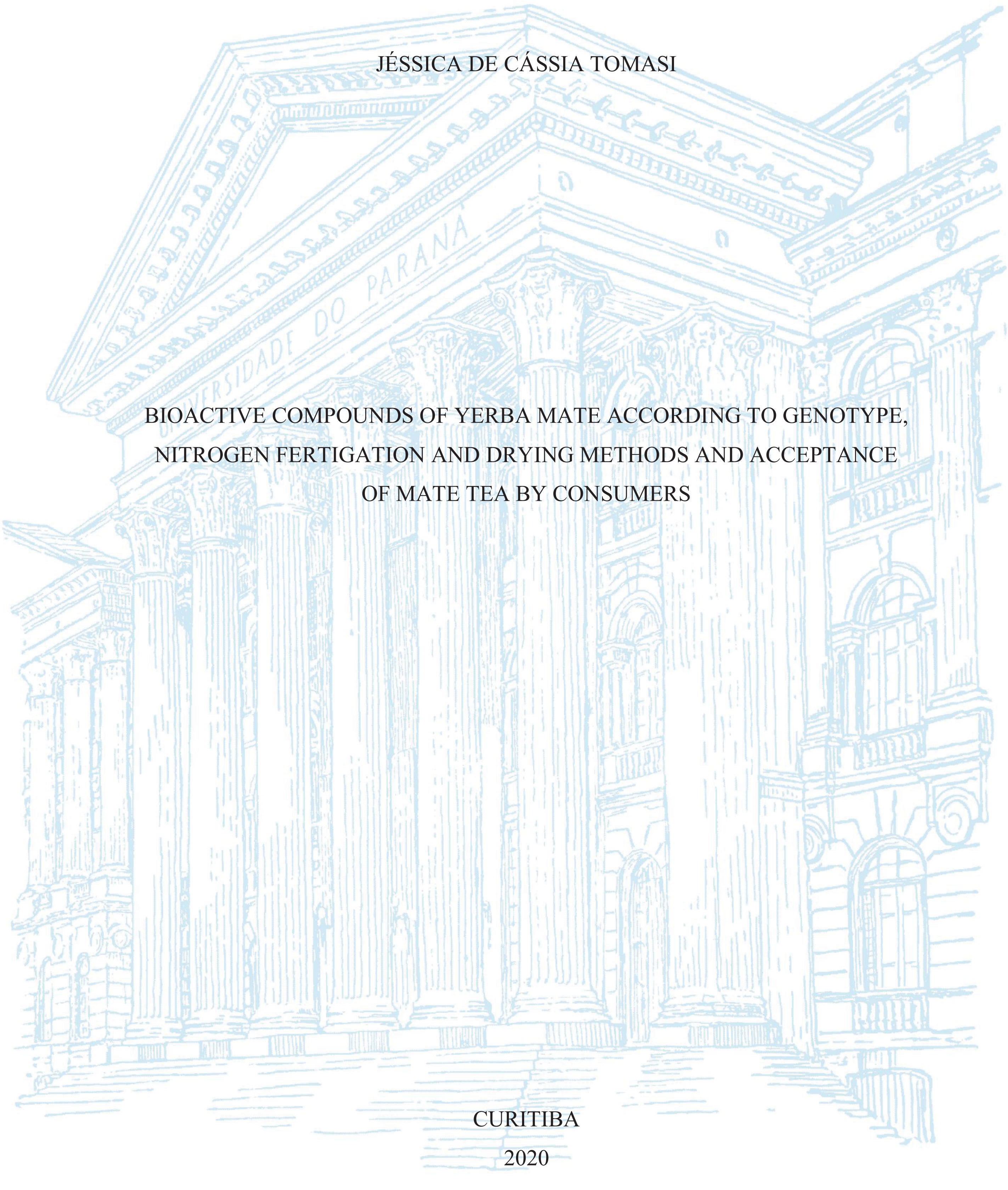
UNIVERSIDADE FEDERAL DO PARANÁ

JÉSSICA DE CÁSSIA TOMASI



BIOACTIVE COMPOUNDS OF YERBA MATE ACCORDING TO GENOTYPE, NITROGEN FERTIGATION AND DRYING METHODS AND ACCEPTANCE OF MATE TEA BY CONSUMERS

CURITIBA
2020

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanitarismo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Doutora em Ciências.

Orientador: Dr. Cícero Deschamps

Coorientadores: Dr. Ivar Wendling

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **JESSICA DE CASSIA TOMASI** intitulada: **BIOACTIVE COMPOUNDS OF YERBA MATE ACCORDING TO GENOTYPE, NITROGEN FERTIGATION AND DRYING METHODS AND ACCEPTANCE OF MATE TEA BY CONSUMERS**, sob orientação do Prof. Dr. CICERO DESCHAMPS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 13 de Março de 2020.

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Aos meus pais e irmão:

Edson José Tomasi

Márcia Andréia Steinbrenner e

Willian Edson Tomasi

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Não são as espécies mais fortes que sobrevivem, nem as mais inteligentes, e sim
as mais suscetíveis a mudanças
(CHARLES DARWIN)

RESUMO

Ilex paraguariensis A. St.-Hil é uma espécie arbórea, nativa e cultivada no Brasil. Popularmente é conhecida por erva-mate e apresenta importância econômica, ambiental, social e cultural para região Sul. Tradicionalmente as folhas secas são matéria prima para o preparo de bebidas conhecidas como chimarrão e tereré. Nos últimos anos, a erva-mate tem ganhado destaque mundial com produtos diferenciados na linha de bebidas, alimentos, cosméticos e diversos outros produtos manufaturados. A visibilidade da cultura se deve aos compostos bioativos presentes nas suas folhas, benéficos para a saúde humana. Dentre eles destacam-se as metilxantinas (e.g. cafeína e teobromina) e os ácidos cafeoilquínicos da classe dos compostos fenólicos. Todavia, o teor desses compostos é alterado por muitos fatores (e.g. ambientais, genéticos, processamento). Tendo em vista a demanda de mercado por produtos à base de mate, incluindo o chá mate, métodos alternativos de secagem das folhas é uma demanda crescente, assim como, a aceitação dos produtos desenvolvidos por esses novos processos. Com isso, no capítulo 1 foram testados os métodos tradicionais de secagem das folhas (secador rotativo e esteira) e alternativos (secadores a 40, 60 e 80°C, liofilizador e microondas) na alteração do teor dos compostos bioativos, características nutricionais, capacidade antioxidante e coloração. No capítulo 2 as folhas secas em todos os métodos supracitados foram tostadas e utilizadas no preparado de chá mate quente e gelado para verificação da aceitação do consumidor. Os resultados confirmam que o microondas é uma alternativa eficiente tecnicamente de secagem das folhas do mate e a aceitação do chá mate foi satisfatória para essa tecnologia de secagem. Nos capítulos 3 e 4 foi proposto um novo sistema de cultivo (semi-hidropônico) visando a produção de folhas ao longo do ano todo e maior controle do perfil fitoquímico. Para tanto, foram testados dois genótipos (EC22 e EC40), doses de nitrogênio (D1-114, D2-206, D3-380, D4-760 e D5-1142 mg.L^{-1}), tipo de folha (jovem e madura) e sazonalidade (11 colheitas durante dois anos de cultivo). Os resultados de produtividade (capítulo 3) demonstraram não haver diferença na produtividade de folhas jovens e maduras e confirmaram a superioridade do genótipo EC40 na utilização do N, uma vez que, a dose ideal foi D1-114 mg.L^{-1} ao passo que para EC22 foi 206 mg.L^{-1}. Em relação a sazonalidade, pouca oscilação foi observada ao longo das colheitas, confirmando o potencial do sistema em termos de produção contínua. No capítulo 4, seguiu-se a mesma condução e tratamentos do capítulo 3, entretanto foram avaliados os teores dos compostos bioativos (cafeína, teobromina, ácido 3-cafeoilquínico, ácido 4-cafeoiquínio, ácido 5-cafeoilquínino, compostos fenólicos totais) e a capacidade antioxidante frente aos radicais ABTS e DPPH. Com base nos resultados, conclui-se haver influência da sazonalidade no teor dos compostos e capacidade antioxidante, sendo o genótipo EC40 superior ao EC22 para todas as variáveis analisadas. Comportamento diferencial foi verificado na síntese das metilxantinas, sendo o genótipo EC40 superior em termos de cafeína e EC22 superior em termos de teobromina. A dose de nitrogênio influenciou no teor dos compostos, sendo que, a maior dose (D5-1142 mg.L^{-1}) correspondeu ao teor elevado de metilxantinas totais, cafeína e teobromina para ambos os genótipos; já para os ácidos cafeoilquínicos, resposta inversa foi observada. De modo geral essa pesquisa trouxe inovação em termos de processo de secagem e cultivo da erva-mate, assim como, maior compreensão dos fatores que afetam os compostos bioativos de interesse presentes nas folhas da espécie. Sendo, portanto, um trabalho inédito e com vasto potencial de continuidade em pesquisas futuras.

Palavras-chave: *Ilex paraguariensis*. Cafeína. Sistema semi-hidropônico. Compostos fenólicos. Ácidos cafeoilquínicos.

ABSTRACT

Ilex paraguariensis A. St.-Hil is a tree species, native and cultivated in Brazil. It is popularly known as yerba mate and has economic, environmental, social and cultural importance for the Southern region. Traditionally, species dry leaves are used to prepare drinks known as “chimarrão” and “tereré”. In the last years, it has received more attention because of the potential for production of differentiated products like drinks, food and cosmetics. The visibility of the crop is due to the bioactive compounds present in the mate leaves that are beneficial to human health. The main compounds belong to methylxanthines (e.g. caffeine and theobromine) and phenolic (caffeoylquinic acids) classes. However, the content of these compounds is altered by many factors (e.g. environmental, genetic, processing). In view of the market demand for new products based on mate, including mate tea, the development of alternative methods of leaf drying is very important ensure the quality of the raw material as well as the acceptance of the products generated by these processes. In chapter 1, the traditional methods of leaf drying (rotative dryer and conveyor dryer) and alternative (dryers at 40, 60 and 80 ° C, freeze dryer and microwave oven) were tested on the content of the bioactive compounds, nutritional characteristics, antioxidant capacity and coloring. In chapter 2, the leaves dried in all methods above mentioned were toasted and used in the prepared hot and iced mate tea to verify the consumer acceptance. The results of the first two chapters showed that the microwave oven represents an efficient alternative for drying mate leaves and the acceptance of mate tea was also satisfactory using this drying technology. In chapters 3 and 4, a new cultivation system (semi-hydroponic) which would allow the leaf production throughout the year and the monitoring of the phytochemical profile was evaluated. Two genotypes (EC22 and EC40) were compared in relation to the effect of nitrogen dose (D1-114, D2-206, D3-380, D4-760 and D5-1142 mg.L^{-1}), leaf type (young an mature) and seasonality (11 harvests during two years of cultivation). Production results of both genotypes (chapter 3) demonstrated no difference in the production of young and matures leaves, but the EC40 genotype was more efficient in the nitrogen use since the D1 ideal dose was -114 mg.L^{-1} and 206 mg.L^{-1} for EC22. In terms of seasonality, there was little variation over time, which confirm the system potential for continuous leaf production. In chapter 4, the same system and treatments as mentioned for chapter 3 were compared for both genotypes in relation to the the bioactive compound contents(caffeine, theobromine, 3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, total phenolic compounds) and the antioxidant capacity by ABTS and DPPH radicals. Based on the results, we conclude that of the seasonality affects the content of the compounds and antioxidant capacity, with the EC40 genotype being superior to the EC22 for all analyzed variables. Different results were verified for the methylxanthines accumulation where the caffeine of EC40 genotype was higher than in EC22 genotype, but lower in theobromine content. The nitrogen dose affects the content of the compounds, and the highest dose (1142 mg.L^{-1}) resulted in increase of total methylxanthines, caffeine and theobromine for both genotypes and decrease for caffeoylquinic acids. This research brought innovation for process of drying and cultivation system to yerba mate, as well as a better understanding of the factors that influence the content of bioactive compounds present in species leaf. Therefore, this work is original and present hight potential for future researches.

