariensis): a randomized, double-blind, placebo-controlled clinical trial. BMC Complement Altern Med 15:338. https://doi.org/10.1186/s12906-015-0859-1

14. Colpo A, Rosa H, Lima ME, Pazzini CEF, de Camargo VB, Bas- sante FE, Folmer V (2016) Yerba mate (Ilex paraguariensis St. Hill.)-based beverages: How successive extraction in uences the extract composition and its capacity to chelate iron and scavenge free radicals. Food Chem 209:185–195. https://doi.org/10.1016/j. foodchem.2016.04.059

15. Yu SI, Yue SW, Liu Z, Zhang T, Xiang N, Fu H (2015) Yerba mate (Ilex paraguariensis) improves microcirculation of vol- unteers with high blood viscosity: a randomized, double-blind, placebo-controlled trial. Exp Gerontol 62:14–22. https://doi. org/10.1016/j.exger.2014.12.016

16.Fernandes ES, Machado MO, Becker AM, Andrade F, Maraschin M, Silva EL (2012) Yerba mate (Ilex paraguariensis) enhances the gene modulation and activity of paraoxonase-2: In vitro and in vivo studies. Nutr 28:1157–1164. https://doi.org/10.1016/j. nut.2012.04.011

17. Ribeiro MC, Santos Â, Riachi LG, Rodrigues ACB, Coelho GC, Marcellini PS, de Maria CAB (2017) The e ects of roasted yerba mate (Ilex paraguariensis A. St. Hil.) consumption on glycemia and total serum creatine phosphokinase in patients with traumatic brain injury. J Funct Food 28:240–245. https://doi.org/10.1016/j. j .2016.11.024

18. Arçari DP, Bartchewsky W, Dos Santos TW, Oliveira KA, Funck A, Pedrazzoli J, De Souza MF, Saad MJ, Bastos DH, Gambero A, Carvalho PO, Ribeiro ML (2009) Antiobesity e ects of yerba maté extract (Ilex paraguariensis) in high-fat diet-induced obese mice. Obesity 12:2127–2133. https://doi.org/10.1038/ oby.2009.158

19. Schinella G, Fantinelli JC, Mosca SM (2005) Cardioprotec- tive e ects of Ilex paraguariensis extract: evidence for a nitric oxide-dependent mechanism. Clin Nutr 24:360–366. https://doi. org/10.1016/j.clnu.2004.11.013

20. Arçari DP, Porto VB, Rodrigues ERV, Martins F, Lima RJ, Sawaya ACHF, Carvalho PO (2011) E ect of mate tea (Ilex para- guariensis) supplementation on oxidative stress biomarkers and LDL oxidisability in normo and hyperlipidaemic humans. J Funct Food 3:190–197. https://doi.org/10.1016/j.j .2011.04.001

21. Martinet A, Hostettmann K, Schutz Y (1999) Thermogenic e ects of commercially available plant preparations aimed at treating human obesity. Phytomed 6:231–238. https://doi.org/10.1016/ S0944-7113(99)80014-2

22. Chen JH, Ho CT (1997) Antioxidant activities of ca eic acid and its related hydroxycinnamic acid compounds. J Agric Food Chem 45:2374–2378. https://doi.org/10.1021/jf970055t

23. Ooi KL, Muhammad TST, Tan ML, Sulaiman SF (2011) Cytotoxic, apoptotic and anti-α-glucosidase activities of 3,4- di-O-ca eoyl quinic acid, an antioxidant isolated from the polyphenolic- rich extract of Elephantopus mollis Kunth. J Ethnopharmacol 135:685–695. https://doi.org/10.1016/j.jep.2011.04.001

24. Ong KW, Hsu A, Song L, Huang D, Tan BKH (2011) Polyphe- nols-rich Vernonia amygdalina shows anti-diabetic e ects in streptozotocin-induced diabetic rats. J Ethnopharmacol 133:598– 607. https://doi.org/10.1016/j.jep.2010.10.046

25. Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK (2010) Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced obese mice. Food Chem Toxicol 48:937–943. https://doi.org/10.1016/j. fct.2010.01.003

26. Filip R, Sebastian T, Ferraro G, Anesini C (2007) E ect of Ilex extracts and isolated compounds on peroxidase secretion of rat submandibulary glands. Food Chem Toxicol 45:649–655. https:// doi.org/10.1016/j.fct.2006.10.014

27. Bastos DHM, Fornari AC, Queiroz YS, Torres EAFS (2006) Bioactive compounds content of Chimarrão infusions related to the moisture of yerba mate (Ilex paraguariensis) leaves. Braz Arch Biol Technol 49:399–404. https://doi.org/10.1590/ S1516-89132006000400007

28. Heck CI, Schmalko M, Gonzalez de Mejia E (2008) E ect of growing and drying conditions on the phenolic composition of mate teas (Ilex paraguariensis). J Agric Food Chem 56:8394– 8403. https://doi.org/10.1021/jf801748s

29. Petti S, Scully C (2009) Polyphenols, oral health and dis- ease: A review. J Dent 37:413–423. https://doi.org/10.1016/j. jdent.2009.02.003

30. Augusti PR, Conterato GMM, Denardin CC, Prazeres ID, Serra AT, Bronze MR, Emanuelli T (2021) Bioactivity, bio- availability, and gut microbiota transformations of dietary phenolic compounds: implications for covid-19. J Nutr Biochem97:108787. https://doi.org/10.1016/j.jnutbio.2021.108787

31. Piovezan-Borges ACP, Valério-Júnior C, Gonçalves IL, Miel- niczki-Pereira AA, Valduga AT (2016) Antioxidant potential of yerba mate (Ilex paraguariensis St. Hil.) extracts in Saccharo- myces cerevisae de cient in oxidant defense genes. Braz J Biol 76:539–544. https://doi.org/10.1590/1519-6984.01115

32. Mateos R, Baeza G, Sarriá B, Bravo L (2018) Improved LC-MSn characterization of hydroxycinnamic acid derivatives and avo- nols in di erent commercial mate (Ilex paraguariensis) brands. Quanti cation of polyphenols, methylxanthines, and antioxidant activity. Food Chem 241:232–241. https://doi.org/10.1016/j. foodchem.2017.08.085

33. Rzasa-Duran E, Kryczyk-Poprawa A, Drabicki D, Podkowa A, Sułkowska-Ziaja K, Szewczyk A, Kała K, Opoka W, Zieba P, Fidurski M, Muszynska B (2022) Yerba Mate as a source of ele- ments and bioactive compounds with antioxidant activity. Anti- oxidants 11: 371. https://doi.org/10.3390/antiox11020371

34. Duarte GS, Farah A (2011) E ect of simultaneous consumption of milk and co ee on chlorogenic acids' bioavailability in humans. J Agric Food Chem 59:7925–7931. https://doi.org/10.1021/ jf201906p

35. Marques V, Farah A (2009) Chlorogenic acids and related com- pounds in medicinal plants and infusions. Food Chem 113:1370– 1376. https://doi.org/10.1016/j.foodchem.2008.08.086

36. Kogiannou DAA, Kalogerolopoulos N, Kefalas P, Polissiou MG, Kalior AC (2013) Herbal infusions, their phenolic pro le, antioxidant and anti-in ammatory e ects in HT29 and PC3 cells. Food Chem Toxicol 61:152–159. https://doi.org/10.1016/j. fct.2013.05.027

37. Catalkaya G, Venema K, Lucini L, Rocchetti G, Delmas D, Daglia M, De Filippis A, Xiao H, Quiles JL, Xiao J, Capanoglu E (2020) Interaction of dietary polyphenols and gut microbiota: Microbial metabolism of polyphenols, in uence on the gut microbiota, and implications on host health. Food Front 1:109–133. https://doi. org/10.1002/ t2.25

38. Kasprzak-Drozd K, Oniszczuk T, Stasiak M, Oniszczuk A (2021) Bene cial e ects of phenolic compounds on gut microbiota and metabolic syndrome. Int J Mol Sci 22:3715. https://doi. org/10.3390/ijms22073715

39. Leonard W, Zhang P, Ying D, Fang Z (2021) Hydroxycinnamic acids on gut microbiota and health. Compr Rev Food Sci Food Saf 20:710–737. https://doi.org/10.1111/1541-4337.12663

40. Mas AL, Brigante FI, Salvucci E, Ribotta P, Martinez ML, Wun- derlin DA, Baroni MV (2022) Novel cookie formulation with defatted sesame our: evaluation of its technological and sensory properties. Changes in phenolic pro le, antioxidant activity, and gut microbiota after simulated gastrointestinal digestion. Food Chem doi. https://doi.org/10.1016/j.foodchem.2022.133122

41. Sánchez-Tapia M, Tovar AR, Torres N (2019) Diet as regulator of gut microbiota and its role in health and disease. Arch Med Res 50:259–268. https://doi.org/10.1016/j.arcmed.2019.09.004

42. Jamar G, Estadella D, Pisani LP (2017) Contribution of anthocy- anin-rich foods in obesity control through gut microbiota interac- tions. BioFactors 43:507–516. https://doi.org/10.1002/biof.1365

43. Duda-Chodak A, Tarko T, Satora P, Sroka P (2015) Interaction of dietary compounds, especially polyphenols, with the intesti- nal microbiota: A Review. Eur J Nutr 54:325–341. https://doi. org/10.1007/s00394-015-0852-y

44. Loo TY, Howell K, Chan M, Zhang P, Ng K (2020) Modulation of the human gut microbiota by phenolics and phenolic ber-rich foods. Compr Rev Food Sci Food Saf 19: 1268–1298. https://doi. org/10.1111/1541-4337.12563

45. Lee SY, Lee DY, Kang JH, Kim JH, Jeong WJ, Kim HW, Oh DH, Yoon SH, Hur SJ (2022) Relationship between gut micro- biota and colorectal cancer: Probiotics as a potential strategy for prevention. Food Res Int 156:111327. https://doi.org/10.1016/j. foodres.2022.111327

46. Davinelli S, Scapagnini G (2022) Interactions between dietary polyphenols and aging gut microbiota: A review. BioFactors 48:274–284. https://doi.org/10.1002/biof.1785

47. Zhang W, Qi S, Xue X, Naggar TA, Wu L, Wang K (2021) Under- standing the gastrointestinal protective e ects of polyphenols using foodomics-based approaches. Front Immunol 12:671150. https://doi.org/10.3389/ mmu.2021.671150

48. Selma MV, Espin JC, Tomas-Barberan FA (2009) Interaction between phenolics and gut microbiota: Role in human health. J Agric Food Chem 57:6485–6501. https://doi.org/10.1021/ jf902107d

49. Aravind SM, Wichienchot S, Tsao R, Ramakrishnan S, Chakkara- varthi S (2021) Role of dietary polyphenols on gut microbiota, their metabolites and health bene ts. Food Res Int 142:110189. https://doi.org/10.1016/j.foodres.2021.110189

50. Cueva C, Gil-Sánchez I, Moreno-Arribas MV, Bartolomé B (2016) Interactions between wine polyphenols and gut micro- biota. In: Moreno-Arribas MV, Bartolomé B (eds) Wine: safety, consumer preferences and impact on human health. Springer International Publishing, Switzerland, pp 259–278

51. Parkar SG, Trower TM, Stevenson DE (2013) Fecal micro- bial metabolism of polyphenols and its e ects on human gut microbiota. Anaerobe 23:12–19. https://doi.org/10.1016/j. anaerobe.2013.07.009

52. Ozdal T, Sela DA, Xiao J, Boyacioglu D, Chen F, Capanoglu E (2016) The reciprocal interactions between polyphenols and gut microbiota and e ects on bioaccessibility. Nutrients 8:78. https:// doi.org/10.3390/nu8020078

53. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary anti- oxidants in human health and disease. Oxid Med Cell Longev 2:270–278. https://doi.org/10.4161/oxim.2.5.9498

54. Gil-Sánchez I, Cueva C, Sanz-Buenhombre M, Guadarrama A, Moreno-Arribas MV, Bartolomé B (2018) Dynamic gastrointes- tinal digestion of grape pomace extracts: Bioaccessible phenolic metabolites and impact on human gut microbiota. J Food Compos Anal 68:41–52. https://doi.org/10.1016/j.jfca.2017.05.005

55. Nash V, Ranadheera CS, Georgousopoulou EN, Mellor DD, Pan- agiotakos DB, McKune AJ, Naumovski N (2018) The e ects of grape and red wine polyphenols on gut microbiota–A systematic review. Food Res Int 113:277–287. https://doi.org/10.1016/j. foodres.2018.07.019

56. Ramírez-Pérez O, Cruz-Ramón V, Chinchilla-López P, Mén- dez-Sánchez N (2017) The role of the gut microbiota in bile acid metabolism. Ann Hepatol 16:21–26. https://doi. org/10.5604/01.3001.0010.5672

57. Boaventura BCB, da Silva EL, Liu RH, Prudêncio ES, Di Pietro PF, Becker AM, Amboni RDDMC (2015) E ect of yerba mate (Ilex paraguariensis A St. Hil.) infusion obtained by freeze con- centration technology on antioxidant status of healthy individuals. LWT – Food Sci Technol 62:948–954. https://doi.org/10.1016/j. lwt.2015.02.028

58. Stalmach A, Steiling H, Williamson G, Crozier A (2010) Bioavail- ability of chlorogenic acids following acute ingestion of co ee by humans with an ileostomy. Arch Biochem Biophys 501:98–105. https://doi.org/10.1016/j.abb.2010.03.005

59. Gómez-Juaristi M, Martínez-López S, Sarria B, Bravo L, Mateos R (2018) Absorption and metabolism of yerba mate phenolic compounds in humans. Food Chem 240:1028–1038. https://doi. org/10.1016/j.foodchem.2017.08.003

60. Cani PD, Possemiers S, Van de Wiele T et al (2009) Changes in gut microbiota control in ammation in obese mice through a mecha- nism involving GLP-2-driven improvement of gut permeability. Gut 58:1091–1103. https://doi.org/10.1136/gut.2008.165886

61. Gordon JI (2012) Honor thy gut symbionts redux. Science 336:1251–1253. https://doi.org/10.1126/science.1224686

62. Frolinger T, Sims S, Smith C, Wang J, Cheng H, Faith J, Ho L, Hao K, Pasinetti GM (2019) The gut microbiota composition a ects dietary polyphenols mediated cognitive resilience in mice by modulating the bioavailability of phenolic acids. Sci Rep 9:3546. https://doi.org/10.1038/s41598-019-39994-6

63. Filosa S, Di Meo F, Crispi S (2018) Polyphenols-gut micro- biota interplay and brain neuromodulation. Neural Regen Res 13:2055–2059. https://doi.org/10.4103/1673-5374.241429

64. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano- Tixier AS, Abert-Vian M (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combi- nations, protocols and applications. Rev Ultrason Sonochem 34:540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035

65. Mandal V, Mohan Y, Hemalatha S (2007) Microwave assisted extraction - An innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev 1:7–18

66. Laborde JL, Bouyer C, Caltagirone JP, Gérard A (1998) Acous- tic cavitation eld prediction at low and high frequency ultra- sounds. Ultrasonics 36:581–587. https://doi.org/10.1016/ S0041-624X(97)00106-6

67. Kuang SS, Oliveira JC, Crean AM (2010) Microencap- sulation as a tool for incorporating bioactive ingredients into food. Crit Rev Food Sci 50:1913–1918. https://doi. org/10.1080/10408390903044222

68. Hu B, Liu X, Zhang C, Zeng X (2017) Food macromolecule based nanodelivery systems for enhancing the bioavailability of poly- phenols. J Food Drug Anal 25:3–15. https://doi.org/10.1016/j. jfda.2016.11.004

69. Zhao D, Simon JE, Wu Q (2020) A critical review on grape poly- phenols for neuroprotection: Strategies to enhance bioe cacy. Crit Rev Food Sci Nutr 60:597–625. https://doi.org/10.1080/104 08398.2018.1546668

70. Chen KN, Chen MJ, Liu JR, Lin CW, Chiu HY (2005) Optimi- zation of incorporated prebiotics as coating materials for probi- otic microencapsulation. J Food Sci 70:M260–M266. https://doi. org/10.1111/j.1365-2621.2005.tb09981.x

71. Peptu CA, Ochiuz L, Alupei L, Peptu C, Popa M (2014) Car- bohydrate based nanoparticles for drug delivery across biologi- cal barriers. J Biomed Nanotechnol 10:2107–2148. https://doi. org/10.1166/jbn.2014.1950

72. Fang Z, Bhandari B (2011) E ect of spray drying and storage on the stability of bayberry polyphenols. Food Chem 129:1139– 1147. https://doi.org/10.1016/j.foodchem.2011.05.093

73. Berté KAS, Beux MR, Spada PK, Salvador M, Ho mann-Rib- ani R (2011) Chemical composition and antioxidant activity of yerba-mate (Ilex paraguariensis A.St.-Hil., Aquifoliaceae) extract as obtained by spray drying. J Agric Food Chem 59:5523–5527. https://doi.org/10.1021/jf2008343

74. Becker AL, Cunha HP, Lindenberg AC, de Andrade F, de Carv- alho T, Boaventura BCB, da Silva EL (2019) Spray-Dried yerba mate extract capsules: Clinical evaluation and antioxidant poten- tial in healthy individuals. Plant Foods Hum Nutr 74:495–500. https://doi.org/10.1007/s11130-019-00764-4

Table S1. Compilation list of the most recent studies demonstrating the beneficial effects of Yerba Mate on health through a brief presentation of samples, measured activities, methods, results and references

Samples	Activity measured	Methods	Results	Reference
Extract of YM fruits	Antioxidant and hypolipemiant potential	Male Wistar rats, DPPH, blood tests	Reduced total cholesterol and triglycerides	Fernandez *et al.* [1]
Frozen concentrated Mate infusion	Antioxidant activity	31 healthy individuals / blood samples	Improvement on antioxidant parameters	Boaventura *et al.* [2]
YM extract	Antioxidant activity	NO, DPPH	Antioxidant capacity	Colpo *et al.* [3]
YM extract	Protective effect against oxidative damage	In vitro in rat brain	Prevention of oxidative damage and mitochondrial dysfunction	de Lima *et al.* [4]
Frozen-dried YM extract	Influence on lipid metabolism	30 Wistar rats in high-fat diet	Potential anti-obesity effect	de Rezende *et al.* [5]
YM extract	Anti-proliferative and cytotoxic effect	Human cell lines	Reduction on viability/ proliferation of cancer cells	Amigo-Benavent *et al.* [6]
Mate tea	Hepatoprotective effect	32 mice on high-fat diet	Anti-inflammatory effect / Prevented insulin increase / HDL decrease	Barroso *et al.* [7]
YM infusion	Antioxidant on aluminium toxicity. In Alzheimer's disease	*Caenorhabditis elegans* / Folin-ciocalteu's	Al elicits AD characteristics in AD / YM infusions modifies cholinergic nervous systems	Bortoli *et al.* [8]
YM extract	Effect on type 1 diabetes induced by *streptozotocin*	32 male Swiss mice (25-35 g)	Reduced hyperglycemia / normalised oxidative stress parameters / prevented peripheral neuropathy	de Lima *et al.* [9]
Exhausted Mate leaves	Antioxidant activity	ABTS, DPPH	High antioxidant potential	Gullón *et al.* [10]
Purified YM polysaccharide	Antioxidant, antimicrobial, cytotoxicity	ABTS, DPPH, MIC, MBCS, MFCS	Anti-inflammatory effect / Prevented insulin increase / HDL decrease	Kungel *et al.* [11]
YM infusion	Bioavailability of phenolic compounds	12 individuals / Urine samples / Metabolite	YM polyphenols partially bioavailable and partially metabolised	Gómez-Juaristi *et al.* [12]
YM leaves	Antioxidant activities of nanoparticles	Topical use in pig skin / DPPH	Topical antioxidant effect	dos Santos *et al.* [13]

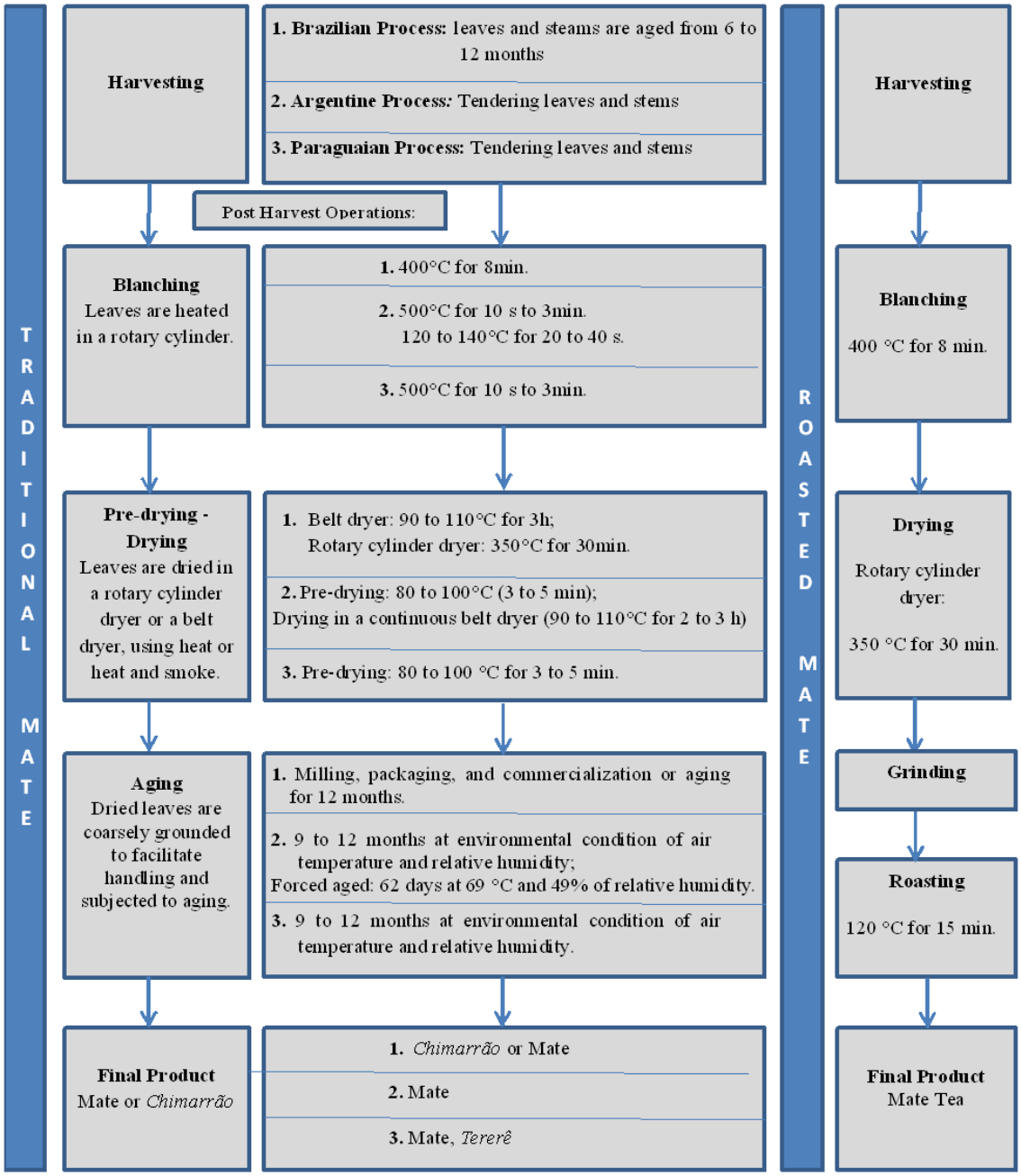

Figure S1: Process flow diagram for yerba mate in: Brazil (1) [14], Argentina (2) [15,16], and Paraguay (3) [17]. Adapted from Cardozo Junior and Morand [17]

References

1. Fernandes ES, Machado MO, Becker AM, Andrade F, Maraschin M, Silva EL (2012) Yerba mate (Ilex paraguariensis) enhances the gene modulation and activity of paraoxonase-2: In vitro and in vivo studies. Nutr 28:1157–1164. https://doi.org/10.1016/j.nut.2012.04.011
2. Boaventura BCB, da Silva EL, Liu RH, Prudêncio ES, Di Pietro PF, Becker AM, Amboni RDDMC (2015) Effect of yerba mate (Ilex paraguariensis A St. Hil.) infusion obtained by freeze concentration technology on antioxidant status of healthy individuals. LWT – Food Sci Technol 62:948–954. https://doi.org/10.1016/j.lwt.2015.02.028
3. Colpo A, Rosa H, Lima ME, Pazzini CEF, de Camargo VB, Bassante FE, Folmer V (2016) Yerba mate (Ilex paraguariensis St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. Food Chem 209:185–195. https://doi.org/10.1016/j.foodchem.2016.04.059
4. de Lima ME, Colpo AC, Maya-López M, Rosa H, Túnez I, Galván-Arzate S, Santamaría A, Folmer V (2017) Protective effect of Yerba Mate (Ilex paraguariensis St. Hill.) against oxidative damage in vitro in rat brain synaptosomal/mitocondrial P2 fractions. J Funct Foods 34:447-452. https://doi.org/10.1016/j.jff.2017.05.026
5. de Resende PE, Kaiser S, Pittol V, Hoefel AL, Silva RDA, Marques CV, Ortega GG (2015) Influence of crude extract and bioactive fractions of Ilex paraguariensis A. St. Hil. (Yerba Mate) on the Wistar rat lipid metabolism. J Funct Foods 15:440–451. https://doi.org/10.1016/j.jff.2015.03.040
6. Amigo-Benavent M, Wang S, Mateos R, Sarriá B, Bravo L (2017) Antiproliferative and cytotoxic effects of green coffee and yerba mate extracts, their main

hydroxycinnamic acids, methylxanthine and metabolites in different human cell lines. Food Chem Toxicol 106:125-138. https://doi.org/10.1016/j.fct.2017.05.019

7. Barroso MV, Graça-Reis A, Cattani-Cavalieri I, Gitirana LB, Valencia SS, Lanzetti M (2019) Mate tea reduces fat diet-induced liver and metabolic disorders in mice. Biomed Pharmacother 109:1577-1555. https://doi.org/10.1016/j.biopha.2018.11.007
8. Bortoli P, Kaizer R, Alves C, Costa E, Vanin AP, Sofiatti J, Siqueira D, Dallago R, Treichel H, Vargas G (2017) Ilex paraguariensis: Potential antioxidant on aluminium toxicity, in an experimental model of Alzheimer's disease. J Inorg Biochem. 181:104-110. https://doi.org/10.1016/j.jinorgbio.2017.11.001
9. de Lima ME, Colpo AZ, Rosa H, Salgueiro ACF, da Silva MP, Noronha DS, Folmer V (2018) Ilex paraguariensis extract reduce blood glucose, peripheral neuropathy and oxidative damage in male mice exposed to streptozotocin. J Funct Foods. 44:9-16. https://doi.org/10.1016/j.jff.2018.02.024
10. Gullón B, Eibes G, Moreira MT, Herrera R, Labidi J, Gullón P (2018) Yerba mate waste: A sustainable resource of antioxidant compounds. Ind Crop Prod 113:398-405. https://doi.org/10.1016/j.indcrop.2018.01.064
11. Kungel PT, Correa VG, Corrêa RC, Peralta RA, Sokovic´ M, Calhelha RC, Peralta RM (2018) Antioxidant and antimicrobial activities of a purified polysaccharide from yerba mate (Ilex Paraguariensis). Int J Biol Macromol 114:1161-1167. https://doi.org/10.1016/j.ijbiomac.2018.04.020
12. Gómez-Juaristi M, Martínez-López S, Sarria B, Bravo L, Mateos R (2018) Absorption and metabolism of yerba mate phenolic compounds in humans. Food Chem 240:1028-1038. https://doi.org/10.1016/j.foodchem.2017.08.003
13. dos Santos LP, Caon T, Battisti MA, da Silva CHB, Simões CMO, Reginatto FH, de Campos AM (2017) Antioxidant polymeric nanoparticles containing standardized

extract of Ilex paraguariensis A. St.-Hil. for topical use. Ind Crop Prod 208:738-747. https://doi.org/10.1016/j.indcrop.2017.07.035

14. Esmelindro MC, Toniazzo G, Waczuk A, et al (2022) Caracterização físico-química da erva-mate: Influência das etapas do processamento industrial. Ciênc Tecnol Aliment 22:193–204. https://doi.org/10.1590/S0101-20612002000200016
15. Schmalko ME, Alzamora SM (2001) Color, chlorophyll, caffeine, and water content variation during yerba mate processing. Dry Technol 19:599–610. https://doi.org/10.1081/DRT-100103937
16. Valerga J, Reta M, Lanari MC (2012) Polyphenol input to the antioxidant activity of yerba mate (Ilex paraguariensis) extracts. LWT-Food Sci Technol 45:28–35. https://doi.org/10.1016/j.lwt.2011.07.022
17. Cardozo Junior E, Morand C (2016) Interest of mate (Ilex paraguariensis A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review. J Funct Foods 21:440–454. https://doi.org/10.1016/j.jff.2015.12.010

5. CHAPTER II

Manuscript 2

Recent overview on the relationship of Yerba Mate (*Ilex paraguariensis* A. St. Hil), diet and Alzheimer's Disease

Running title: The chemical properties of yerba mate (Ilex paraguariensis A. St. Hil) and Alzheimer's disease

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Abstract

Yerba Mate (YM) is a food product derived from *Ilex paraguariensis*, a tree of the aquifoliaceae family, originated from South America. The association between its stimulating effect and its potential antioxidant function has created an increasing market with a growing presence in the world. Different chemical components responsible for YM's health benefits have been identified such as phenolic compounds, organic acids, xanthines, saponins, and cellulose. Studies have shown several health effects related to this plant, such as cardiovascular and bone protection, weight reduction, diabetes and

insulin control, cholesterol improvement, thermogenic effects, gene modulation and an important antioxidant activity, which may explain most of the benefits. More recently, diseases such as Alzheimer have also been positively connected to YM consumption. A diet high in simple carbohydrates and saturated fatty acids is a known environmental risk factor for Alzheimer's disease (AD), but a comprehensive understanding of the interconnected mechanisms by which such a diet may contribute to AD pathogenesis is lacking. The present article aims to conduct a review of the recent scientific literature, in addition to pointing out issues in need of further evaluation along with new perspectives to this functional food product and its relationship with AD.

Keywords: tea, infusion, antioxidant, stimulant, phenolics, Alzheimer disease.

Introduction

Ilex paraguariensis A. St. Hil. (mate) is an Aquifoliaceae green tree originally from South America. After raw mate leaves and branches are industrially processed, Yerba Mate (YM) is obtained as the main product, reaching consumers in several countries around the world, being mainly consumed as energy drinks and teas, as well as cosmetics, and in the pharmaceutical industry. Their central reason for being so attractive is the taste and stimulant properties [1, 2].

However, the health benefits of Yerba Mate have been widely described in recent research, showing the ability of this herb to aid in the treatment of numerous cardiovascular and neurodegenerative diseases and to promote systemic improvement in the health of patients who make regular use of this plant.

The main factors responsible for this significant biological activity are the compounds present in the extracts of YM, namely, xanthines, phenolics, flavonols, minerals and other compounds present in smaller quantities. More than that, phenolic

compounds have been linked to interactions with the gut microbiota, promoting changes that minimize dysbiosis and promote a decrease in the risk of neuroinflammation processes, a likely precursor of diseases such as AD [3].

Nevertheless, a complete understanding of the mechanisms by which AD develops and how to prevent it is lacking. In this work, we bring in a consistent and comprehensive way, the main directions received from recent research showing a clear potential of Yerba Mate to assist the treatment and prevention of AD.

Alzheimer Disease (AD)

In 2015, there were roughly 47 million dementia sufferers worldwide, and by 2050, there are expected to be 152 million (World Health Organization, 2020). Notedly, in Europe, there were 9.8 million dementia sufferers as of 2018, and that number is expected to rise to 11.4 million by 2025 and 18.8 million by 2050 [4]. In fact, dementia is a real and current concern worldwide, and, although it is a general term for an irreversible neurological condition, most of the cases are frequently caused by Alzheimer's disease (AD) [5].

Memory loss, cognitive impairment, and changes in personality and behavior are some of the clinical manifestations of AD neurodegeneration, which begins in the temporal region of the brain, in the hippocampus, and spreads to other brain areas. According to Aisen et al. [6] and Alzheimer's disease facts and figures 2021, Alzheimer's disease (AD) gradually progresses to the prodromal stage in patients with mild cognitive impairment (MCI), and then to mild, moderate, and finally severe dementia. This is reflected in an increasing degree of inability to perform daily activities.

In addition to genetic factors, conjectures such as the cholinergic, mitochondrial cascade and neurovascular hypothesis, oxidative stress, calcium dyshomeostasis and lymphatic system impairment may act together on AD development, in a multifactorial process driven by complex molecular mechanisms [7]. Likewise, bacterial lipopolysaccharide (LPS), a pro-inflammatory component of gram-negative bacteria's cell walls, has been found in the brain of AD patients. It is hypothesized that LPS has the ability to cause systemic inflammation and amyloid accumulation, which could result in the deposition of amyloid [8, 9].

Furthermore, growing evidences suggest that modifiable environmental factors, such as an imbalanced diet and physical inactivity, have a significant role in the development of AD by generating conditions like diabetes, obesity, hypercholesterolemia, or hypertension. Nearly 40% of all risk variables for AD are modifiable [10], and they have the potential to significantly influence how the preclinical stage develops in asymptomatic persons through midlife.

Tables 1 and 2 contain information about what is new in terms of potential treatments and findings about the mechanism by which neurodegenerative diseases such as Alzheimer's, Parkinson's and Mild Cognitive Impairment evolve.