Keywords: *Ilex paraguariensis*. Caffeine. Semi-hydroponic system. Phenolic compounds. Caffeoylquinic acids

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CHAPTER 4

1 GENERAL INTRODUCTION

Yerba mate or mate (*Ilex paraguariensis* A. St.-Hil.) is a native and cultivated plant from South American countries. Brazil is the world's largest producer (619 thousand tonnes per year), followed by Argentina (291 thousand tonnes per year) and Paraguay (105 thousand tonnes per year) (RIACHI; DE MARIA, 2017; FAOSTAT, 2017). Of all crops classified as permanent cultivation in the southern and central-western regions of Brazil, mate represents 21.8 % of the total harvested area, with average yield of 7.6 tonnes.ha^{-1} (IBGE, 2018).

The habit of consuming drinks prepared with the leaves and fine branches of yerba mate has been adopted for centuries by the native inhabitants, the guaranis, and has become traditional in its region of occurrence, being consumed mainly as chimarrão and tereré (BRACESCO et al., 2011; BLUM-SILVA et al., 2015; CARDOZO JUNIOR; MORAND, 2016). Another beverage is the mate tea, produced with dried and toasted leaves, with has been consumed in other countries, including the United States, Germany and Syria (CARDOZO JUNIOR, MORAND, 2016; DE GODOY et al., 2013). More recently, there has been an increasing interest in the development of new forms of yerba mate consumption, such as capsules, tablets, pills, and other manufactured products (BECKER et al., 2019).

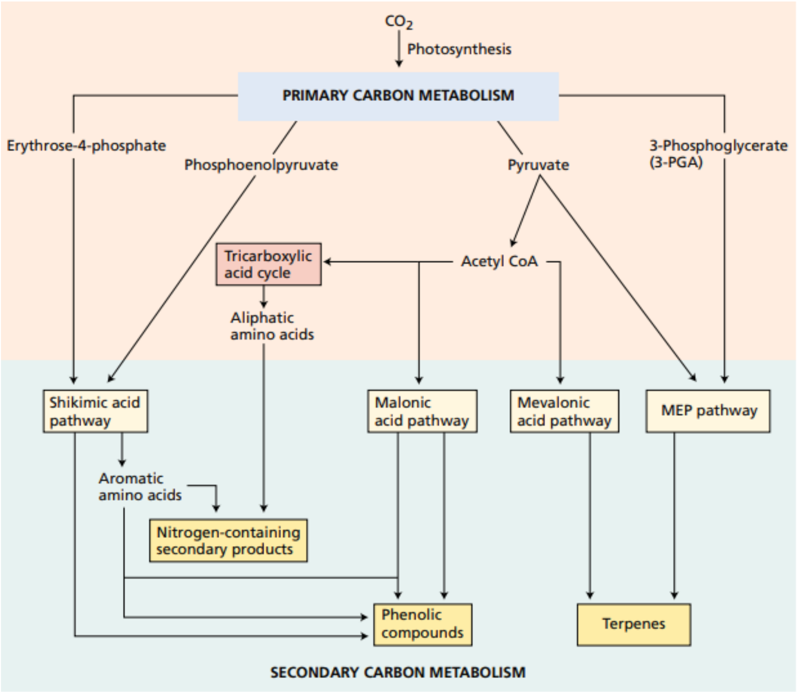
The potential of yerba mate is due to its bioactive compounds. Among the 100 most consumed plants in Brazil, mate is one of the main sources of methylxanthines (caffeine and theobromine) (MEINHART et al., 2019), monocaffeoylquinic and dicaffeoylquinic acids (MEINHART et al., 2017). with antioxidant, anti-inflammatory, antiatherogenic, anticarcinogenic, antiobesity, antiglycogen, diuretic, energetic properties actions (HECK; DE MEJIA, 2007; CARDOZO JUNIOR; MORAND, 2016). In addition mate has a nutritional potential value, because the presence of proteins, fibers carbohydrates, lipids and minerals (ESMELINFRO et al., 2002; EFINGE et al., 2009; SOUZA et al., 2015).

In industrial terms, the content of bioactive compounds, as well as the aroma and taste, are influenced mainly by post-harvest (drying) that directly interferes with the final quality of raw material that will be used in various industrial sectors (LEE; CHAMBERS, 2010). Traditionally, post-harvest techniques applied to yerba mate leaves comprised of two steps: the pre-drying, also known as “roasting” which uses fire as a heat source (± 400 °C for 2-8 min), following by drying either in a rotatory dryer (± 350°C in the inlet and ± 110°C in the output for 30 min) or in conveyor dryer (90 - 110°C for 3 h), and finally, the dried material is milled (ISOLABELLA et al., 2010). This processes require large spaces for allocation and operate in conditions to produce traditional drinks like “chimarrão and tererê” which demands specific process in order to acquire aroma and taste characteristics.

Thus, other drying methods must be studied for different industrial segments such as tea. In this context many alternatives may be tested for yerba mate drying with: dryers, freezing drying and microwave oven. As a complement, in order to prove the efficiency of a drying method, in addition to the content of its bioactive compounds, sensory analysis is extremely important for the final market acceptance (YANG; LEE, 2019).

The bioactive compounds described above are part of the plants secondary metabolism. Secondary metabolites have no generally recognized, direct roles in the processes of photosynthesis, respiration, solute transport, translocation, protein synthesis, nutrient assimilation, differentiation, or the formation of carbohydrates, proteins, and lipids (TAIZ; ZEIGER, 2013). These metabolites are synthesized in response to various external signals. Coordinated transcriptional control of biosynthetic genes emerges as a major mechanism dictating the accumulation of secondary metabolites in plant cells (DUTTA; SEN; DESWAL, 2007). Based on biosynthetic pathway, secondary metabolites are classified into three major groups: terpenes, phenolic compounds and nitrogen containing compounds (FIGURE 1) (VERMA; SHUKLA, 2015).

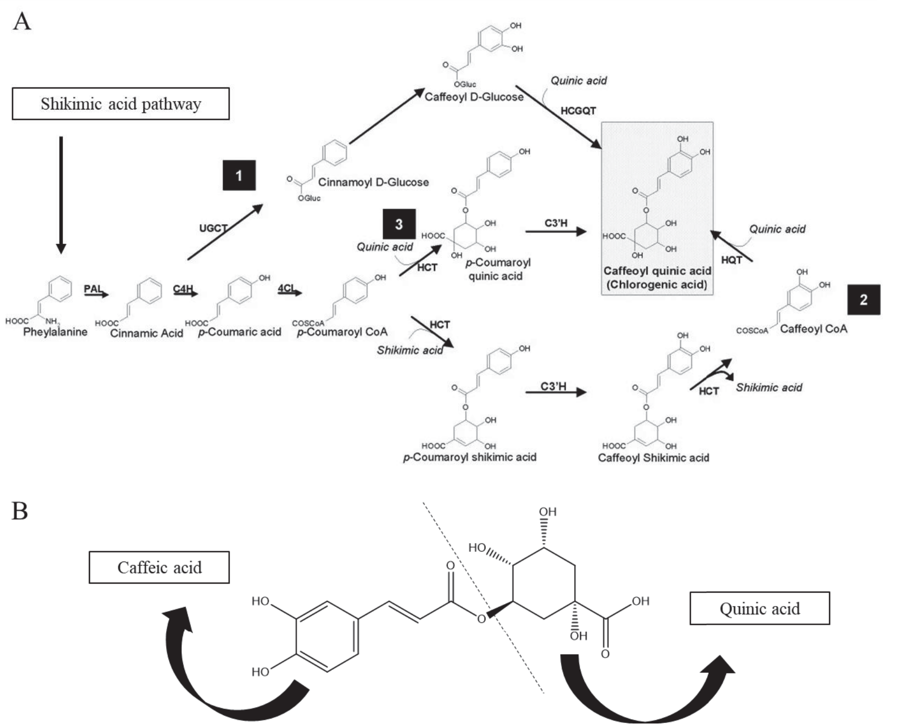
FIGURE 1 – MAJOR PATHWAYS OF SECONDARY METABOLITE BIOSYNTHESIS AND THEIR INTERRELATIONSHIPS WITH PRIMARY METABOLISM.



SOURCE: Taiz; Zeiger (2013)

For yerba mate the main compounds of the phenolic compounds class are caffeoylquinic acids (monocaffeoylquinic acids – CQAs and dicaffeoylquinic acids - diCQAs). Their metabolic routes are expressed in the figure (FIGURE 2A). The CQAs and diCQAs are formed by esterification among quinic acid and caffeic acid (FIGURE 2B). Quinic acid has axial hydroxyl groups on carbons 1 and 3 and equatorial hydroxyl groups on carbons 4 and 5. CQAs are categorized according to the number or the position of caffeoyl groups esterified in one or more carbons of quinic acid (WOŹNIAK et al., 2020).

FIGURE 2 - PATHWAYS OF CAFFEOYLQUINIC ACIDS

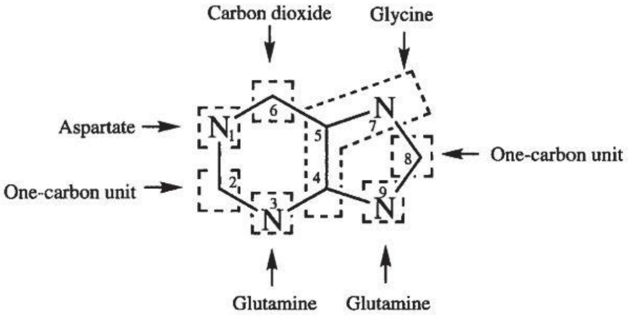


SOURCE: A - Comino et al., (2007) with few modifications; B – The author (2020)
SUBTITLE: A - The three routes for caffeoylquinic acid (labelled 1, 2 and 3) synthesis in plants. Enzymes involved in the pathway are: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-hydroxycinnamoyl CoA ligase; HCT, hydroxycinnamoyl CoA shikimate/quinate hydroxycinnamoyl transferase; C3H, p-coumarate 3'-hydroxylase; HQT, hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase; UGCT, UDP glucose: cinnamate glucosyl transferase; HCGQT, hydroxycinnamoyl D-glucose: quinate hydroxycinnamoyl transferase; B: 3-caffeoylquinic acid formed by esterification of quinic acid and caffeic acid.

Other groups of secondary metabolites with extremely importance for yerba mate is the nitrogen containing compounds such as methylxanthines class (are known as purine alkaloids). Caffeine and theobromine are the best known and abundance purine alkaloids present in yerba mate leaves and traces of theophylline (ASHIHARA; CROZIER, 1999;

ASHIHARA; SANO; CROZIER, 2008; YIN; KATAHIRA; ASHIRARA, 2015). The methylxanthines are derived from purine nucleotides. The purine ring is assembled from several small molecules. The N-1 atom originates from aspartate, C-4, C-5 and N-7 are from glycine, N-3 and N-9 come from the amide group of the side chain of glutamine, C-2 and C-8 are from activated derivatives of tetrahydrofolate, and C-6 is from CO_2 (FIGURE 3) (ASHIHARA; CROZIER, 1999).

FIGURE 3 - ORIGINS OF THE N AND C ATOMS IN THE PURINE RING

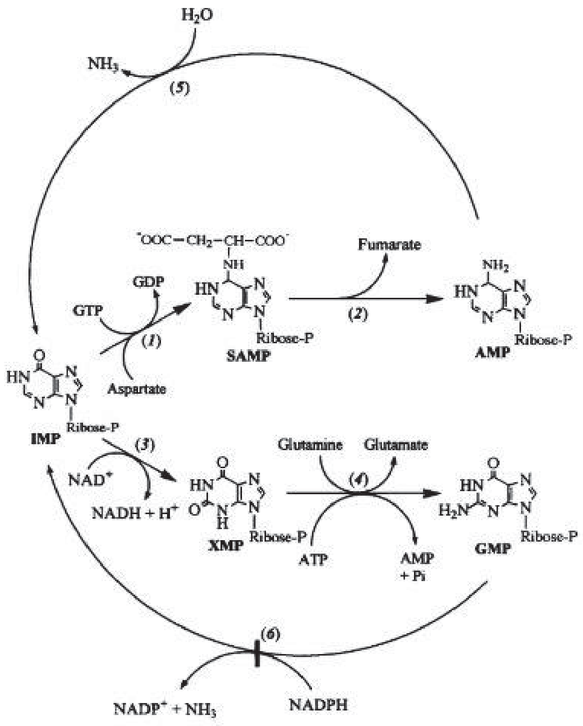


SOURCE: Ashihara e Crozier (1999)

The biosynthetic pathway of purine nucleotides in plants is know as *de novo*. The *de novo* pathway is usually defined as the pathway that is responsible for the synthesis of IMP (inosine-5-monophosphate) from 5-phosphoribosylamine (PRA). PRA is formed from 5-phosphoribosyl- 1-pyrophosphate (PRPP) and this is synthesized from ribose-5-phosphate, an intermediate of the pentose phosphate pathway (primary metabolism). IMP produced by *de novo* purine biosynthesis is converted to AMP (adenosine-5-monophosphate) and GMP (guanosine-5-monophosphate) (FIGURE 4) (ASHIHARA; CROZIER, 1999). The nucleotides formed (IMP, GMP, AMP) (Figure 4) are the basis for the synthesis of methylxanthines (purine alkaloids).

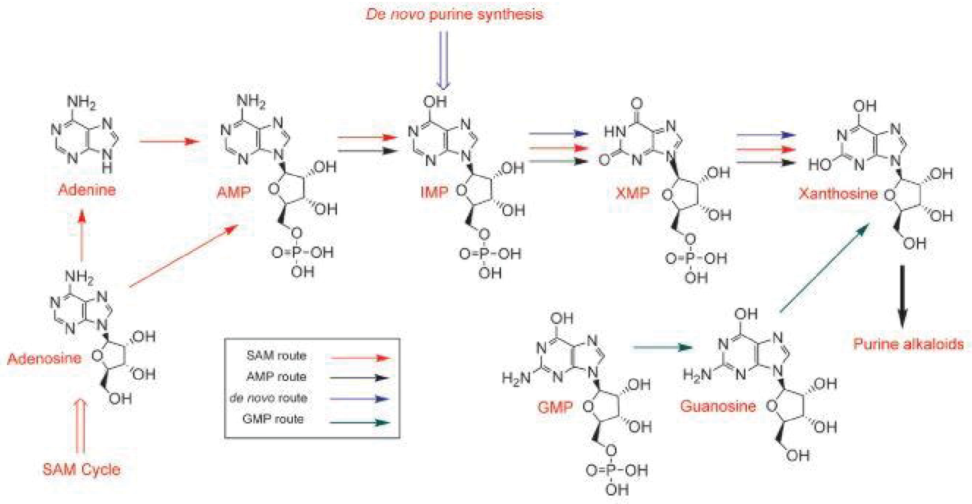
Thus, the initial substrate of purine alkaloid synthesis is xanthosine. It is supplied by at least four different pathways: *de novo* purine biosynthesis (*de novo* route), the degradation pathways of adenine nucleotides (AMP route) and guanine nucleotides (GMP route), and the S-adenosyl-L-methionine (SAM) cycle (SAM route) (FIGURA 5) (ASHIHARA; SANO; CROZIER, 2008). Thus, the xanthosine is used as substrate for caffeine, theobromine and theophylline synthesis in yerba mate leaves (FIFURE 6).

FIGURA 4 - INTERCONVERSION OF PURINE NUCLEOTIDES IN HIGHER PLANTS



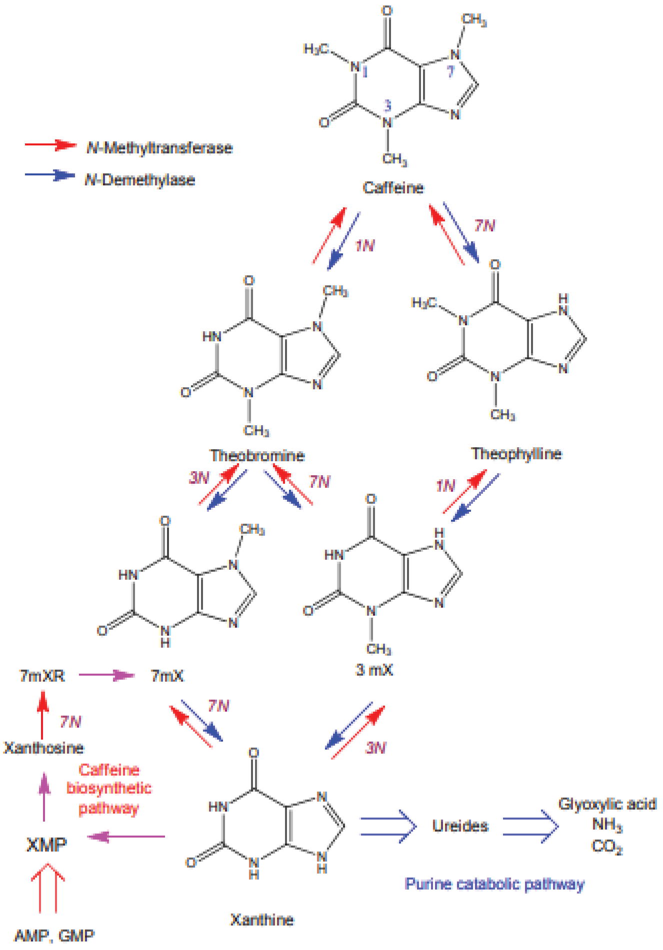
SOURCE: Ashihara e Crozier (1999)
SUBTITLE: IMP (inosine-5-monophosphate); SAMP (adenylosuccinate); AMP (adenosine-5-monophosphate); GMP (guanosine-5-monophosphate) and XMP (xanthosine-5-monophosphate).

FIGURA 5 - SYNTHESIS ROUTES OF PURINE ALKALOIDS



SOURCE: Ashihara, Sano e Crozier (2008)
SUBTITLE: IMP (inosine-5-monophosphate); AMP (adenosine-5-monophosphate); GMP (guanosine-5-monophosphate) and XMP (xanthosine-5-monophosphate).

FIGURA 6 - SYNTHESIS OF PURINE ALKALOIDS IN MATE LEAVES



SOURCE: Katahira e Ashirara (2015)
SUBTITLE: AMP (adenosine-5-monophosphate); GMP (guanosine-5-monophosphate); XMP (xanthosine-5-monophosphate); 7mXR: 7-methylxanthosine; 7mX: 7-methylxanthine 3mX: 3-methylxanthine.

The concentration and accumulation of secondary metabolites are influenced by genetic factors (genetic regulation), ontogenic factors (developmental stages), morphogenetic factors (tissues which performed specialized functions) and environmental factors (abiotic and biotic) (VERMA; SHUKLA, 2015). In case of the environmental many abiotic factors are involved in fluctuation of secondary metabolites with water, light, temperature, soil and chemicals (minerals/fertilizers) and can redirect the metabolism to consequently regulate the production of active constituents. Because plants have a complex and variable chemical composition, the type and content of secondary metabolites as well as biological effects were often determined according to the change of environment (YANG et al., 2018).

In view of the genetic factor effect, not only the phytochemicals but also in terms of leaf mass production characteristics and the economic, social, environmental importance of the yerba mate, the EMBRAPA Forests started in 1997 the breeding program for the species (RESENDE et al., 2000), aiming selecting potential genotypes to compose cultivation systems, highly productive and with a phytochemical standard. An example of this are the high and low caffeine EC40 and EC22 genotypes, respectively. According to ANVISA's resolutions n° 277 a decaffeinated product has a maximum of 0.1 % (g/100g) of caffeine, extracted with water (BRASIL, 2005).

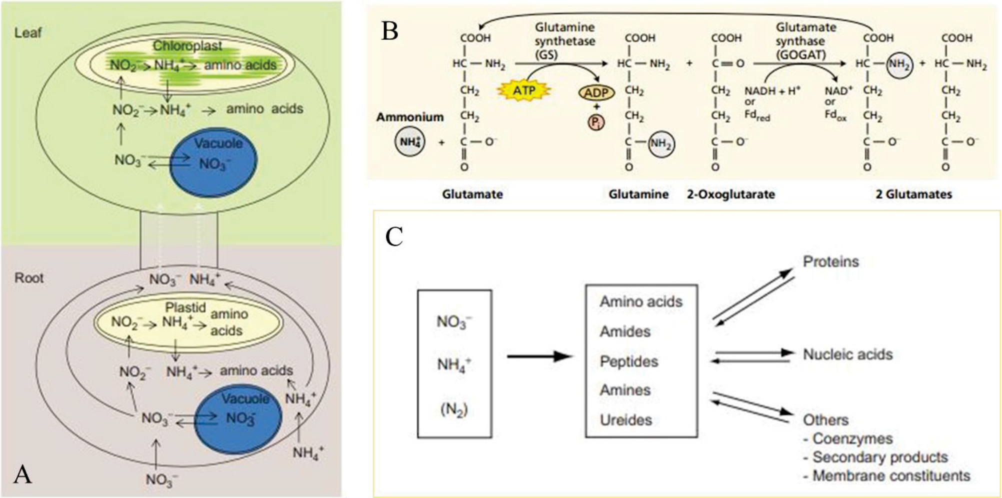
Currently, yerba mate is exploited in natural systems (extractivism with harvest every 24 to 48 months), pure production (monoculture with harvest every 12, 18 to 24 months) or in agroforestry systems (SANTIN et al., 2017a, 2017b, 2019; WESTPHALEN et al., 2019). The use of the semi-hydroponic system is feasible for the conduction of yerba mate ministumps for vegetative propagation (WENDLING; DUTRA; GROSSI, 2007), however, no research has been conducted in order to establish the system as a cultivation option, focusing on the production of raw material for various industrial sectors.