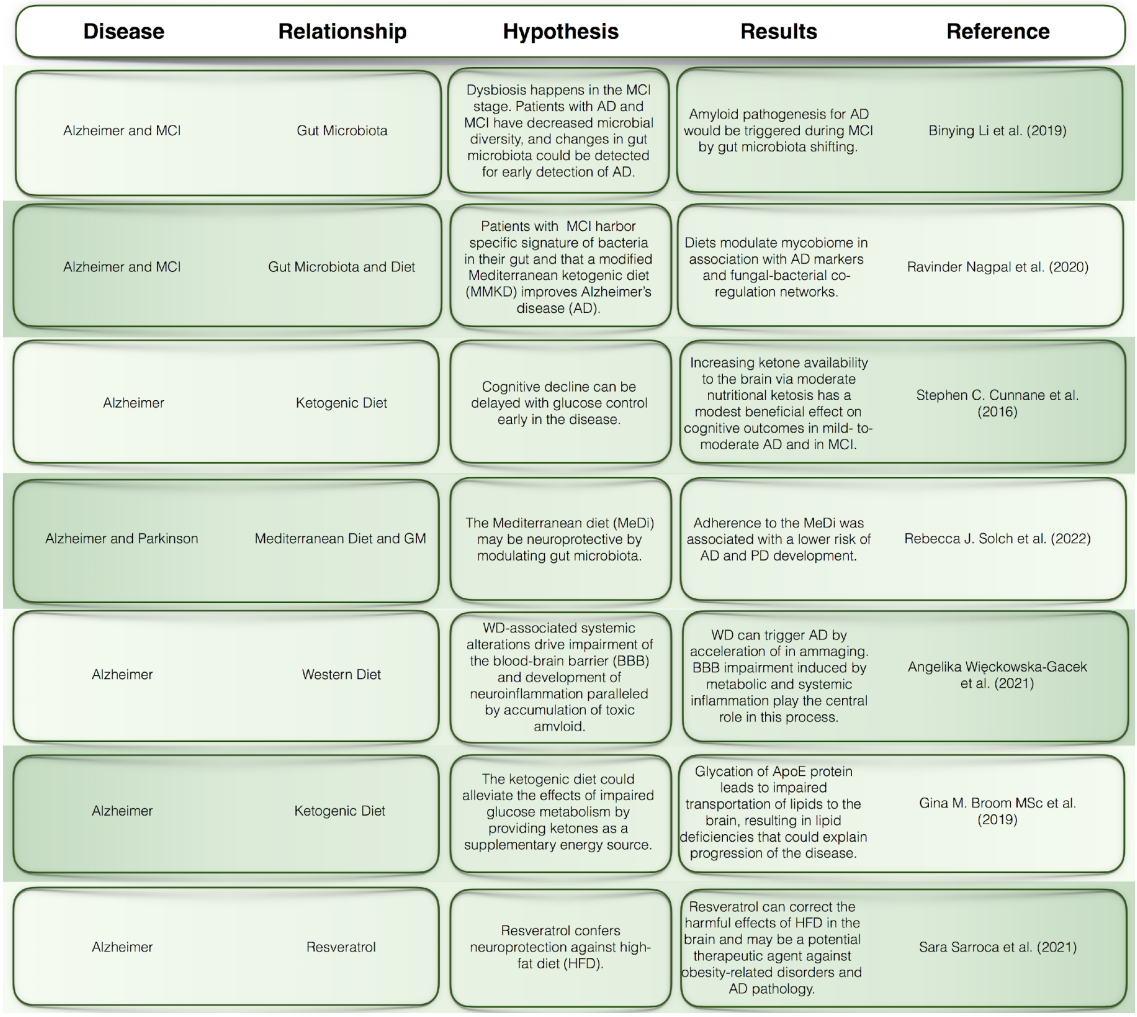
The World Health Organization (WHO), in 2019, strongly encouraged emphasis on AD prevention through changes in modifiable risk factors, such as food. This is because prevention is better than cure and because age, genetic background, and family history cannot be changed. In other words, in absence of curative treatments, emphasis should be placed on prevention measures like alterable lifestyle choices, especially food [11].

Designing a good nutritional plan, however, necessitates a better, holistic understanding of the cross talk of body organs, cells, and signaling pathways connecting

poor diet with the development of AD, especially in light of the complexity and dynamic aspects of AD development [12].

Notwithstanding the direct impact of imbalanced diet and physical inactivity on AD emergence, an imbalance in the microbial ecology, has also been hypothesized as a mechanism for neuroinflammation leading to AD [13]. In fact, recent data point to the importance of the gut microbiome in the pathogenesis of neurological disorders [14, 15, 16, 17, 18, 19, 20]. Although, whether and how the gut microbiota affects the host's cognitive function is still unknown, diverse aspects of host health and disease have been linked to the gut-brain axis, an integrated network where the central nervous system and microbiome interact via endocrine, immunological, and neurological signaling pathways [21].

Table 1. Potential treatments and recent findings as to Alzheimer's, Parkinson's and their relationship with Diet, Gut Microbiota and Phenolic Compounds.



Disease	Relationship	Hypothesis	Results	Reference
Alzheimer and MCI	Gut Microbiota	Dysbiosis happens in the MCI stage. Patients with AD and MCI have decreased microbial diversity, and changes in gut microbiota could be detected for early detection of AD.	Amyloid pathogenesis for AD would be triggered during MCI by gut microbiota shifting.	Binying Li et al. (2019)
Alzheimer and MCI	Gut Microbiota and Diet	Patients with MCI harbor specific signature of bacteria in their gut and that a modified Mediterranean ketogenic diet (MMKD) improves Alzheimer's disease (AD).	Diets modulate mycobiome in association with AD markers and fungal-bacterial co-regulation networks.	Ravinder Nagpal et al. (2020)
Alzheimer	Ketogenic Diet	Cognitive decline can be delayed with glucose control early in the disease.	Increasing ketone availability to the brain via moderate nutritional ketosis has a modest beneficial effect on cognitive outcomes in mild- to-moderate AD and in MCI.	Stephen C. Cunnane et al. (2016)
Alzheimer and Parkinson	Mediterranean Diet and GM	The Mediterranean diet (MeDi) may be neuroprotective by modulating gut microbiota.	Adherence to the MeDi was associated with a lower risk of AD and PD development.	Rebecca J. Solch et al. (2022)
Alzheimer	Western Diet	WD-associated systemic alterations drive impairment of the blood-brain barrier (BBB) and development of neuroinflammation paralleled by accumulation of toxic amyloid.	WD can trigger AD by acceleration of in ammaging. BBB impairment induced by metabolic and systemic inflammation play the central role in this process.	Angelika Więckowska-Gacek et al. (2021)
Alzheimer	Ketogenic Diet	The ketogenic diet could alleviate the effects of impaired glucose metabolism by providing ketones as a supplementary energy source.	Glycation of ApoE protein leads to impaired transportation of lipids to the brain, resulting in lipid deficiencies that could explain progression of the disease.	Gina M. Broom MSc et al. (2019)
Alzheimer	Resveratrol	Resveratrol confers neuroprotection against high-fat diet (HFD).	Resveratrol can correct the harmful effects of HFD in the brain and may be a potential therapeutic agent against obesity-related disorders and AD pathology.	Sara Sarroca et al. (2021)

Table 2. Potential treatments and recent findings as to Alzheimer's, Parkinson's and their relationship with Diet, Gut Microbiota and Phenolic Compounds (continuation).

Disease	Relationship	Hypothesis	Results	Reference
Alzheimer and Parkinson	Olive leaves phenolic compounds	Olive leaves phenolic compounds have neuroprotective effects against neurodegenerative disorders.	Agreement on the great impact of olive tree leaves phenolic compounds on many metabolic syndromes as well as on the most prevalent neurodegenerative diseases	Fatma Hadrich et al (2022)
Alzheimer	Polar phenolic compounds	Natural polar phenolic compounds have the capacity to abolish Alzheimer's disease-associated pathogenic effects of apoE4 forms.	Natural compounds could modify the structure of apoE4 forms and ameliorate AD-related pathogenic effects of apoE4 forms.	Christina Mountaki et al. (2021)
Alzheimer	Phenolic compounds	Phenolic Compounds Prevent Alzheimer's Pathology through Different Effects on the Amyloid- Aggregation Pathway.	Oral administration of phenolic compounds prevented the development of AD pathology by affecting different A aggregation pathways *in vivo*	Tsuyoshi Hamaguchi et al. (2009)
AD, oxidative stress, melasma and Hyperglycemia	Phenolics and Stachys critic subsp. T. Smyrnaea	Antioxidant activity of S. cretica subsp. Smyrnaea and key enzymes inhibitory potential of the herb is positively associated with Alzheimer's disease, hyperglycemia, and hyperpigmentation	Promising antiradical activity and metal ion reducing power. *S. cretica* manifested strong antityrosinase and extraordinary anti-amylase and anti-glucosidase effects.	Mir Babak Bahadori et al. (2019)
Alzheimer	Multi-targets therapeutics	Multi-target-directed ligands (MTDLs) strategy and multi-target compounds with/ without ChEs based on natural active products and active fragments as the promising strategy to treat AD.	MTDLs strategy serves as the best pharmacological option for tackling the multifactorial nature of AD and for halting the progression of the disease.	Zhipei Sang et al. (2022)
Alzheimer	Herbal medicines	Neuroprotective potential of *Scutellaria baicalensis*, *Ginkgo biloba*, *Hypericum perforatum*, *Curcuma longa*, *Lavandula angustifolia*, *Trigonella foenum-graecum* and *Rosmarinus of cinalis*.	Due to the presence of flavonoids, phenolic acids, curcuminoids and phenolic diterpenoids all extracts were able to scavenge free radicals and were moderate glycogen synthase kinase-3β inhibitors.	Filipe Fernandes et al. (2022)
Epilepsy	Yerba Mate	Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate on pentylenetetrazol-induced seizures in Wistar rats.	YM provides new perspectives for therapeutic approaches with natural compounds to reduce the convulsions' frequency and to minimize the neuronal damage associated with recurrent seizures.	Cátia dos Santos Branco et al. (2013)
Alzheimer	Yerba Mate	Potential antioxidant on aluminium toxicity in an experimental model of Alzheimer's disease using strains of Caenorhabditis elegans.	The extract protected against the Al toxicity through of an upregulated the AChE.	Pâmela M. Bortoli (2018)

For this reason, maintaining an eubiotic gut environment may be crucial for healthy brain aging, as well as to maintain intestinal barrier function and permeability. Consuming a diet high in fiber and phenolics may reduce gut dysbiosis by boosting the number of healthy gut bacteria, once fiber is the primary energy source for the gut microbiota [14].

Roseburia and *F. prausnitzii,* for instance, two bacteria that produce short chain fatty acids (SCFA), become less prevalent on diets deficient in fiber [22]. Following a diet rich in phenolics and fiber may support the maintenance of gut eubiosis by modulating these bacteria similarly [23].

In addition, a buildup of phenolics in the colon promotes microbial fermentation and the expansion of helpful microorganisms like *Lactobacillus* while reducing the number of genera that contain pathogens like *Clostridium, Shigella, and Escherichia* [24, 25, 26].

That is where the phenolic compounds (PC) from YM play an important role. The interaction between GM and PC has been extensively addressed by a number of studies using animal models or in-vitro colonic models. The findings reveal that dietary PC increases the number of beneficial bacteria and antimicrobial activity against pathogenic bacteria on GM [27].

Furthermore, among the health benefits of YM compounds, its antioxidant potential may help to decrease the risk of neurodegenerative diseases related to oxidative process, such as Alzheimer's disease (AD) [28]. However, the plant present high aluminum (Al) fixation and naturally grows on soils with acidic pH, where Al ions are easily solubilized as basic salts [29]. Al demonstrates high biological reactivity providing a wide range of potential binders able to induce neurotoxicity [30] and its consumption has been correlated to neurological and behavioral pathologies, such as Parkinson and Alzheimer [28].

Nevertheless, Bortoli et al. [28] have observed that long-term exposure to mate and aluminum ions provided an increase in the acetylcholinesterase (AChE) activity (which hydrolyses acetylcholine, neurotransmitter molecule that acts on the passage of the nerve impulse from the neurons to the muscle cells) in *Caenorhabditis elegans* strains. This result has been generated as a response to the selective loss of cholinergic neurons, characteristic of AD [31], and imply that YM extract is able to protect neurons against the Al toxicity through an upregulation of AChE. Likewise, research conducted by Exley *et al*. [30] evidenced that the brain toxicity of Al will only manifest at critical or high daily exposure to this metal.

More recently a plethora of studies provided evidence that phenolic compounds play an important role in the control of AD. For instance, compounds such as quercetin, kaempferol and gallic acid (found in most YM extracts) revealed anti-AChE activity, which might be attractive to applications in AD treatments due to their neuroprotective effect [32]. These phenolic compounds and flavonoids together with rutin and luteolin (also found in YM extracts) have been extensively studied due to their antioxidant, anti-inflammatory and neuroprotective effect on the Central Nervous System (CNS). Not only have they been assigned to treatment of AD, but also in another neurodegenerative disease, Parkinson [33]. Figure 1 shows the anti-AChE activity of phenolic compounds found in three different herbs.

All this evidence provides the necessary arguments for further studies on the impact of phenolic compounds from YM in the treatment of AD and other neurodegenerative diseases

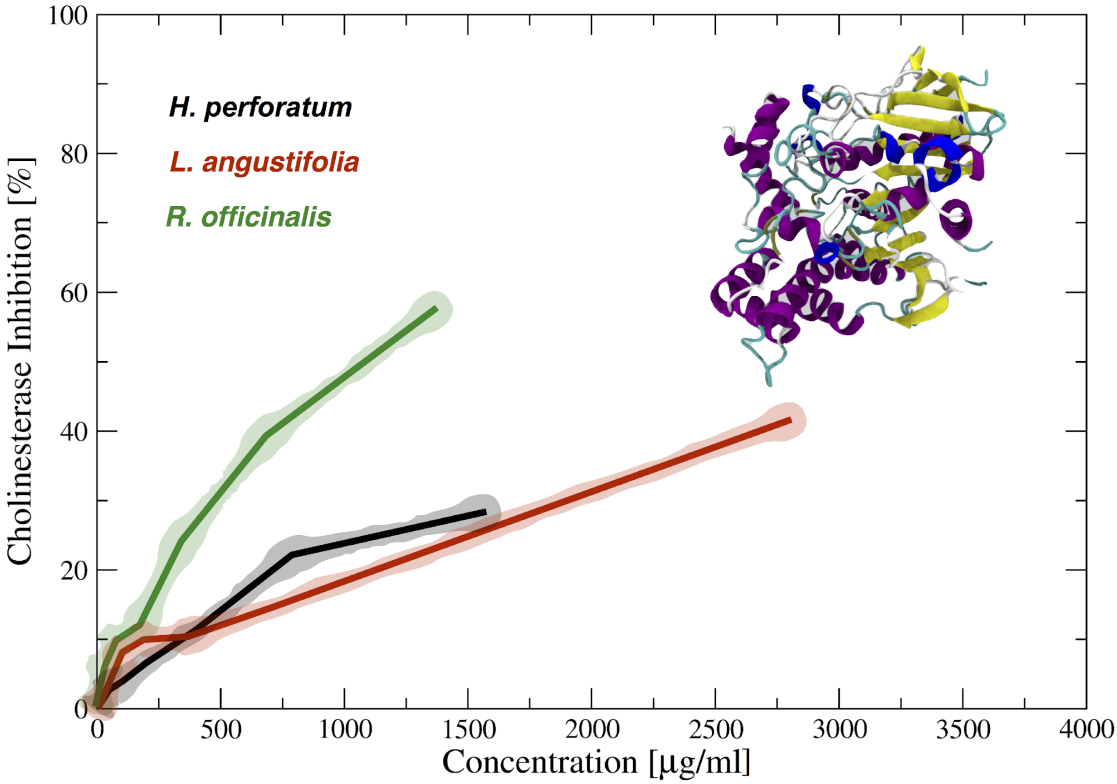


Figure 1. Anti-AChE activity of phenolic compounds found in *Hypericum perforatum, Lavandula angustifolia* and *Rosmarinus officinalis*. Data from Fernandes et al.,[37].

Yerba mate beneficial health effects

In the past twenty years, clinical trials have exploited the utilization of YM for the prevention and treatment of numerous diseases [34]. Studies have also linked YM to a large number of health benefits among its antioxidant properties [35], vasodilator functions [36], gene modulation and protection against induced DNA damage [37], cardioprotective effects [38], cholesterol improvement [39], hypoglycemic effects [40], anti-obesity and weight loss properties [41], and thermogenic effects [42] among other already known benefits.

Nevertheless, YM extracts are also rich on minerals (**Table 3**), and it seems that its phenolic compounds (5-caffeoylquinic acid and 3,5 dicaffeoylquinic acid) and

metabolites (ferulic acid, caffeic acid, dehydrocaffeic acid, dehydroferulic acid) may reduce cancer cell proliferation and viability in carcinoma cells in humans [43, 44].

Table 3. Minerals present on 900 ml of YM leaf infusions and Chimarrão [45].

Minerals	YM leaf infusion [mg]	Tea mate [mg]
P	24.00	12.00
K	79.80	49.00
Ca	7.90	3.40
Mg	51.20	18.90
Cu	249.00	183.50
Mn	0.98	0.90
Fe	0.83	0.75
Zn	0.15	0.12
Na	6.23	5.40
B	0.08	0.07

Many of the biological activities of mate are related to the presence of caffeoylquinic acids, especially 5-caffeoylquinic acid (5- CQA) [46]. Fortunately, the main phenolic compounds present in YM are derivatives of caffeoyl such as chlorogenic, 3,5-dicaffeoylquinic, 4,5-dicaffeoylquinic, 3,4-dicaffeoylquinic and caffeic acids [47].

For instance, chlorogenic acids (CGA) present in YM extracts are widely distributed in plant materials as they are derived from hydroxycinnamic acid esters (HCAs) with quinic acids (QA). **Figure 2** shows the chemical structures of these compounds. They, although neglected, have high bioavailability, are dietary compounds

and their hydrolytic products, QA and caffeic acid (CA), are significant as chemical products of high interest with great commercial value.

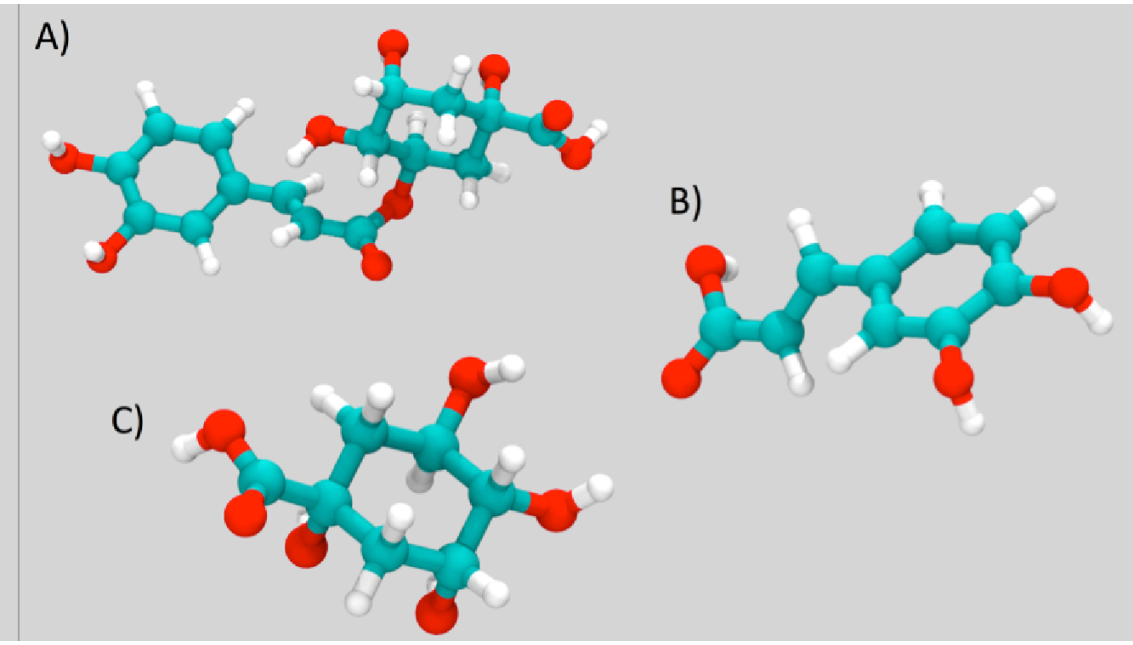


Figure 2. 3D representation of the chemical structure of Chlorogenic Acid (A), Hidrocinnamic Acid (B) and Quinic Acid (C). Oxygen atoms are represented in red, while carbon atoms in green and hydrogen in white.

Antioxidant Activity

The generation of reactive oxygen species (ROS) is a physiological process originated by the aerobic metabolism. However, excessive production of ROS can cause an imbalance in the proportion of oxidant and antioxidant molecules processed by antioxidant defense system, leading to a condition called oxidative stress. In this context, when the antioxidant system is overwhelmed, a series of events can occur leading to cell death, process with a significant role in the etiology of numerous human diseases such as diabetes and cancer, as well as certain neurodegenerative and cardiovascular diseases

[48]. It is widely known that antioxidants protect the body against the harms caused by the degenerative diseases and free radicals.

In this regard, one of the most important YM compounds, CGA, has gained attention due to its large distribution in the human diet presenting potential health effects [49]. Macedo et al. [50] reported that CGA improves Low Density Lipoproteins (LDL) resistance to lipid peroxidation. Moreover, it was found to inhibit atherosclerosis acceleration through the enhancement of paraoxonase 2 (PON2) expression and activity [37].

Nevertheless, the protective effects of YM might not be only due to CGAs but, instead, the medicinal and therapeutic effects of mate seem to be due to a coordinated action between the different compounds in the extract. In this concern, the capacity of YM to prevent chemically induced ROS generation was correlated with glutathione balance disruption, mitochondrial dysfunction and lipid peroxidation in rat brain [35].

Authors [38, 51] have studied the antioxidant activity of YM using chemical models and lipoproteins. They found that the antioxidant effect is primarily due to the phenolic compounds in the extract that displaces electrons forming intramolecular hydrogen bonds. Consequently, the compounds in YM have the capacity to suppress chain reactions and restore lesions induced by ROS.

Stimulant Effect

Theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine) and Caffeine (1,3,7-trimethylxanthine) are widely used as stimulants around the world [52]. **Figure 3** shows the chemical structures of these compounds. Those purine alkaloids are found in approximately 100 species in the nature [53], but only a small number are considered

sources of methylxanthines worldwide: coffee (*Coffea sp*), tea (*Camelia sinensis*), mate (*Ilex paraguariensis*), cocoa (*Theobroma cacao*), guarana seeds (*Paulinia cupana*) and cola seeds (*Cola nitida*) [54].

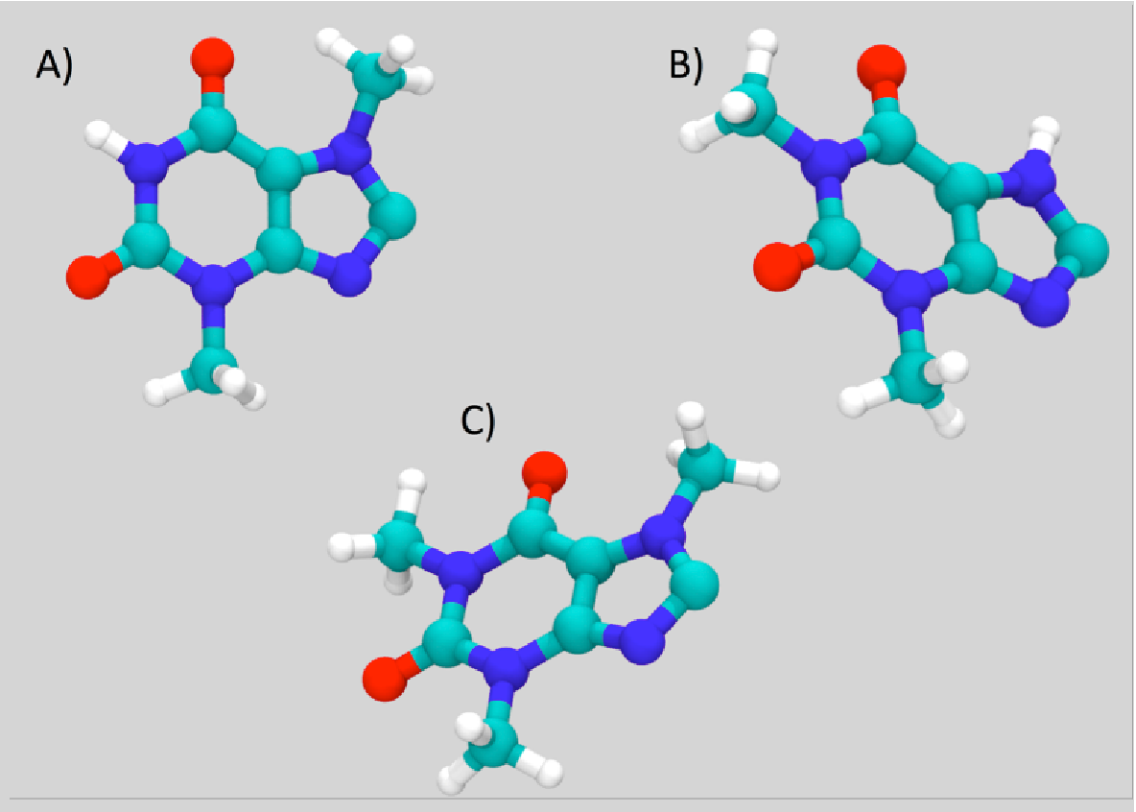


Figure 3. Representation of methylxanthine derivatives found in yerba mate with greater stimulant effect. 3D representation of the chemical structure of Theobromine (A), Theophylline (B) and Caffeine (C). Oxygen atoms are represented in red, while carbon atoms in green, nitrogen in blue and hydrogen in white.

Methylxanthines have presented not only stimulant effects but also a plethora of biological properties, such as peripheral vasoconstriction, central nervous system and myocardial stimulation, smooth muscle relaxation, neuroprotective, hypoglycemic, anti-inflammatory, diuretic and cardio protective effects, among other benefits [55].

For instance, Halliwell *et al*. [56] demonstrated that theobromine was able to suppress Poly (ADP-ribose) polymerase 1 (PARP-1) activity, reducing nicotinamide adenine (NAD+) levels in cells with extensive DNA damage. For instance, after treatment with irradiation, theobromine could assist in maintaining the cells viability. Theobromine and caffeine also attenuated hydroxyl radical formation and oxidative DNA damage by hydroxyl radicals [57].

Investigations regarding caffeine ingestion through mate consumption have made clear that the total intake of caffeine (25–175 mg) and theobromine (6–28.5 mg) through mate ingestion will depend on the way of preparation, raw materials and the amount consumed [58]. In fact, YM extracts end up providing reduced quantity of caffeine when compared with different tea beverages (Figure 4). However, considering that mate ingestion can be up to 6 L per day, heavy drinkers can ingest up to 1 g of caffeine daily [59]. This quantity is expressively higher than the average caffeine intake by adults, about 180 mg/day [60].

Although the undeniable presence of methylxanthines in YM extracts, their concentrations were observed to vary not only in relation to the extraction method, but also concerning the region and the way mate trees were grown.

For instance, when comparing hot mate and *tererê* drinks, Meinhart *et al.* [58], verified that *tererê* presented six times more caffeine than mate. Authors suggested that the higher amount of caffeine in drinks produced with cold water might be due to the complete infusion of YM that is only partial when dealing with mate in hot beverages.

Moreover, when comparing brands from the three main countries that consume YM, Uruguayan brands contain the highest caffeine concentrations, followed by Argentinian and finally Brazilian brands. A study by Jacques *et al*. [61] demonstrated that trees growing in shady areas have more caffeine, sometimes even twice as much as the ones

growing in full sunlight. Another study reported that low caffeine contents indicate increased theobromine, as the theobromine/caffeine relationship is probably due to the methylxanthine biosynthesis [62].

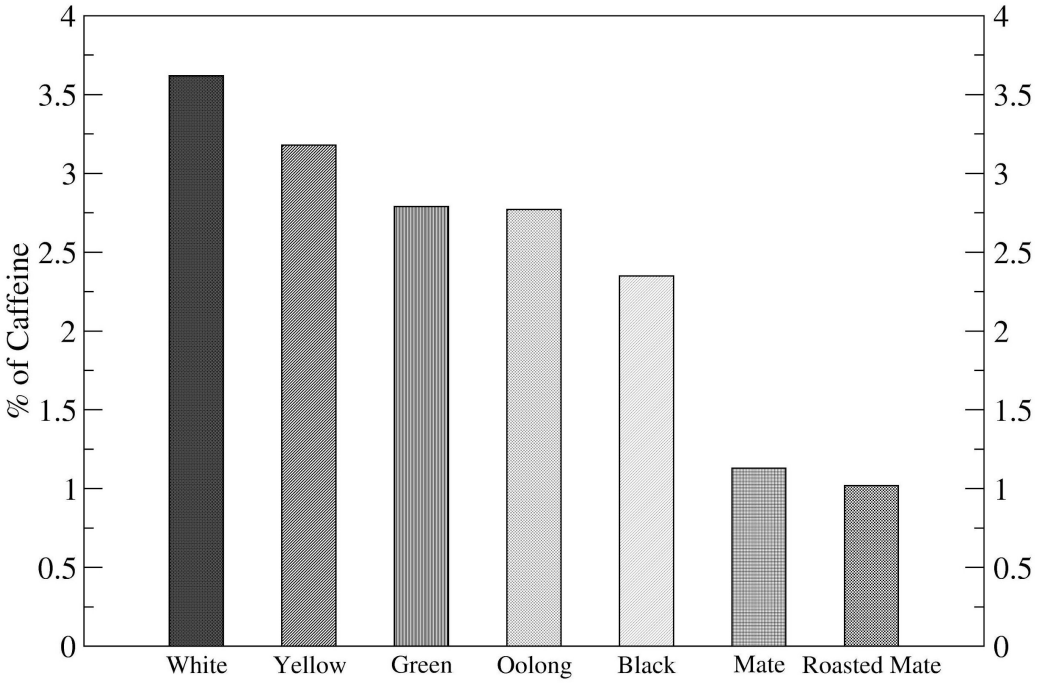


Figure 4. Caffeine contents of teas and mate. teas. Values obtained by HPLC analysis with liquid extractions of 20 g of dry samples [72].

Future Perspectives

Numerous researches have shown that oxidative stress plays a significant role in the etiology of neurodegenerative illnesses, including Alzheimer's disease, and that phenolic compounds are beneficial for preventing or reducing the damage brought on by oxidative stress in cell and animal experiments [63]. The effectiveness of phenolic antioxidants in clinical AD patients is still up for discussion. There have been a variety of likely explanations put out for this, including the small number of participants in the trials, short-

term antioxidant supplementation, antioxidant dosage, brain bioavailability, and starting the intervention too late in the course of the disease [64, 65].

But because the blood-brain barrier prevents substantial levels of phenolic antioxidants from entering the brain, delivery methods must be devised to boost the compounds' availability in the brain. Modifications to compound structure, production of a prodrug, and encapsulation in various nano systems, such as liposomes or micelles, are methods used to increase the bioavailability of phenols in the brain [66].

In addition, it is well known that phenolic acids and flavonoids are extensively metabolized after they are ingested and absorbed by the gastrointestinal system, passing through the several compartments of the gastrointestinal tract due to enzymatic actions, metabolic activity of the intestinal microbiota and pH alterations [67]. Thereby, phenolic acids and flavonoids are transformed into bioavailable plasma metabolites, although presenting a lower antioxidant effect than the precursor molecules.

Since the health benefits related to the consumption of YM-based beverages may be associated with the extracted components and their bioavailability, determining the amount of nutrients present in the different products, the extent of the migration of the compounds to the water, such as the multiple extractions affect the phenolic compounds in beverages and the final portion of nutrients absorbed and used by the human body is of highest importance.

Studies suggest that in order to account for confounders and the fluctuation of microbial composition and further prove causality, it is necessary to examine food, microbiota, and AD risk within people in well-designed prospective studies. It is possible that nutrition supports gut microbiome and brain health through two different (confusing) pathways; or that dietary patterns are determined by intestinal dysbiosis and AD or that AD itself causes intestinal dysbiosis (reverse causation).

On the other hand, it is well known that obesity, non-alcoholic fatty liver disease (NAFLD), hypercholesterolemia, and insulin resistance are all caused by poor eating habits and collectively make up the metabolic syndrome and diet causes dysbiosis in the gut microbiota. These factors worsen a low-grade systemic inflammatory state, impair the blood-brain barrier's ability to regulate amyloid fluxes into and out of the brain, and increase the amount of amyloid in the brain.

Even though there is some data, future research should focus on how specific dietary elements, such as fatty acids, cholesterol, and carbohydrates, affect the advancement of AD pathogenesis at various stages and how bioactive compounds such as phenolic compounds can help prevent and treat it. The interaction between environmental and genetic risk factors for AD should also be covered in future research. The epigenetic modifications that environmental AD risk factors like diet may cause are particularly intriguing, as is the idea that these changes may pass to future generations as disease-promoting rather than disease-protecting predispositions [12].

Although studies link the interaction of the diet and microbiome with the cerebrospinal fluid (CSF) markers of AD in patients with MCI, more thorough research will be needed to elucidate the potential mechanisms and practical implications of these associations with respect to the pathogenesis of AD and human aging. This research would clarify new prognostic markers based on MCI and AD microbiomes and could eventually result in the discovery of new probiotic bacteria with therapeutic potential. [68].

According to studies, yerba mate offers more knowledge about natural neuroprotective substances and can help build pharmaceutical therapy strategies for brain-damaged individuals. In addition, it would be fascinating to research products from various locations, given the possible variations between yerba mate originating from

various regions. Finally, research on yerba mate isolates may shed light on the primary bioactive components [69].

It's also crucial to remember that mate is usually taken as tea (*chimarrão*), which is made by adding successive amounts of water to the same quantity of the herb. Even with the last addition of water, the most diluted serving of tea is likely to contain antioxidant potential like that from the first made tea because the presence of these compounds is maintained during the consumption of the beverage [70]. Therefore, more research is required to determine whether the daily consumption of yerba mate tea is safe even though many people do so.

Furthermore, as natural antioxidants are significantly sensitive, in order to improve their effectiveness, they should be protected from the surrounding environment. Recent techniques such as encapsulation may be valuable options for this purpose. For instance, the stability of phenolics can be improved using microencapsulation technology, such as spray drying [71]. Fortunately, several technologies have already been developed in this concern and are widely used on pharmaceutical and nutritional domains representing a growing trend.

Conclusion

Ilex paraguariensis is a plant native to South America and has been shown to have numerous health benefits for the human body, being an ally in the prevention and treatment of numerous degenerative diseases, such as AD, mainly due to the presence of phenolic compounds. Although many of these effects are not yet well established, several studies have shown that the plant may be a promising functional food to increase antioxidant activity and, consequently, protect the body's cells from oxidative damage, in

order to prolong life and improve the health.

Moreover, recent studies provided evidence that compounds such as quercetin, kaempferol and gallic acid (found in most YM extracts) revealed anti-AChE activity, which might be attractive to applications in AD treatments in accordance with the cholinergic hypothesis. Phenolic compounds as these and other found in YM extracts promotes microbial fermentation and the expansion of helpful microorganisms like Lactobacillus while reducing the number of genera that contain pathogens, being important to the maintenance of eubiotic gut environment, crucial for healthy brain aging, as well as to maintain intestinal barrier function and permeability.

Future studies should examine the feasibility of different types of dietary approaches, such as the ketogenic diet as a treatment for AD, as glycation prevention via carbohydrate control may be a key element in the management of the disease.

Conflicts of Interest. The authors declare no conflict of interest.

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REFERENCES

3. Bastos, D. H. M., Fornari, A. C., Queiroz, Y. S., Torres, E. A. F. S., 2006. Bioactive compounds content of *Chimarrão* infusions related to the moisture of yerba mate (*Ilex paraguariensis*) leaves. *Brazilian Archives of Biology and Technology*, 49, 399–404. doi: 10.1590/S1516-89132006000400007

4. Reichert, C. L., Friedrich, J. C., Cassol, G. H., Pensin, C. F., Mitsui, M. L., Donaduzzi, C. M., Cardozo-Junior, E. L., 2013. Chemical stability and dissolution study of mate (*Ilex paraguariensis St. Hil*.) extract and some formulations. *International Journal of Pharmaceutical Science and Technology*, 8(1), 33–49.

5. Vasconcellos, A. C., Frazon, J., Noreña, C. P. Z. Phenolic Compounds Present in Yerba Mate Potentially Increase Human Health: A Critical Review. Plant Foods for Human Nutrition. 77(4):495-503. doi; 10.1007/s11130-022-01008-8.

6. Georges, J., Miller, O., Bintener, C. (2020). Dementia in Europe Yearbook 2019 Estimating the prevalence of dementia in Europe. Dementia in Europe Yearbooks.

7. Dumurgier J, Sabia S. 2020. Nouvelles tendances épidémiologiques de la maladie d'Alzheimer [Epidemiology of Alzheimer's disease: latest trends]. Rev Prat. 70(2), 149-151. French. PMID: 32877124.

8. Aisen, P.S., Cummings, J., Jack, C.R., Morris, J.C., Sperling, R., Frolich, L., Jones, R.W., Dowsett, S.A., Matthews, B.R., Raskin, J., Scheltens, P., Dubois, B., 2017. On the path to 2025: understanding the Alzheimer's disease continuum. Alzheimers Res. Ther. 9, 60. doi: 10.1186/s13195-017-0283-5.

9. Breijyeh, Z., Karaman, R., 2020. Comprehensive review on Alzheimer's disease: causes and treatment. Molecules, 25, 5789. doi: 10.3390/molecules25245789.

10. Zhao Y., Cong L., Jaber V., Lukiw W. J. 2017a. Microbiome-Derived Lipopolysaccharide Enriched in the Perinuclear Region of Alzheimer's Disease Brain. Front Immunol. 8,1064. doi: 10.3389/fimmu.2017.01064.

11. Zhao Y., Jaber V., Lukiw W. J. 2017b. Secretory Products of the Human GI Tract Microbiome and Their Potential Impact on Alzheimer's Disease (AD): detection of Lipopolysaccharide (LPS) in AD Hippocampus. Front Cell Infect Microbiol, 7, 318.

12. Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfeld, J., Cooper, C., Costafreda, S.G., Dias, A., Fox, N., Gitlin, L.N., Howard, R., Kales, H.C., Kivimaki, M., Larson, E.B., Ogunniyi, A., Orgeta, V., Ritchie, K., Rockwood, K., Sampson, E.L., Samus, Q., Schneider, L.S., Selbæk, G., Teri, L., Mukadam, N., 2020. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet 396, 413–446. doi: 10.1016/S0140-6736(20)30367-6.

13. Solch, R. J., Aigbogun, J. O., Voyiadjis, A. G., Talkington, G. M., Darensbourg, R. M., O'Connell, S., Pickett, K. M., Perez, S.P., Maraganore, D. M. 2022. Mediterranean diet adherence, gut microbiota, and Alzheimer's or Parkinson's disease risk: A systematic review. Journal of the Neurological Sciences, 434, 120166. doi: 10.1016/j.jns.2022.120166.

14. Więckowska-Gacek, A., Mietelska-Porowska, A., Wydrych, M., Wojda, U. 2021. Western diet as a trigger of Alzheimer’s disease: From metabolic syndrome and systemic inflammation to neuroinflammation and neurodegeneration, Ageing Research Reviews, 70, 101397. doi: 10.1016/j.arr.2021.101397.

15. Kagan, B. L., Jang, H., Capone, R., Teran, Arce, F., Ramachandran, S., Lal, R., Nussinov, R. 2012. Antimicrobial properties of amyloid peptides. Mol Pharm. 9(4), 708-17. doi: 10.1021/mp200419b.

16. Sampson, T. R., Mazmanian, S. K. 2015. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe. 17(5):565-76. doi: 10.1016/j.chom.2015.04.011.

17. Sampson T. R., *et al.* 2016. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell. 167(6), 1469-1480.e12. doi: 10.1016/j.cell.2016.11.018.

18. Wu, SC., Cao, ZS., Chang, KM. Juang, JL. 2017. Intestinal microbial dysbiosis aggravates the progression of Alzheimer’s disease in Drosophila. Nat. Commun. 8, 24. doi: 10.1038/s41467-017-00040-6

19. Kumar, D. K. V., Choi, S. H., Washicosky, K. J., Eimer, W. A., Tucker, S., Ghofrani, J., Lefkowitz, A., McColl, G., Goldstein, L. E., Tanzi, R. T., Moir, R. D. 2016. Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. Sci. Transl. Med. 8 (340):340ra72. doi: 10.1126/scitranslmed.aaf1059.

20. Berer K, et al. 2017. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc. Natl. Acad. Sci. USA, 114 (40), 10719–24.

21. Cekanaviciute E, et al. 2017. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc Natl Acad Sci USA, 114(40):10713–8. doi: 10.1073/pnas.171123511.

22. Sharon G, et al. 2019. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell, 177(6), 1600-1618.e17. doi: 10.1016/j.cell.2019.05.004.

23. Cox L. M., Weiner H. L. 2018. Microbiota Signaling Pathways that Influence Neurologic Disease. Neurotherapeutics, 15(1), 135–45. doi: 10.1007/s13311-017-0598-8

24. Benus, R.F.J., van der Werf, T.S., Welling, G.W., Judd, P.A., Taylor, M.A., Harmsen, H.J. M., Whelan, K., 2010. Association between Faecalibacterium prausnitzii and dietary fbre in colonic fermentation in healthy human subjects, Br. J. Nutr. 104(5), 693–700. doi: 10.1017/S0007114510001030.

25. Gallè, F., et al. 2020. Mediterranean diet, physical activity and gut microbiome composition: a cross-sectional study among healthy young Italian adults, Nutrients 12(7), 2164. doi: 10.3390/nu12072164.

26. Maskarinec, G. *et al.* 2019. Fecal microbial diversity and structure are associated with diet quality in the multiethnic cohort adiposity phenotype study, J. Nutr. 149(9), 1575–1584. doi: 10.1093/jn/nxz065

27. Zhu, C., Sawrey-Kubicek, L., Beals, E., Rhodes, C.H., Houts, H.E., Sacchi, R., Zivkovic, A. M. 2020. Human gut microbiome composition and tryptophan metabolites were changed differently by fast food and Mediterranean diet in 4 days: a pilot study, Nutr. Res. 77, 62–72.

28. Ma, G., Chen, Y. 2020. Polyphenol supplementation benefts human health via gut microbiota: a systematic review via meta-analysis, J. Funct. Foods 66, 103829.

29. Clifford MN, Hooft JJ, Crozier A (2013) Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. Am J Clin Nutr 98:1619S–1630S. doi: 10.3945/ajcn.113.058958.

30. Bortoli, P., Kaizer, R., Alves, C., Costa, E., Vanin, A. P., Sofiatti, J., Siqueira D.; Dallago, R., Treichel, H., Vargas, G., 2018. *Ilex paraguariensis*: Potential antioxidant on aluminium toxicity, in an experimental model of Alzheimer's disease. *Journal of Inorganic Biochemistry*, 181, 104-110. doi: 10.1016/j.jinorgbio.2017.11.001

31. Gaiad, S., Rakocevic, M., Reissmann, C.B., 2006. N sources affect growth, nutrient content, and net photosynthesis in maté (*Ilex paraguariensis St. Hil.*). *Brazilian Arch. Biol. Technol.* 49, 689–697. doi: 10.1590/S1516-89132006000600001.

32. Exley, C., Mold, M., Shardlow, E., Shuker, B., Ikpe, B., Wu, L., Fraser, P.E., 2012. Copper is a potent inhibitor of the propensity for human ProIAPP 1-48 to form amyloid fibrils *in vitro*. *J. Diabetes Res. Clin. Metab.* 1(1), 3. doi:10.7243/2050-0866-1-3

33. V.N. Talesa, V. N. 2001. Acetylcholinesterase in Alzheimer's disease, Mech. Ageing Dev. 122, 1961–1969. doi: 10.1016/S0047-6374(01)00309-8.

34. Li, N., Jiang, H., Yang, J., Wang, C., Wu, L., Hao, Y., Liu, Y. (2021) Characterization of phenolic compounds and anti-acetylcholinase activity of coconut shells, Food Bioscience, 42, 101204, doi: 10.1016/j.fbio.2021.101204.

35. Hadrich, F., Chamkha, M., Sayadi, S. (2022) Protective effect of olive leaves phenolic compounds against neurodegenerative disorders: Promising alternative for Alzheimer and Parkinson diseases modulation. *Food and Chemical Toxicology*, 159, 112752. doi: 10.1016/j.fct.2021.112752.

36. Petrilli, A., Souza, S., Teixeira, A., Pontilho, P., Souza, J., Luzia, L., Rondó, P., 2016. Effect of chocolate and yerba mate phenolic compounds on inflammatory and oxidative biomarkers in HIV/AIDS individuals. *Nutrients*, 8(5), 132. doi: 10.3390/nu8050132

37. de Lima, M.E., Colpo, A.C., Maya-López, M., Rosa, H., Túnez, I., Galván-Arzate, S., Santamaría, A., Folmer, V., 2017. Protective effect of Yerba Mate (*Ilex paraguariensis St. Hill.*) against oxidative damage *in vitro* in rat brain synaptosomal/mitocondrial P2 fractions. *Journal of Funcional Foods* 34, 447-452. doi:10.1016/j.jff.2017.05.026

38. Yu, S.I., Yue, S.W., Liu, Z., Zhang, T., Xiang, N., Fu, H., 2015. Yerba mate (Ilex paraguariensis) improves microcirculation of volunteers with high blood viscosity: a randomized, double-blind, placebo-controlled trial. Exp. Gerontol. 62, 14-22. doi: 10.1016/j.exger.2014.12.016

39. Fernandes, E. S., Machado, M. O., Becker, A. M., Andrade, F., Maraschin, M., Silva, E. L., 2012. Yerba mate (Ilex paraguariensis) enhances the gene modulation and activity of paraoxonase-2: In vitro and in vivo studies. Nutrition, 28(8), 1157–1164.
doi: 10.1016/j.nut.2012.04.011

40. Schinella, G., Fantinelli, J. C., Mosca, S. M., 2005. Cardioprotective effects of *Ilex paraguariensis* extract: evidence for a nitric oxide-dependent mechanism. *Clinical Nutrition*, 24, 360-366. doi:10.1016/j.clnu.2004.11.013.

41. Arçari, D. P., Porto, V. B., Rodrigues, E. R. V., Martins, F., Lima, R. J., Sawaya, A. C. H. F., Carvalho, P. O., 2011b. Effect of mate tea (*Ilex paraguariensis*) supplementation on oxidative stress biomarkers and LDL oxidisability in normo and hyperlipidaemic humans. Journal of Functional Foods, 3, 190–197.
doi: 10.1016/j.jff.2011.04.001.

42. Ribeiro, M. C., Santos, Â., Riachi, L. G., Rodrigues, A. C. B., Coelho, G. C., Marcellini, P. S., de Maria, C. A. B., 2017. The effects of roasted yerba mate (*Ilex paraguariensis A. St. Hil.*) consumption on glycemia and total serum creatine phosphokinase in patients with traumatic brain injury. *Journal of Functional Foods*, 28, 240–245. doi: 10.1016/j.jff.2016.11.024.

43. Kim, S. Y., Oh, M. R., Kim, M. G., Chae, H. J., Chae, S. W., 2015. Anti-obesity effects of Yerba Mate (*Ilex Paraguariensis*): a randomized, double-blind, placebo-controlled clinical trial. *BMC Complementary and Alternative Medicine*, 15(1), 338. doi:10.1186/s12906-015-0859-1.

44. Martinet, A., Hostettmann, K., and Schutz, Y., 1999. Thermogenic effects of commercially available plant preparations aimed at treating human obesity. *Phytomedicine*, 6(4), 231–238. doi:10.1016/S0944-7113(99)80014-2

45. de Mejia, E. G., Song, Y. S., Heck, C. I., Ramirez-Mares, M., 2010. Yerba mate tea (*Ilex paraguariensis*): Phenolics, antioxidant capacity and *in vitro* inhibition of colon cancer cell proliferation. *Journal of Functional Foods,* 2, 23-34. doi:10.1016/j.jff.2009.12.003

46. Amigo-Benavent, M., Wang, S., Mateos, R., Sarriá, B., Bravo, L., 2017. Antiproliferative and cytotoxic effects of green coffee and yerba mate extracts, their main hydroxycinnamic acids, methylxanthine and metabolites in different human cell lines. Food and chemical toxicology, 106, 125-138.
doi: 10.1016/j.fct.2017.05.019

47. Bastos, M.C., Cherosim, V. F., Reissmann, C, B., Kaseker, J. F., Gaiad, S., 2018. Yerba Mate: Nutrient levels and quality of the beverage depending on the harvest season. Journal of Food Composition and Analysis, 69, 1-6. doi: 10.1016/j.jfca.2018.01.019

48. Bains, Y., Gugliucci, A., 2017. *Ilex paraguariensis* and its main component chlorogenic acid inhibit fructose formation of advanced glycation endproducts with amino acids at conditions compatible with those in the digestive system. *Fitoterapia*, 117, 6–10. doi: 10.1016/j.fitote.2016.12.006.

49. Streit, N. M., Hecktheuer, L. H. R., do Canto, M.W., Mallmann, C.A., Streck, L., Parodi, T.V., Canterle, L.P., 2007. Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (*Ilex paraguariensis*). *Elsevier*, 102, 560-564. doi: 10.1016/j.foodchem.2006.05.028

50. Halliwell, B., 2011. Free radicals and antioxidants—quo vadis? *Trends Pharmacol.Sci*. 32, 125–130. doi: 10.1016/j.tips.2010.12.002

51. Upadhyay, R., Mohan Rao, J., 2013. An outlook on chlorogenic acids – Occurrence, chemistry, technology, and biological activities. *Critical Reviews in Food Science and Nutrition*, 53(9), 968–984. doi: 10.1080/10408398.2011.576319

52. Macedo, J. A., Battestin, V., Ribeiro, M. L., Macedo, G. A., 2011. Increasing the antioxidant power of tea extracts by biotransformation of polyphenols. *Food Chemistry*, 126, 491–497. doi:10.1016/j.foodchem.2010.11.026

53. Heck, C. I., Mejia, E. G., 2007. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science*, 72, 138–151. doi: 10.1111/j.1750-3841.2007.00535.x

54. Pauwels, R.A., Buist, A.S., Calverley, P.M., Jenkins, C.R., Hurd, S.S., 2001. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonar disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *American Journal of Respiratory Critical Care Medicine*, 163(2), 1256–1276.

55. Zulak, K.G., Liscome, D.K., Ashihara, H., Facchini, P.J., 2006. Alkaloids. In: Crozier, A., Clifford, M.N., Ashihara, H. (Eds.), *Plant secondary metabolites: occurrence, structure and role in the human diet.* Blackwell, Oxford, USA, 102–131

56. Tarka, M.S., Hurst, W.J., 1998. Introduction to the chemistry, isolation, and biosynthesis of methylxanthines. In: Spiller, G.A. (Ed.), *Caffeine*. CRC Press, Boca Raton, Florida, USA, 13–22. doi:10.1201/9781420050134.ch1

57. Martínez-Pinilla, E., Oñatibia-Astibia, A., Franco, R.,2015. The relevance of theobromine for the beneficial effects of cocoa consumption. *Frontiers in pharmacology*, 6, 30. doi:10.3389/fphar.2015.00030

58. Halliwell, B., Gutteride, J. M. C., 2007. Free radicals in biology and medicine (4th ed.). *New York: Oxford University Press Inc.* (Chapters 4 and 10).

59. Azam, S., Hadi, N., Khan, N. U., Hadi, S. M., 2003. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. *Medical Science Monitor*, 9, 325–330.

60. Meinhart, A. D., Bizzotto, C. S., Rybka, A. C. P., Sobrinho, M. R., Filho, J.T., Ballus C. A. Godoy, H. T., 2010. Methylxanthines and phenolics content extracted during the consumption of mate (*Ilex paraguariensis* St. Hil) beverages. Journal of Agricultural and Food Chemistry*,* 58, 2188-2193. doi: 10.1021/jf903781w

61. Bastos, D. H. M., Oliveira, D. M., Matsumoto, R. L. T., Carvalho, P. O., Ribeiro, M. L., 2007. Yerba maté: Pharmacological properties, research and biotechnology. *Medicinal and Aromatic Plant Science and Biotechnology*, 1(1), 37–46.

62. Temple, J. L., Bernard, C., Lipshultz, S. E., Czachor, J. D., Westphal, J. A., Mestre, M. A. 2017. The Safety of Ingested Caffeine: A Comprehensive Review. Frontiers in Psychiatry, 8, 1-19. doi: 10.3389/fpsyt.2017.00080.

63. Jacques, R.A., Krause, L.C., Freitas, L.S., Dariva, C., Oliveira, J.V., Caramão, E.B., 2007a. Influence of drying methods and agronomic variables on the chemical composition of mate tea leaves (*Ilex paraguariensis A. St.-Hil*) obtained from high-pressure CO2 extraction. *J. Agric. Food Chem*. 55, 10081–10085. doi: 10.1021/jf071544o

64. Isolabella, S., Cogoi, L., López, P., Anesini, C., Ferraro, G., Filip, R., 2010. Study of the bioactive compounds variation during yerba mate (Ilex paraguariensis) processing, Food Chemistry, 122(3), 695-699. doi: 10.1016/j.foodchem.2010.03.039.

65. Uddin, M.S., et al. 2020. Neuroprotective role of polyphenols against oxidative stress-mediated neurodegeneration, Eur. J. Pharmacol. 886, 173412. doi: 10.1016/j.ejphar.2020.173412.

66. Arslan, J., Jamshed, H., Qureshi, H., 2020. Early detection and prevention of alzheimer's disease: role of oxidative markers and natural antioxidants, Front. Aging Neurosci., 12, 231. doi: 10.3389/fnagi.2020.00231

67. Ferreira, M.E., de Vasconcelos, A.S., da Costa Vilhena, T., da Silva, T.L., da Silva Barbosa, A., Gomes, A.R., Dolabela, M.F., Percario, S. 2015. Oxidative stress in alzheimer's disease: should we keep trying antioxidant therapies? Cell. Mol. Neurobiol. 35, 595–614. doi: 10.1007/s10571-015-0157-y.

68. Pandareesh, M.D., Mythri, R.B., Srinivas Bharath, M.M., 2015. Bioavailability of dietary polyphenols: factors contributing to their clinical application in CNS diseases, Neurochem. Int. 89,198–208. doi: 10.1016/j.neuint.2015.07.003

69. Boaventura, B. C. B., da Silva, E. L., Liu, R. H., Prudêncio, E. S., Di Pietro, P. F., Becker, A. M., Amboni, R. D. D. M. C., 2015. Effect of yerba mate (*Ilex paraguariensis A St. Hil*.) infusion obtained by freeze concentration technology on antioxidant status of healthy individuals. *LWT – Food Science and Technology*, 62(2), 948–954. doi: 10.1016/j.lwt.2015.02.028

70. Nagpal, R. et al. 2020. Gut mycobiome and its interaction with diet, gut bacteria and alzheimer's disease markers in subjects with mild cognitive impairment: A pilot study. E. Bio. Medicine, 59, 102950. doi: 10.1016/j.ebiom.2020.102950

71. dos Santos Branco, C. et al. 2013. Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (Ilex paraguariensis St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. Brain Research Bulletin 92, 60–68. doi: 10.1016/j.brainresbull.2012.11.008

72. Colpo, A.Z.C. 2012. Perfil fitoquímico e capacidade e capacidade antioxidante de extratos de erva-mate (Ilex paraguariensis A.St. Hill.), UNIPAMPA, (2012), p. 87.

73. Yurdagel, U., 2007. Microencapsulation of anthocyanin pigments of black carrot (Daucus carota L.) by spray drier. *J. Food Eng*. 80, 805–812. doi:10.1016/j.jfoodeng.2006.07.009

74. Cardozo Junior, E. L., Morand, C. 2016. Interest of mate (Ilex paraguariensis A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review. Journal of Functional Foods, 21, 440-454. doi: 10.1016/j.jff.2015.12.010

75. Komes, D. et al. 2018. Determination of Caffeine Content in Tea and Maté Tea by Using Different Methods. Czech Journal of Food Sciences, 27.

76. Li, B., He, Y., Ma, J., Huang, P., Du, J., Cao, L., Wang, Y., Xiao, Q., Tang, H., Chen, S. 2019. Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota. Alzheimer's & Dementia, 15, 1357-1366. doi: 10.1016/j.jalz.2019.07.002

77. Cunnane S.C., Courchesne-Loyer A., St-Pierre V., Vandenberghe C., Pierotti T., Fortier M., Croteau E., Castellano C.A. 2016. Can ketones compensate for deteriorating brain glucose uptake during aging? Implications for the risk and treatment of Alzheimer's disease. Ann N Y Acad Sci., 1367(1), 12-20. doi: 10.1111/nyas.12999.

78. Sarroca, S., Gatius, A., Rodríguez-Farré, E., Vilchez, D., Pallàs, M., Griñán-Ferré, C., Sanfeliu, C., Corpas, R. 2021. Resveratrol confers neuroprotection against high-fat diet in a mouse model of Alzheimer's disease via modulation of proteolytic mechanisms, The Journal of Nutritional Biochemistry, 89, 108569. doi: 10.1016/j.jnutbio.2020.108569.

79. Broom, G. M., Shaw, I. C., Rucklidge, J. J. 2019. The ketogenic diet as a potential treatment and prevention strategy for Alzheimer's disease, Nutrition, 60, 118-121, doi: 10.1016/j.nut.2018.10.003.

80. Mountaki, C., Dafnis, I., Panagopoulou, E. A., Vasilakopoulou, P. B., Karvelas, M., Chiou, A., Karathanos, V. T., Chroni, A. 2021. Mechanistic insight into the capacity of natural polar phenolic compounds to abolish Alzheimer's disease-associated pathogenic effects of apoE4 forms, Free Radical Biology and Medicine, 171, 284-301, doi: 10.1016/j.freeradbiomed.2021.05.022.

81. Hamaguchi, T., Ono, K., Murase, A., Yamada, M. 2009. Phenolic Compounds Prevent Alzheimer's Pathology through Different Effects on the Amyloid-Aggregation Pathway. The American Journal of Pathology, 175(6), 2557. doi: 10.2353/ajpath.2009.090417

82. Bahadori, M. B., Kirkan, B., Sarikurkcu, C. 2019. Phenolic ingredients and therapeutic potential of Stachys cretica subsp. smyrnaea for the management of oxidative stress, Alzheimer's disease, hyperglycemia, and melasma, Industrial Crops and Products, 127, 82-87. doi: 10.1016/j.indcrop.2018.10.066.

83. Sang, Z., Wang, K., Dong, J., Tang, L. 2022. Alzheimer's disease: Updated multi-targets therapeutics are in clinical and in progress. European Journal of Medicinal Chemistry, 238, 114464. doi: 10.1016/j.ejmech.2022.114464.

6. CHAPTER III

Manuscript 3

Identification and quantification of phenolic compounds from yerba mate (*Ilex Paraguariensis*) in different extraction methods by LC-DAD-ESI-MS/MS

Running Title: **HPLC identification and quantification of phenolic compounds from yerba mate**

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Category: Food Science and Technology

Abstract

The high content in phenolic compounds by hypothesis accounts for *in vitro* and *in vivo* antioxidant capacity of the extracts obtained from yerba mate (YM); on the other hand, the high complexity of the samples extracted, depending on the method employed, may preclude complete resolution by conventional HPLC techniques. Due to the variety of products, drinking practices, and the transfer rates of the chemicals into the infusions, evaluating their profiles that represent the real dietary intake may provide analytical and

interpretative challenges. This study aimed to identify the phenolics present in YM in three different extraction methods (aqueous, microwave and methanol), and posteriorly determining their contents by HPLC-DAD-MS. A significant variety of phenolics was obtained as a result of each extraction, and mainly composed of caffeoylquinic acids and a multitude of different hydroxycinnamic acids, such as caffeic acid (CA), ferulic acid (FA) and p-coumaric acid (pCA).

Keywords: tea, polyphenols, antioxidant activity, antioxidant capacity, phenolic compounds

Introduction

There is growing interest related to rapid screening and full characterization of the constituents of plants with medicinal properties; among these, South America's subtropical climates are home to the plant *Ilex Paraguariensis* (St. Hil.), and its derivative yerba mate (YM), the principal beverage substitute for coffee or tea in nations like Brazil, Argentina, Paraguay, or Uruguay, where daily YM consumption has been reported at more than 1 L/person, historically made from the plant's leaves.

Due to its health benefits and rising popularity as a result of being recognized as a functional food, YM has gained popularity on a global scale (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011). The plant has been used for centuries and for the treatment and prevention of many different diseases in indigenous tribal medicine. Indeed, this fact has stimulated a number of recent research to describe the pharmacological effects of this drink, suggesting its antioxidant, anti-inflammatory, anti-

obesity, and anti-carcinogenic characteristics both *in vitro* and *in vivo* (Arçari et al., 2009; de Mejía, Song, Heck, & Ramírez-Mares, 2010).

Herbal infusions have long been a staple of human diets, and the discovery of their high phenolic content has attracted notice as a potential source of nutrients that may improve health (Cory et al., 2018).The nutritional and health benefits associated to the intake of YM have been attributed to its high content in bioactive components, such as methylxanthines and phenolic compounds, but still most therapeutic applications of YM have been associated with its phenolic composition (Del Rio et al., 2013).

Among the phenolic compounds of YM, hydroxycinnamate esters (chlorogenic acids) are prominent, with high concentrations of caffeoylquinic and dicaffeoylquinic acids (Bravo et al., 2007; Filip et al., 2001). The main phenolic compounds in coffee, for example, are chlorogenic acids (CGAs) as well, with high amounts caffeoyquinic acids and also containing ferruloylquinic and dicaffeoylquinic acids (Clifford, 2017).

The beneficial effects of phenolic-rich diets are well documented, but the required amounts of each specific compound and its matrix (within foods or as a supplement) are not defined (Cory et al., 2018). As well, recent other factors, such as compounds bioavailability in the human body and the gut microbiota has brought scientists attention.

Analytical procedures for the characterization of the bioactive compounds in YM leaves (Marx et al., 2003) have been carried out by several extraction methods, including protocols using organic solvents and their aqueous mixtures with maceration, ultrasound assisted extraction and supercritical fluid extractions (Dugo et al., 2009; Marques & Farah, 2009; Dartora et al., 2011; Lima et al., 2016; Perrenoud et al., 2016; Konieczynski et al., 2017; Souza et al., 2021). However, the evaluation of aqueous infusions representing the actual dietary intake present analytical and interpretation challenges due

to the diversity of available products, drinking procedures, transfer rates into the infusions and bioavailability (Bravo et al., 2007; Theuma & Attard, 2020; Panzl et al., 2022).

Therefore, the main objective of this work was to study different extraction methods such as aqueous extraction (AE), microwave-assisted extraction (MAE) and methanol solvent extraction (MSE) considering the quality and quantity of phenolic compounds recovered from YM using ultra-high performance liquid chromatography with diode array detection (UHPLC-DAD).

Materials and Methods

Material

Samples of ground YM were purchased from a local supermarket (30°02'41.2"S 51°12'01.6"W), respecting the use of a single well-established brand on the market and the same batch. The packed YM was kept at room temperature, aerated, and light-protected. Only distilled water was employed as a solvent in the MAE and AE procedures, whereas methanol and water were utilized in the MSE method. All of the reagents were of analytical grade.

Chemicals

Standards of caffeic, 5-caffeoylquinic acid, ellagic, ferulic, gallic, hydroxybenzoic and p-coumaric acids, (+)-catechin, daidzein, (−)-epicatechin, epicatechin gallate, epigallocatechin, kaempferol, quercetin, quercetin 3-O-glucoside, resveratrol and quercetin 3-rutinoside (97–99% purity) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and methanol both of HPLC grade were used, from J. T. Baker (Phillipsburg, NJ). Chloridric and formic acid were purchased from Merck (Darmstadt, Germany). Methanol (P.A.) and potassium phosphate dibasic were purchased from Neon Comercial (São Paulo, Brazil). Fluorescein sodium salt, 6-hydroxy-2,5,7,8-

tetramethylchroman-2-carboxylic acid (Trolox) and α,α′-Azodiisobutyramidine dihydrochloride (AAPH) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium carbonate and sodium phosphate monobasic monohydrate were acquired from Dinamica (São Paulo, Brazil) and Vetec (São Paulo, Brazil), respectively. Ultrapure water (Milli-Q) was generated by the Millipore System (Molsheim, FR). The samples and solvents were filtered through cellulose acetate (aqueous solutions) or polytetrafluoroethylene (organic solutions) membranes of 0.22 μm (Mallmann, 2019).

Extraction methods

Microwave assisted extraction (MAE)

MAE was performed according to Cassol and Noreña (2019). YM was mixed in 1:20 w/w distilled water at room temperature and immediately placed in a conventional microwave oven (Electrolux, MEF41) at 2450MHz, using a microwave power of 700 W for 1 min (T1) and 1000 W 40 seconds (T2). Those combinations were determined by previous assays, where several batch times and powers were tested respecting the maximum temperature of 80 °C. Subsequently, the extract was vacuum filtered on Whatman paper n. 01 for solid waste separation and frozen at -18 ºC for later analysis.

Aqueous extraction (AE)

The simulation of the traditional infusion extraction method was carried out as described in Torterolo et al. (2014) with modifications. YM was mixed in pure water at a rate of 5 g of herb to 100 ml of water (1:20 w/w) previously heated to 80 °C, and kept stirring at constant temperature for 15 (T3), 30 (T4) and 60 minutes (T5) in a magnetic heated stirrer (Fisatom 752a, São Paulo). The maximum heating point was 80 ºC, since it is the temperature advised by the product label and at which the beverage is usually

consumed. The mixture was then cooled, and stored in an aluminum-lined glass vial at room temperature and protected from light for 20 h. Thereafter, the extract was vacuum filtered on Whatman paper n. 01 for solid waste separation. The filtered extract was frozen at -18 ºC for later analysis.

Methanol solvent extraction (MSE)

A MSE (T6) was also performed to compare the effects of MAE and AE. In a Falcon tube, 2 g of product was added to 10 mL of acidified methanol:water (8:2, v/v) (1% HCl, w/v) at 80 °C. Subsequently, the tube was vortexed (Quimis, Q920-A2) for 5 minutes (Rodriguez et al., 2014) and then centrifuged (Sigma, 4K15) at 3000×g for another 5 minutes, and the supernatant was set aside. The supernatants were mixed together and the solvent was evaporated using a rotary evaporator (Heidolph, Laborot 4000) at 40 °C.