Supplemental plant mineral nutrition may provide a means not only to stimulate plant growth but also influence the content of secondary metabolites (YANG et al., 2018). Nitrogen (N) is an essential macronutrient and a key factor limiting agricultural productivity. It is a critical nutrient since is needed to synthesize amino acids, which are the building elements of protein, nucleotides, chlorophyll, and numerous other metabolites and cellular components. The nitrate (NO_3^-) and ammonium (NH_4^+) in plants, are taken up by the roots, and assimilated in roots or shoots. Assimilation of inorganic nitrogen is energetically costly, requiring reducing equivalents, ATP and C skeletons and many adaptations observed in plants in response to changing N supply are at least partly due to changes in gene expression (VIDAL; GUTIÉRREZ, 2008; NUNES-NESI; FERNIE; STITT, 2010).

The uptake of nitrate and ammonium into plant roots is mediated by transport proteins with different affinities. The high-affinity transport systems (HATS) operate at low concentrations (<0.5mM) of external nitrate or ammonium. At higher concentrations, >0.5mM, uptake is primarily via the low affinity transport systems (LATS), allowing large influxes of substrate at high substrate availability. Both uptake systems have inducible and constitutive components. After absorption, nitrogen uptake in NO_3^- form needs to be reduced in roots or shoots because the nitrogen assimilation occurs in NH_4^+ form. The reduction of nitrate to ammonium is mediated by two enzymes: nitrate reductase, with reduction of nitrate to nitrite and nitrite reductase, which transforms nitrite to ammonium (FIGURA 7A) (HAWKESFORD et al., 2011).

The assimilation of ammonium (NH_4^+) into essential amino acids is an enzymatic process that involves two enzymes Glutamine synthetase (GS) and glutamate synthase (GOGAT). Glutamine synthetase (GS) combines ammonium with glutamate to form glutamine. Plants contain two classes of GS, one in the cytosol and the other in root plastids or shoot chloroplasts. Elevated plastid levels of glutamine stimulate the activity of glutamate synthase (GOGAT). This enzyme transfers the amide group of glutamine to 2-oxoglutarate, yielding two molecules of glutamate. Plants contain two types of GOGAT: One accepts electrons from NADH; the other accepts electrons from ferredoxin (FIGURA 7B) (TAIZ; ZEIGER, 2013). The inorganic N assimilated into glutamate and glutamine can readily be used for the synthesis of other amides as well as amino acids, ureides, amines, peptides, proteins, nucleic acids and other N-containing compounds (FIGURA 7C) (HAWKESFORD et al., 2011).

FIGURA 7 - OVERVIEW OF N UPTAKE AND N ASSIMILATION IN PLANTS



SOURCE: A and C: Hawkesford et al. (2011); B: Taiz; Zeiger (2013)

Based on this introduction, this thesis was divided into four chapters: chapter 1, aiming to find an alternative drying method for yerba mate based mainly on maintaining high levels of bioactive compounds. Chapter 2, aiming to verifiys the content of bioactive compounds, antioxidant capacity and acceptance of hot and iced mate tea produced with toasted raw material obtained from different drying processes. The chapter 3 and 4 aimed to confirm the efficiency of the semi-hydroponic system as an new cultivation system for yerba mate by evaluating leaf production, bioactive compounds and antioxidant capacity of young and mature leaves of two genotypes submitted to fertigation with different nitrogen doses for two years.

REFERENCES

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC n. 277, de 22 de setembro de 2005. Regulamento técnico para café, cevada, chá, erva-mate e produtos solúveis. **Diário Oficial [da] República Federativa do Brasil, Brasília**, DF, 22 set. de 2005.

ASHIHARA, H.; CROZIER, A. Biosynthesis and metabolism of caffeine and related purine alkaloids in plants. **Advances in Botanical Research**, v. 30, p. 117-205, 1999.

ASHIHARA, H.; SANO, H.; CROZIER, A. Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. **Phytochemistry**, v. 69, n. 4, p. 841–856, 2008.

BECKER, A. M. et al. Spray-dried yerba mate extract capsules: clinical evaluation and antioxidant potential in healthy individuals. **Plant Foods for Human Nutrition**, v. 74, n. 4, p. 495–500, 2019.

BLUM-SILVA, C. H. et al. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. **Revista Brasileira de Farmacognosia**, v. 25, n. 1, p. 1–6, 2015.

BOLTON D. **Market research reflects and predicts growth**. 2019. Available from: https://worldteanews.com/market-trends-data-andinsights/market-research-reflects-and-predicts-growth. Accessed 1 November.

BRACESCO, N. et al. Recent advances on *Ilex paraguariensis* research: minireview. **Journal of Ethnopharmacology**, v. 136, n. 3, p. 378–384, 2011.

CARDOZO JUNIOR, E. L.; 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**, v. 21, p. 440–454, 2016.

COMINO, C. et al. Isolation and functional characterization of a cDNA coding a hydroxycinnamoyl transferase involved in phenylpropanoid biosynthesis in *Cynara cardunculus* L. **BCD Plant Biology**, n. 14, 2007.

DE GODOY, R. C. B. et al. Consumer perceptions, attitudes and acceptance of new and traditional mate tea products. **Food Research International**, v. 53, n. 2, p. 801–807, 2013.

DUTTA, A.; SEN, J.; DESWAL, R. Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L). G. Don. **Plant Cell Reports**, v. 26, n. 10, p. 1869–1878, 2007.

EFING, L. C. et al. Caracterização química e capacidade antioxidante da erva-mate**. Boletim do Centro de Pesquisa de Processamento de Alimentos**, v. 27, p. 241–246, 2009.

ESMELINDRO, M. C. et al. Caracterização físico-química da erva-mate: influência das etapas do processamento industrial. **Ciência e Tecnologia de Alimentos**, v. 22, p. 193–204, 2002.

FAOSTAT. **CROPS**. 2017. Available from: http://www.fao.org/faostat/en/#data/QC. Accessed 5 November, 2019.

HAWKESFORD, M. et al. Function of macronutrients. In: Marschner, H. **Mineral nutrition of higher plants.** 3nd ed**.** Academic Press, 2011. p. 135-189.

HECK, C. I.; DE MEJIA, E. G. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**, v. 72, n. 9, 2007.

IBGE. **Produção Agrícola Municipal (PAM)**. 2018. Available from https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9117 producao-agricola-municipal-culturas-temporarias-e. Accessed 5 November, 2019.

ISOLABELLA, S. et al. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. **Food Chemistry**, v. 122, n. 3, p. 695–699, 2010.

KOVÁCIK, J. et al. Variation of antioxidants and secondary metabolites in nitrogen-deficient barley plants. **Journal of Plant Physiology**, v. 171, p. 260–268, 2014.

LEE, J.; CHAMBERS, D. H. Descriptive analysis and U.S. consumer acceptability of 6 green tea samples from China, Japan, and Korea. **Journal of Food Science**, v. 75, n. 2, 2010.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, p. 522–530, 2017.

MEINHART, A. D. et al. Methylxanthines in 100 brazilian herbs and infusions: Determination and consumption. **Emirates Journal of Food and Agriculture**, v. 31, n. 2, p. 125–133, 2019.

NUNES-NESI, A.; FERNIE, A. R.; STITT, M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. **Molecular Plant**, v. 3, n. 6, p. 973–996, 2010.

RAMAKRISHNA, A.; RAVISHANKAR, G. A. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signaling and Behavior**, v. 6, n. 11, p. 1720–1731, 2011.

RIACHI, L. G.; DE MARIA, C. A. B. Yerba mate: an overview of physiological effects in humans. **Journal of Functional Foods**, v. 38, p. 308–320, 2017.

SANTIN, D. et al. Adubação nitrogenada e intervalos de colheita na produtividade e nutrição da erva-mate e em frações de carbono e nitrogênio do solo. **Ciência Florestal**, v. 29, n. 3, p. 1199, 2019.

SANTIN, D. et al. Intervalos de colheita e adubação potássica influenciam a produtividade da erva-mate (*Ilex paraguariensis*) no Estado do Paraná. Floresta, v. 46, n. 4, p. 509–518, 2017a.

SANTIN, D. et al. Manejo de colheita e adubação fosfatada na cultura da erva-mate (*Ilex paraguariensis*) em fase de produção. **Ciência Florestal**, v. 27, n. 3, p. 783–797, 2017b.

SOUZA, A. H. P. et al. Phytochemicals and bioactive properties of *Ilex paraguariensis*: an *in-vitro* comparative study between the whole plant, leaves and stems. **Food Research International**, v. 78, p. 286–294, 2015.

TAIZ, L.; ZEIGER, E. **Plant Physiology**, Sunderland: Sinauer Associates Inc, 2013.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, n. 4, p. 105–113, 2015.

VIDAL, E. A.; GUTIÉRREZ, R. A. A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis*. **Current Opinion in Plant Biology**, v. 11, n. 5, p. 521–529, 2008.

WENDLING, I.; DUTRA, L. F.; GROSSI, F. Produção e sobrevivência de miniestacas e minicepas de erva-mate cultivadas em sistema semi-hidrônico. **Pesquisa Agropecuária Brasileira**, v. 42, n. 2, p. 289–292, 2007.

WESTPHALEN, D. J. et al. Impact of different silvicultural techniques on the productive efficiency of *Ilex paraguariensis* A.St.Hill. **Agroforestry Systems**, 2019.

WOŹNIAK, D. et al. Caffeoylquinic acids. **Handbook of Dietary Phytochemicals**, p. 1–40, 2020.

YANG, J.-E.; LEE, J. Consumer perception and liking, and sensory characteristics of blended teas. **Food Science and Biotechnology**, 2019.

YANG, L. et al. Response of plant secondary metabolites to environmental factors. **Molecules**, v. 23, n. 4, p. 1–26, 2018.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of mate (*Ilex paraguariensis*). **Natural Product Communications**, v. 10, n. 5, p. 707–712, 2015.

CHAPTER 1

2 MICROWAVE OVEN: AN EFFICIENT ALTERNATIVE FOR *Ilex paraguariensis* LEAVES DRYING TO MAINTAIN THE PHYTOCHEMISTRY, NUTRITIONAL, AND COLOR CHARACTERISTICS

ABSTRACT

The increase in consumption of *Ilex paraguariensis* leaves is especially due to its bioactive compounds beneficial to the human health. However, the drying process is the most important post-harvest technique for maintaining the quality of *Ilex paraguariensis* raw material. Therefore, we studied the effect of various drying processes on phytochemical, nutritional and leaf color characteristics. The processes were composed of the typical drying techniques used for *I. paraguariensis* leaves (roasting + rotary dryer and roasting + conveyor dryer) and cutting-edge techniques for food processing (microwave oven, freeze-drying and oven dryer). The results indicate that microwave oven can be an alternative technique for drying yerba mate leaves because the content of phenolic compounds, antioxidant capacity (DPPH and ABTS), metilxantines and caffeoylquinic acids were similar or higher to the traditional processes. The nutritional analysis was satisfactory for protein, dietary fibre, ash, lipid and moisture for the microwave oven dried material with a higher or similar content, being yerba mate a potential source of fibre and protein. Furthermore, microwave oven technique preserved the green leaf color, resulting in good attractive quality, suggesting potential to integrate a new drying method for yerba mate productive chain.