HPLC analysis

A Shimadzu (Kyoto, Japan) HPLC apparatus connected in series to a DAD detector (SPD-M20A) and a mass spectrometer (MS) with Quadrupole-Time-of Flight (QTOF) analyzer and an electrospray ionization source (ESI) (Bruker Daltonics, micrOTOF-Q III model, Bremen, Germany) were used to identify and quantify the phenolic compounds.

The chromatography conditions used to phenolic compound separation were previously described by Mallmann et al. (2023). The separation was carried out with a Phenomenex C18 (250 mm × 4.6 mm, 4 μm) column. Solvents were classified as A (water acidified with 0.1% formic acid) and B (acetonitrile acidified with 0.1% formic acid). The samples were eluted according to the binary gradient which began with 5% B as the initial

condition and reached 50.1% B at 46 minutes. The flow rate was 0.5 mL min^{-1} and the injection volume was 20 μL. After the separation of the phenolic compounds in the column, the eluate entered the ESI interface. The ESI source was operated under the following conditions: negative mode, capillary voltage: 3500 V, scan range of m/z 50 to 1500, dry temperature and gas flow (N2): 310 °C and 8 L min^{-1}, nebulizer gas pressure: 4 bar. MS2 spectra were acquired in Auto-MS2 mode (data-dependent acquisition) and the collision energy was estimated dynamically based on appropriate values for the *m/z* and with an appropriate mass width to ensure good quality fragmentation spectra.

Before analysis, an external instrument calibration was performed using a syringe pump (kdScientific, Holliston, USA) directly connected to the ionization source. A solution of 10 mM sodium formate cluster was used for a calibrant as recommended by Bruker Daltonics (Bremen, Germany) for molecules up to m/z 1500. A calibration curve was obtained with 20 cluster masses each differing by 68 Da in the negative mode. This external calibration was used to automatically calibrate each analysis in the equipment software.

The phenolic compounds were identified or tentatively identified using the following parameters: elution order in the C18 column, UV–vis absorption characteristics, accurate mass and fragmentation pattern in comparison to standards analyzed under the same conditions or data available in the literature.

The quantification of the phenolic compounds was performed by analytical curves of 2 phenolic standards (chlorogenic acid and rutin at 320 and 360 nm, respectively). The analytical curves were constructed from the analysis of fifteen concentrations in a 1 to 20 mg L^{-1} range. The method was validated by analyzing the following parameters: linearity (R2), limits of detection (LOD) and quantification (LOQ), (**Table 1**). The LOD and LOQ

were determined according to the methodology of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 2005). This method estimates the limits using the analytical curves through the relationship between the standard error (s) and the slope of the curve (b).

$$LOD = 3{,}3 \times s/b \quad (1)$$

$$LOQ = 10 \times s/b \quad (2)$$

Table 1. HPLC-DAD method validation parameters for phenolic compounds quantification.

Standard	**Quantification (nm)**	**Calibration Curve**	**R^2**	**Linearity range (mg/L)**	**LOD**	**LOQ**
Chlorogenic Acid	320	y = 90213,714x + 14796,576	0.9923	1 – 20	3.06	9.28
Rutin	360	y = 55653,314x + 3310,521	0.9983	1 – 20	1.42	4.31

Statistical analysis

ANOVA was used to analyze the data, and the Tukey test was employed to compare means (at 5 percent significance level). SAS System for Windows version 9.3 software was used for statistical analysis.

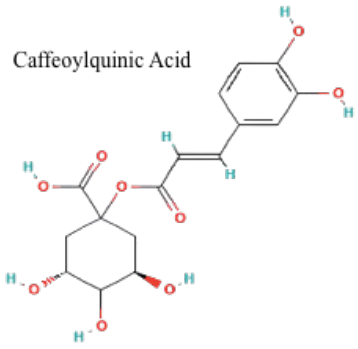
Results and Discussion

HPLC qualitative analysis

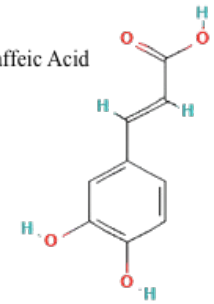
The phenolic compounds found in each extract are described in **Tables 2 to 7** . A significant variety of PCs were obtained as a result of each extraction method, and mainly composed of caffeoylquinic acid and its dimers and a multitude of different hydroxycinnamic acids, such as ferulic acid (FA), p-coumaric acid (pCA) and caffeic acid (CA) and its derivatives. Caffeic acid, for example, was detected in all extracts. It is considered an important biosynthetic precursor representing the main hydroxycinnamic fraction of the phenolic fraction, forming isomers of mono and dicaffeoylquinic acid, also observed in the extracts. These molecules represented most of the phenolics found in the extracts, being also observed as isomers, dimers or linked to carbohydrate molecules such as caffeic acid hexoside I (324 - 326 nm, 15.9 - 18.7 min).

Flavonols, on the other hand, were obtained in the form of rutin, kaempferol, quercetin and also as combinations with carbohydrates such as Kaempferol rutinoside (329 - 340 nm, 29.2 - 29.4 min). **Figure 1** shows the structures of the main phenolic compounds found in YM extract.

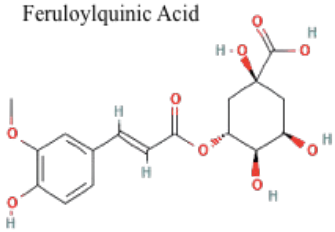
Caffeoylquinic Acid



Caffeic Acid



Feruloylquinic Acid



Dicaffeoylquinic Acid

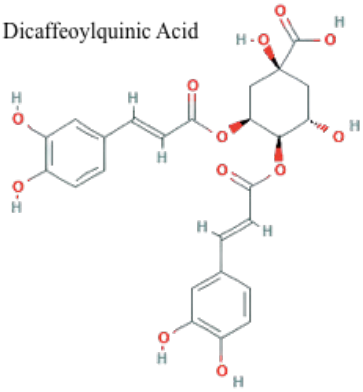


Fig. 1. Main phenolic compounds found in YM by LC-DAD-ESI-MS/MS analysis.

Most of the constituents displayed similar spectral behavior with maximum absorption peak at 320–330 nm and a shoulder at 290–300 nm, being characterized as hydroxycinnamic acid derivatives. A small group of compounds showed the maximum of absorbance at 312 nm, corresponding to p-coumaroylquinic acid derivatives (Alonso-Salces, Guillou, & Berrueta, 2009). The rest of the minor constituents of YM were characterized as flavonols attending to UV-spectra characteristics with two maximum absorption peaks at 255–265 and 345–375 nm (Dugo et al., 2009).

A total of 29 phenolic compounds were identified in YM, and around 16 to 18 compounds for each extraction, being mostly hydroxycinnamic acid derivatives. Four of this compounds in YM extracts had an UV spectrum compatible with a flavonoid structure, specifically of flavonols, which is characterized by two absorption maxima at 255–265 and 345–367 nm. **Tables 2-7** list the phenolic compounds identified in YM, ordered according to peak elution, and their chemical characterization: retention time (RT), UV absorption maximum from DAD, quasimolecular ion [M–H]–, MS/MS fragment ions with relative abundance (RA) and tentative nomenclature. Some compounds overlapped in the same chromatographic peak and thus were quantified jointly considering the most abundant.

Elution/retention time of the compounds in YM is closely related to their hydrophobicity, which depends on the number, position, and nature of the cinnamoyl moieties. The equatorial (C4 and C5) hydroxyl groups in the quinic or shikimic acids give more hydrophobicity to the molecule than free axial (C1 and C3) hydroxyl groups (Clifford et al., 2005), thus the hydroxycinnamate esters with hydroxyl

groups at position 3 were the most hydrophilic derivatives. **Figure 2** demonstrates the chromatograms, obtained by LC-DAD-ESI-MS/MS, for the identification of the phenolic compounds from YM in the 6 different treatments.

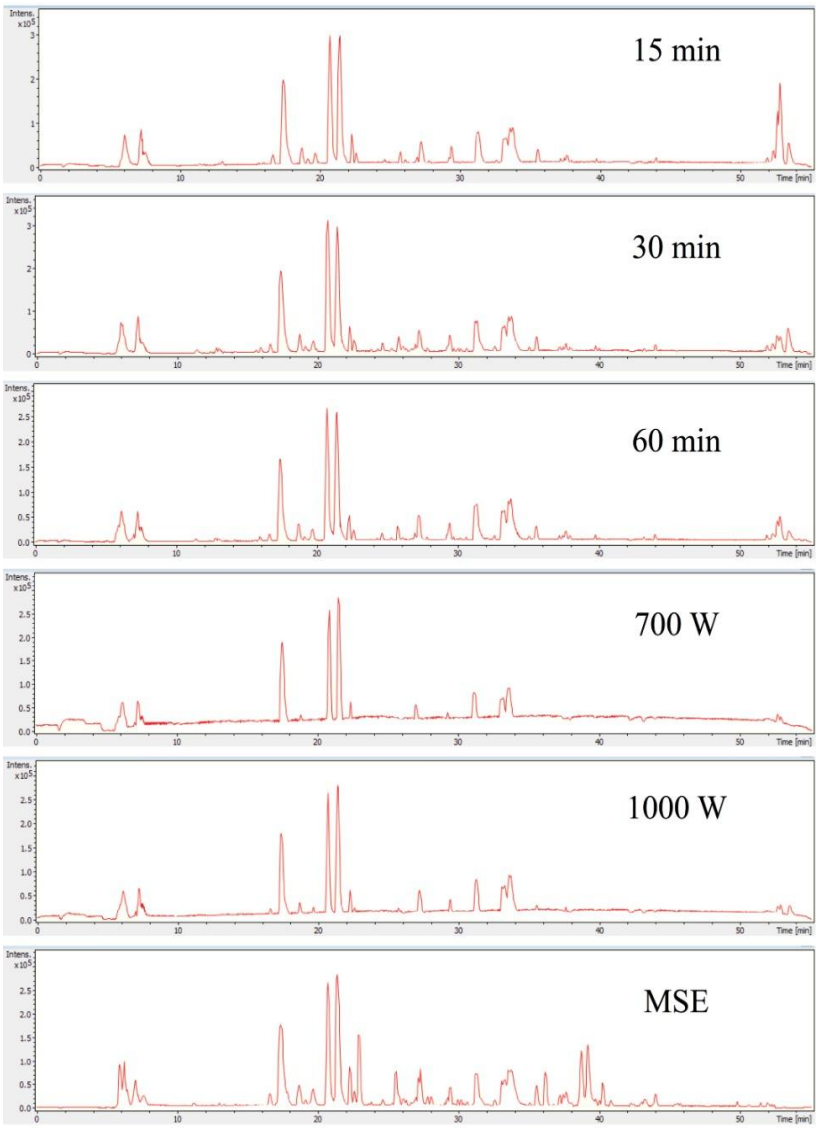


Fig. 2. Chromatograms, obtained by LC-DAD-ESI-MS/MS, of the phenolic compounds from YM in the 6 different treatments.

HPLC quantitative analysis

Variations of the chemical profiles and total content of phenolics can be observed among the different YM extractions, with a typical relative distribution among the main

caffeoyl and dicaffeoylquinic acid isomers. The content of the analytes in the aqueous infusions of different types of YM extraction samples are shown in **Tables 2 to 7**, where values are also presented in mg g^{-1} of YM used for the infusion, according to Panzl, MV et al. (2022).

When we analyze the total amounts of compounds found in the different extraction methods we can verify that AE 15'and AE 60' had no significant difference between the contents, with total amounts of 7020 mg L^{-1} (14,04 mg g^{-1}) and 6756 mg L^{-1} (13,51mg g^{-1}), respectively. AE 30' showed a significant difference with a total amount of 12275 mg L^{-1} (24,55 mg g^{-1}). MAE 700 W and 1000 W showed total amounts of 5811 mg L^{-1} (11,62 mg g^{-1}) and 6793 mg L^{-1} (13,58 mg g^{-1}) and MSE presented a significant different total amount of 25739 mg L^{-1} (51,47 mg g^{-1}), even though those values may not be replicable since consumers make their own extractions with water and never with methanol.

Caffeoylquinic acids were the major constituents of the phenolic fraction of YM, representing more than 50% of all phenolics. In particular, 2-caffeoylquinic acid was the major compound in all extracts, followed by caffeoylquinic acid dimer and 4-caffeoylquinic acids. These representations were similar to the results previously reported by others authors (Bravo et al., 2007, Heck et al., 2008, Marques and Farah, 2009). Dicaffeoylquinic acids were the second most abundant group of phenolics, present in more than 30% of the phenolic fraction, being 2-dicaffeoylquinic acid the main isomer presents YM extracts**.**

The estimated total sum of caffeoylquinic and di-caffeoylquinic acids contents were of 12,42 mg g^{-1} (AE 15'), 23,59 mg g^{-1} (AE 30'), 12,64 mg g^{-1} (AE60'), 7,27 mg g^{-1} (MAE 700 W), 12,61 mg g^{-1} (MAE 1000 W) and 33,97 mg g^{-1} (MSE), in accordance

with Panzl, MV et al. (2022)., who found a total sum of 12.4 mg g^{-1} for roasted YM. **Figures 1 and 2** express a comparison of the amounts of Caffeoylquinic and Dicaffeoylquinic acids in the different extracts.

Minor amounts of some dihydroxycinnamic acid derivatives, such as caffeoyl-feruloylquinic and caffeoyl-*p*-coumaroylquinic acids were also determined. Caffeic acid was mainly esterified with quinic acid, although a minimal amount of free caffeic acid was also quantified. Flavonols were another important group of phenolics identified in YM, being rutin the most abundant, even more than other hydroxycinnamic acid derivatives, in agreement with other authors (Bravo et al., 2007, Heck et al., 2008, da Silveira et al., 2016). **Figure 3** expresses the amounts of rutin and kaempferol rutinoside found between the different extracts.

When comparing mate with coffee, for the equivalent to the traditional drinking manner (25 g per serving), the intake of caffeine is between 172 and 342 mg, whereas the sum of caffeoylquinic acids is between 803 to 1404 mg. The ratio of caffeine to the sum of caffeoylquinic acids (CQAs) is between 0.15 to 0.27, values that are comparable to green or lightly roasted coffee and indicate that YM is a good source of CQAs with a lower intake of caffeine (Center for Science in the Public Interest, n.d.). For coffee shops brews, the amount of caffeine ranges from 150 mg in an espresso (1.5 oz) to 410 mg in a 20 oz serving of coffee. Brewed bags of black tea provide 55 to 60 mg of caffeine and green teas 35-58 mg. Thus, YM (25 mg serving) provides an amount of caffeine comparable to coffee brews (Clifford, M.N and Madala, N.E, 2017).

YM provides a high amount of CGAs relative to the caffeine intake as reflected by low ratios of this parameter and may be considered a good source of CGAs in the diet. These infusions provided a good intake of CGAs depending on the form of consumption.

Besides that, all extraction methods may be of great use to obtain phenolic compounds from YM, although MAE seems to be a fast, easy and cheap method to extract similar amounts of phenolic compounds, such as caffeoylquinic acids and dicaffeoylquinic acids from YM in comparison to the longer times from the aqueous extractions and the impossibility to replicate MSE in the daily consumption of the plant. As well, the use of a green technology, avoiding the application of organic solvents seems to be efficient for the extraction of most phenolic compounds.

In the present study, target compounds were quantitatively extracted in fast steps using a small amount of sample, and the analysis was performed by UHPLC-DAD in a chromatographic method with a rapid separation of the analytes. The analytical method seems to be useful for the evaluation of the chemical profiles of YM infusions, with the advantages of small consumption of samples and chromatographic solvents and short extraction and analysis times.

It is important to highlight that this study was performed in commercial mate products; processing might have altered the initial phenolic composition of mate leaves, since high temperatures during drying and roasting, and the prolonged storage during the "estacionado" of the dried leaves may have caused oxidation and chemical transformations of the phenolic compounds. Therefore, it cannot be inferred whether the identified phenolic compounds were initially present in the fresh mate leaves or formed during processing and storage. Nevertheless, since the analyzed mates are the products actually used by consumers, we consider of interest the chemical characterization of the phenolic fraction here provided.

Table 2. Identification and quantification of phenolic compounds in sample **15'** analyzed by LC-ESI-MS.

Peak	Compound	tR (min)a	λmáx (nm)	Molecular Formula	Theoretical [M-H]-	Experimental [M-H]-	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeic acid hexoside I	15.9	324	C15H18O9	341.0873	341.0889	161(100), 135(25), 133(14), 179(14)	12,58 ± 0,59	0,02 ± 0,59
2	Caffeoylquinic acid I	16.6	321	C16H18O9	353.0873	353.0904	191(100), 135(72), 179(41), 161(5)	0,84 ± 0,23	0,001± 0,23
3	Caffeoylquinic acid II	17.4	329	C16H18O9	353.0873	353.0902	191(100), 135(73), 179(46), 161(4)	1937,70 ± 1,34	3,87± 1,34
4	Caffeic acid hexoside III	18.7	325	C15H18O9	341.0873	341.0909	161(100), 135(61), 179(55), 177(31)	45,22 ± 0,73	0,09± 0,73
5	Caffeoylquinic acid dimer I	19.1	327	C32H35O18	707.1829	707.1848	191(100), 203(19), 463(13), 243(12)	13,72 ±0,39	0,02±0,39
6	Caffeoylquinic acid III	19.7	324	C16H18O9	353.0873	353.0901	135(100), 191(94), 173(87), 179(70)	42,20 ± 1,10	0,08± 1,10
7	Caffeoylquinic acid IV	20.6	329	C16H18O9	353.0873	353.0900	135(100), 191(98), 173(78), 179(59)	642,50 ± 1,15	1,2± 1,15
8	Caffeoylquinic acid dimer II	21.4	329	C32H35O18	707.1829	707.1846	191(100), 353(9), 161(1), 193(1)	1110,43 ± 0,77	2,22± 0,77
9	Feruloylquinic acid I	22.3	324	C17H20O9	367.1029	367.1037	134(100), 193(65), 117(8), 135(8)	32,15 ± 1,35	0,06± 1,35
10	Caffeic acid	24.6	323	C9H8O4	179.0344	179.0412	135(100), 134(78), 136(9), 117(7)	2,93 ± 1,08	0,005± 1,08
11	Coumaroylquinic acid	25.5	280/324	C16H18O8	337.0924	337.0981	191(100), 93(23), 163(19), 119(16)	11,53 ± 0,17	0,02± 0,17
12	Feruloylquinic acid II	26.9	325	C17H20O9	367.1029	367.1105	191(100), 93(26), 134(25), 193(20)	7,47 ± 1,10	0,01± 1,10
13	Rutin	27.2	349	C27H30O16	609.1456	609.1475	300(100), 301(71), 302(10), 179(3)	262,50 ± 0,80	0,5± 0,80
14	Kaempferol rutinoside	29.4	329	C27H30O15	593.1506	593.1559	285(100), 284(50), 286(12), 327(2)	68,00 ± 0,66	0,13 ± 0,66
15	Dicaffeoylquinic acid II	33.2	329	C25H24O12	515.1190	515.1268	191(100), 179(71), 353(21), 135(9)	1685,47 ± 0,66	3,37± 0,66
16	Dicaffeoylquinic acid III	33.7	327	C25H24O12	515.1190	515.1258	173(100), 179(79), 353(35), 191(29)	733,33 ± 1,15	1,46 ± 1,15
17	Caffeoyl-feruloylquinicacid	34.9	324	C26H26O12	529.1346	529.1425	193(100), 173(77), 179(51), 155(23)	10,05 ±0,25	0,02±0,25
18	Dicaffeoylquinic acid IV	35.5	325	C25H24O12	515.1190	515.1268	173(100), 179(85),191(34), 353(34)	10,54 ± 0,36	0,02± 0,36

[a]Retention time on the C_{18}Synergi (4 ⌠m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

Table 3. Identification and quantification of phenolic compounds in sample **30'** analyzed by LC-ESI-MS.

Peak	Compound	tR (min)a	λ máx (nm)	Molecular Formula	Theoretical [M-H]-	Experimental [M-H]-	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeic acid hexoside I	15.9	325	C15H18O9	341.0873	341.0881	161(100), 135(26), 133(14), 179(11)	11,09 ± 0,2	0,02 ± 0,2
2	Caffeoylquinic acid I	16.6	322	C16H18O9	353.0873	353.0892	191(100), 135(73), 179(39), 192(9)	0,11 ± 0,15	0,0002± 0,15
3	Caffeoylquinic acid II	17.3	328	C16H18O9	353.0873	353.0916	191(100), 135(76), 179(46), 192(7)	1746,20 ± 1,09	3,49 ± 1,09
4	Caffeic acid hexoside II	18.8	325	C15H18O9	341.0873	341.0916	161(100), 135(63), 179(55), 177(35)	44,64 ± 1,16	0,08± 1,16
5	Caffeoylquinic acid III	19.7	324	C16H18O9	353.0873	353.0920	135(100), 173(87), 191(82), 179(67)	39,22 ± 0,60	0,07± 0,60
6	Caffeoylquinic acid IV	20.7	331	C16H18O9	353.0873	353.0931	135(100), 191(90), 173(71), 179(49)	6196,89 ± 1,94	12,39± 1,94
7	Caffeoylquinic acid dimer II	21.4	327	C32H35O18	707.1829	707.1797	191(100), 353(9), 192(7), 354(1)	1114,14 ± 0,29	2,22 ± 0,29
8	Feruloylquinic acid I	22.3	324	C17H20O9	367.1029	367.1078	134(100), 193(66), 117(10), 135(8)	30,30 ± 1,02	0,06± 1,02
9	Caffeic acid	24.6	323	C9H8O4	179.0344	179.0401	135(100), 134(85), 136(9), 117(7)	1,42 ± 0,76	0,002± 0,76
10	Feruloylquinic acid II	25.7	325	C17H20O9	367.1029	367.1053	173(100), 134(37), 193(22), 93(20)	9,60 ± 0,81	0,01 ± 0,81
11	Feruloylquinic acid III	27.0	326	C17H20O9	367.1029	367.1078	191(100), 93(27), 134(21), 193(19)	6,65 ± 1,63	0,01 ± 1,63
12	Rutin	27.2	348	C27H30O16	609.1456	609.1426	300(100), 301(74), 302(11), 179(2)	281,23 ± 1,30	0,56± 1,30
13	Kaempferol rutinoside	29.4	330	C27H30O15	593.1506	593.1522	285(100), 284(48), 286(13), 287(2)	62,28 ± 1,94	0,12± 1,94
14	Dicaffeoylquinic acid I	31.3	325	C25H24O12	515.1190	515.1205	173(100), 179(95), 191(40), 353(32)	334,58 ± 1,51	0,66± 1,51
15	Dicaffeoylquinic acid dimer	33.1	328	C50H47O24	1031.2458	1031.2396	353(100), 354(16), 515(6), 191(3)	1663,87 ± 1,87	3,32± 1,87
16	Dicaffeoylquinic acid II	33.6	328	C25H24O12	515.1190	515.1218	173(100), 179(83), 353(38), 191(31)	722,74 ± 1,52	1,44± 1,52
17	Caffeoyl-feruloylquinicacid	35.1	324	C26H26O12	529.1346	529.1378	193(100), 173(72), 179(57), 335(21)	10,90 ± 0,26	0,02± 0,26

[a]Retention time on the C_{18}Synergi (4 ⌠m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

Table 4. Identification and quantification of phenolic compounds in sample **60'** analyzed by LC-ESI-MS.

Peak	Compound	tR (min)a	λ máx (nm)	Molecular Formula	Theoretical [M-H]-	Experimental [M-H]-	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeic acid hexoside I	15.9	325	C15H18O9	341.0873	341.0833	161(100), 135(30), 133(17), 179(15)	10,95 ± 0,15	0,02± 0,15
2	Caffeoylquinic acid I	16.5	322	C16H18O9	353.0873	353.0858	191(100), 135(80), 179(44), 192(7)	1,21 ± 0,50	0,002± 0,50
3	Caffeoylquinic acid II	17.4	328	C16H18O9	353.0873	353.0891	191(100), 135(76), 179(45), 192(8)	1799,66 ± 0,95	3,59± 0,95
4	Caffeic acid hexoside II	18.8	325	C15H18O9	341.0873	341.0895	161(100), 135(68), 179(50), 177(38)	48,52 ± 0,33	0,09± 0,33
5	Caffeoylquinic acid III	19.7	324	C16H18O9	353.0873	353.0880	135(100), 173(77), 191(73), 179(62)	35,02 ± 0,31	0,07± 0,31
6	Caffeoylquinic acid IV	20.7	328	C16H18O9	353.0873	353.0908	135(100), 173(77), 191(73), 179(62)	732,62 ± 1,19	1,52± 1,19
7	Caffeoylquinic acid dimer II	21.4	328	C32H35O18	707.1829	707.1759	191(100), 353(9), 192(7), 354(1)	1102,00 ± 1,14	2,20 ± 1,14
8	Feruloylquinic acid I	22.2	324	C17H20O9	367.1029	367.1067	134(100), 193(70), 135(10), 117(9)	29,03 ± 1,31	0,05± 1,31
9	Caffeic acid	24.6	323	C9H8O4	179.0344	179.0401	135(100), 134(76), 136(12), 117(8)	7,26 ± 1,10	0,01± 1,10
10	Feruloylquinic acid II	25.7	326	C17H20O9	367.1029	367.1043	173(100), 134(39), 193(23), 93(19)	9,45 ± 0,53	0,01± 0,53
11	Feruloylquinic acid III	27.0	326	C17H20O9	367.1029	367.1051	191(100), 93(27), 134(21), 193(21)	4,72 ± 0,49	0,009± 0,49
12	Rutin	27.2	350	C27H30O16	609.1456	609.1396	300(100), 301(72), 302(11), 179(3)	257,41 ± 1,54	0,51± 1,54
13	Kaempferol rutinoside	29.4	336	C27H30O15	593.1506	593.1449	285(100), 284(46), 286(12), 287(2)	71,07 ± 1,47	0,14± 1,47
14	Dicaffeoylquinic acid I	31.3	325	C25H24O12	515.1190	515.1136	173(100), 179(83), 191(38), 353(28)	406,36 ±1,70	0,81±1,70
15	Dicaffeoylquinic acid dimer	33.1	328	C50H47O24	1031.2458	1031.2414	353(100), 354(16), 515(6), 191(4)	1408,17 ± 0,75	2,81 ± 0,75
16	Dicaffeoylquinic acid II	33.6	328	C25H24O12	515.1190	515.1183	173(100), 179(81), 353(36), 191(29)	810,03 ± 1,87	1,62± 1,87
17	Caffeoyl-feruloylquinic acid	35.1	325	C26H26O12	529.1346	529.1349	193(100), 173(66), 179(55), 161(19)	11,71 ± 0,19	0,01 ± 0,19
18	Dicaffeoylquinic acid IV	35.5	325	C25H24O12	515.1190	515.1176	173(100), 179(74),191(34), 353(30)	10,81 ± 1,65	0,02± 1,65

[a]Retention time on the C_{18}Synergi (4 ⌈m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

Table 5. Identification and quantification of phenolic compounds in sample **MAE 700 W** analyzed by LC-ESI-MS.

Peak	Compound	tR (min)a	λ máx (nm)	Molecular Formula	Theoretical [M-H]-	Experimental [M-H]-	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeoylquinic acid I	17.4	325	C16H18O9	353.0873	353.0870	191(100), 179(46), 135(46)	1,20 ± 0,71	0,002± 0,71
2	Caffeic acid hexoside I	17.8	326	C15H18O9	341.0873	341.0856	161(100), 135(27), 179(16), 133(16)	1625,43 ± 0,83	3,25± 0,83
3	Caffeic acid hexoside II	18.7	326	C15H18O9	341.0873	341.0856	161(100), 135(72), 179(47), 221(23)	47,47 ± 1,05	0,09 ± 1,05
4	Caffeoylquinic acid II	19.7	325	C16H18O9	353.0873	353.0860	135(100), 191(98), 173(84), 179(79)	32,71 ± 0,98	0,06± 0,98
5	Caffeoylquinic acid IV	20.8	325	C16H18O9	353.0873	353.0884	135(100), 191(93), 173(72), 179(58)	493,33 ± 1,62	0,98± 1,62
6	Caffeoylquinic acid dimer II	21.4	329	C32H35O18	563.1401	563.1402	149(100), 517(50), 205(47), 191(29)	894,88 ± 1,29	1,78± 1,29
7	Feruloylquinic acid I	22.3	324	C17H20O9	367.1029	367.1036	134(100), 193(96), 117(8), 135(7)	34,55 ± 1,39	0,06± 1,39
8	Caffeic acid	24.4	324	C9H8O4	179.0344	179.0311	135(100), 134(91)	0,76 ± 0,64	0,001± 0,64
9	Feruloylquinic acid II	25.4	326	C17H20O9	367.1029	367.1039	173(100), 134(39), 93(17), 193(16)	5,55 ± 1,59	0,01± 1,59
10	Feruloylquinic acid III	26.7	326	C17H20O9	367.1029	367.1047	191(100), 93(24), 134(23), 193(19)	4,17 ± 1,31	0,008± 1,31
11	Rutin	26.9	349	C27H30O16	609.1456	609.1484	300(100), 301(64), 302(10), 343(2)	291,97 ± 0,86	0,58± 0,86
12	Kaempferol rutinoside	29.2	340	C27H30O15	593.1506	593.1514	285(100), 284(47), 593(38), 286(13)	66,95 ± 1,59	0,13± 1,59
13	Isorhamnetin rutinoside	29.5	336	C28H32O16	623.1612	623.1624	315(100), 314(38), 623(29), 316(18)	n.q	n.q
14	Dicaffeoylquinic acid I	31.1	327	C25H24O12	515.1190	515.1220	173(100), 179(100), 191(39), 353(34)	280,93 ± 1,33	0,56± 1,33
15	Dicaffeoylquinic acid II	33.0	329	C25H24O12	515.1190	515.1223	191(100), 179(73), 353(21), 135(9)	1419,38 ± 0,67	2,83± 0,67
16	Dicaffeoylquinic acid III	33.6	327	C25H24O12	515.1190	515.1220	173(100), 179(84), 353(39), 191(32)	534,79 ± 11,45	1,06± 11,45

[a]Retention time on the C_{18}Synergi (4 ⌠m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

Table 6. Identification and quantification of phenolic compounds in sample **MAE 1000 W** analyzed by LC-ESI-MS.

Peak	Compound	tR (min)a	λ máx (nm)	Molecular Formula	Theoretical [M-H]-	Experimental [M-H]-	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeoylquinic acid I	16.6	321	C16H18O9	353.0873	353.0834	191(100), 135(77), 179(41), 192(6)	9,92 ± 0,43	0,01± 0,43
2	Caffeoylquinic acid II	17.4	329	C16H18O9	353.0873	353.0855	191(100), 135(78), 179(46), 192(7)	1,20 ± 0,71	0,002± 0,71
3	Caffeic acid hexoside I	17.6	325	C15H18O9	341.0873	341.0846	161(100), 135(26), 133(16), 179(16)	47,47 ± 1,41	0,09± 1,41
4	Caffeic acid hexoside II	18.7	325	C15H18O9	341.0873	341.0853	161(100), 135(63), 179(59), 177(36)	32,70 ± 0,98	0,06± 0,98
5	Caffeoylquinic acid III	19.6	325	C16H18O9	353.0873	353.0870	135(100), 173(91), 191(82), 179(66)	1625,43 ± 0,83	3,25 ± 0,83
6	Caffeoylquinic acid IV	20.7	328	C16H18O9	353.0873	353.0869	135(100), 191(91), 173(72), 179(51)	36,02 ± 0,18	0,07± 0,18
7	Caffeoylquinic acid dimer II	21.4	327	C32H35O18	707.1829	707.1890	191(100), 353(9), 192(7), 354(1)	1090,35 ± 1,62	2,18± 1,62
8	Feruloylquinic acid I	22.2	324	C17H20O9	367.1029	367.1014	134(100), 193(70), 117(9), 135(9)	33,71 ± 1,84	0,06± 1,84
9	Caffeoylquinic acid V	22.5	321	C16H18O9	353.0873	353.0853	191(100), 192(7), 161(3), 173(2)	695,19 ± 1,44	1,39± 1,44
10	Caffeic acid	24.6	323	C9H8O4	179.0344	179.0405	135(100), 134(84), 136(10), 109(7)	5,45 ± 1,44	0,01± 1,44
11	Feruloylquinic acid III	26.9	326	C17H20O9	367.1029	367.1016	191(100), 93(26), 134(18), 193(17)	4,60 ± 0,62	0,009± 0,62
12	Rutin	27.1	349	C27H30O16	609.1456	609.1436	300(100), 301(71), 302(11), 179(2)	278,92 ± 1,34	0,55± 1,34
13	Quercetin glucose	29.1	340	C21H20O12	463.0877	463.0852	300(100), 301(37), 302(7), 97(6)	n.q	n.q
14	Kaempferol rutinoside	29.4	331	C27H30O15	593.1506	593.1594	285(100), 284(50), 286(12), 327(2)	68,35 ± 1,81	0,13± 1,81
15	Dicaffeoylquinic acid I	31.3	325	C25H24O12	515.1190	515.1178	173(100), 179(98), 191(42), 353(31)	376,99 ± 1,26	0,75± 1,26
16	Dicaffeoylquinic acid dimer	33.1	328	C50H47O24	1031.2458	1031.2414	353(100), 354(16), 515(6), 191(3)	1697,67 ± 1,76	3,39± 1,76
17	Dicaffeoylquinic acid II	33.6	329	C25H24O12	515.1190	515.1183	173(100), 179(87), 353(41), 191(34)	789,63 ± 0,30	1,57± 0,30

[a]Retention time on the C_{18}Synergi (4 ⌠m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

Table 7. Identification and quantification of phenolic compounds in sample **MSE** analyzed by LC-ESI-MS.