Keywords: Yerba mate. Caffeine. HPLC. Total phenolic compounds. Caffeoylquinic acids. Protein.

2.1 INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.-Hil) is a native tree species from the South American countries Brazil, Paraguay, and Argentina (ISOLABELLA et al., 2010). Leaves are commonly used as raw material to prepare traditional beverages commonly named as “chimarrão” and “tererê” (CARDOZO JUNIOR; MORAND, 2016). More recently, there has been an increasing interest in developing new forms of yerba mate consumption, such as capsules, tablets, pills, and other manufactured products (BECKER et al., 2019). The many reasons for this interest are due to bioactive compounds with antioxidant activity, anti-inflammatory, antiatherogenic, anticarcinogenic, antiobesity, and anti-glycogen activities, diuretic and energetic properties (HECK; DE MEJIA, 2007; CARDOZO JUNIOR; MORAND, 2016) and its nutritional value (SOUZA et al., 2015).

The bioactive compounds are mainly attributed to methylxanthines (e.g. theobromine, caffeine) (MEINHART et al., 2019) and phenolic compounds related to flavonoids and phenolic acids (ZORIĆ et al., 2014). Within the phenolic acid class, the yerba mate contains caffeoylquinic acids (e.g. 3-caffeoylquinic, 4-caffeoylquinic, 5-caffeoylquinic acids) and dicaffeoylquinic acids (e.g.3,4 -dicaffeoylquinic, 3,5 – dicaffeoylquinic, 4,5 - dicaffeoylquinic acids) (MEINHART et al., 2017) pertaining to a group of a few species in nature that contains these compounds in abundance.

Many industrial segments seek for alternative strategies to obtain higher levels of phenolic compounds, antioxidant properties, and caffeine content (BRACESCO, 2019); therefore, it is wise to choose an optimum methodology for drying leaves, as it will determine the content of bioactive compounds and nutritional properties (LIN et al., 2012).

The quality of processed leaves is improved by reducing or inactivating enzymatic (oxidases) reactions, while also able to reduce microbial and physiological degradation (HOSSAIN; BARRY-RYAN; MARTIN-DIANA, 2010; WOJDYŁO et al., 2014) that directly interferes into leaf color and final product acceptance (SOYSAL, 2004).

Traditionally, post-harvest techniques applied to yerba mate leaves are needed for the beverage market, and are comprised of two steps: the pre-drying, also known as “roasting” which uses fire as a heat source (± 400 °C for 2-8 min), followed by drying either in a rotatory dryer (± 350°C in the inlet and ± 110°C in the output for 30 min) or in conveyor dryer (90 - 110°C for 3 h). Finally, dried material is milled and stored for posterior consumer use or targeted for different industrial purposes (ESMELINDRO et al., 2002; ISOLABELLA et al., 2010).

As noticed, the methodology currently used for yerba mate leaf-drying could have potential drawbacks and be unsafe for consumer usage since the smoke used during leaf-drying (maimed in roasting) is absorbed by leaves, and are known to contain dangerous compounds such as polycyclic aromatic hydrocarbons (PHAs) (VIEIRA et al., 2010; THEA et al., 2016). PHAs are reported to have a strong association with reactive oxygen species, tumour initiating activity and to induce oxidative stress (CIEMNIAK et al., 2019).

Although it has been suggested a variety of drying techniques at industry-scale for many plant materials, such as oven dryer (SOYSAL, 2004), freeze-drying (WOJDYŁO et al., 2014), and microwave oven (PASSARDI et al., 2006; ŚLEDŹ et al., 2013); studies on the usages of possible alternatives for leaf-drying techniques for yerba-mate are scarce, as the current methodology is used since the 19th century (GERHARDT, 2011) without signs to change.

The microwave oven is a drying technology that have seen an expansion in recent years and already have been developed in industrial scale (GUO et al., 2017). Microwave oven for drying yerba mate leaves has been reported before (PASSARDI et al., 2006); tough from our knowledge, no study have been conducted about the maintenance of the bioactive compounds, antioxidant capacity, nutritional levels and leaf post-processing color while also comparing with traditional drying methods. Therefore, in this study, we compared the microwave oven with freeze dryer, oven dryer and traditional yerba mate drying processes and its effects in order to maintain the phytochemicals content, nutritional levels, and leaf color with the aim to present alternative strategies of yerba mate drying.

2.2 MATERIAL AND METHODS

2.2.1 Plant material and drying processes

A total of 15 kg of yerba mate leaves were used for each drying process (n =3) supplied by Ervateira Capimar – União da Vitória, Parana State, Southern Brazil. After that, we applied seven drying processes to leaves: roasting for 3 min at 400 °C and drying in a rotary dryer for 30 min at 150 – 180 °C; roasting for 3 min at 400 °C and drying with conveyor dryer for 4h at 100 °C; microwave oven for 5 min (Electrolux®1000 W, 2450 MHz); oven dryer at 40 °C; oven dryer at 60 °C; oven dryer at 80 °C and freeze-drying for 72 h at -40 °C (Liotop®L101, 200 mbar).

The yerba-mate leaves submitted to oven dryer (New Technique®00ND) were removed only after it reached constant weight (± two days). After each treatment, leaf samples were processed in a coffee grinder (Cadence®) (classified as fine grinding – sieve 0.25 mm) and stored at -20°C for analysis.

2.2.2 Aqueous extracts preparation

For the preparation of aqueous extracts (use of water as a solvent because it is usual in plant-based drinks), we diluted 10 mg of powder sample from each process with 2 mL of MilliQ water (concentration 5 mg.L^{-1}). The material was mixed in a vortex Genie2® for 30 s, and the extraction was carried out in a Thermomixer Eppendorf® equipment at 450 rpm for 1 h at 60 ºC. The extracts were then filtered through a 0.22 µm filter paper.

2.2.3 Content of total phenolic compounds

We quantified total phenolic compounds (TPC) by Folin-Ciocalteau spectrophotometric method (SINGLETOM; ROSSI, 1965) with minor modifications. In a volumetric flask, we added 0.1 mL of the extract with 6.0 mL of distilled water and 0.5 mL of the Folin-Ciocalteau reagent, followed by one min of manually stirring. Afterward, 2 mL of 15 % aqueous Na_2CO_3 in water (w/v) solution was added, followed by stirring for 30 s. The final volume was adjusted with distilled water to reach 10 mL. After 2 hours of reaction in a dark environment, the absorbance was measured at 760 nm in a Shimadzu®-1800 UV/VIS spectrophotometer. As a reference, we established an analytical curve with gallic acid within the range of 0.25-13 mg.L^{-1}. Results were expressed in mg gallic acid equivalent (GAE) per gram of sample (mg GAE.g^{-1}) on a dry basis.

2.2.4 Antioxidant capacity (DPPH and ABTS radicals)

The antioxidant capacity of extracts by free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined with minor modifications. First 0.1 mL of sample was added to 3.9 mL of DPPH methanolic solution (0.06 mmol.L^{-1}), the reaction occurred in the dark at room temperature for 30 min, and the absorbance was then measured at 515 nm (BRAND-WILLIANS; CUVELLIER; BERSET, 1995).

The extracts antioxidant capacity by free radicals ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) was determined by reacting 10 mL ABTS (7 mmol.L^{-1}) with 176 µL potassium persulfate (140 mmol.L^{-1}). The reaction occurred in the dark at room temperature for 16 h. An aliquot of 1 mL of this solution was added in 100 mL of sodium acetate buffer (20 mmol.L^{-1}) pH 4.5. The absorbance was adjusted to 0.7 ± 0.05. From this adjusted solution, 3 mL was added to 30 µL of the extract. After 2 h the absorbance was measured (RE et al., 1999; YIM et al., 2013) with minor modifications.

All antioxidant activity assays were performed in a spectrophotometer UV/VIS (Shimadzu, model 1800, Kioto, JPN). The results were compared with a standard curve

(Trolox 0-1000 µmol.L^{-1} for DPPH and 0–2500 µmol.L^{-1} for ABTS) and expressed in µmol Trolox equivalent per gram of sample (TEAC µmol.g^{-1}) on dry basis.

2.2.5 High performance liquid chromatography HPLC-UV

For chromatographic analyses, we used a Shimadzu® liquid chromatograph (UFLC) controlled by the LC solution software and equipped with an automatic injector and UV detector (SPD-20A). Separation of the compounds was performed using Shim-Pack CLC-ODS (M) C18 column (250 x 4.6 mm i.d., 5 µm particle size), protected by Shim-Pack CLC G-ODS pre-column (100 x 4.0 mm id) both Shimadzu (Kyoto, Japan). The separation of compounds presents in the aqueous extract (20 µL of injection) was conducted at 30°C with a flow rate of 0.5 mL. min^{-1}. The mobile phase consisted of a gradient elution of water with acetic acid (99.9:0.1, v/v) (solvent A) and acetonitrile Merck® 100 % (solvent B). Compound detection was performed at a fixed wavelength of 280 nm. The gradient elution program was: 0-15 min (3-3 % B), 15-20 min (3-20 % B), 20-40min (20-20 % B), 40-45min (20-30 % B), 45-55 min (30-100 % B), 55-75 (100-100 % B), 75-80 (100-3 % B) and 80-95 (3-3 % B).

The identification and quantification of 1,3,7-trimethylxanthine (caffeine - CF) and 3,7-dimethylxanthine (theobromine - TB) was performed using an analytical curve of caffeine and theobromine Sigma® standards at a range of 0 to 1.0 mg.mL^{-1} and 0 to 0.5 mg.mL^{-1} respectively.

The identification of the caffeoylquinic acids (3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA)) and 3,5-dicaffeoylquinic acid (3,5-DQA) was performed by Sigma® standards and semi-quantification was performed using an analytical curve at a range of 0 to 10 mg.mL^{-1} of the 3-CQA Sigma®. The results were expressed as mg of compound per g sample (mg.g^{-1}) on a dry basis.

2.2.6 Analysis of the colorimetric components L^* a^* b^*

The color analysis was performed on a Konica Minolta®CR-400 colorimeter in the CIE system L* a* b*, with the coordinate L* (brightness where: 0 = black; 100 = white), a* (red / green intensity where: +a* = degree of red; -a* = degree of green), b* (yellow/blue intensity where: +b* = degree of yellow; -b* = degree of blue). The readings were performed on the final samples (dried and milled).

2.2.7 Nutritional characteristics

Nutrition analysis was carried out according to AOAC (2016) methods. The moisture and ash content were determined gravimetrically by mass loss with heating at 105 °C and 550 °C for 12 and 4 hours, respectively. The lipid (ether extract) content was determined by extraction with ethyl ether in a lipid extracting system. The total protein content was determined by the Kjeldahl conventional method (digestion, distillation, and titration) and the protein content determined by multiplying the content of total nitrogen by factor 6.25 (universal factor). Total dietary fiber content was determined by the gravimetric-enzymatic method using the Megazyme kit®. The results were expressed as percentages (g. $100g^{-1}$) (dry basis).

2.2.8 Statistical analysis

The experimental design was completely randomized, with three repetitions per drying process. The data were tested for homogeneity of variances by the Bartlett test and normality by the Shapiro-Wilk test. Analysis of variance was performed, and the means were submitted to the Tukey test at 1 % probability with the ASSISTAT® software 7.7 (SILVA; AZEVEDO, 2016). Pearson correlations were performed using the R software (R CORE TEAM, 2016).