Peak	Compound	t_R (min)[a]	λ máx (nm)	Molecular Formula	Theoretical $[M-H]^-$	Experimental $[M-H]^-$	MS/MS	mg.L^{-1}	mg g^{-1}
1	Caffeoylquinic acid I	16.6	320	$C_{16}H_{18}O_9$	353.0873	353.0826	191(100), 135(72), 179(36), 192(6)	16,67 ± 1,83	0,03± 1,83
2	Caffeoylquinic acid II	17.4	329	$C_{16}H_{18}O_9$	353.0873	353.0859	191(100), 135(73), 179(44), 192(6)	5801,93 ± 1,76	11,60± 1,76
3	Caffeic acid hexoside I	17.6	325	$C_{15}H_{18}O_9$	341.0873	341.0846	161(100), 135(25), 133(13), 179(13)	20,74 ± 0,72	0,04± 0,72
4	Caffeic acid hexoside II	18.7	325	$C_{15}H_{18}O_9$	341.0873	341.0870	161(100), 135(69), 179(56), 221(33)	163,48 ± 0,50	0,32± 0,50
5	Caffeoylquinic acid III	19.7	324	$C_{16}H_{18}O_9$	353.0873	353.0854	135(100), 191(89), 173(84), 179(75)	147,16 ± 1,79	0,29± 1,79
6	Caffeoylquinic acid IV	20.7	328	$C_{16}H_{18}O_9$	353.0873	353.0867	135(100), 191(94), 173(76), 179(53)	1390,34 ± 1,93	1,39 ± 1,93
7	Caffeoylquinic acid dimer II	21.4	328	$C_{32}H_{35}O_{18}$	707.1829	707.1882	191(100), 353(9), 192(7), 354(1)	2932,01 ± 0,87	5,86± 0,87
8	Feruloylquinic acid I	22.1	324	$C_{17}H_{20}O_9$	367.1029	367.1019	134(100), 193(63), 135(11), 117(11)	153,26 ± 0,58	0,30± 0,58
9	p-coumaroylquinic acid	24.2	320	$C_{16}H_{18}O_8$	337.0924	337.0911	173(100), 93(47), 163(35), 119(34)	n.q	n.q
10	Caffeic acid	24.5	323	$C_9H_8O_4$	179.0344	179.0381	135(100), 134(78), 117(10), 89(9)	20,68 ± 0,97	0,04± 0,97
11	Feruloylquinic acid III	25.5	326	$C_{17}H_{20}O_9$	367.1029	367.1012	161(100), 133(10), 162(9), 135(9)	20,80 ± 1,47	0,04± 1,47
12	Rutin	27.1	357	$C_{27}H_{30}O_{16}$	609.1456	609.1437	300(100), 301(71), 302(10), 179(3)	964,04 ± 177	1,92± 177
13	Caffeoyl-quinolactone	28.0	326	$C_{16}H_{16}O_8$	335.0767	335.744	161(100), 135(17), 133(15), 162(9)	n.q	n.q
14	Quercetin glucose	29.1	340	$C_{21}H_{20}O_{12}$	463.0877	463.0809	300(100), 301(49), 97(15), 302(8)	n.q	n.q
15	Kaempferol rutinoside	29.4	340	$C_{27}H_{30}O_{15}$	593.1506	593.1594	285(100), 284(50), 286(12), 287(2)	244,17 ± 1,02	0,48± 1,02
16	Dicaffeoylquinic acid I	31.3	329	$C_{25}H_{24}O_{12}$	515.1190	515.1126	173(100), 179(94), 191(38), 353(28)	956,30 ± 1,91	1,91± 1,91
17	Dicaffeoylquinic acid II	33.6	329	$C_{25}H_{24}O_{12}$	515.1190	515.1154	173(100), 179(86), 353(37), 191(34)	6446,23 ± 1,47	12,89± 1,47
18	Tricaffeoylquinic acid	40.2	327	$C_{34}H_{30}O_{15}$	677.1507	677.1447	353(100), 515(50), 173(31),179(25)	n.q	n.q

[a]Retention time on the C_{18}Synergi (4 ⌠m) column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.**n.q**= below the limit of quantification.

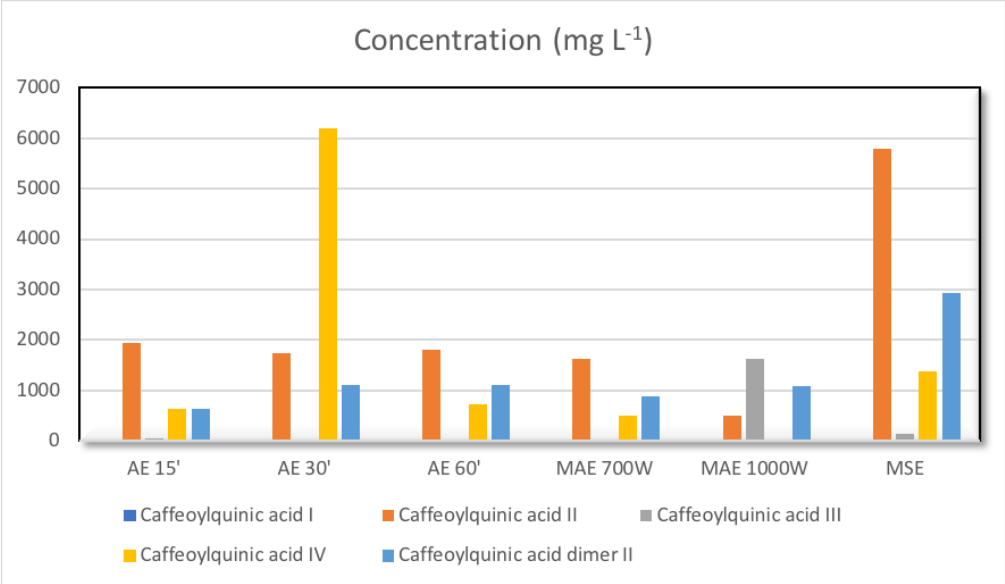


Figure 3. expresses the concentrations of caffeoylquinic acids in the different extracts.

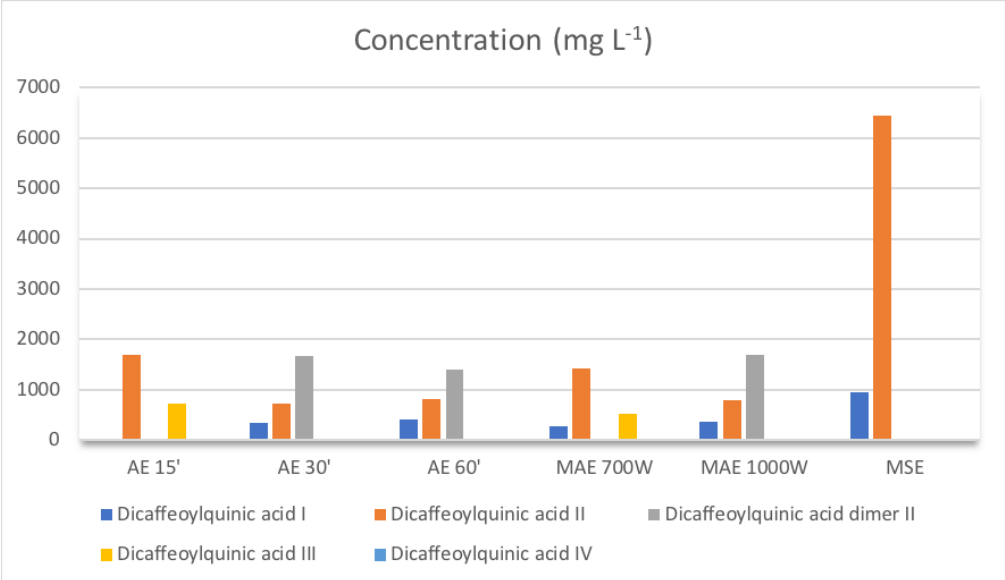


Figure 4. expresses the concentrations of dicaffeoylquinicacids in the different extracts.

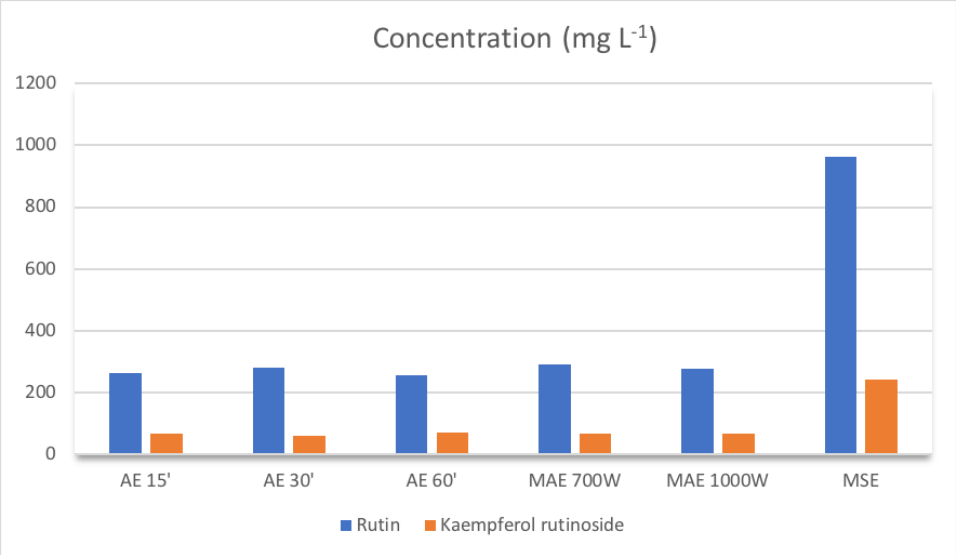


Figure 6. expresses the concentrations of rutin and kaempferol rutinosidein the different extracts.

Conclusion

In order to evaluate the amounts and types of phenolic compounds and other bioactive compounds in human diets, there is a need for fast and robust analytical methods that can be applied to the evaluation of teas with different particle sizes, degree of processing and type of product and extraction. The results of this study increment experimental databases of food bioactive compounds, thus contributing to further research on the consumption of YM products in different populations required to evaluate their potential health-promoting effects. In conclusion, the results revealed that *Ilex Paraguariensis* is an important source of phenolic compounds with a moderate content of methylxanthines and, therefore, with high antioxidant potency mainly associated with the phenolic content.

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Authors' Contributions

Conceptualization: Frazzon, J.; Noreña, C.P.Z. Data acquisition: Vasconcellos, A.C. Data analysis: Vasconcellos, A.C.; Mallmann, L; Noreña, C.P.Z. Design of the methodology: Frazzon, J.; Noreña, C.P.Z.; Mallmann, L. Writing and editing: Vasconcellos, A.C.; Noreña, C.P.Z.

REFERENCES

Alonso-Salces, R. M., Guillou, C., & Berrueta, L. A. (2009). Liquid chromatography cou- pled with ultraviolet absorbance detection, electrospray ionization, collision-induced dissociation and tandem mass spectrometry on a triple quadrupole for the on-line characterization of polyphenols and methylxanthines in green coffee beans. Rapid Communications Mass Spectrometry, 23, 363–383.

Arçari, D. P., Bartchewsky, W., Santos, T. W., Oliveira, K. A., Funck, A., Pedrazzoli, J., ... Ribeiro, M. L. (2009). Antiobesity effects of yerba maté extract (Ilex paraguariensis) in high-fat diet-induced obese mice. Obesity, 17(12), 2127–2133.

Bracesco, N.; Sanchez, A.G.; Contreras, V.; Menini, T.; Gugliucci, A. 2011. Recent advances on Ilex paraguariensis research: Mini review. Journal of Ethnopharmacology 136: 378–384.

Bravo, L., Goya, L., & Lecumberri, E. (2007). LC/MS characterization of phenolic con- stituents of mate (Ilex paraguariensis, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Research International, 40, 393–405.

Cassol, L.; Rodrigues, E.; Noreña, C.P.Z. 2019. Extracting phenolic compounds from Hibiscus sabdariffa L. calyx using microwave assisted extraction. Industrial Crops and Products 133: 168-177.

Clifford, M. N., & Madala, N. E. (2017). Surrogate standards: A cost-effective strategy for identification of phytochemicals. Journal of Agricultural and Food Chemistry, 65(18), 3589–3590. 10.1021/acs.jafc.7b01588.

Clifford, M. N., Knight, S., & Kuhnert, N. (2005). Discriminating between the six isomers of dicaffeoylquinic acid by LC-MSn. Journal of Agricultural and Food Chemistry, 53, 3821–3832.

Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The role of polyphenols in human health and food systems: A mini-review. Frontiers in Nutrition, 5(September), 1–9. 10.3389/fnut.2018.00087.

da Silveira, T. F., Meinhart, A. D., de Souza, T. C., Teixeira Filho, J., & Godoy, H. T. (2016). Phenolic compounds from yerba mate based beverages-A multivariate optimisation. Food Chemistry, 190, 1159–1167.

Dartora, N., De Souza, L. M., Santana-Filho, A. P., Iacomini, M., Valduga, A. T., Gorin, P. A. J., & Sassaki, G. L. (2011). UPLC-PDA-MS evaluation of bioactive compounds from leaves of Ilex paraguariensis with different growth conditions, treatments and ageing. Food Chemistry, 129(4), 1453–1461. 10.1016/j.foodchem.2011.05.112.

de Mejía, E. G., Song, Y. S., Heck, C. I., & Ramírez-Mares, M. (2010). Yerba mate tea (Ilex paraguariensis): Phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. Journal of Functional Foods, 2, 23–34.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly) phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxidants & Redox Signaling, 18, 1818–1892.

Dugo, P., Cacciola, F., Donato, P., Jacques, R. A., Caramao, E. B., & Mondello, L. (2009). High efficiency liquid chromatography techniques coupled to mass spectrometry for the characterization of mate extracts. Journal of Chromatography A, 1216, 7213–7221.

Filip, R., López, P., Giberti, G., Coussio, J., & Ferraro, G. (2001). Phenolic compounds in seven South American Ilex species. Fitoterapia, 72(7), 774–778. 10.1016/S0367-326X(01)00331-8.

Heck, C. I., Schmalko, M., & Gonzalez de Mejia, E. (2008). Effect of growing and drying conditions on the phenolic composition of mate teas (Ilex paraguariensis). Journal of Agricultural and Food Chemistry, 56, 8394–8403.

Konieczynski, P., Viapiana, A., & Wesolowski, M. (2017). Comparison of infusions from black and green teas (Camellia sinensis L. Kuntze) and Erva-mate (Ilex paraguariensis A. St.-Hil.) based on the content of essential elements, secondary metabolites, and antioxidant activity. Food Analytical Methods, 10(9), 3063–3070. 10.1007/s12161-017-0872-8.

Lima, J. DP., Farah, A., King, B., De Paulis, T., & Martin, P. R. (2016). Distribution of major chlorogenic acids and related compounds in Brazilian green and toasted Ilex paraguariensis (Maté) leaves. Journal of Agricultural and Food Chemistry, 64(11), 2361– 2370. 10.1021/acs.jafc.6b00276.

Mallmann, L. P. (2019). ESTUDO DOS COMPOSTOS FENÓLICOS NÃO EXPLORADOS DO ARAÇÁ AMARELO E VERMELHO POR LC-DAD-ESI-MS/MS. UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL.

Mallmann, L.P; Rios, A.O; Rodrigues, E., 2023. MS-FINDER and SIRIUS for phenolic compound identification from high-resolution mass spectrometry data. Food Research International, 163 (112315). https://doi.org/10.1016/j.foodres.2022.112315.

Marques, V., & Farah, A. (2009). Chlorogenic acids and related compounds in medicinal plants and infusions. Food Chemistry, 113, 1370–1376.

Marx, F., Janssens, M. J. J., Urfer, P., & Scherer, R. (2003). Caffeine and theobromine com- position of mate (Ilex paraguariensis) leaves in five plantations of misiones, Argentina. Plant Foods for Human Nutrition, 58(3), 1–8. 10.1023/B:QUAL.0000041144.28025.fc.

Panzl, M. V., Almeida, J. M. S., Pedrozo-Peñafiel, M., Menchaca, D., Aucélio, R. Q., & Rodríguez-Haralambides, A. (2022). Evaluation of polycyclic aromatic hydrocarbons in dried leaves of yerba mate (Ilex paraguariensis) and their extraction into infusions. Polycyclic Aromatic Compounds, 0(0), 1–15. 10.1080/10406638.2022.2030770.

Perrenoud, A. G., Guillarme, D., Boccard, J., Veuthey, J., Barron, D., & Moco, S. (2016). Ultra-high performance supercritical fluid chromatography coupled with

quadrupole- time-of-flight mass spectrometry as a performing tool for bioactive analysis. Journal of Chromatography A, 1450, 101–111. 10.1016/j.chroma.2016.04.053.

Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., Mena, P., Del Rio, D., and Crozier, A., 2014. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. Archives of Toxicology, 88(10), 1803–1853. doi: 10.1007/s00204-014-1330-7.

Souza, M. C., Silva, L. C., Chaves, J. O., Salvador, M. P., Sanches, V. L., Cunha, D. T., Carneiro, T. F., & Rostagno, M. A. (2021). Simultaneous extraction and separation of compounds from mate (Ilex paraguariensis) leaves by pressurized liquid extraction coupled with solid-phase extraction and in-line UV detection. Food Chemistry: Molecular Sciences, 2(September 2020), Article 100008. 10.1016/j.fochms.2020.100008.

Theuma, M., & Attard, E. (2020). From herbal substance to infusion: The fate of polyphenols and trace elements. Journal of Herbal Medicine, 21(November 2019). 10.1016/j.hermed.2020.100347.

Torterolo, P., Falconi, A., Benedetto, L., Rodriguez-Haralambides, A., Rufo, C., & Bracesco, N. (2014). Yerba mate : efectos sobre la vigilia y el sueño. AnFaMed, 1(1), 28–40.

7. CHAPTER IV

Manuscript 4

Extraction of phenolic compounds from Yerba Mate through a rapid green technology method

Running Title: **Bioactive compounds from mate**

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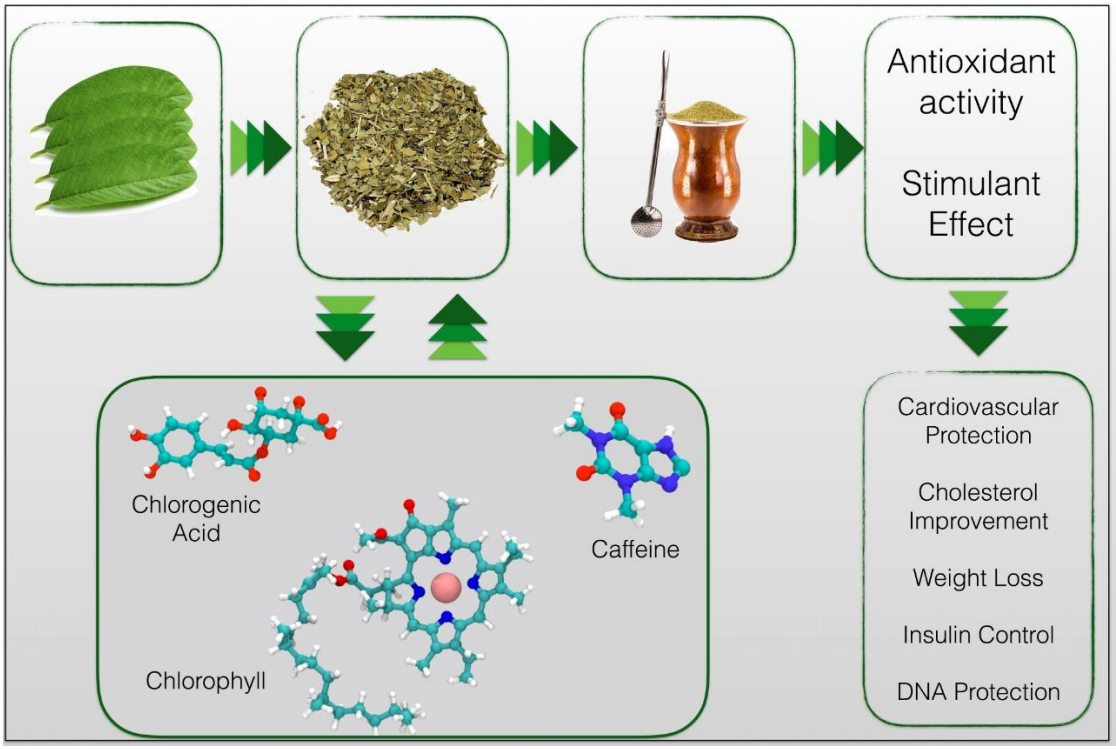

Fig0. Graphic Abstract.

Abstract

Yerba mate (YM) is an industrially processed product derived from the leaves and twigs of *Ilex paraguariensis*. The purpose of this study was to demonstrate the effects of using water as solvent in the process of extraction of phenolic and chlorophyll compounds during infusion at 80 ºC, the temperature at which most YM beverages are consumed, in different extraction methods. The antioxidant capacity of YM was investigated. Methanol, microwave-assisted and aqueous extraction were carried out for comparison purposes. Aqueous infusion for 60 minutes yielded the best results, with values of 48.3 mg GAE g^{-1} and 2.0g chlorophyll g^{-1}, even though microwave produced very similar results in around 60 seconds. In terms of antioxidant capacity, methanol solution produced the greatest yields of DPPH and ABTS, with values of 515.9 and 3630.8 mol TE g^{-1}, respectively. However, HRSA revealed that aqueous infusion provided greater protection against hydroxyl radicals.

Keywords: tea, polyphenols, antioxidant activity, phenolic compounds

Introduction

Ilex Paraguariensis A. St. Hil. (Mate, Aquifoliaceae) is a green tree from South America. Yerba mate (YM) is the main product generated when mate branches and leaves are processed and reach consumers in several countries worldwide (Bastos *et al*., 2006). Mate's pharmacological activities have recently been investigated and documented in scientific papers, highlighting an important antioxidant capacity, as well as a plethora of

beneficial health effects, such as anti-carcinogenic, anti-obesity, and anti-inflammatory properties, prevention of cardiovascular diseases, control of Alzheimer's disease, glucose, insulin and Diabetes, DNA damage protection, improvement of bone mineral density and atherosclerosis decrease (Heck and Mejia, 2007; Bracesco *et al*., 2011; Bastos *et al*., 2007).

Several authors (Anesini *et al*., 2012 *et al*.; Bracesco *et al*., 2011; Heck and Mejia, 2007; Schinella *et al*., 2005) investigated the properties of mate using chemical models and lipoprotein studies *in vivo*, demonstrating that its antioxidant capacity is mainly because of the phenolics present in the extract. However, different chemical components such as enzymes, organic acids, minerals, vitamins, amino acids, xanthines, saponins, lignin, lutein and cellulose were also identified as responsible for mate health benefits (Bastos *et al*., 2006; Bastos *et al*. 2007; Bravo *et al*., 2007; Mateos *et al*., 2018; Silveira *et al*., 2016).

Among all these compounds found in YM extracts some important molecules deserve further investigation motivated by their wide range of applications. Chlorophylls (Chl), for instance, are pigments mostly present in foods as Chl *a* and *b* (Pérez-Gálvez *et al*., 2017). Their photochemically active complexes have been related to antimutagenicity, antigenotoxicity, and antioxidant action (Senge *et al*., 2014). However, their application is restricted due to structural instability and oxidation vulnerability, requiring their extraction via the application of non-destructive methods.

Therefore, the main objective of this study was to compare different extraction methods such as aqueous extraction (AE), methanol solvent extraction (MSE) and microwave-assisted extraction (MAE) in relation to the quality of bioactive components recovered from YM, such as phenolic compounds (TPC) and Chl, as well as to assess their antioxidant capacity using DPPH, ABTS, and HRSA methods.

Materials and Methods

Material

Samples of ground YM were purchased from a local supermarket (30°02'41.2"S 51°12'01.6"W), respecting the use of a single well-established brand on the market and the same batch. Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) and gallic acid, as well as ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)), DPPH (2,2-difenil-1-picrilhidrazil) and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Saint Louis, MO). The packed YM was kept at room temperature, aerated, and light protected. Only distilled water was employed as a solvent in the MAE and AE procedures, whereas methanol and water were utilized in the SE method. All the reagents were of analytical grade.

Extraction methods

Microwave assisted extraction (MAE)

MAE was performed according to Cassol and Noreña (2019). YM was mixed in 1:20 w/w distilled water at room temperature and immediately placed in a conventional microwave oven (Electrolux, MEF41) at 2450 MHz, using a microwave power of 700 W for 1 min (T1) and 1000 W for 40 seconds (T2). Those combinations were determined by previous assays, where several batch times and powers were tested respecting the maximum temperature of 80 °C. Subsequently, the extract was vacuum filtered on Whatman paper n. 01 for solid waste separation and frozen at -18ºC for later analysis.

Aqueous extraction (AE)

The aqueous extraction was performed according to Kuck and Noreña (2016), with minor modifications. YM was mixed in distilled water at a rate of 5 g of herb to 100 ml of water (1:20 w/w) previously heated to 80 °C and kept stirring at constant temperature for 15' (T3), 30' (T4) and 60' minutes (T5) in a magnetic heated stirrer (Fisatom 752a, São Paulo). The maximum heating point was 80 ºC, since it is the temperature advised by the product label and at which the beverage is usually prepared by consumers. The mixture was then cooled and stored in an aluminum-lined glass vial at room temperature protected from light for 20 h. Thereafter, the extract was vacuum filtered on Whatman paper n. 01 for solid waste separation. The filtered extract was frozen at -18 ºC for later analysis.

Methanol solvent extraction (MSE)

A MSE (T6) was also performed to compare the effects of MAE and AE. In a Falcon tube, 2 g of product was added to 10 mL of acidified methanol: water (8:2, v/v) (1% HCl, w/v) at 80 °C. Subsequently, the tube was vortexed (Quimis, Q920-A2) for 5 minutes (Rodrigues *et al*., 2013) and then centrifuged (Sigma, 4K15) at 3000×g for another 5 minutes, and the supernatant was set aside. The supernatants were mixed, and the solvent was evaporated using a rotary evaporator (Heidolph, Laborot 4000) at 40 °C.

Chemical analysis

Total phenolic content (TPC)

The Folin-Ciocalteu technique, developed by Singleton *et al.* (1965), was used to determine the TPC content of the extracts. The method was comprised by the mix of 1 ml of the diluted sample, 0.5 ml of Folin-Ciocalteu reagent, and 2.5 ml of sodium carbonate buffer. The combination was placed in a test tube and left alone in the dark for 2 hours. Posteriorly, the UV-Vis spectrophotometer was used to collect measurements at 765 nm (Thermo Scientific, Genesys S10). Finally, it was built a standard curve, and the results were reported in milligrams of gallic acid per gram of dry material.

Chlorophyll content

The analysis was performed according to Lichtenthaler *et al.* (1987), where 1 g of sample was weighed into a falcon tube and 5 ml of 80% acetone was added. The material was centrifuged (Sigma, 4K15) for 15 min at 3000 rpm. Posteriorly, the supernatant was shifted to a 25 ml flask, completing the volume with 80% acetone. Absorbance readings were taken on a UV-Vis spectrophotometer at wavelengths of 647 and 663 nm.

The tests were done in triplicate and the results were expressed in $\mu g.g^{-1}$ on a dry basis, by the use of equations (1), (2) and (3).

$$Total\ Chl = 7.15 \times A_{663} + 18.71 \times A_{647} \quad (1)$$

$$Chl'a' = 12.25 \times A_{663} - 2.79 \times A_{647} \quad (2)$$

$$Chl'b' = 21.50 \times A_{647} - 5.10 \times A_{663} \quad (3)$$

Where A_{663} and A_{647} refers to the data absorbances of 663 and 647nm wavelength, respectively. *Total Chl, Chl'a' and Chl 'b',* refers to the concentrations of chlorophyll total*, a* and *b,* respectively.

Antioxidant capacity

DPPH (2,2-difenil-1-picril-hidrazil)

The determination of antioxidant capacity by DPPH was performed according to the procedures developed by Brand-Williams *et al*. (1995) with minor modifications. This method is based on the reduction of the violet DPPH radical through the action of antioxidants. The absorbance of DPPH on solution was measured before adding the sample and after 30 minutes of reaction, followed by absorbance readings (515nm) on an UV-Vis spectrophotometer. Values were expressed as percentage of DPPH radical inhibition in comparison to the control values without the extracts. All tests were performed in triplicate. A standard curve was defined with trolox and the results were expressed as mmol-trolox equivalents (TE) per g^{-1} of sample on a dry basis.

ABTS (2,2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))

The determination of the antioxidant capacity by ABTS was done in accordance with the procedures described by Re *et al*. (1999). The procedure began with the preparation of the ABTS stock solution, which was kept at room temperature and protected from light for 16 h. An aliquot of the stock was diluted with distilled water to prepare the ABTS radical solution. The resulting mixture of the ABTS solution and sample rested in the dark for 20 minutes, followed by absorbance readings (734nm) on

an UV-Vis spectrophotometer. Results were expressed by the standard Trolox curve equation as mmol-trolox equivalents (TE) per g^{-1} of sample on a dry basis.

Hydroxyl radical (OH•) scavenging capacity (HRSA)

The extract's ability to protect deoxyribose from hydroxyl radical generation by the Fenton reaction is measured by its hydroxyl radical scavenging capacity. The HRSA of YM extracts was evaluated applying the deoxyribose assay (Halliwell *et al*., 1987). After the treatment with thiobarbituric acid (TBA) at 1 percent (w/v), the formation of a malondialdehyde from the reaction between deoxyribose and OH radical species was verified using a UV-Vis spectrophotometer at wavelength of 532 nm. The extract was tested at three different concentrations (1.0 mg mL^{-1}; 2.5 mg mL^{-1} and 5.0 mg mL^{-1}), and the results were presented as a percentage of hydroxyl radical scavenging.

The HRSA was calculated according to the following equation:

$$Hydroxil\ radical\ scavening\ (\%) = 100 - \left[100 \times \frac{A_{1-}A_2}{A_0}\right] \qquad (4)$$

Where A_0 is the absorbance of deoxyribose in the absence of extract, A_1 is the absorbance of deoxyribose added with the extract and A_2 is the absorbance of extract in the absence of deoxyribose.

Color

Color parameters, *L*, a** and *b*,* were determined using a colorimeter (Chroma Meter CR 410, Japan) and the *CIELab* scale. All measurements were performed in triplicate. According to the method, *L ** indicates lightness and ranges from 0 (black) to

100 (white); and the chromaticity coordinates represented by *a * and b **, go from green (-60) to red (+60); and from blue (-60) to yellow (+60), respectively.

Thereafter, the values of *Chroma* and *Hue* angle were determined according to equations (5) and (6):

$$Croma = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (5)$$

$$Hue = tg^{-1}\left(\frac{b^*}{a^*}\right) \quad (6)$$

Statistical analysis

ANOVA was used to analyze the data, and the Tukey test was employed to compare means (at 5 percent significance level). SAS System for Windows version 9.3 software was used for statistical analysis.

Results and Discussion

Chemical Analysis

Total phenolic compounds (TPC)

Figure 1 depicts the TPC values obtained from YM using various extraction methods. TPC contents of the samples varied from 26.9 (T1) to 48.3 (T5) mg GAE g^{-1} d.b. As can be seen, values for aqueous extractions (AE) at 15, 30 and 60 minutes were higher than all MAE treatments. In fact, the lowest values were obtained from microwave-assisted extractions (T1 and T2) without significant difference among them ($p>0.05$) meaning that neither power nor duration had an influence on the extraction.

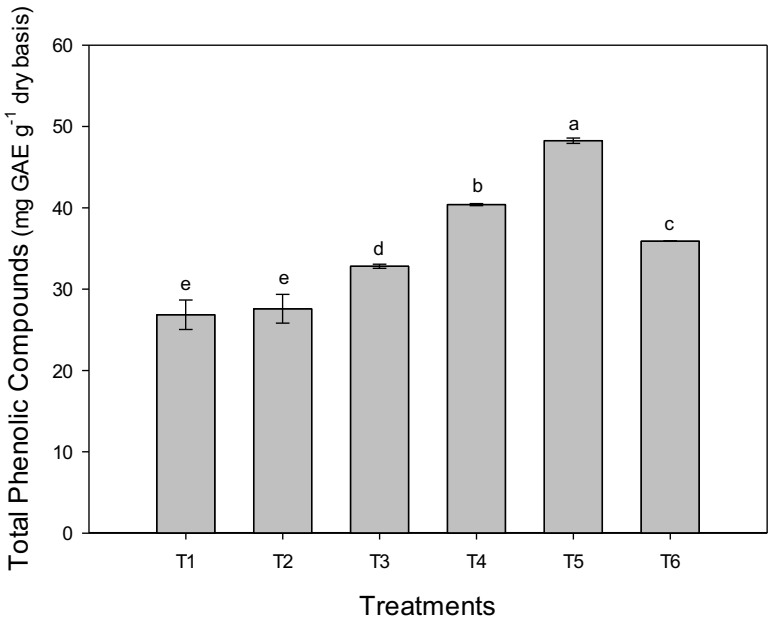

Figure 1 - Total phenolic compounds, where treatments are described as T1 (MAE 700 W×1min); T2 (MAE 1000 W×40sec); T3 (AE 15 min); T4 (AE 30 min); T5 (AE 60 min) and T6 (MSE). Statistically different results were separated by letters a, b, c, d and e.

However, the TPC obtained from aqueous extractions are still lower than those reported in the literature, ranging between 50 and 70 mg GAE g^{-1} d.b (Correa *et al*., 2017; Deladino *et al*. 2013). The same can be observed for the TPC obtained by using ethanol solution (60%, v/v), 193.9 mg GAE g^{-1} d.b (Martin *et al*., 2013).