2.3 RESULTS AND DISCUSSIONS

2.3.1 Phenolic content and antioxidant capacity

Depending on the drying method used for drying, significant differences can be detected. We observed higher content of phenolic compounds and antioxidant capacity by DPPH and ABTS radicals in leaf samples dried by traditional methods, roasting + rotary dryer, roasting + conveyor dryer and microwave oven ($p \leq 0.01$) (TABLE 1). The antioxidant activity is primarily attributed to polyphenol compounds in the extract, which delocalize electrons and form intramolecular hydrogen bonds (COLPO et al., 2016).

The differences obtained from methodologies can be attributed to the heat flow inherit of each process; a faster drying process can reduce the amount of time to inactivate the enzymes responsible for oxidation, which contributes to compound conservation and, consequently, higher antioxidant capacity (ISOLABELLA et al., 2010; CHEN; MARTYNENKO, 2018). The traditional method inactivates the enzymes during the roasting stage (3 min at 400°C); in the case of microwave oven drying, it is reported to be a combination of factors, but mainly fast drying with homogeneous energy distribution (OZKAN; AKBUDAK; AKBUDAK, 2007).

TABLE 1 - CONTENT (DRY BASIS) OF TOTAL PHENOLIC COMPOUNDS (TFC) AND ANTIOXIDANT CAPACITY OF AQUEOUS EXTRACTS BY DPPH AND ABTS RADICALS OF YERBA MATE LEAVES DRIED BY VARIOUS METHODOLOGIES.

Drying Process	mgEAG[a].g^{-1}	TEAC[b] μm.g^{-1}	
	TFC[c]	Radical DPPH	Radical ABTS
Roasting + rotary dryer	$90.34^{A} \pm 5.98$	$389.97^{A} \pm 0.75$	$1070.45^{A} \pm 2.91$
Roasting + conveyor dryer	$83.67^{A} \pm 3.13$	$383.67^{A} \pm 0.24$	$1059.31^{A} \pm 7.49$
Microwave oven	$90.07^{A} \pm 5.12$	$380.93^{A} \pm 5.30$	$1001.74^{A} \pm 6.46$
Oven dryer 40°C	$31.81^{BC} \pm 0.41$	$109.96^{B} \pm 5.75$	$362.06^{BC} \pm 3.91$
Oven dryer 60°C	$5.23^{D} \pm 1.29$	$27.24^{C} \pm 1.17$	$129.38^{C} \pm 2.56$
Oven dryer 80°C	$16.93^{CD} \pm 0.27$	$98.08^{B} \pm 4.28$	$247.42^{BC} \pm 5.03$
Freeze drying	$37.34^{B} \pm 0.65$	$95.66^{B} \pm 7.55$	$426.32^{B} \pm 3.11$

SOURCE: The author (2020).
NOTE: Values are reported as mean ± stantard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.01$).
SUBTITLE: [a] gallic acid equivalent; [b] antioxidant capacity equivalent to Trolox; [c] total phenolic compounds.

The reduced levels of total phenolic compounds and antioxidant capacity in the oven dryer process (40, 60, and 80 °C) (TABLE 1) can be related to the actuation of enzymes that have not been inactivated as polyphenol oxidases (PPO) and peroxidases (POD). The PPO catalyzes the oxidation of total phenolic compounds by molecular oxygen (MAYER, 2006; PERONE; CAPOBIANCO; PAPARELI, 2009), while peroxidases (POD) are extremely specific in the reduction of hydrogen peroxide (RICHARD-FORGET; GAUILLARD, 1997). The optimum PPO activation temperature is between 20 to 40 °C (more than 80 % of activity), while the POD showed higher activity (more than 80 %) at temperatures of 20, 40, and 60 °C. Both enzymes showed high thermal resistance, being PPO completely inhibited after 5 min at 80 °C and POD reducing the activity; however, keeping 20 % activity after 30 min at 80 °C (CENI et al., 2008; LIN et al., 2012).

The significantly lower content of total phenolic compounds was also presented in the freeze-drying process when compared to traditional methods and microwave oven process ($p \leq 0.01$) (TABLE 1). This could be attributed to the fact that low or intermediate molecular weight compounds, such as phenolics, are removed as a result of the vacuum applied during freeze-drying (VAN SUMERE et al., 1983). Also, since there is no thermal heat, compounds bindings that are conjugated remains unchanged, resulting in reduced antioxidant activity (WU et al., 2013; XIONG et al., 2019). However, the number of compounds obtained from freeze-drying was similar to oven drying at 40 ºC (TABLE 1). Similar results in the content of phenolic compounds between the drying process at 40 °C and freeze-drying were found for *Carica papaya* leaves (RAJA et al., 2017).

2.3.2 Identification and quantification of caffeoylquinic acids by HPLC

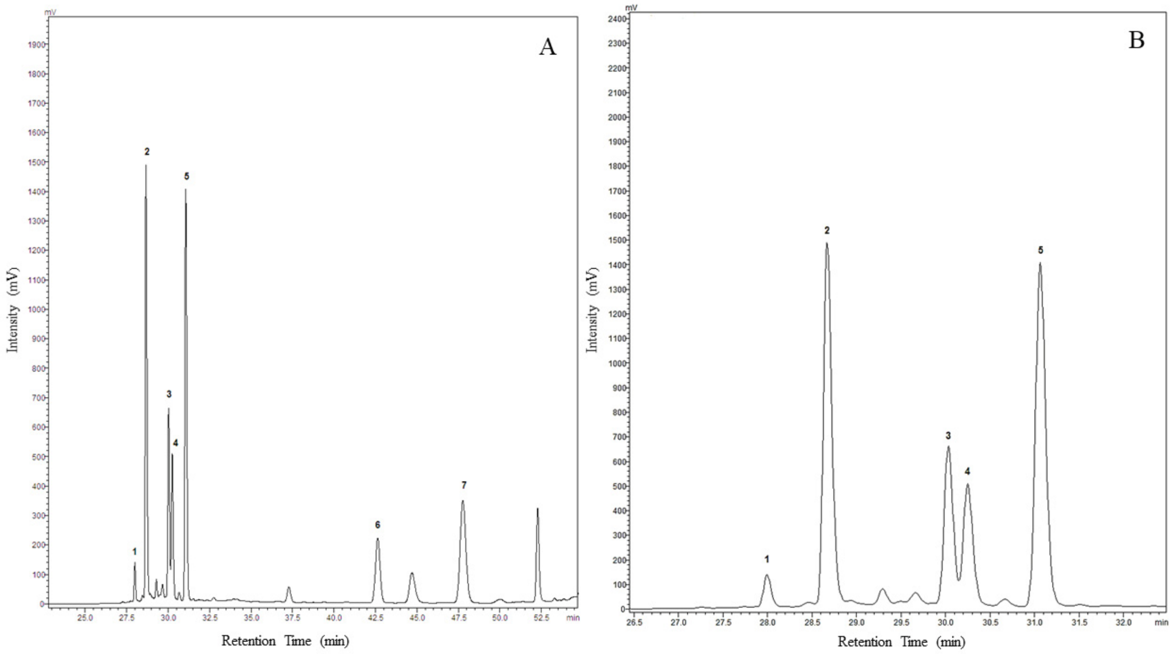
The chromatogram with the compounds identified in this work is in Figure 1. The content of caffeine presented a small variation between the drying methods, ranging from 10.22 to 13.83 $mg.g^{-1}$ (TABLE 2); whereas the lowest amount was observed in leaves submitted to the traditional drying method ($p \leq 0.01$) which relates with thermal degradation due to the high temperature of roasting (400 °C) (ESMELINDRO et al., 2002; ISOLABELLA et al., 2010). Although oven drying and freeze-drying had a high content of caffeine, microwave oven also presented values within the same range (13 $mg.g^{-1}$) – even though presenting the lowest amount of total phenolic compounds and antioxidant capacity between the studied processes. For theobromine, we did not observe differences between methods, as it is known that yerba mate leaves have lower quantities of this compound in dried form (COLPO et al., 2016). Methylxanthines (caffeine and theobromine) showed a positive correlation (FIGURE 2), those are the key compounds for the beverage industry that seeks stimulant compounds in its manufactured products. It is possible to maintain high levels with the correct usage of drying method.

The caffeoylquinic acids (3-CQA, 4-CQA and 5-CGA) and dicaffeoylquinic acid (3,5-DQA) are the main phenolic compounds responsible for the antioxidant capacity of yerba mate leaf extracts (DE MEJÍA et al., 2010; MEINHART et al., 2017). In this study the 3-CQA, 4-CQA and 5-CGA contents were approximately ten times superior ($p \leq 0.01$) in leaves dried by traditional methods and microwave oven compared to other drying methods (TABLE 2).

Furthermore, microwave oven drying method resulted in higher 3.5-DQA content (8.75 mg.g^{-1}); however, with no statistical difference from leaves dried with roasting + conveyor (TABLE 2). Therefore, microwave oven may be considered an alternative for drying yerba mate leaves, resulting in an aqueous extract rich in caffeoylquinic and dicaffeoylquinic acids. According to Butiuk et al. (2016), these compounds are of significant value and relevant to the pharmaceutical industry.

Significant correlation between caffeoylquinic acids (3-CGA, 4-CQA and 5-CQA), 3,5-DQA, and phenolic compounds were detected between them and with DPPH and ABTS radicals (FIGURE 2). These results confirms that caffeoylquinic acids and dicaffeoylquinic acid are the main phenolic compounds responsible for the hindrance, or inhibition, of free radicals in the oxidation process, as reported by Berté et al. (2011) and Mateos et al (2018); in addition to their anti-inflammatory and neuroprotective action Cittadini et al. (2019).

FIGURE 1 – REPRESEATIVE HPLC-UV CHROMATOGRAM SHOWING THE COMPOUNDS IDENTIFIED FROM YEBA MATE AQUEOUS EXTRACTS



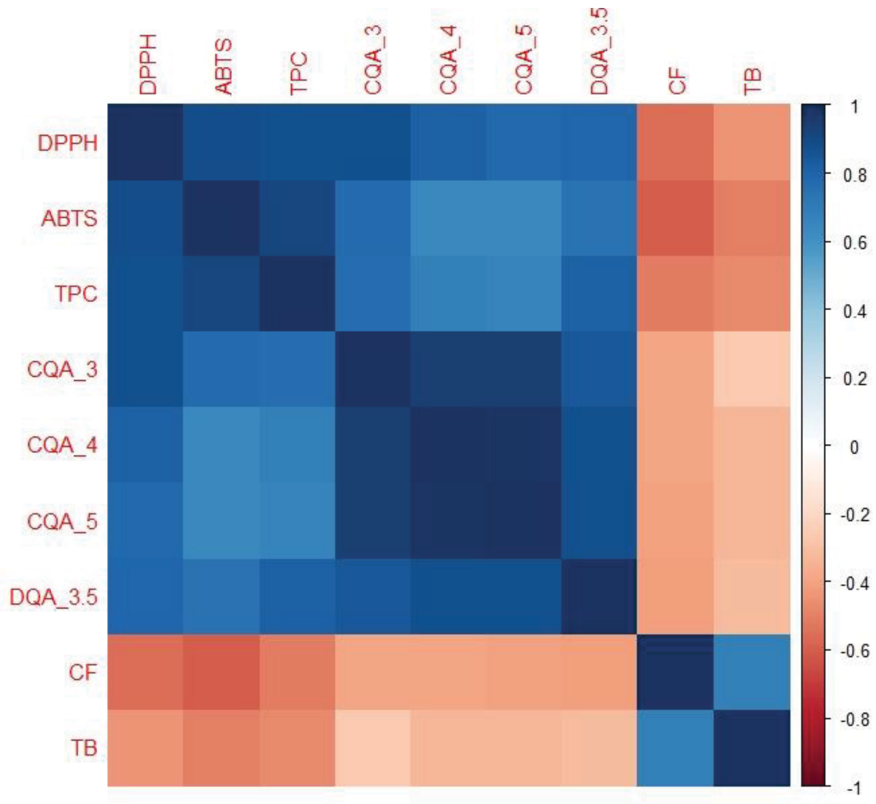
SOURCE: The author (2020)
FIGURE DESCRIPTION: A – complete chromatogram; B – expansion of the first part. Compounds identified 1= theobromine (RT 28,0 min), 2 = 5-caffeoylquinic acid (RT 28.6 min), 3 = 3-caffeoylquinic acid (RT 30,0 min), 4 = 4-caffeoylquinic acid (RT 30.2 min) 5 = caffeine (RT 31.0 min), 6 = Umbelliferone / internal standard (RT 42,5 min), 7 = 3,5-dicaffeoylquinic acid (RT 47,5 min).