It is worth noting that all extractions were carried out until the extract reached and maintained a constant temperature of 80 °C; as a result, the phenolic compounds content might not have been entirely extracted. This temperature was deemed the end point of the extraction procedure in order to minimize degradation of bioactive components, and is mostly because mate infusion is often consumed at temperatures below 80 °C.

According to Sadeghi *et al*. (2017), water molecules absorb energy in microwave heating, causing dipole rotation and subsequent internal heating, resulting in water vaporization and higher pressure inside the plant cells. Since this method directly applies an electromagnetic field to the sample, there is an increase of cell breakdown releasing

substances into the solvent (Sparr Eskilsson and Bj¨orklund, 2000; Bouras *et al*., 2015; Rezaei *et al*., 2013).

In contrast, in traditional extraction, heat is transferred by conduction from the heating media to the water, then to the surface, and finally to the interior of the YM powder. In other words, heating rate in conventional heating is determined by thermal conductivity while in microwave heating it is governed by dielectric loss. In addition, according to Mao *et al*. (2021) the presence of the extract positively influences dielectric loss at the same time thermal conductivity is negatively impacted. As a result, the incorporation of biomass into the water increased dielectric loss and decreased thermal conductivity, speeding microwave volumetric heating and delaying traditional heating. Therefore, the reduced batch time applied in the MAE methodology, in order to respect the maximum sample temperature of 80 ºC, caused microwaved extracts to present the lowest TPC content.

On the other hand, for MSE extracts (T6) where the mixing procedure lasted only 5 min, the TPC content, 35.9 mg GAE g^{-1} d.b, was higher than that presented by the aqueous extractions carried out for 15 min (T3). It suggests that better results in phenolic compound extractions might be obtained with longer mixing batch times and solvent/solute affinity.

According to Figure 1, the highest concentration of phenolic compounds was found in aqueous extractions carried out with the longest exposure time (T5, 48.3 mg GAE g^{-1} d.b). However, as previously mentioned, this value is still lower than those reported in the literature. Thermal degradation caused by higher temperatures and longer times could be attributed to phenolic oxidation, decomposition, and polymerization reactions, according to Alonso-Carrillo *et al*., (2017). Temperature seems to have varying impacts on food TPC. Some phenolics have been shown to have better heat stability,

which causes phenolic compounds to rise (Juániz *et al*., 2016). TPC depletion may also occur at high temperatures as a result of phenolics degradation (Turkmen *et al*., 2005).

i.

Chlorophyll content

Figure 2 shows total Chl, Chl *a* and Chl *b* contents of YM extracts obtained by microwave assisted extraction, aqueous extraction and methanol solvent extraction.

For MAE total Chl, Chl *a*, and Chl *b* contents were the lowest values obtained by extraction, while the greatest was found in the AE T5. It is worth noting that unlike the TPC results no significant differences were observed between T3, T4 and T6 (MSE).

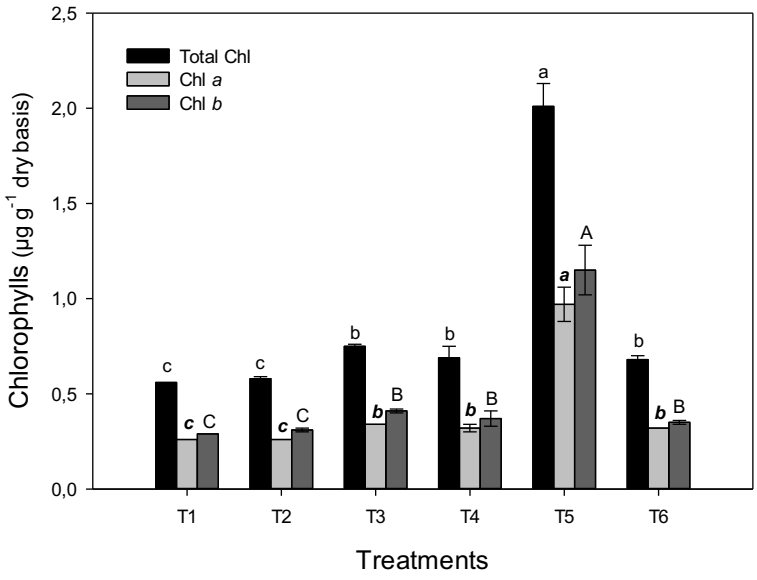

Figure 2 - Total Chlorophyll, Chlorophyll *a* and Cholophyll *b* contents of YM extracts obtained by microwave assisted extraction, aqueous extraction and methanol solvent extraction. T1 (MAE 700 W×1min); T2 (MAE 1000 W×40sec); T3 (AE 15 min); T4 (AE 30 min); T5 (AE 60 min), T6 (MSE). Statistically different results were separated by letters a, b and c.

Because of the presence of a long chain of conjugated carbon double bonds, Chls are sensitive to acid, light, oxygen, and heat deterioration (Karadeniz *et al*., 2007). Temperature and heat treatment time are the two most critical parameters determining the amount of chlorophylls retained after heating (Schwartz *et al*., 1981). On the other hand, heating inactivates enzymes responsible for promoting the rapid green color loss. (Karadeniz *et al*., 2007).

It has been shown that Chl *b* is thermally more stable than Chl *a*. In fact, our results revealed that the Chl *a* level in the YM extracts was lower when compared to Chl *b*. However, enzyme activity is also largely responsible for the degradation of chlorophyll molecules (chlorophyllase, chlorophyll oxidase, magnesium dechelatase, peroxidase, etc.) (Yamauchi and Watada, 1991; Heaton and Marangoni, 1996). Therefore, it is likely that chlorophyllase or some other remaining enzymatic activity destroyed Chl *a* more quickly than Chl *b* in the samples.

In most situations, the Chl content decreases as the heating duration increases. However, when the power on MAE was increased, the contents of Chl *a* and *b* did not change.

According to Schwartz *et al*. (1983), blanching vegetables, which is a heating process, can induce the formation of Chls *a′* and *b′*, respectively, the CIO epimers of Chls *a* and *b*. These findings suggest that the pyropheophytin production is affected by the intensity of the heat treatment, which might explain why the concentrations of Chls *a* and *b* were considerably greater on T5.

Furthermore, based on these results, conventional heating at T5 (AE 60 min) resulted in the highest extraction and preservation of Chls for YM extracts ($p<0.05$). However, Weemaes *et al*. (1999) have reported that the dietary matrix may have a significant impact on the resistance of Chls *a* and *b* to heat degradation, with different rates for various fruits

and vegetables. For instance, Benlloch-Tinoco *et al*. (2015) observed that using a microwave heating method (1000 W×340sec) on the Chl pigments of kiwifruit puree compared to a normal heat treatment allowed for better Chl preservation.

In vitro antioxidant capacity

The antioxidant capacity of YM extracts was assessed by deoxyribose methods as well as ABTS and DPPH. Phenolic compound's ability to donate electrons may be seen, for example, when the purple color solution produced by the DPPH radical lights. According to Zarin *et al*. (2016), in the presence of the radical, the degree of color change is proportional to the concentration of antioxidants.

The synthetic radical DPPH is commonly used to evaluate radical scavenging abilities of bioactive chemicals. It is more stable than superoxide and hydroxyl radicals and is not affected by side reactions like enzyme inhibition or metal ion chelation (Koehnlein *et al*., 2012).

The antioxidant ability assessed by DPPH (Figure 3) suggests that all treatments could inhibit DPPH free radical. In particular, T6 (MSE) showed greater ability to inhibit DPPH radical oxidation (515.9 μmol TE g^{-1} d.b), compared to MAE and AE.

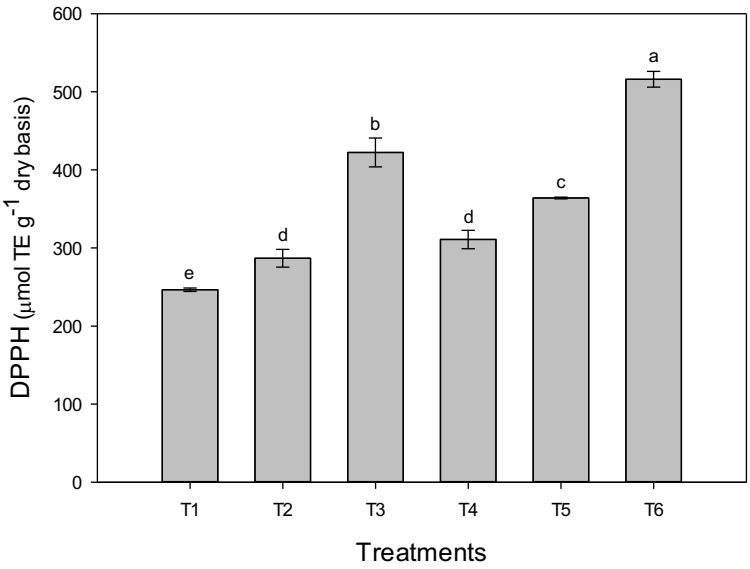

Figure 3 - Antioxidant capacity by DPPH, where treatments are described as T1 (MAE 700 W×1min); T2 (MAE 1000 W×40sec); T3 (AE 15 min); T4 (AE 30 min); T5 (AE 60 min) and T6 (MSE). Statistically different results were separated by letters a, b, c, d and e.

On the other hand, MAE treatments showed the lower values for DPPH inhibition, as well as for TPC content. In its turn, aqueous extraction resulted in better values, with AE 15 min (T3), obtaining the highest yield (422.3 µmol TE g^{-1} d.b), compared to those provided by MAE, with values ranging from 246.5 to 286.8 µmol TE g^{-1} d.b).

The antioxidant capacity of YM extracts assessed by ABTS method evaluates the ability of the extracts to decrease the ABTS+ radical while in aqueous solutions (Re *et al*., 1999). Likewise, the previous analysis, MSE samples exhibited the greatest antioxidant capacity by ABTS (3630.8 mol TE g^{-1} d.b), while T4 and T5 AE, despite having lower values, yielded better results than MAE, which exhibited the lowest extraction yields once again (Figure 4).

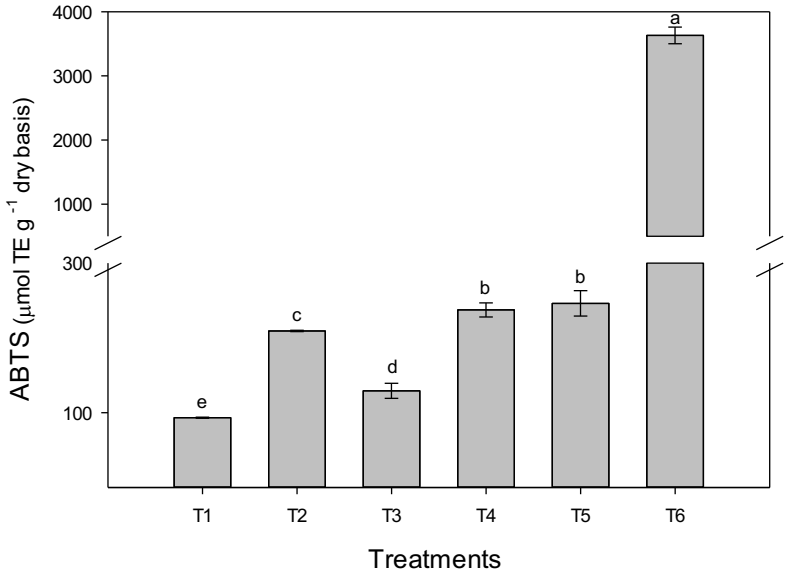

Figure 4 - Antioxidant capacity by ABTS, where treatments are described as T1 (MAE 700 W×1min); T2 (MAE 1000 W×40sec); T3 (AE 15 min); T4 (AE 30 min); T5 (AE 60 min) and T6 (MSE). Statistically different results were separated by letters a, b, c, d and e.

In other analysis, the extract's ability to protect deoxyribose from hydroxyl radical production by the Fenton reaction was evaluated by their hydroxyl radical scavenging capacity, HRSA (Halliwell *et al*., 1987). According to Gio *et al*. (2008), after the insertion of TBA in the deoxyribose solution, the antioxidants coming from the extracts compete for hydroxyl radicals, resulting in less deoxyribose fragmentation.

During these analyses, different extract concentrations (1.0; 2.5; 5.0 mg mL^{-1}) were used to verify hydroxyl radical scavenging ability. The highest concentration of extract (5.0 mg mL^{-1}) showed the greatest efficiency on deoxyribose protection and in percentages of hydroxyl radical scavenging. For this reason, only these results will be presented.

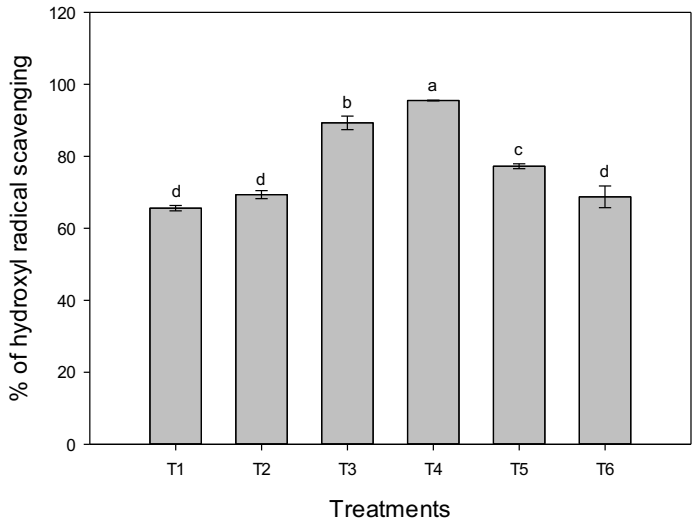

Figure 5 - Antioxidant capacity by HRSA, where treatments are described as T1 (MAE 700 W×1min); T2 (MAE 1000 W×40sec); T3 (AE 15 min); T4 (AE 30 min); T5 (AE 60 min) and T6 (MSE). Statistically different results were separated by letters a, b, c and d.

On the deoxyribose assay (Figure 5), aqueous extraction for 30 minutes (T4) showed the highest percentage of hydroxyl radical scavenging (OH •) (99.1%) and consequent inhibition of deoxyribose degradation, followed by aqueous extraction for 15 minutes (T3) (89.3%). When compared to the lowest yields found with MAE, T1, T2 and MSE did not demonstrate a meaningful difference (65.6, 69.3 and 68.7%, respectively).

Hydroxyl radical scavenging is critical because it decreases lipid peroxidation, radical's effects on cell membranes and DNA damage (Halliwell, 2015). The hydroxyl radical seems to connect nucleotides in DNA, causing DNA strand breaking, conducting to mutagenesis, carcinogenesis, and cytotoxicity (Swarna *et al*., 2013).

Our findings revealed that YM extracts had varied antioxidant profiles when comparing DPPH, ABTS, and hydroxyl radical scavenging tests, suggesting that not all extracts behaved similarly in all techniques. This is most likely owing to the various mechanisms of neutralization and reactivity of each type of radical with the chemicals in the samples.

Furthermore, the primary component present in the sample does not necessarily define their antioxidant ability, but rather the interaction between the minor and major components, which is commonly determined by the type of method used to assess the antioxidant capacity. Furthermore, the antioxidant activity of phenolic compounds is linked to their chemical structure, which may respond differently in each technique. (Apak *et al*., 2013; Dawidowicz and Olszowy, 2014).

Despite what the results suggest, the total antioxidant capacity of plant extracts does not accurately translate what occurs in vivo in plant cells, because there is a dynamic activity of an antioxidant system formed by enzymes (catalase, superoxide dismutase, ascorbate reductase, etc.) and low molecular weight compounds such as flavonoids, tannins, cinnamic, ascorbic acids, tocopherol, etc (Neill *et al*, 2002). Therefore, the correlation between antioxidant activity and oxidation potential is not always straightforward and sometimes requires parallel testing for better understanding.

Despite the widespread use of in vitro antioxidant tests to determine which antioxidants and foods are the most effective, time has revealed that the assays have both technical and conceptual limitations (Apak *et al*., 2013). Although antioxidant tests were developed to discover which antioxidants could be expected to give the highest protection against free radicals in vivo, the radical scavenging found in test tubes most likely does not occur in vivo. Further investigations focusing on diverse trials should be carried out to clarify the antioxidant activity of these compounds.

For instance, Endo *et al*. (1985) proposed that a transfer of singlet excited Chl energy to oxygen, resulting in reactive oxygen species, could explain the pro-oxidant activity of Chls under light. They also suggested that Chls and pheophytins give protection by inhibiting the autoxidation of vegetable oils, as well as a method for donating hydrogen that breaks the radical chain events.

Wanasundara *et al*. (1998) demonstrated a pro-oxidant action of aqueous-ethanol extracts of green tea in edible oils, most likely because of the catalytic activity of their chlorophylls. In fact, Chl seems to exhibit significant antioxidant activity, and although this activity is expressed *in vitro* only at high concentrations of approximately 1 mmol/L, its large amounts found in plants implies that Chl plays a role in protecting against lipid oxidation. (Lanfer-Marquez *et al*., 2005).

Color

The colorimetric parameters of extracts obtained by MAE, AE and MSE are presented in Table 1. The luminosity (*L*),* indicated values ranging from 53.77 to 64.64, being the darkest value assigned to T6 (MSE). This may be related to the type of phenolic compounds and Chls extracted by methanol in contrast to all other methodologies applying only water as solvent. In fact, by using the colorimetric parameters to perform a cluster analysis, it is possible to observe two groups of extracts, one composed by those obtained only through water heating, and the other acquired using the organic solvent, MSE (Figure 6).

Table 1 - Colorimetric parameters of YM extracts obtained by microwave assisted extraction, aqueous extraction and methanol solvent extraction.

	L*	***a****	***b****	***Hue (°)***	***Chroma***
T1	64.64 ± 0.45^{a}	-4.88 ± 0.02^{b}	16.50 ± 0.24^{d}	106.48 ± 0.15^{a}	17.20 ± 0.24^{d}
T2	63.84 ± 0.20^{b}	-5.13 ± 0.09^{a}	17.24 ± 0.33^{c}	106.57 ± 0.02^{a}	17.99 ± 0.30^{c}
T3	63.49 ± 0.41^{b}	-5.17 ± 0.14^{a}	$17.49\pm0.50^{b,c}$	106.46 ± 0.03^{a}	$18.24\pm0.51^{b,c}$
T4	63.52 ± 0.21^{b}	-5.05 ± 0.05^{a}	18.08 ± 0.18^{b}	105.60 ± 0.00^{b}	18.77 ± 0.19^{b}
T5	64.06 ± 0.18^{a}	-4.79 ± 0.02^{b}	18.04 ± 0.06^{b}	104.87 ± 0.02^{c}	18.67 ± 0.06^{b}

T6	53.77±0.36[c]	-3.56±0.19[c]	45.92±0.6[a]	94.43±0.022[d]	46.06±0.68[a]

However, in general, for *a** parameter, all treatments presented negative values, meanwhile for *b** all treatments presented positive values, suggesting their presence in the second quadrant of the chromatic circle (greenness and yellowness). This is owing to the significant prevalence of Chls. For *Hue* angle, expressing tonality, all samples, including the solvent extraction, presented values ranging from 94.43 to 106.57º, which confirmed that the colors are in the second quadrant of the chromatic circle. For color saturation, indicated by *Chroma*, T6 (MSE) presented the highest values.

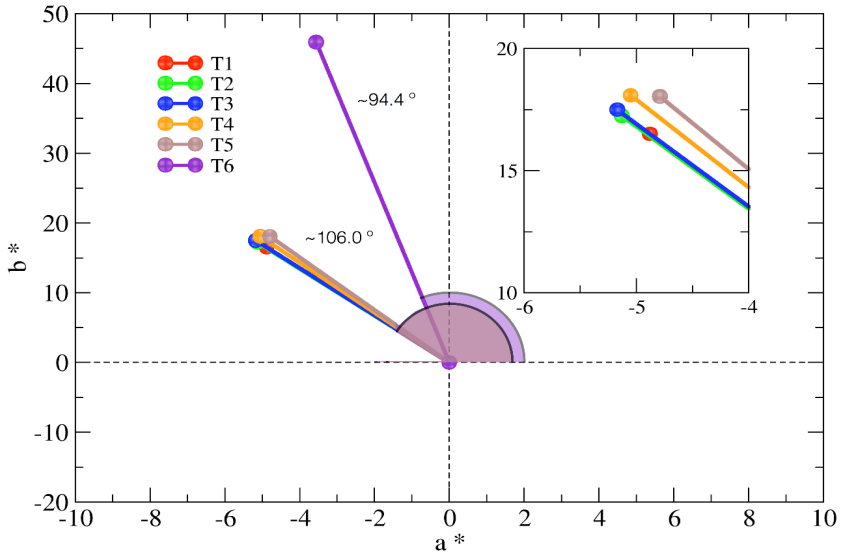

Figure 6 - Colorimetric parameters (*a** and *b**) of YM extracts obtained by microwave assisted extraction, aqueous extraction and methanol solvent extraction, depicted in the third quadrant of the chromatic circle. Samples T1, T2, T3, T4 and T5 are displayed in more detail on the inlet graph.

Conclusion

The optimum extraction conditions for phenolic and chlorophyll components were in water at 80 ºC and time of 60 minutes, obtaining values of 48.3 mg GAE g^{-1} and 2.0 g chlorophyll g^{-1}, respectively. It is noteworthy that all treatments were able to extract more Chl *b* than Chl *a*. In terms of antioxidant activity measured by DPPH and ABTS, the methanol solution extraction separated a greater number of compounds with higher activity; however, it was unable to separate compounds that protected deoxyribose against hydroxyl radicals; in this case, water extractions yielded higher values for HRSA.

Our study demonstrates the separation of chemicals with beneficial characteristics at the temperature at which mate is typically consumed. MAE provided great conditions in terms of time and temperature for separation of phenolic and Chls compounds, but additional research is needed to determine the best separation parameters.

REFERENCES

1. Alonso-Carrillo, N.; Aguilar-Santamaría, M.A.; Vernon-Carter, E.J.; Jiménez-Alvarado, R.; Cruz-Sosa, F.; Román-Guerrero, A. 2017. Extraction of phenolic compounds from Satureja macrostema using microwave-ultrasound assisted and reflux methods and evaluation of their antioxidant activity and cytotoxicity. **Industrial Crops and Products** 103: 213-221.
2. Anesini, C.; Turner, S.; Cogoi, L.; Filip, R. 2012. Study of the participation of caffeine and polyphenols on the overall antioxidant activity of mate (Ilex paraguariensis**). LWT-Food Science and Technology** 45: 299-304.
3. Apak, R.; Gorinstein, S.; Böhm, V.; Schaich, K.M.; Özyürek, M.; Güçlü, K. 2013. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). **Pure and Applied Chemistry** 85: 957-998.
4. Bastos, D.H.M.; Fornari, A.C.; Queiroz, Y.S.; Torres, E.A.F.S. 2006. Bioactive compounds content of chimarrão infusions related to the moisture of yerba mate (Ilex paraguariensis) leaves. **Brazilian Archives of Biology and Technology** 49: 399–404.

5. Bastos, D.H.M.; Oliveira, D.M.; Matsumoto, R.L.T.; Carvalho, P.O.; Ribeiro, M.L. 2007. Yerba maté: Pharmacological properties, research and biotechnology. **Medicinal and Aromatic Plant Science and Biotechnology** 1: 37–46.
6. Benlloch-Tinoco, M.; Kaulmann, A.; Corte-Real, J.; Rodrigo, D.; Martínez-Navarrete, N.; Bohn, T. 2015. Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage. **Food Chemistry** 187: 254–262.
7. Bouras, M.; Chadni, M.; Barba, F.J.; Grimi, N.; Bals, O.; Vorobiev, E. 2015. Optimizationof microwave-assisted extraction of polyphenols from Quercus bark. **Industrial Crops and Products** 77: 590–601.
8. Bracesco, N.; Sanchez, A.G.; Contreras, V.; Menini, T.; Gugliucci, A. 2011. Recent advances on Ilex paraguariensis research: Mini review. **Journal of Ethnopharmacology** 136: 378–384.
9. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. **LWT - Food Science and Technology** 28: 25–30.
10. Bravo, L.; Goya, L.; Lecumberri, E. 2007. LC/MS characterization of phenolic constituents of mate (Ilex paraguariensis A. St.-Hill) and its antioxidant activity compared to commonly consumed beverages. **Food Reseach International** 40: 303-405.
11. Cassol, L.; Rodrigues, E.; Noreña, C.P.Z. 2019. Extracting phenolic compounds from Hibiscus sabdariffa L. calyx using microwave assisted extraction. **Industrial Crops and Products** 133: 168-177.
12. Correa, V.G.; Gonçalves, G.A.; de Sá-Nakanishi, A.B.; Ferreira, I.C.; Barros, L.; Dias, M.I.; Koehnleine, E.A.; de Souza, C.G.M.; Bracht, A.; Peralta, R.M. 2017. Effects of in vitro digestion and in vitro colonic fermentation on stability and functional properties of yerba mate (Ilex paraguariensis A. St. Hil.) beverages. **Food Chemistry** 237: 453-460.
13. Dawidowicz, A.L.; Olszowy, M. 2014. Do antioxidant properties of the main component of essential oil reflect its antioxidant properties? The comparison of antioxidant properties of essential oils and their main components. **Natural Product Research** 28: 1952–1963.
14. Deladino, L.; Teixeira, A.S.; Reta, M.; García, A.D.M.; Navarro, A.S.; Martino, M.N. 2013. Major phenolics in yerba mate extracts (Ilex paraguariensis) and their

contribution to the total antioxidant capacity. **Food and Nutrition Sciences** 4: 154-162.

15. Endo, Y.; Usuki, R.; Kaneda, T. 1985. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. **Journal of the American Oil Chemists Society** 62: 1387–1390.
16. Halliwell, B., 2015. Free radicals and other reactive species in disease. **Enc. Life Sci. (ELS)**. 1-9.
17. Halliwell, B.; Gutteridge, J.M.C.; Aruoma, O.I. 1987. The deoxyribose method: a simple ‘‘test tube’’ assay for determination of rate constants for reactions of hydroxyl radicals. **Analytical Biochemistry** 165: 215-219.
18. Heaton J.W.; Marangoni A.G. 1996. Chlorophyll degradation in processed food and senescent plant tissues. **Trends in Food Science and Technology** 7: 705- 708.
19. Heck C.I.; Mejia E.G. 2007. Yerba mate tea (Ilex paraguariensis): A comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science** 72: 138–151.
20. Juániz, I.; Ludwig, I.A.; Huarte, E.; Pereira-Caro, G.; Moreno-Rojas, J.M.; Cid, C.; De Peña, M.P. 2016. Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. **Food Chemistry** 197: 466–473.
21. Kuck, L.S.; Noreña, C.P.Z. 2016. Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. **Food Chemistry** 194: 569-576.
22. Lanfer-Marquez, U.M.; Barros, R.M.C.; Sinnecker, P. 2005. Antioxidant activity of chlorophylls and their derivatives. **Food Research International** 38: 885–891.
23. Lichtenthaler, H.L. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods Enzymology** 148: 350-382.
24. Mao, Y.; Robinson, J.; Binner, E. 2021. Understanding heat and mass transfer processes during microwave-assisted and conventional solvent extraction. **Chemical Engineering Scienc** 233: 116418.
25. Martin, J.G.P.; Porto, E.; de Alencar, S.M.; da Glória, E.M.; Corrêa, C.B.; Cabral, I.S.R. 2013. Antimicrobial activity of yerba mate (Ilex paraguariensis St. Hil.) against food pathogens. **Revista Argentina de microbiologia** 45: 93-98.

26. Mateos, R.; Baeza, G.; Sarriá, B.; Bravo, L. 2018. Improved LC-MSn characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (Ilex paraguariensis) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry** 241: 232-241.
27. Neill, S.O.; Gould, K.S.; Kilmartin, P.A.; Mitchell, K.A.; Markham, K.R. 2002. Antioxidant activities of red versus green leaves in Elatostema rugosum. **Plant, Cell & Environment** 25: 539-547.
28. Pérez-Gálvez, A.; Viera, I.; Roca, M. 2017. Chemistry in the bioactivity of chlorophylls: an overview. **Current Medicinal Chemistry** 24: 4515-4536.
29. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Riceevans, C. 1999. Antioxidant activity applyng an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine** 26: 1231-1237.
30. Rezaei, S.; Rezaei, K.; Haghighi, M.; Labbafi, M. 2013. Solvent and solvent to sample ratio as main parameters in the microwave-assisted extraction of polyphenolic compounds from apple pomace. **Food Science and Biotechnology** 22: 1–6.
31.
32. Sadeghi, A.; Vaid. H.; Behnam, K. 2017. Microwave assisted extraction of bioactive compounds from food: a review. **International Journal of Food Sciences and Nutritional Engineering** 7: 19-27.
33. Schinella, G.; Fantinelli, J.C.; Mosca, S.M. 2005. Cardioprotective effects of Ilex paraguariensis extract: evidence for a nitric oxide-dependent mechanism. **Clinical Nutrition** 24: 360-366.
34. Schwartz, S.J.; Woo, S.L.; von Elbe, J.H. 1981 High- performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. **Journal of Agricultural and Food Chemistry** 29: 533-535.
35. Schwartz, S.J.; Von Elbe, J.H. 1983. Kinetics of chlorophyll degradation to pyropheophytin in vegetables**. Journal of Food Science** 48: 1303–1306.
36. Senge, M.O.; Ryan, A.A.; Letchford, K.A.; MacGowan, S.A.; Mielke, T. 2014. Chlorophylls, symmetry, chirality, and photosynthesis. **Symmetry** 6: 781–843.
37. Silveira, T.F.F.; Meinhart, A.D.; Coutinho, J.P.; de Souza, T.C.L.; Cunha, E.C.E.; de Moraes, M.R.; Godoy, H.T. 2016. Content of lutein in aqueous extracts of yerba mate (Ilex paraguariensis St. Hil). **Food Research International** 82: 165-171.

38. Singleton, V.L.; Rossi, J.A. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. **American Journal of Enology and Viticulture** 16: 144–158.
39. Sparr Eskilsson, C.; Bj¨orklund, E. 2000. Analytical-scale microwave-assisted extraction. **Journal of Chromatography A** 902: 227-250.
40. Swarna, J.; Lokeswari, T.S., Smita, M.; Ravindhran, R. 2013. Characterisation and determination of in vitro antioxidant potential of betalains from Talinum triangulare (Jacq.) Willd. **Food Chemistry** 141: 4382–4390.
41. Turkmen, N.; Sari, F.; Velioglu, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. **Food Chemistry** 93: 713–718.
42. Wanasundara, U.N.; Shahidi, F. 1998. Antioxidant and prooxidant activity of green tea extracts in marine oils. **Food Chemistry** 63: 335–342.
43. Weemaes, C.A.; Ooms, V.; Van Loey, A.M.; Hendrickx, M.E. 1999. Kinetics of chlorophyll degradation and color loss in heated broccoli juice. **Journal of Agricultural and Food Chemistry** 47: 2404-2409.
44. Yamauchi, N.; Watada, A.E. 1991. Regulated chlorophyll degradation in spinach leaves during storage. **Journal of the American Society for Horticultural Science** 116: 58-62.
45. Zarin, M.A.; Wan, H.Y.; Isha, A.; Armania, N. 2016. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from Leucaena leucocephala hybrid-Rendang. **Food Science and Human Wellness** 5: 65-75.

8. CHAPTER V - ANNEX

Manuscript 5

Other analyzes that were carried out

Encapsulation of Yerba Mate extract using Gum Arabic, Guar Gum and Polydextrose as wall material: Characterization of physicochemical, structural and bioactive properties of microparticles

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Abstract

Yerba mate extracts (YM) have been proven in several studies to be an ally in preventing the development of a plethora of diseases as well as defending against free radical reactivity, mainly because of its antioxidant capabilities. Thereby, as a natural antioxidant and defined as GRAS (generally regarded as safe), YM might be a viable low-cost natural antioxidant for extending the shelf life of foods or serving as a functional ingredient in the food sector. However, its compounds are particularly sensitive to changes in the medium and must be protected in order to preserve or increase antioxidant activity. Encapsulation is a recent approach that might be useful for this reason. More

specifically, microencapsulation techniques can pack particles, prolonging and protecting the inside component and its functioning. The purpose of this study was to use dietary fibers, such as Guar gum, Polydextrose and Gum Arabic, which are carbohydrate polymers with a degree of polymerization greater than 3, as wall materials for the chemical encapsulation of YM extracts. To do so, rheological properties of the YM extract dispersions were carried out to confirm the feasability of those dietary fibers as wall materials in the extract for the development of microparticules by encapsulation using lyophilization and atomization methods

KEYWORDS: microencapsulation, antioxidant, dietary fibers, bioavailability.

Introduction

Yerba Mate (YM) is the major product derived from *Ilex paraguariensis* A. St. Hil., an Aquifoliaceae green tree originated from South America obtained after the operation process where the raw mate leaves and branches are processed (Bastos *et al*., 2007).

Phenolic Compounds (chlorogenic acid), methylxanthines (caffeine and theobromine), saponins, and over 200 phytochemicals are found in the leaves of YM, all of which contribute to the antioxidant activity of its extracts (Mateos *et al*., 2018; Bravo *et al*., 2007; Heck & De Mejia, 2007).