TABLE 2 - – CONTENT (ON A DRY BASIS) OF CAFFEINE, THEOBROMINE, CAFFEOYLQUINIC ACIDS (3-CQA, 4-CQA, 5-CQA) AND DICAFFEOYLQUINIC ACID (3,5-DQA) IN AQUEOUS EXTRACTS OF YERBA MATE LEAVES DRIED BY THE STUDIED PROCESS.

Drying process	Content $mg.g^{-1}$					
	Caffeine	Theobromine	Caffeoylquinic acids			Dicaffeoylquinic acids
			3-CQA	4-CQA	5CQA	3,5-DQA
Roasting + rotary dryer	$10.22^{C} \pm 0.06$	$0.79^{A} \pm 0.01$	$5.13^{A} \pm 0.03$	$3.92^{A} \pm 0.02$	$9.57^{A} \pm 0.04$	$6.97^{B} \pm 0.06$
Roasting + conveyor dryer	$10.65^{BC} \pm 0.09$	$0.84^{A} \pm 0.01$	$4.95^{A} \pm 0.04$	$3.70^{A} \pm 0.03$	$10.09^{A} \pm 0.08$	$7.21^{AB} \pm 0.03$
Microwave oven	$13.04^{ABC} \pm 0.06$	$0.84^{A} \pm 0.03$	$5.27^{A} \pm 0.05$	$4.40^{A} \pm 0.02$	$11.19^{A} \pm 0.05$	$8.75^{A} \pm 0.05$
Oven dryer 40°C	$12.65^{ABC} \pm 0.03$	$0.84^{A} \pm 0.01$	$0.72^{B} \pm 0.05$	$0.33^{B} \pm 0.01$	$1.07^{B} \pm 0.02$	ND
Oven dryer 60°C	$13.25^{ABC} \pm 0.05$	$0.92^{A} \pm 0.01$	$0.13^{B} \pm 0.01$	$0.03^{B} \pm 0.01$	$0.06^{B} \pm 0.01$	ND
Oven dryer 80°C	$13.83^{A} \pm 0.05$	$0.97^{A} \pm 0.01$	$0.44^{B} \pm 0.03$	$0.15^{B} \pm 0.01$	$0.42^{B} \pm 0.01$	ND
Freeze drying	$13.49^{AB} \pm 0.08$	$0.95^{A} \pm 0.01$	$0.19^{B} \pm 0.01$	ND^{b}	$0.04^{B} \pm 0.01$	ND
Retention Time (min)	31.0	28.0	30.0	30.2	28.6	47.5

SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.01$);
SUBTITLE: ND: not detected.

FIGURE 2 – PEARSON MATRIX OF CORRELATION FOR THE TOTAL PHENOLIC COMPOUNDS (TPC), ANTIOXIDANT ACTIVITY OF THE RADICALS ABTS AND DPPH, CAFFEOYLQUINIC ACIDS (3-CAFFEOYLQUINIC ACID (CQA_3)), 4-CAFFEOYLQUINIC ACID (CQA_4)), 5-CAFFEOYLQUINIC ACID (CQA_5)), 3,5-DICAFFEOYLQUINIC (DQA_3.5), CAFFEINE (CF) AND THEOBROMINE (TB).



SOURCE: The author (2020).

2.3.3 Colorimetric test

The values for colorimetric tests components were obtained after various drying methods and milling of yerba mate leaves (Table 3).

TABLE 3 – COLORIMETRIC COMPONENTS OF L* A* B* FOR YERBA MATE LEAVES PROCESSED TROUGHT DIFFERENT DRYING METHODS.

Drying process	Colorimetric Components		
	L*	a*	b*
Roasting and rotary dryer	$28.49^{BCD} \pm 0.35$	$-7.02^{C} \pm 0.11$	$19.25^{BC} \pm 0.46$
Roasting and conveyor dryer	$30.17^{BCD} \pm 0.19$	$-10.06^{B} \pm 0.10$	$21.33^{B} \pm 0.32$
Microwave oven	$30.78^{BC} \pm 2.24$	$-12.66^{A} \pm 0.48$	$26.12^{A} \pm 0.94$
Oven dryer 40°C	$31.56^{B} \pm 0.47$	$-5.90^{C} \pm 0.15$	$17.27^{CD} \pm 0.30$
Oven dryer 60°C	$24.53^{CD} \pm 1.14$	$-0.15^{D} \pm 0.04$	$14.52^{DE} \pm 0.36$
Oven dryer 80°C	$23.62^{D} \pm 2.54$	$0.38^{D} \pm 0.08$	$12.29^{E} \pm 0.97$
Freeze drying	$41.13^{A} \pm 1.03$	$-9.51^{B} \pm 0.10$	$18.71^{BC} \pm 0.11$

SOURCE: The author (2019).
NOTE: Values are reported as mean ± stantard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.01$).
SUBTITLE: L*(degree of brightness: 0: black; 100: white); a* (+a*: degree of red color; -a*: degree of green color); b* (+b*: degree of yellow; -b*: degree of blue color).

Leaves dried with microwave oven were significantly better ($p \leq 0.01$) at maintaining the green color, represented by component colorimeter a* (-12.66), as well as by the values obtained in the component L* and b*, which represents light colors of the spectrum (TABLE 3). The maintenance of these colors in leaves dried with microwave oven are related to their mechanism. The water that is internally stored in the leaf is pushed through the surface, which is then converted to steam in a fast process reaction that maintains the overall temperature of the system, without overheat the surface which contributes to the color maintenance (OZKAN; AKBUDAK; AKBUDAK, 2007; CENI et al., 2009). As a comparison, freeze-drying method exhibited the most-resemblance colors of the raw material, but with a whitish green coloration due to the higher value of lighter component of L* (TABLE 3).

Traditional drying methods resulted in darker green colors, observed by increasing a* component and a decrease in b*. In the oven dryers, darkening of the raw material was perceived as the temperature increases from 40 °C to 80 °C; therefore, colorimetric resulted in component values of L* and a* increase for dark and red spectrum respectively, and b* decrease for blue spectrum (TABLE 3). Several mechanisms may be involved in color change, to name a few: the conversion of chlorophylls to pheophytins, due to the magnesium of the chlorophyll molecule replaced by hydrogen (RUDRA et al., 2008); enzymatic process

(CENI et al., 2008) and non-enzymatic process known as Maillard reaction (GUPTA et al., 2018).

Even though there are interesting characteristics within each drying method, it is in best interest for the beverage industry to obtain a dried leaf color that is not modified by the drying process, without the need to add another chemical for the beverage to look acceptable for the consumer. Therefore, the usage of a microwave oven for drying can also be used to maintain the color from fresh leaves (SOYSAL, 2004; ŚLEDŹ et al., 2013; GUO et al., 2017).

2.3.4 Nutrition characteristics

To understand the effect of the various drying processes on yerba mate leaves, we studied moisture and nutritional characteristics on the leaves of each process (TABLE 4).

Moisture content of dried leaves from different drying techniques exhibited the highest moisture with oven dryer at 40°C (7.52 %), followed by traditional drying method, roasting (5.78), and microwave oven (5.71 %). The treatment that showed the lowest moisture content was freeze-drying (2.44 %), and this can be inferred to the effective water removal that occurs during sublimation, as compared to evaporation that occurs in all the other drying methods (LIN et al., 2012). Furthermore, the moisture content in all drying methods is within the standard of commercial samples of yerba mate, not exceeding 6 % (BRAGHINI et al., 2014). A low moisture is important to reduce microbial growth and degradation.

The lipid content and compounds soluble in ethyl ether (pigments) of yerba mate leaves varied depending on the drying method ($p \leq 0.01$) from 4.30 % to 6.55 %, whereas the lowest content was obtained with traditional process. This reduction in lipid content may be a result of enzymatic oxidative reactions (lipoxygenases) and non-enzymatic processes such as auto-oxidation and photo-oxidation (BARRIUSO; ASTIASARÁN; ANSORENA, 2013) which is also in line with colorimetric results and in agreement with other works for this species (4.25 – 4.9 %) (BERTÉ et al., 2011; SOUZA et al., 2015).

The protein content remained unchanged in leaf samples dried using the various methods (mean 16.70 %), suggesting that it is unaffected by drying temperatures used herein. This average value is within the range mentioned in others works (7.97-26.1 %) (BERTÉ et al., 2011; SOUZA et al., 2015). Other important point is the fact of the yerba mate can be a potential source of vegetable protein and, according Ortolá et al. (2019), the increasing dietary intake of vegetable protein may delay unhealthy aging when replacing carbohydrates, fats, or animal protein, especially from meat and dairy.

The dietary fibre content was affected ($p \leq 0.01$) and the lowest content (47.99 %) occurred in traditional process (roasting + conveyor dryer) (TABLE 4). It is important to mention that in this work, we analysed the total dietary fibre content - composed by soluble and insoluble fraction (BERNAUD; RODRIGUES, 2013) - and it is possible that some key characteristics of this process (roasting by 3 min to 400°C and drying by 4 hours to 100°C) may have weakened some fibres linkage. Nonetheless, the obtained value for dietary fibre is within the range obtained by another work using similar drying processes (52.97 %) (BERTÉ et al., 2011).

The inorganic residue (ash content) did not modified in leaf samples dried in the majority of the processes. Only leaves dried using traditional method showed reduction in the ash content ($p \leq 0.01$); and the ash contents found in this work were similar to the ones reported before, between 5 and 7 % (ESMELINDRO et al., 2002; BRAGHINI et al., 2014; SOUZA et al., 2015).