Over the last two decades, clinical trials have explored the use of YM in the prevention and treatment of a variety of conditions (Kim *et al*., 2015), such as atherosclerosis, Alzheimer, diabetes, chronic kidney disease (Gugliucci *et al*., 2009), cancer (Ronco *et al*., 2016) and obesity (Martinet *et al*., 1999). Several authors have

associated YM to a wide range of health benefits, including antioxidant capabilities (Colpo *et al*., 2016, de Lima *et al*., 2017), vasodilators functions (Yu *et al*., 2015), gene modulation and DNA damage defense (Fernandes *et al*., 2012), hypoglycemic effects (Ribeiro *et al*., 2017), anti-obesity and weight loss properties (Kim *et al*., 2015; Arçari *et al*., 2009), cardioprotective effects (Schinella *et al*., 2005) cholesterol improvement (Arçari *et al*., 2011) and thermogenic effects (Martinet *et al*., 1999).

Antioxidants are commonly used in the food industry to reduce changes in scent, nutritional value, flavor, and color (Schinella *et al*., 2000). Synthetic antioxidants are often used and are thought to be stable and effective; yet, their use is restricted in many countries since they might be hazardous to human health (Schinella *et al*., 2000). YM is GRAS (generally regarded as safe) and might be a viable low-cost natural antioxidant for extending the shelf life of foods or serving as a functional ingredient in the food sector. In this context, natural antioxidants have emerged as a viable replacement for synthetic food additives, which have been linked to several harmful health consequences (Moure *et al*., 2001).

The antioxidant capacity of YM *in vitro* is well documented, and it has been strongly linked to its phenolic content; nevertheless, the influence of the phenolic fraction and the mechanism by which it expresses antioxidant properties *in vivo* remains unknown. Because most compounds are particularly sensitive to changes in the medium once isolated, they become more labile and should be protected before application. Various variables, including oxygen, light, pH, and temperature, may quickly degrade these molecules (Bakowska *et al*., 2003).

Due to the sensitivity, protection from the surrounding medium can be achieved using a variety of modern techniques in order to preserve or increase antioxidant activity. Furthermore, because a significant amount of phenolic compounds are not absorbed by

the human body and its metabolites pass through the small and large intestine, the potential interactions between phenolics and the gut microbiota are also important to understand and improve the antioxidant mechanism (Del Rio *et al*., 2010).

In general, a viable candidate for disease prevention or health promotion should be both bioavailable and bioactive at the site of action. "(oral) bioavailability," as defined by the FDA, refers to "the rate and degree to which the active ingredient or active moiety is absorbed from a drug product and becomes accessible at the site of action" (FDA, 2003). Even though most dietary PC are xenobiotics with low bioavailability, it is already known that the result of phenolic rich-food consumption is beneficial to the human body, with a high potential for health promotion (Rein *et al*. 2013), the mechanisms involved in the process are still unknown.

Some PCs with *in vitro* bioactivity have limited intestinal absorptivity and quick clearance rates *in vivo*, leading in little systemic impact. Moreover, even the most abundant PC in the diet, as well as their primary metabolites, may not reach high enough concentrations at the site of action to trigger any biological response. The gap between most PC's low bioavailability and their *in vivo* biological effects is causing researchers to focus on the Gut Microbiota (GM). The wide variation in PC absorption, distribution, metabolism, and excretion across individuals has been a subject of worry. Individuals with polymorphisms in intestinal enzymes or xenobiotic transporters may absorb more than others (Enokido *et al*. 2014).

Encapsulation, for example, is a recent approach that might be useful for this reason. It is well recognized that microencapsulation techniques, such as spray drying, can increase the stability of phenolic compounds. The microencapsulation technique uses an encapsulating substance to pack particles, prolonging and protecting the inside component and its functioning. It also provides a way to change some of the product's

features, such as its look or the product's interactions with the carrier food matrix (Kuang *et al*., 2010; Hu *et al*., 2017).

Microencapsulation enhanced the bioactivity of the yerba mate distilled water extract, according to recent research by Vargas *et al*. (2021). The *in vivo* research lasted 30 days and involved 32 male Wistar rats divided into four groups (n = 8): control, yerba mate extract, microparticles, and empty microparticles, using sodium alginate as the wall material. When compared to the other groups, the rats that received the YM microparticles had significantly greater antioxidant activity and lower lipid peroxidation in their plasma and brains, respectively. These findings revealed that the YM extract's bioactivity had been enhanced via microencapsulation.

Wall Materials

Fibers, proteins, and gums are some of the wall materials that may be utilized to encapsulate food (Shahidi and Han, 1993). It's vital to understand that using multiple encapsulating agents for microcapsule synthesis might result in varying physical qualities, depending on the structure and features of each agent (Tonon *et al*., 2009), as well as changing the microcapsules' functional properties (Chen *et al*., 2005). In this sense, there has been an increase in the use of prebiotics as encapsulating agents to protect these compounds due to their characteristics to the food industry as well as their nutritional value, when they also provide the body with the fiber's prebiotic benefits. As well, coatings with prebiotics activity have been suggested as encapsulation agents for bioactive compounds.

Dietary fibers, which are carbohydrate polymers with a degree of polymerization greater than 3, are a form of wall material utilized for chemical encapsulation that is neither digested nor absorbed in the small intestine, meaning they are resistant to

breakdown by food enzymes found in the human body (Phillips *et al*., 2008; Phillips and Williams, 2008). Dietary fibers have the following characteristics: they shorten intestinal transit time and increase stool volume; they are fermentable by the colon microbiota; they lower LDL cholesterol levels; and they lower postprandial blood glucose and/or insulin levels (Phillips *et al*., 2008).

Whey protein, gum arabic, chitosan, and polydextrose were employed as wall materials to encapsulate garlic extract by Tavares, Santos e Noreña (2021). Their findings suggest that this research may be utilized to improve the quality and shelf life of bioactive compound-rich powders by adjusting their processing and storage settings. Because their powders included large levels of phenolic chemicals, they were able to use them in food items.

Guar gum is a galactomannan isolated from the endosperm of guar seed, used as a soluble fiber supplement (Slavin, 2003). It is a natural polymer extracted from the seed of the plant *Cyamopsis tetragonalobus* and is commercially cultivated in parts of the Indian Subcontinent as well as in North Africa and South America (Gong *et al*, 2012). The mechanism of action of guar gum and other soluble fibers is based on their action of sequestering bile acids in the duodenum. Studies suggest that it directly inhibit the absorption of cholesterol and its production. Although with proven physiological effects, its high viscosity limits the use of guar gum or its incorporation into foods (Farmer *et al*., 1995)

This polysaccharide produces very viscous solutions, even in low concentrations, due to its high molecular weight and the presence of extensive intermolecular associations through hydrogen bonds (Gong *et al*, 2012). It has the characteristic of having a high hydrophilic character attributed to the interaction of hydroxyls present in galactose with water forming bonds of hydrogen (Thombare *et al*, 2016) which is one of the factors that

limits its use in a wider range of industrial areas. Guar gum is an organic compound consisting of ß-1,4-glycosidic mannose units and one α-1,6-galactose unit every two units of mannose, forming branches (Oliveira *et al*, 2015).

Partially hydrolyzed guar gum (PHGG) has its viscosity significantly reduced, making this fiber easily added to foods and accepted by consumers. Its use as a modulator of intestinal transit, improving both diarrhea and constipation, has been proven by several studies. However, it is not clear that, after loss of viscosity, all effects of guar gum (including cholesterol-lowering and hypoglycaemic effects) are maintained (Alam *et al*., 2000; Spapen *et al*., 2001).

Polydextrose is a biopolymer that has a low caloric content and is considered to be a dietary fiber with probiotic activity, due to its low digestibility in the small intestine, passing directly to the colon where it is gradually fermented by the endogenous microbiota (Craig *et al*., 1999), and has many positive effects on human health, including reducing cholesterol and serum glucose levels, inducing apoptosis of prostate cancer cells and in stimulating immune responses (Jackson *et al*., 2007; Mohnen, 2008). Polydextrose is a highly branched, randomly bonded glucose polymer with an average degree of polymerization of 12, ranging from 2 to 120. The molecule contains all possible combinations of α- and β-linked 1→2 (Lahtinen *et al*., 2010; Flood *et al*., 2004; Murphy *et al*., 2001).

Gum Arabic is a dietary fiber with antioxidant properties, effects against liver, kidney and cardiac toxicity and is used in the treatment of kidney disease and diabetes (Ali *et al*., 2009). This gum is used as a wall material in several encapsulation processes, largely due to its low cost, non-toxicity, biocompatibility, biodegradability, good ability to act as a stabilizer, emulsifier and protection against oxidation processes (Fernandes *et*

al., 2016; Eratte *et al*., 2014; Garcia-Saldaña *et al*., 2016; Kuck and Noreña, 2016; Premi and Sharma, 2017).

Gum arabic, a natural polysaccharide derived from exudates of *Acacia senegal* and *Acacia seyal* trees, is a commonly used food hydrocolloid. The complex chemical structure of the gum has been widely studied revealing a multifraction material consisting mainly of a highly branched polysaccharide and a protein–polysaccharide complex (GAGP) as a minor component. It is recognized by many researchers that GA consists of mainly three fractions:**1**-**4** (1) The major one is a highly branched polysaccharide consisting of β-(1→3) galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid (found in nature as magnesium, potassium, and calcium salt). (2) A smaller fraction (∼10 wt% of the total) is a higher molecular weight arabinogalactan–protein complex (GAGP –GA glycoprotein) in which arabinogalactan chains are covalently linked to a protein chain through serine and hydroxyproline groups. The attached arabinogalactan in the complex contains ∼13% (by mole) glucoronic acid. (3) The smallest fraction (∼1% of the total) having the highest protein content (∼50 wt %) is a glycoprotein which differs in its amino acids composition from that of the GAGP complex (Randall *et al*., 1988; Fenyo *et al*., 1990; Islam *et al*., 1997; Idris *et al*., 1998).

Rheology

Sequentially, prior to drying the dispersion for encapsulation, it is necessary to analyze the rheological properties of these dispersions in order to understand the structural qualities of the extract and the wall materials, as well as the flow characteristics during spray drying. In this context, rheology is the study that investigates how fluids

flow and deform, as well as how materials react to applied stress or strain (Rao, 2014; Steffe, 1996).

Rheological analyses provide useful information about molecular interactions between polymers and encapsulated compounds, as well as the formation of network structures between them, allowing researchers to assess specific molecular mechanisms of the encapsulation process and predict the final properties of microparticles (Tavares *et al*., 2021; Wandrey *et al*., 2010). It's also a useful tool for relating the physical characteristics, structure, and texture of foods composed mostly of biopolymers (proteins, polysaccharides, and lipids) and aqueous solutions containing dissolved sugars and ions (Rao, 2014; Upadhya *et al*., 2020). Food scientists can utilize rheological characterisation to help with ingredient selection, as well as determining and optimizing production techniques, designing packaging and storage solutions (Dogan et el., 2011).

The aim of this study was to evaluate chemical and rheological properties of the YM extract dispersions using guar gum, polydextrose and gum arabic in order to suggest the feasability of those fibers as wall materials in the extract for the development of microparticules by encapsulation using lyophilization and atomization methods.

Materials and Methods

Material

Samples of ground YM were purchased from a local supermarket (30°02'41.2"S 51°12'01.6"W), respecting the use of a single well-established brand on the market and the same batch). The packed YM was kept at room temperature, aerated, and light protected. Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) and gallic acid, as well as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), DPPH

(2,2-difenil-1-picrilhidrazil) and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Saint Louis, MO). All the reagents were of analytical grade.

Aqueous extract preparation from Yerba mate (AE)

The preparation of the UM extract was carried out through an aqueous extraction at 80 °C over a 60 min stirring bath as described by Kuck and Noreña (2016). Previous analysis of TPC, chlorophyll maintenance, color and antioxidant activityhave shown this as the best methodology concerning all those parameters.

YM was mixed in distilled water at a rate of 5 g of herb to 100 ml of water (1:20 w/w) previously heated to 80 °C and kept stirring at constant temperature for 60 minutes in a magnetic stirrer with heating (Fisatom 752a, São Paulo). The maximum heating temperature was 80 ºC, since it is the temperature advised by the product label and at which the beverage is usually prepared by consumers. It was then cooled and stored in an aluminum-lined glass vial at room temperature protected from light for 20h. Following, the extract was vacuum filtered on Whatman paper n. 01 for solid waste separation.

Subsequently, the filtered extract was frozen at -18ºC for later analysis. Before the dispersions were prepared, chemical analysis for the evaluation of total phenolic content (TPC) and antioxidant capacity of the extract by ABTS and DPPH were performed.

Total phenolic content (TPC)

The Folin-Ciocalteu technique, developed by Singleton *et al.* (1965), was used to determine the TPC content of the extract. For this, 1 ml of the diluted sample, 0.5 ml of Folin-Ciocalteu reagent, and 2.5 ml of sodium carbonate buffer. The combination was placed in a test tube and left in the dark for 2 hours. Subsequently, the UV-Vis spectrophotometer was used to collect measurements at 765 nm (Thermo Scientific,

Genesys S10). Finally, a standard curve was built, and the results were reported in milligrams of gallic acid per gram of dry basis.

ABTS anioxidant capacity

The determination of the antioxidant capacity of the extract by ABTS (2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was done in accordance with the procedures described by Re *et al*. (1999). The procedure started with the preparation of the ABTS stock solution, which was kept at room temperature and protected from light for 16 h. An aliquot of the stock was diluted with distilled water to prepare the ABTS radical solution. The resulting mixture was left in the dark for 20 minutes, followed by absorbances readings (734nm) on an UV-Vis spectrophotometer. Results were expressed by the standard Trolox curve equation as mmol-trolox equivalents (TE) per g^{-1} of sample on a dry basis.

DPPH antioxidant capacity

The determination of antioxidant capacity of the YM extract by DPPH (2,2-difenil-1-picril-hidrazil) was done according to the procedures developed by Brand-Williams *et al.* (1995) with minor modifications. This method is based on the reduction of the violet DPPH radical through the action of antioxidants. The DPPH radical was prepared in pure methanol. For the analyses, 100 µl of yerba mate extract and 3.9 ml of radical were used.

Then, after 30 minutes of reaction, absorbance readings (515nm) on an UV-Vis spectrophotometer were performed. Values were expressed as percentage of DPPH radical inhibition in comparison to the control values without the extract. All tests were

performed in triplicate. It was defined as a standard curve with Trolox, and the results were expressed as mmol-trolox equivalents (TE) per g^{-1} of sample on a dry basis.

Preparation of the Dispersions

After the TPC and antioxidant capacity analysis of the extract, three dispersions were prepared in a total proportion of 15% of wall material to 85% of extract, being: T1: GG POLI EXT (7.5% guar gum and 7.5% polydextrose), T2: GA POLI EXT (7.5% gum arabic and 7.5% polydextrose), T3: GGGA EXT (7.5% guar gum and 7.5% gum arabic). The dispersions were stirred at 6500 rpm for 5 min using an Ultra-Turrax (T25, IKA). For comparison purposes, all dispersions were prepared in duplicate but using distilled water in place of the extract, being: T4: GG POLI (7.5% guar gum and 7.5% polydextrose), T5: GA POLI (7.5% gum arabic and 7.5% polydextrose), T6: GGGA (7.5% guar gum and 7.5% gum arabic). Subsequently, the prepared dispersions were analyzed.

ζ-potential analysis

A ZetaPALS (Brookhaven Instruments, 31450) was used to determine the ζ-potential of dispersions. Each mixture had their pH measured and then put into a ζ-potential cell. Measurements were carried out in duplicate at 20 °C.

Rheological measurements

Rheological properties were determined according to the procedures described by Noreña *et al*. (2015), with modifications. The tests were carried out in an oscillatory regime in a Thermo Scientific HAAKE MARS 40/60 modular Rheometer, with parallel plate geometry (35 mm and gap 1 mm) varying the shear rate from 0.1 to 100 s^{-1}. The procedure started with the definition of a distance of 0.7 mm between the plates.

Afterwards, the samples for each dispersion were loaded in the lower plate of the Rheometer and the results of the analysis were obtained through the software.

The power law (Ostwald–de Waele model) was used to fit the experimental data and to determine the rheological parameters (K and n) of the dispersions, according to Eq. 1:

$$\tau = K\dot{\gamma}^n \qquad (1)$$

Where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index and *n* is the flow behavior index.

The storage or elastic modulus (G') and the loss or viscous modulus (G") were determined varying the frequency from 0.1 to 100 Hz. All measurements were performed in triplicate at 20 °C.

The viscoelastic properties of the dispersions were determined using two tests: dynamic and static measurements. For the assessment of dynamic viscoelastic properties, strain amplitude sweeps (0.01–500 Pa) were applied under a constant angular frequency (1 Hz) in order to determine the linear viscoelastic region (LVR).

Constant strain (0.05 Pa), within the LVR, was chosen to be used in the frequency sweep tests (from 0.1 to 100 Hz) at 20 °C. The values of the storage modulus (G′) and the loss modulus (G") were registered as functions of frequency.

For the measurement of static properties, creep and recovery tests were performed by applying a constant tension of 1 Pa to the sample for 180 seconds and allowing the deformation to recover for the same time, after the load was removed.

Color

Color was determined for the extract and its dispersions, using a colorimeter (Chroma Meter CR 410, Japan) adapted for liquids, using the *CIELab* scale, to measure the parameters *L*, a** and *b**. All measurements were performed in triplicate. According to the method, *L** indicates lightness and ranges from 0 (black) to 100 (white); and the chromaticity coordinates, *a*,* goes from green (-60) to red (+60); and *b*,* goes from blue (-60) to yellow (+60)

The values of *Chroma* and *Hue* angle were determined according to equations (1) and (2):

$$Croma = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (1)$$

$$Hue = tg^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

Statistical analysis

Statistical analysis was performed using SAS software (v. 9.3). Data were subjected to analysis of variance (ANOVA), and mean comparison of different treatments was carried out using Tukey's test ($p < 0.05$).

Results and Discussion

TPC and antioxidant activity

Hartwig *et al*., (2012) studied 10 different mate brands and found TPC values ranging from 4.98 to 6.92 g Garlic Acid Equivalents (GAE) % d.b for hot mate, from 1.45 to 3.00 g GAE % d.b for cold mate and from 9.91 to 11.00 g GAE % d.b for mate bags, using water as solvent. These results are close to those reported by González de Mejia *et*

al. (2005) and Bravo *et al*. (2007) for mate decoctions, ranging from 9.0 to 17.6 g and 9.07 to 9.90 g GAE % dm, respectively. The TPC and antioxidant activity of the YM extract is shown in Table 1.

Table 1. Antioxidant properties of YM extracts obtained from 60 minutes aqueous extraction at 80 °C

Antioxidant Properties	**Sample**
	YM Extract
ABTS (μmol TE. g^{-1} d.b)	181.7 ± 0.2
DPPH (μmol TE. g^{-1} d.b)	205.3 ± 0.4
TPC (mg GAE g^{-1} d.b)	28.1 ± 0.5
TPC retention (%)	50.83

Martin *et al*. (2013) found values of 193.9 mg GAE g^{-1} d.b using ethanol solution (60%, v/v). According to Bouras *et al*. (2015), the polarity of the solvent is an important factor in separation. The use of water, for instance, facilitates the extraction of the phenolic compound. It is worth noting that all extractions were carried out until the extract reached and maintained a constant temperature of 80 °C and only with distilled water as solvent; as a result, the phenolic compounds content was not entirely extracted. This temperature was deemed the end point of the extraction procedure in order to minimize degradation of bioactive components, and is mostly because mate infusion is often consumed at water temperatures less than 80 °C.

The antioxidant capacity of YM extracts was assessed by ABTS and DPPH. The ability of phenolic compounds to donate electrons can be seen, for example, when the purple-colored solution produced by the DPPH radical lights up.

The synthetic radical DPPH is commonly used to evaluate radical scavenging abilities of bioactive chemicals. It is more stable than superoxide and hydroxyl radicals

and is not affected by side reactions like enzyme inhibition or metal ion chelation (Koehnlein *et al*., 2012).

The antioxidant ability assessed by DPPH showed a mean value of 205.32 μmol Trolox Equivalent (TE) /g d.b suggesting that the compounds present in the extract could inhibit DPPH free radical. On the other hand, ABTS evaluates the ability of the extracts to decrease the $ABTS^{+}$ radical while in aqueous solutions (Re *et al*., 1999). The extract showed a mean value of 181.66 μmol TE/g d.b.

Our findings revealed that YM extracts had varied antioxidant profiles when comparing DPPH and ABTS. Furthermore, the primary component present in the sample does not necessarily define their antioxidant ability, but rather its interaction with minor components, which is commonly determined by the type of method used to assess the antioxidant capacity (Apak *et al*., 2013). Also, the antioxidant activity of phenolic compounds, mostly caffeic acid and its derivatives (accounting for over 90% of the total phenolic content of YM extracts), is linked to their chemical structure, which may respond differently in each technique (Dawidowicz *et al*.,2014; Piovezan-Borges *et al*., 2016; Yu *et al*., 2015; Moon and Terao, 1998; Chen and Ho, 1997).

ζ-potential analysis

The ζ-potential analysis can be helpful in the determination of the net charge of the complexes present in the dispersions, confirming the charge neutralization by electrostatic interactions. As a generally accepted principal dispersion systems presenting ζ-potential values higher than 30 mV or less than -30 are considered to have sufficient repulsive force to attain better physical colloidal stability (Cabuk *et al*., 2016).

The ζ-potentials for the extract as well as for the dispersions in distilled water and in the extract are shown on Table 2. It is worth mention that all values are significantly different ($p > 0.05$).

As can be seen, all dispersions are far from presenting themselves as agglomerates. In fact, ζ-potentials values far zero mean that biopolymers composing the dispersions are charged. Surprisingly, all values regarding the dispersions containing PD presented lower absolute values of ζ-potentials than the others.

Table 2: ζ−potential and pH for YM extracts obtained from 60 minutes aqueous extraction at 80 °C using Guar Gum, Gum Arabic and Polydextrose.

Samples	ζ−potential (mV)	pH
GA	-29.36 ± 1.0^{h}	5,0
GG	30.81 ± 0.6^{c}	6,3
POLI	23.67 ± 0.3^{b}	3,8
EXTRACT	61.03 ± 1.^{7d}	6,0
GG POLI	-16.58 ± 2.1^{j}	5,2
GG POLI EXTRACT	-31.78 ±1.0^{g}	5,5
GA POLI	16.06 ± 2.1^{a}	4,9
GA POLI EXTRACT	-18.04 ± 3.3^{i}	5,1
GGGA	-46.59 ± 1.0^{f}	5,2
GGGA EXTRACT	-84.75 ± 5.9^{e}	5,3

Rheological properties

Viscosity

The behavior of the apparent viscosity for all dispersions is presented on Figure 1. As a general pattern, the apparent viscosity decreases as the shear rate goes up. However, each profile tends asymptotically to a constant value after the shear rate reaches 60 s^{-1}. All the dispersions showed the typical shear-thinning behavior of a pseudoplastic fluid (non-Newtonian behavior), where the viscosity decreases with an increase in shear rate (Huang *et al*., 2015).

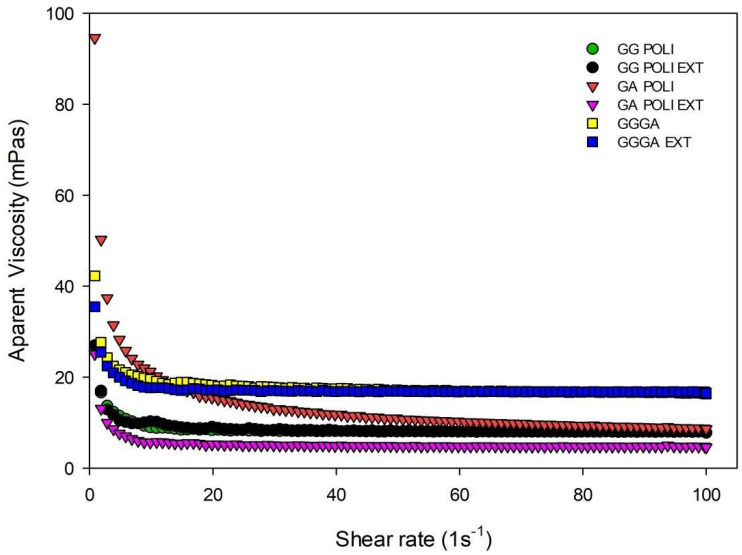

Figure 1: Steady-state flow curves for guar gum and polydextrose (GG POLI), Gum arabic and polydextrose (GA POLI) and guar gum and gum Arabic (GGGA) in distilled water and in the extract at 20 °C.

This behavior is the result of a direction effect, in which the increase of the shear rate causes alignment of the long chain of polymer molecules and randomly positioned chains in the orientation of flow, causing less interaction between adjacent polymer chains. All samples behaved like a near-Newtonian fluid, with flow behavior index (*n*)

values less than 1, confirming the shear-thinning (pseudoplastic) behavior (Koocheki *et al*., 2009; Tavares *et al*., 2020; Tavares, Santos e Noreña, 2021).

Table 3: Consistency index (K) and the flow behavior index (*n*) calculated for all dispersions presented on Figure 1.

Samples	k	n	R^2
GG POLI	0,008274	0,990409	0,999898
GA POLI	0,044126	0,645855	0,999151
GGGA	0,022022	0,936807	0,999925
GG POLI EXTRACT	0,009820	0,955251	0,999497
GA POLI EXTRACT	0,006945	0,946678	0,999366
GGGA EXTRACT	0,017861	0,985724	0,999948

Effect of temperature on apparent viscosity

The effect of temperature on the apparent viscosity of the dispersions was examined from the temperature of 20 to 100 °C at a fixed shear rate ($\dot{\gamma}$) of 0.1 s^{-1}, (**Figure 2**). The viscosity of all dispersions decreased with increased temperature. This decrease can be attributed to the disruption of the entanglement of the chains and the increase in intermolecular spaces causing thermal expansion, reduction of intermolecular forces and, consequently, a decrease in the viscosity of the sample. (Rosas-Flores *et al*., 2013; Xiao *et al*., 2012; Toğrul *et al.,* 2004; Tavares *et al*., 2020).

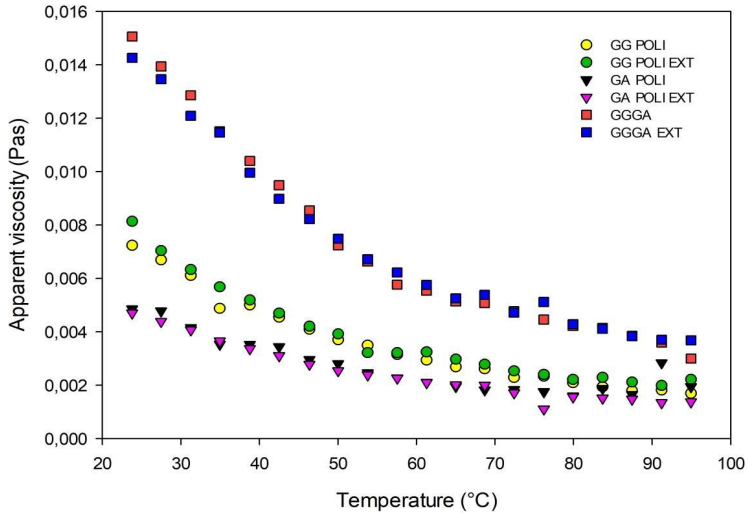

Figure 2: Apparent viscosity versus temperature from 20 to 100 °C for guar gum and polydextrose (GG POLI), Gum arabic and polydextrose (GA POLI) and guar gum and gum Arabic (GGGA) in distilled water and in the extract at 20 °C.

The results suggested that GGGA has a more flexible molecular conformation compared to the other dispersions. The greater variation in apparent viscosity with increasing temperature implies greater disruption of the entanglement of their chains and network structure (Tavares, Santos e Noreña, 2021).

Therefore, GGGA seems to be more protected against thermal stress than the other dispersions, which might indicate that GGGA can be suitable to be applied in food products that require high-temperature heating, and also products susceptible to high temperature changes during the processing, handling and storage.

Dynamic oscillatory tests

Dynamic rheological measurements were used to study dispersion containing guar gum, gum Arabic and polydextrose and mixtures (Figures 3, 4 and 5). In all cases, the magnitude of storage modulus (G′) and loss modulus (G″) increased with increasing frequency, suggesting that the polymer chains composing the dispersions are entangled.

Furthermore, as G″ values were higher than G′ in the entire frequency range analyzed in the tests, it is possible to infer that all dispersions presented weakly viscoelastic fluid behavior (Bastos *et al*., 2010).

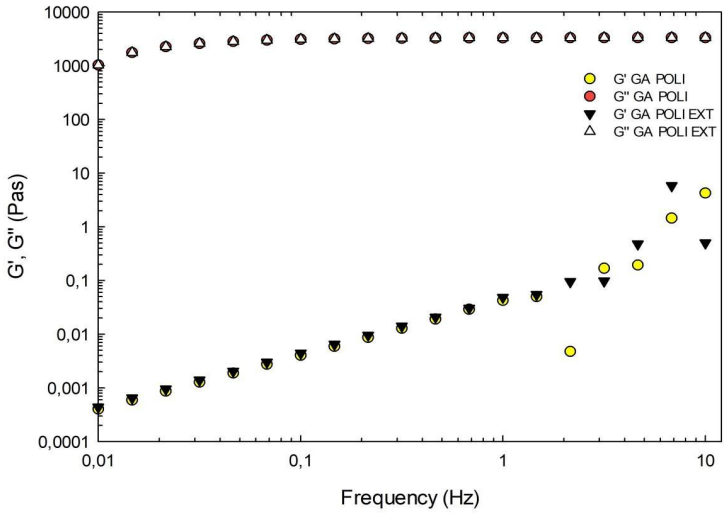

Figure 3. Dynamic oscillatory rheological curves for mixture of gum Arabic and polydextrose in distilled water and in the extract. Storage modulus (G') and lost modulus (G") are represented in yellow and red for distilled water dispersion (●), and in black and white for extract (▲), respectively.

The similar values presented by the dispersions composed with distilled water and YM extract reveal that the phenolic compounds do not interfere in the arrangement of the polymer network, nor do they imply the formation of a stronger network. Small differences are observed only in the parameter G', for frequencies above 2 Hz.

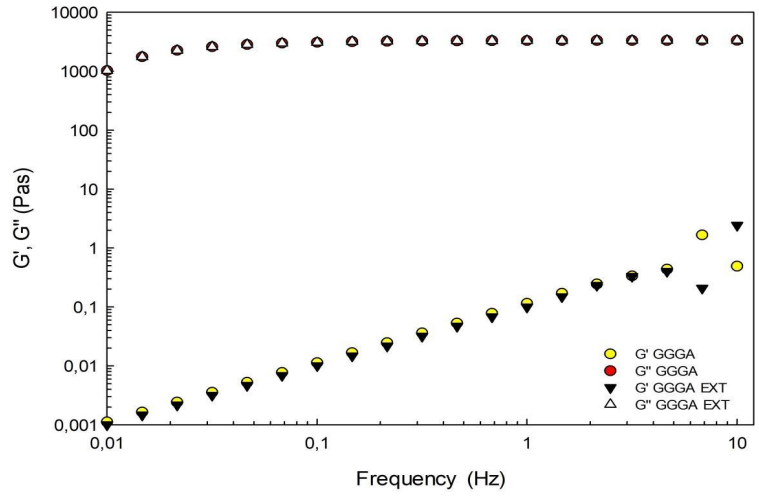

Figure 4: Dynamic oscillatory rheological curves of mixture of guar gum and gum Arabic in distilled water and in the extract. Storage modulus (G') and lost modulus (G") are represented in yellow and red for distilled water dispersion (●), and in black and white for extract (▲), respectively.

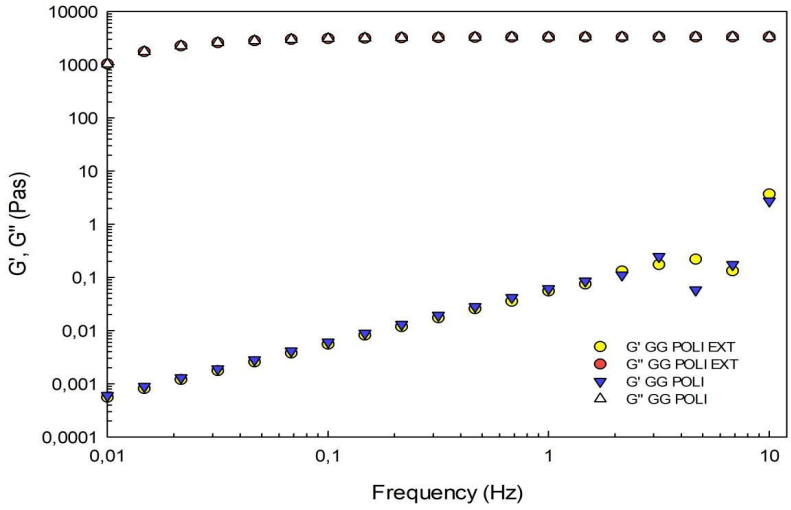

Figure 5: Dynamic oscillatory rheological curves mixture of guar gum and polydextrose in distilled water and in the extract. Storage modulus (G') and lost modulus (G") are represented in yellow and red for distilled water dispersion (●), and in black and white for extract (▲), respectively.

Temperature sweep test

The temperature sweep curves with variation G′ and G″ moduli upon heating from 20 to 70 °C at a fixed frequency (1 Hz) are shown in Figures 6, 7 and 8.

Analyzing the profile exhibited by GGGA and GG POLI dispersions, it is possible to infer that as G" is greater than G' within the entire range of temperatures studied, these dispersions do not form a strong gel-like network structure with increasing temperature, and therefore, presented a predominantly liquid-like viscous behavior, even though it did not exhibit a crossover point.

On the other hand, GA POLI dispersion displayed crossover points in temperatures greater than 55 ºC, with G' modulus becoming greater than G", indicating that this structure was predominantly elastic and remained in the solid-like behavior during the whole range of investigated temperatures.

In GGGA and GG POLI profiles, the decreasing initial values of the G" modulus may be related to the increase in fluidity with increasing temperature, which can be attributed to the movement of energy dissipation of the molecules and reduction of intermolecular interactions (Hesarinejad *et al*., 2014). Moreover, temperatures in the range from 60 to 70 °C indicate the point where GGGA and GG POLI chains start to disentangle inverting the decreasing trend of the elastic modulus.

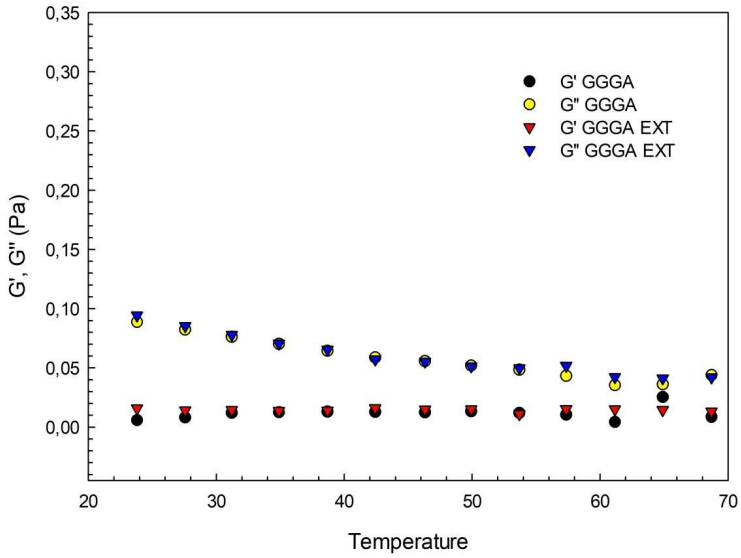

Figure 6: Temperature sweep test for dispersion made with mixture of GGGA dispersed in distilled water and extract, determined at constant frequency of 1 Hz and fixed stress of 1 Pa. Storage modulus (G') and lost modulus (G") are represented in black and yellow for distilled water dispersion (●), and in red and blue for extract (▲), respectively.

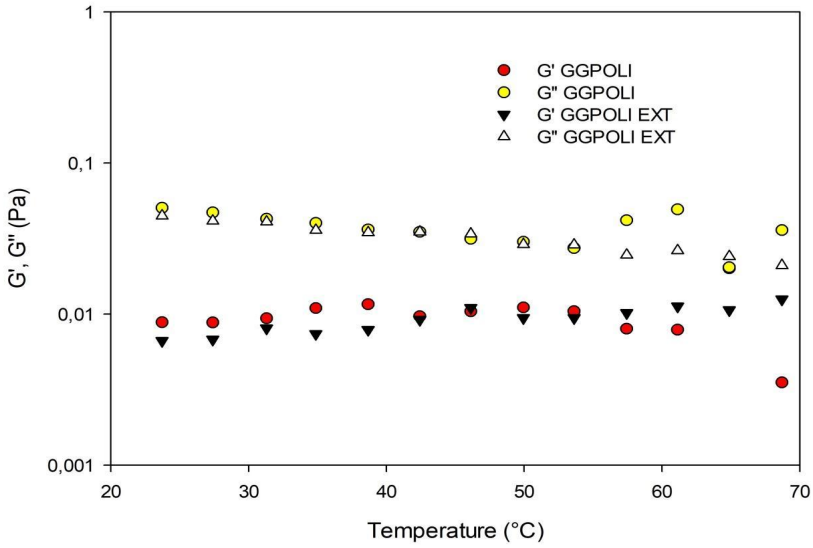

Figure 7: Temperature sweep test for dispersions made of mixtures of GG POLI dispersed in distilled water and extract, determined at constant frequency of 1 Hz and fixed stress of 1 Pa. Storage modulus (G') and lost modulus (G") are represented in yellow and red for distilled water dispersion (●), and in black and white for extract (▲), respectively.

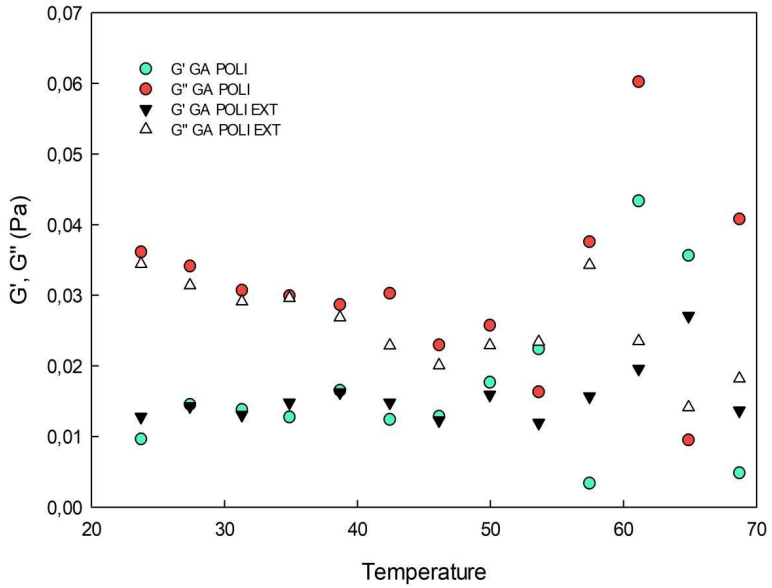

Figure 8: Temperature sweep test for dispersion made with mixtures of GA POLI dispersed in distilled water and extract, determined at constant frequency of 1 Hz and fixed stress of 1 Pa. Storage modulus (G') and lost modulus (G") are represented in green and red for distilled water dispersion (●), and in black and white for extract (▲), respectively.

Creep and recovery behavior

Creep and recovery tests were performed to determine the values of compliance (J(t)), which represent the deformation of samples caused by the application of shear stress (Ibarz *et al.,* 2002). In these tests, the total recovery shape and magnitude of material deformation depends on the test duration, extent of stress applied and the temperature of the sample (Cabuk *et al*., 2016).

Creep and recovery tests have been employed to study the internal structure of a material, and its structural changes associated with the introduction of some alteration on the initial composition (Dolz *et al*., 2008). As a general behavior, samples with stronger,

compact and stable structures have lower J_t values, while samples with weaker network structure have higher J_t values (Huang *et al.*, 2016).

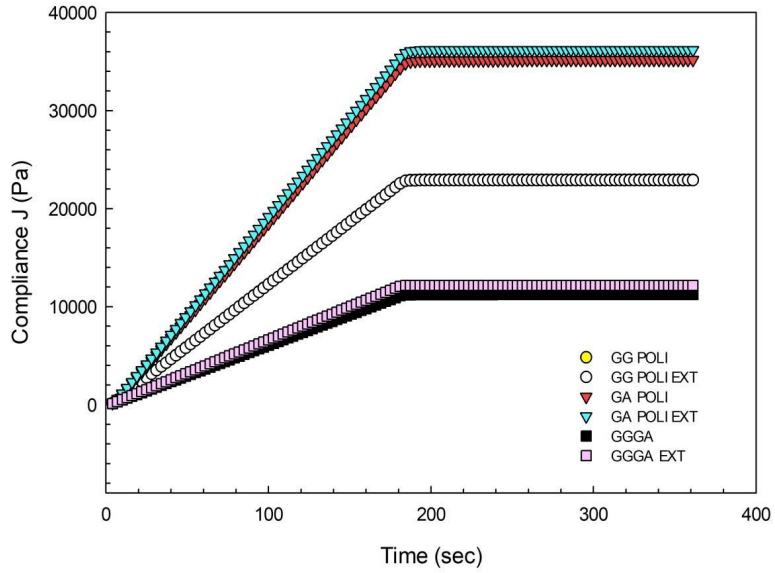

Figure 9. Compliance results for the creep and recovery behaviors of the dispersions. The creep test was performed during a time interval from 0 to 180 sec, and the recovery from 180 to 360 s.

For the creep phase, constant stress of 1 Pa was applied to samples for 180 s, and the increase of creep compliance (J_c) corresponds to the deformation of the structures caused by the applied stress within the LVR. After the creep period, the recovery phenomenon was monitored (applied stress = 0). In this period, only purely elastic systems could fully regenerate after application of constant stress. (Yilmaz *et al.*, 2012). However, superior viscoelastic properties and fewer deformations are also observed in viscous dispersions presenting strong network structures (Ma *et al.*, 2013).

As can be observed in Figure 4, GGGA dispersions with both distilled water and extract showed lower magnitudes of J_c, 11000 – 10000 Pa, respectively, indicating the formation of a stronger and stable polymer structure, while the higher values of J_c

obtained for GA POLI dispersions with both distilled water and extract, 36000 – 35000 Pa, respectively, indicating a weaker network structure.

In general, compliance results for the dispersions revealed similar creep and recovery curve shapes related to the same test conditions. A viscous behavior can be inferred from all profiles once they exhibit high compliance and no recovery after load removal.

Color

The colorimetric parameters obtained for the extract and its dispersions are shown in Table 4 . The luminosity (*L*),* exhibited values ranging from 86.14 to 94.88, referring to the darkest values presented by GG POLI and GAGG and extract. These darkest extracts may be related to their amount of phenolic compounds and chlorophyll (Chls).

For *a** parameter, all treatments presented negative values, meanwhile for *b** all treatments presented positive values, suggesting their presence in the second quadrant of the chromatic circle (greenness and yellowness). For *Hue* angle, expressing tonality, all samples presented similar values ranging from 103,31 to 104.3º, which confirmed the colors as present in the second quadrant of the chromatic circle

Table 4: Colorimetric parameters of YM extracts obtained from 60 minutes aqueous extraction at 80 °C.

	*L**	*a**	*b**	*Hue (°)*	*Chroma*
GG POLI	94.88±0.02[c]	-10.4±0.2[c]	40.97±0.02[c]	104,2±0.2[b]	42.27±0.04[c]
GA POLI	86.14±0.03[a]	-9.03±0.02[a]	38.18±0.02[b]	103,31±0.02[a]	39.23±0.02[b]
GAGG	86.18±0.03[a]	-8.85±0.03[b]	37.04±0.02[a]	103,44±0.03[a]	38.08±0.02[a]
EXTRACT	87.16±0.02[b]	-9.6±0.1[a]	37.6±0.1[a]	104,3±0.1[b]	38.8±0.1[a]

Conclusions

The final considerations of this manuscript are still under development, since important analyzes are being carried out. These analyzes are fundamental for a better understanding of the relationships between the materials and phenomena being observed and cannot be ruled out by a partial conclusion.

REFERENCES

1. Alam, N.H, Meier R, Schneider H, Sarker Sa, Bardhan Pk, Mahalanabis D, *et al*. Partially Hydrolyzed Guar Gum-Supplemented Oral Rehydration Solution In The Treatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000; 31(5):503-7.
2. Ali, B. H.; Ziada, A.; Blunden, G. Biological effects of gum arabic. A review of some recent research. Food and Chemical Toxicology, v.47, n.1, p. 1-8, 2009.

3. Apak, R.; Gorinstein, S.; Böhm, V.; Schaich, K.M.; Özyürek, M.; Güçlü, K. 2013. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure and Applied Chemistry 85: 957-998.
4. Arçari, D. P., Bartchewsky, W., Dos Santos, T. W., Oliveira, K. A., Funck, A., Pedrazzoli, J., De Souza, M. F., Saad, M. J., Bastos, D. H., Gambero, A., Carvalho, P. O., Ribeiro, M. L., 2009. Antiobesity effects of yerba maté extract (*Ilex paraguariensis*) in high-fat diet-induced obese mice. *Obesity*, **12**, 2127–2133. doi: 10.1038/oby.2009.158
5. Arçari, D. P., Porto, V. B., Rodrigues, E. R. V., Martins, F., Lima, R. J., Sawaya, A. C. H. F., Carvalho, P. O., 2011. Effect of mate tea (*Ilex paraguariensis*) supplementation on oxidative stress biomarkers and LDL oxidisability in normo and hyperlipidaemic humans. *Journal of Functional Foods*, **3**, 190–197. doi: 10.1016/j.jff.2011.04.001
6. Bakowska, A.M., Kucharska, A.Z., Oszmianski, J., 2003. The effects of heating, UV irradiation and storage on stability of anthocyanin polyphenol copigment complex. *Food Chem*. **81**, 349–355. doi:10.1016/S0308-8146(02)00429-6
7. BASTOS, D. H. M., Oliveira, D. M., Matsumoto, R. L. T., Carvalho, P. O., Ribeiro, M. L., 2007. Yerba maté: Pharmacological properties, research and biotechnology. *Medicinal and Aromatic Plant Science and Biotechnology*, **1**(1), 37–46.
8. Bastos, Daniele S.; Barreto, Bianca N.; Souza, Hiléia K. S.; Bastos, Margarida; Rocha-Leão, Maria Helena M.; Andrade, Cristina T.; Gonçalves, Maria Pilar. 2010. Characterization of a chitosan sample extracted from Brazilian shrimps and its application to obtain insoluble complexes with a commercial whey protein isolate. Food Hidrocolloides, 24(8), 709-718.

9. Bouras, M.; Chadni, M.; Barba, F.J.; Grimi, N.; Bals, O.; Vorobiev, E. 2015. Optimizationof microwave-assisted extraction of polyphenols from Quercus bark. Industrial Crops and Products 77: 590–601.
10. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. 1995. Use of A Free Radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28: 25–30.
11. Bravo, L., Goya, L., & Lecumberri, E. (2007). Lc/Ms Characterization Of phenolic constituents of mate (Ilex paraguariensis, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Research International*, *40*(3), 393-405.
12. Bravo, L., Goya, L., Lecumberri, E., 2007. Lc/Ms Characterization of Phenolic constituents of mate (*Ilex paraguariensis A. St.-Hill*) and its antioxidant activity compared to commonly consumed beverages. *Food Res. Int.* **40**, 303-405. doi: 10.1016/j.foodres.2006.10.016
13. Cabuk, Mehmet; Yavuz, Mustafa; Unal, Halil Ibrahim. Electrokinetic, electrorheological and viscoelastic properties of Polythiophene-graft-Chitosan copolymer particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, v. 510, p. 231-238, 2016.
14. Chen, K.N., Chen, M.J., Liu, J.R., Lin, C.W., Chiu, H.Y., 2005. Optimization Of incorporated prebiotics as coating materials for probiotic microencapsulation. J. Food Sci. 70, M260–M266. doi:10.1111/j.1365-2621.2005.tb09981.x
15. Clifford, M. N., & Madala, N. E. (2017). Surrogate standards: A cost-effective strategy for identification of phytochemicals. Journal of Agricultural and Food Chemistry, 65(18), 3589–3590. 10.1021/acs.jafc.7b01588.

16. Colpo, A., Rosa, H., Lima, M.E., Pazzini, C.E.F., De Camargo, V.B., Bassante, F.E., Folmer, V., 2016. Yerba mate (*Ilex paraguariensis St. Hill*.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. *Food Chemistry* **209**, 185–195. doi: 10.1016/j.foodchem.2016.04.059

17. Craig, S. *et al*. Polydextrose as soluble fiber and complex carbohydrate. Food Science and Technology, New York, Marcel Dekker, p. 229-248, 1999.

18. Dawidowicz, A.L.; Olszowy, M. 2014. Does Antioxidant Properties of The Main component of essential oil reflect its antioxidant properties? The comparison of antioxidant properties of essential oils and their main components. Natural Product Research 28: 1952–1963.

19. De Lima, M.E., Colpo, A.C., Maya-López, M., Rosa, H., Túnez, I., Galván-Arzate, S., Santamaría, A., Folmer, V., 2017. Protective effect of Yerba Mate (*Ilex paraguariensis St. Hill.*) against oxidative damage *in vitro* in rat brain synaptosomal/mitocondrial P2 fractions. *Journal of Funcional Foods* **34**, 447-452. doi:10.1016/j.jff.2017.05.026

20. Del Rio, D., Costa Lg, Lean Mej, Crozier A (2010) Polyphenols and Health: What compounds are involved? Nutr Metab Cardiovasc Dis 20:1–6 doi: 10.1016/ j.numecd.2009.05.015

21. Dogan, H., & Kokini, J. L. (2011). Measurement and Interpretation of Batter Rheological Properties. In Batters and Breadings in Food Processing (pp. 263–299). AACC International Press.

22. Dolz, M.; Hernández, M. J.; Delegido, J. Creep and Recovery Experimental investigation of low oil content food emulsions. Food Hydrocolloids, v. 22, n. 3, p. 421-427, 2008.

23. Enokido, M., Suzuki A, Sadahiro R, Matsumoto Y, Shibuya N, Goto K, Otani K (2014) Implication of P-Glycoprotein in formation of Depression-Prone personality: Association study between the C3435T MDR1 gene polymorphism and interpersonal sensitivity. Neuropsychobiology 69:89–94 doi: 10.1159/000358063
24. Eratte, D. *et al*. Complex Coacervation With Whey Protein Isolate and Gum Arabic for the microencapsulation of mega-3 rich tuna oil. Food & Functions, v. 5, n. 11, p. 2743-2750.
25. Farmer, J.A, Gotto Am, Jr. Currently Available Hypolipidaemic Drugs and Future therapeutic developments. Baillieres Clin Endocrinol Metab. 1995; 9(4):825-47.
26. FDA (2003) Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products—general considerations. US Food and Drug Administration.
27. Fernandes, E. S., Machado, M. O., Becker, A. M., Andrade, F., Maraschin, M., Silva, E. L., 2012. Yerba mate (*Ilex paraguariensis*) enhances the gene modulation and activity of paraoxonase-2: *In vitro* and *in vivo* studies. *Nutrition*, **28**(8), 1157–1164. doi: 10.1016/j.nut.2012.04.011
28. Fernandes, R.V.D.B. e*t al*. Study Of Ultrasound-Assisted Emulsions On microencapsulation of ginger essential oil by spray drying. Industrial Crops and Products. 94 (2016): 413-423.
29. García-Saldaña, J. S. e*t al*., Microencapsulation of Sulforaphane from broccoli seed extracts by gelatin/gum Arabic ang gelatin/pectin complexes. Food Chemistry, v.201, p. 94-100, 2016.
30. Gonzalez De Mejia, Elvira, *et al*. "Effect Of Yerba Mate (Ilex Paraguariensis) tea on topoisomerase inhibition and oral carcinoma cell proliferation." *Journal of agricultural and food chemistry* 53.6 (2005): 1966-1973.

31. Gugliucci, A., Bastos, D. H., Schulze, J., Souza, M. F., 2009. Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. Fitoterapia, 80, 339–344. doi: 10.1016/j.fitote.2009.04.007

32. Hartwig, Vanessa Graciela, Luis Alberto Brumovsky, And Maria Raquel Fretes. "A total polyphenol content of mate (Ilex paraguariensis) and other plants-derived beverages." *Journal of Food Research* 1.3 (2012): 58-67.

33. Heck, C. I., Mejia, E. G., 2007. Yerba Mate Tea (*Ilex Paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science*, **72**, 138–151. doi: 10.1111/j.1750-3841.2007.00535.x

34. Hesarinejad, Mohammad Ali; Koocheki, Arash; Razavi, Seyed Mohammad Ali. Dynamic rheological properties of Lepidium perfoliatum seed gum: Effect of concentration, temperature and heating/cooling rate. Food Hydrocolloids, v. 35, p. 583-589, 2014.

35. Hu, B., Liu X, Zhang C, Zeng X (2017) Food Macromolecule Based Nanodelivery systems for enhancing the bioavailability of polyphenols. Journal of Food and Drug Analysis 25:3–15 doi: 10.1016/j.jfda.2016.11.004

36. Huang, Jingjing *et al*. Steady, Dynamic, And Creep-Recovery Rheological Properties of myofibrillar protein from grass carp muscle. Food Hydrocolloids, v. 61, p. 48-56, 2016.

37. Ibarz, A., G.V. Barbosa-Cánovas, Unit Operations in Food Engineering (Crc Press, Boca Raton, 2002).

38. Jackson, C. L. *et al*. Pectin Induces Apoptosis in Human Prostate Cancer Cells: correlation of apoptotic function with pectin structure. Glycobiology, v. 17, n.8, p. 805-819, 2007.

39. KIM, S. Y., Oh, M. R., Kim, M. G., Chae, H. J., Chae, S. W., 2015. Anti-obesity effects of Yerba Mate (Ilex Paraguariensis): a randomized, double-blind, placebo-controlled clinical trial. BMC Complementary and Alternative Medicine, 15(1), 338. doi:10.1186/s12906-015-0859-1.

40. Koehnlein, Eloá Angélica, e*t al*. "Antioxidant Activities and Phenolic compounds of raw and cooked Brazilian pinho (Araucaria angustifolia) seeds." African Journal of Food Science 6.21 (2012): 512-518.

41. Koocheki, Arash e*t al*. Rheological Properties of Mucilage Extracted from Alyssum homolocarpum seed as a new source of thickening agent. **Journal of food engineering**, v. 91, n. 3, p. 490-496, 2009.

42. Kosaraju, S. L., Labbett, D., Emin, M., Konczak, I., And Lundin, L., 2008. Delivering polyphenols for healthy ageing. *Nutrition & Dietetics*, **65**, S48-S52. doi: 10.1111/j.1747-0080.2008.00261.x

43. Kuang, S.S, Oliveira Jc, Crean Am (2010) Microencapsulation as A Tool For incorporating bioactive ingredients into food. Crit Rev Food Sci 50:1913–1918 doi: 10.1080/10408390903044222

44. Kuck, L.S.; Noreña, C.P.Z. 2016. Microencapsulation of Grape (Vitis Labrusca Var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. Food Chemistry 194: 569-576.

45. Ma, Z., Boye, J. I., Fortin, J., Simpson, B. K., & Prasher, S. O. (2013). Rheological, physical stability, microstructural and sensory properties of salad dressings

supplemented with raw and thermally treated lentil flours. Journal of Food Engineering, 116, 862-872

46. Martin, José G. Prado, e*t al*. "Antimicrobial Activity of Yerba Mate (Ilex paraguariensis St. Hil.) against food pathogens." *Revista Argentina de microbiologia* 45.2 (2013): 93-98.
47. Martinet, A., Hostettmann, K., And Schutz, Y., 1999. Thermogenic Effects Of commercially available plant preparations aimed at treating human obesity. *Phytomedicine*, **6**(4), 231–238. doi:10.1016/S0944-7113(99)80014-2
48. Mateos, Raquel, *et al*. "Improved Lc-Msn Characterization of Hydroxycinnamic acid derivatives and flavonols in different commercial mate (Ilex paraguariensis) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity." *Food chemistry* 241 (2018): 232-241.
49. Mohnen, D. Pectin Structure and Biosynthesis. Current Opinion in Plant Biology, v.11, n.3, p. 266-277, 2008.
50. Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., José Núñez, M., Parajó, J. C., 2001. Natural antioxidants from residual sources. *Food Chemistry*, **72**(2), 145-171. doi: 10.1016/S0308-8146(00)00223-5
51. Noreña, C. P. Z., Bayarri, S., & Costell, E. (2015). Effects of Xanthan Gum additions on the viscoelasticity, structure and storage stability characteristics of prebiotic custard desserts. Food Biophysics, 10(2), 116-128.
52. Phillips, G. O.; Ogasawara, T.; Ushida, K. The Regulatory and Scientific approach to defining gum arabic (Acacia senegal and Acacia seyal) as a dietary fibre. Food Hydrocolloids, v. 22, p. 24-35, 2008.
53. Phillips, G. O.; William, P. A. Handbook of Hydrocolloids, 2nd Edition, Crc Press, Boca Raton, FL, USA, 2000. 901p.

54. Premi, M.; Sharma, H. Effect of Different Combinations Of Maltodextrin, Gum Arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick.

55. Rao, M.A. In Rheology of Fluid, Semisolid, And Solid Foods: Principles And Applications. ed. by M.A. Rao (Springer, Boston, 2014), pp. 27–61.

56. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Riceevans, C. 1999. Antioxidant activity applyng an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237.

57. Rein, M.J., Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar Sk, Da Silva Pinto M (2013) Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. British journal of clinical pharmacology 75(3):588-602 doi: 10.1111/j.1365-2125.2012.04425.x

58. Ribeiro, M. C., Santos, Â., Riachi, L. G., Rodrigues, A. C. B., Coelho, G. C., Marcellini, P. S., de Maria, C. A. B., 2017. The effects of roasted yerba mate (*Ilex paraguariensis A. St. Hil.*) consumption on glycemia and total serum creatine phosphokinase in patients with traumatic brain injury. *Journal of Functional Foods*, **28**, 240–245. doi: 10.1016/j.jff.2016.11.024

59. Ronco, A. L., Stefani, E., Mendoza, B., Deneo-Pellegrini, H., Vazquez, A., Abbona, E., 2016a. Mate intake and risk of breast cancer in Uruguay: A case-control study. Asian Pacific Journal of Cancer Prevention: APJCP, 17(3), 1453–1461. doi:10.7314/apjcp.2016.17.3.1453

60. Rosas-Flores, Walfred; Ramos-Ramírez, Emma Gloria; Salazar-Montoya, Juan Alfredo. Microencapsulation of Lactobacillus Helveticus And Lactobacillus delbrueckii using alginate and gellan gum. Carbohydrate polymers, v. 98, n. 1, p. 1011-1017, 2013.

61. Schinella, G. R., Troiani, G., Dávila, V., Buschiazzo, P. M., Tournier, H. A., 2000. Antioxidant effects of an aqueous extract of *Ilex paraguariensis. Biochemical and Biophysical Research Communications*, **269**, 357–360. doi:10.1006/bbrc.2000.2293

62. Schinella, G., Fantinelli, J. C., Mosca, S. M., 2005. Cardioprotective Effects of *Ilex paraguariensis* extract: evidence for a nitric oxide-dependent mechanism. *Clinical Nutrition*, **24**, 360-366. doi:10.1016/j.clnu.2004.11.013

63. Shahidi, F.; Han, X.Q., 1993. Encapsulation of Food Ingredients. Critical Reviews in Food Science and Nutrition, **33**(6), 501-547. doi:10.1080/10408399309527645

64. Singleton, V.L.; Rossi, J.A. 1965. Colorimetry of Total Phenolics With Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16: 144–158.

65. Slavin, J. L.; Greenber, N. A. Partially Hydrolyzed Guar Gum: Clinical nutrition uses. Nutrition, v. 19, p. 549-552, 2003.

66. Spapen, Herbert, *et al*. "Soluble Fiber Reduces the Incidence of Diarrhea In Septic patients receiving total enteral nutrition: a prospective, double-blind, randomized, and controlled trial." Clinical Nutrition 20.4 (2001): 301-305.

67. Steffe, J.F. Rheological Methods in Food Process Engineering (Freeman Press, New York, 1996)

68. Tavares, L., Flores, E. E. E., Rodrigues, R. C., Hertz, P. F., & Noreña, C. P. Z. (2020). Effect of deacetylation degree of chitosan on rheological properties and physical chemical characteristics of genipin-crosslinked chitosan beads. Food Hydrocolloids, 106, 105876.

69. Tavares, Loleny; Santos, Lúcia; Noreña, Caciano Pelayo Zapata. Bioactive compounds of garlic: A comprehensive review of encapsulation technologies,

characterization of the encapsulated garlic compounds and their industrial applicability. Trends in Food Science & Technology, 2021.

70. Tavares, Loleny; Souza, H. K. S.; Gonçalves, M. P.; Rocha, C. M. R. (2021) Physicochemical and microstructural properties of composite edible film obtained by complex coacervation between chitosan and whey protein isolate. Food Hydrocolloids 113, 106471.
71. Toğrul, H., N. Arslan, Mathematical Model for Prediction Of Apparent Viscosity of molasses. J. Food Eng. 62, 281 (2004)
72. Tonon, R.V., Brabet, C., Pallet, D., Brat, P., Hubinger, M.D., 2009. Physicochemical and morphological characterization of açai (*Euterpe oleraceae Mart.*) powder produced with different carrier agents. *Int. J. Food Sci. Technol.* **44**, 1950–1958. doi:10.1111/j.1365-2621.2009.02012.x
73. Upadhyay, R., & Chen, J. (2020). Chapter 28 - Rheology and Tribology assessment of foods: A food oral processing perspective. In K. Pal, I. Banerjee, P. Sarkar, D. Kim, W.-P. Deng, N. K. Dubey, & K. B. T.-B.-B. F. Majumder (Eds.) (pp. 697–715). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-816897-4.00028-X
74. Vargas, B. K., *et al*. "Yerba Mate (Ilex Paraguariensis) Microparticles Modulate antioxidant markers in the plasma and brains of rats." *Food Bioscience* 41 (2021): 100999.
75. Wandrey, C. A. Bartkowiak, S.E. Harding, In Encapsulation Technologies for Active Food Ingredients and Food Processing. ed. By N.J. Zuidam, V. Nedovic (Springer, New York, 2010), pp. 31–100
76. Xiao, Qian; Tong, Qunyi; Lim, Loong-Tak. Pullulan-Sodium Alginate Based edible films: Rheological properties of film forming solutions. Carbohydrate Polymers, v. 87, n. 2, p. 1689-1695, 2012.

77. Yilmaz, M. T., Karaman, S., Dogan, M., Yetim, H., & Kayacier, A. (2012). Characterization of O/W model system meat emulsions using shear creep and creep recovery tests based on mechanical simulation models and their correlation with texture profile analysis (TPA) parameters. Journal of Food Engineering, 108, 327-336.

78. Yu, S.I., Yue, S.W., Liu, Z., Zhang, T., Xiang, N., Fu, H., 2015. Yerba Mate (*Ilex paraguariensis*) improves microcirculation of volunteers with high blood viscosity: a randomized, double-blind, placebo-controlled trial. *Exp. Gerontol.* 62, 14-22. doi: 10.1016/j.exger.2014.12.016

79. Gong, Honghong; Liu, Mingzhu; Chen, Jiucun; Han, Fei; Gao Chunmei; Zhang, Bing; Synthesis and Characterization Of Carboxymethyl Guar gum and rheological properties of its solutions. Elsevier, 2012

80. Thombare, N.; Jha, U.; Mishra, S.; Siddiqui, M.Z.; Guar Gum as a Promising Starting Material for Diverse Applications: A Review. International Journal of Biological Macromolecules, 2016.

81. Oliveira, Edilene Gadelha De *et al*. Evaluation of Guar Gum in The Development of theophylline controlled-release matrix tablets. Polímeros, v. 25, n. SPE, p. 54-58, 2015.

82. Randall, R. C.; Phillips, G. O.; Williams, P. A. Food Hydrocolloids 1988, 2, 131.2. FENYO, J. C.; Vandervelde, M. C. In Gum and Sta-bilizer for the Food Industry; IRL Press: Oxford,1990; Vol. 5, pp 17–23.3.

83. ISLAM, A.M.; Phillips, G.O.; Sljivo, A.; Snowden, M.J.; Williams, P. A. Food Hydrocolloids 1997, 11, 493.

84. Idris, O. H. M.; Williams, P. A.; Phillips, G. O. Food Hydrocolloids 1998, 12, 379

85. Lahtinen, S.J.; Knoblock, K.; Drakoularakou, A.; Jacob, M.; Stowell, J.; Gibson, G.R.; Ouwehand, A.C. Effect of molecule branching and glycosidic linkage on the degradation of polydextrose by gut microbiota. Biosci. Biotechnol. Biochem. 2010, 74, 2016–2021.
86. Flood, M.T.; Auerbach, M.H.; Craig, S.A.S. A Review of The Clinical Toleration studies of polydextrose in food. Food Chem. Toxicol. 2004, 42, 1531–1542.
87. Murphy, O. Non-Polyol Low-Digestible Carbohydrates: Food Applications And functional benefits. Br. J. Nutr. 2001, 85, S47–S53.
88. Karaman, Safa; Kayacier, Ahmed. Rheology of Ice Cream Mix Flavored With black tea or herbal teas and effect of flavoring on the sensory properties of ice cream. Food and Bioprocess Technology, v. 5, n. 8, p. 3159-3169, 2012.

9. Concluding Remarks

The South American natural plant *Ilex paraguariensis* has been shown to have several health advantages for people and to be helpful in the treatment and prevention of a wide range of illnesses. Numerous research have suggested that the plant may be a promising functional food product for boosting antioxidant activity and, as a result, shielding body cells from oxidative damage to lengthen life and enhance health.

Despite the fact that YM has been consumed regularly for decades in South America and more recently across the globe, it is crucial to identify the positive and negative effects of its consumption and determine whether it is safe or harmful for the population's health.

Several researches have revealed that the plant has the potential to be an exciting functional food product, mostly because of its phenolic component, even though many of these advantages are not yet fully established. The final fraction of YM chemicals consumed as well as their mode of action in the human body should be better studied in vitro as well as in vivo, in order to better comprehend its interindividual heterogeneity, such as different reactions to PC usage depending on the person, and define its relationship with the GM, brain functions and the whole human system.

It has been already concluded that many of these advantages are not yet firmly proven and given the availability of repetitious and inaccurate information in this area, authors should exercise caution because numerous subpar studies can weaken good research.

Besides human response, the various YM products should also be evaluated in terms of how different extraction methods and consuming styles affect the degree of chemical migration to the water in order to improve it. This is important because nutrients bioavailability and effects are commonly disputed.

With more research efforts, natural antioxidants can therefore be shielded from the environment in a number of ways in order to increase their potency since they are highly sensitive. Using modern techniques such as encapsulation, in addition to encasing, safeguarding, and transporting the required bioactive molecules into the circulatory system, protective or encapsulated delivery vehicles may also enable targeted release in regions like the small intestine.