TABLE 4 - MOISTURE CONTENT AND NUTRIENTS OF YERBA MATE LEAF SAMPLES DRIED BY THE STUDIED PROCESSES

Drying process	% Moisture	% dry basis (g.100g^{-1})			
		Lipid	Protein	Dietary fiber	Total ash
Roasting + rotary dryer	$5.78^{B} \pm 0.32$	$4.70^{CD} \pm 0.48$	$16.22^{A} \pm 0.14$	$53.09^{D} \pm 0.42$	$6.32^{A} \pm 0.11$
Roasting + conveyor dryer	$6.10^{AB} \pm 0.13$	$4.30^{D} \pm 0.26$	$16.38^{A} \pm 0.19$	$47.99^{E} \pm 0.34$	$5.85^{B} \pm 0.05$
Microwave oven	$5.71^{B} \pm 0.11$	$6.11^{AB} \pm 0.25$	$16.45^{A} \pm 0.08$	$52.32^{D} \pm 0.33$	$6.40^{A} \pm 0.07$
Oven dryer 40°C	$7.52^{A} \pm 0.07$	$5.75^{ABC} \pm 0.02$	$17.21^{A} \pm 0.63$	$59.47^{C} \pm 0.58$	$6.44^{A} \pm 0.04$
Oven dryer 60°C	$3.12^{CD} \pm 0.14$	$6.55^{A} \pm 0.16$	$16.83^{A} \pm 0.05$	$69.31^{A} \pm 0.42$	$6.29^{A} \pm 0.02$
Oven dryer 80°C	$3.97^{C} \pm 0.22$	$6.34^{AB} \pm 0.10$	$16.39^{A} \pm 0.26$	$65.74^{B} \pm 0.22$	$6.23^{A} \pm 0.04$
Freeze-drying	$2.44^{D} \pm 0.31$	$5.13^{BCD} \pm 0.36$	$15.59^{A} \pm 0.56$	$57.62^{C} \pm 0.94$	$6.33^{A} \pm 0.08$

SOURCE: The author (2019).
NOTE: Values are reported as mean ± stantard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.01$).

2.4 CONCLUSIONS

Based on the results obtained in this research, we conclude that yerba mate leaves dried in the microwave is a technically viable processes in maintaining the phytochemicals content with antioxidant and stimulating capacity for the various industrial sectors (e.g. food, beverage, cosmetic industry).

The nutritional analyses showed good results for all drying methodologies; however, in nutraceutical terms, the products generated may need higher content of nutrients and phytochemicals as observed in raw material dried in a microwave oven. In addition, the green color was maintained with the usage of these processes.

REFERENCES

AOAC. Official Methods of analysis. **The Association of Official Analytical Chemists International**, v. 38, n. 8, p. 431, 2016.

ASHIHARA, H.; SANO, H.; CROZIER, A. Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. **Phytochemistry**, v. 69, n. 4, p. 841–856, 2008.

BARRIUSO, B.; ASTIASARÁN, I.; ANSORENA, D. A review of analytical methods measuring lipid oxidation status in foods: a challenging task. **European Food Research and Technology**, v. 236, n. 1, p. 1–15, 2013.

BECKER, A. M. et al. Spray-dried yerba mate extract capsules: clinical evaluation and antioxidant potential in healthy individuals. **Plant Foods for Human Nutrition**, v. 74, n. 4, p. 495–500, 2019.

BERNAUD, F. S. R.; RODRIGUES, T. C. Fibra alimentar - ingestão adequada e efeitos sobre a saúde do metabolismo. **Arquivos Brasileiros de Endocrinologia e Metabologia**, v. 57, n. 6, p. 397–405, 2013.

BERTÉ, K. A. S. et al. Chemical composition and antioxidant activity of yerba-mate (*Ilex paraguariensis* A . St . -Hil ., Aquifoliaceae) extract as obtained by spray drying. **Journal of Agricultural and Food Chemistry**, v. 59, p. 5523–5527, 2011.

BRACESCO, N. *Ilex Paraguariensis* as a healthy food supplement for the future world. **Biomedical Journal of Scientific & Technical Research**, v. 16, n. 1, p. 15–18, 2019.

BRAGHINI, F. et al. Physico-chemical composition of mate , before and after simulation of mate. **Pesquisa Agropecuária Gaúcha**, v. 20, p. 7–15, 2014.

BRAND-WILLIANS, W. CUVELLIER, M. E. BERSET. C. Use of a free radical method to evaluate antioxidant activity. **Food Science and Technology**, v. 28, p. 25–30, 1995.

BUTIUK, A. P. et al. Study of the chlorogenic acid content in yerba mate (*Ilex paraguariensis* St. Hil.): effect of plant fraction, processing step and harvesting season. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 3, n. 1, p. 27–33, 2016.

CARDOZO JUNIOR, E. L.; 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**, v. 21, p. 440–454, 2016.

CENI, G. C. et al. Oxidases from mate tea leaves (*Ilex paraguariensis*): Extraction optimization and stability at low and high temperatures. **Bioprocess and Biosystems Engineering**, v. 31, n. 6, p. 541–550, 2008.

CENI, G. C. et al. Influence of application of microwave energy on quality parameters of mate tea leaves (*Ilex paraguariensis* St. Hil.). **Food Technology and Biotechnology**, v. 47, n. 2, p. 221–226, 2009.

CHEN, Y.; MARTYNENKO, A. Combination of hydrothermodynamic (HTD) processing and different drying methods for natural blueberry leather. **LWT - Food Science and Technology**, v. 87, p. 470–477, 2018.

CIEMNIAK, A. et al. Assessing the contamination levels of dried teas and their infusions by polycyclic aromatic hydrocarbons (PAHs). **Journal fur Verbraucherschutz und Lebensmittelsicherheit**, v. 14, n. 3, p. 263–274, 2019.

CITTADINI, M. C. et al. Neuroprotective Effect of *Ilex Paraguariensis* Intake on Brain Myelin of Lung Adenocarcinoma-Bearing Male Balb/c Mice. **Nutrition and Cancer**, v. 71, n. 4, p. 629–633, 2019.

COLPO, A. C. et al. 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**, v. 209, p. 185–195, 2016.

DE MEJÍA, E. G. et al. Yerba mate tea (*Ilex paraguariensis*): phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. **Journal of Functional Foods**, v. 2, n. 1, p. 23–34, 2010.

ESMELINDRO, M. C. et al. Caracterização físico-química da erva-mate: influência das etapas do processamento industrial. **Ciência e Tecnologia de Alimentos**, v. 22, n. 2, p. 193–204, 2002.

GERHARDT, M. Colonos ervateiros: história ambiental e imigração no Rio Grande do Sul. **Revista Esboços**, v. 18, n. 25, p. 73–95, 2011.

GUPTA, R. K. et al. Maillard reaction in food allergy: Pros and cons. **Critical Reviews in Food Science and Nutrition**, v. 58, n. 2, p. 208–226, 2018.

GUO, Q. et al. Microwave processing techniques and their recent applications in the food industry. **Trends and Food Science & Technology**. v. 67, p. 236-247, 2017.

HECK, C. I.; DE MEJIA, E. G. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**, v. 72, n. 9, 2007.

HOSSAIN, M.; BARRY-RYAN, C.; MARTIN-DIANA, A. B. Effect of drying method on the antioxidant capacity of six larniaceae herbs. **Food Chemistry**, v. 123, n. 1, p. 85–91, 2010.

ISOLABELLA, S. et al. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. **Food Chemistry**, v. 122, n. 3, p. 695–699, 2010.

LIN, L. et al. Thermal inactivation kinetics of *Rabdosia serra* (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. **Food Chemistry**, v. 134, n. 4, p. 2021–2029, 2012.

MATEOS, R. et al. Improved LC-MS characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry**, v. 241, p. 232–241, 2018

MAYER, A. M. Polyphenol oxidases in plants and fungi: going places? A review. **Phytochemistry**, v. 67, n. 21, p. 2318–2331, 2006.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, p. 522–530, 2017.

MEINHART, A. D. et al. Methylxanthines in 100 Brazilian herbs and infusions: determination and consumption. **Emirates Journal of Food and Agriculture**, v. 31, n. 2, p. 125–133, 2019.

ORTOLÁ, R. et al. Changes in dietary intake of animal and vegetable protein and unhealthy aging. **The American Journal of Medicine**, v.133, n. 2, p. 231-239, 2019.

OZKAN, I. A.; AKBUDAK, B.; AKBUDAK, N. Microwave drying characteristics of spinach. **Journal of Food Engineering**, v. 78, n. 2, p. 577–583, 2007.

PASSARDI, R. L. et al. Drying of *Ilex paraguariensis* Saint Hilaire by microwave radiation. **Drying Technology**, v. 24, n. 11, p. 1437–1442, 2006.

PERONE, C. A. S.; CAPOBIANCO, M. PETROLINI; PAPARELI, J. S. Determination of polyphenols (tanines) in foods products (teas) using a biosensor of polyphenol oxidase, obtained of crude extract of banana nanica peel (*Musa acuminata*) and characterization this biosensor. **Revista do Instituto de Ciência da Saúde**, v. 27, n. 1, p. 28–34, 2009.

R CORE TEAM. A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. 2016. ISBN 3-900051-07-0. Available in: <http: //www.R-project.org>.

RAJA, K. S. et al. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. **Journal of the Saudi Society of Agricultural Sciences**, p. 1–7, 2017.

RE, R. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9–10, p. 1231–1237, 1999.

RICHARD-FORGET, F. C.; GAUILLARD, F. A. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. **Journal of Agricultural and Food Chemistry**, v. 45, n. 7, p. 2472–2476, 1997.

RUDRA, S. G. et al. Enthalpy entropy compensation during thermal degradation of chlorophyll in mint and coriander puree. **Journal of Food Engineering**, v. 86, n. 3, p. 379–387, 2008.

SILVA, F. DE A. S. E; AZEVEDO, C. A. V. DE. The Assistat Software Version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, n. 39, p. 3733–3740, 2016.

SINGLETON, V.; ROSSI, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v. 16, p. 144-158, 1965.

ŚLEDŹ, M. et al. Selected chemical and physico-chemical properties of microwave-convective dried herbs. **Food and Bioproducts Processing**, v. 91, n. 4, p. 421–428, 2013.

SOUZA, A. H. P. et al. Phytochemicals and bioactive properties of *Ilex paraguariensis*: An in-vitro comparative study between the whole plant, leaves and stems. **Food Research International**, v. 78, p. 286–294, 2015.

SOYSAL, Y. Microwave drying characteristics of parsley. **Biosystems Engineering**, v. 89, n. 2, p. 167–173, 2004.

THEA, A. E. et al. Polycyclic aromatic hydrocarbons (PAHs) in yerba maté (*Ilex paraguariensis* St. Hil) traditional infusions (mate and tereré). **Food Control**, v. 60, p. 215–220, 2016.

VAN SUMERE, C. et al. Freeze-drying and analysis of plant and other biological material. **Analytical Biochemistry**, v. 131, n. 2, p. 530–532, 1983.

VIEIRA, M. A. et al. Occurrence of polycyclic aromatic hydrocarbons throughout the processing stages of erva-mate (*Ilex paraguariensis*). **Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment**, v. 27, n. 6, p. 776–782, 2010.

WOJDYŁO, A. et al. Effect of convective and vacuum-microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. **Food and Bioprocess Technology**, v. 7, n. 3, p. 829–841, 2014.

WU, L. et al. Effects of processing on phytochemical profiles and biological activities for production of sorghum tea. **Food Research International**, v. 53, n. 2, p. 678–685, 2013.

XIONG, Y. et al. Effect of processing on the phenolic contents, antioxidant activity and volatile compounds of sorghum grain tea. **Journal of Cereal Science**, v. 85, p. 6–14, 2019.

YIM, H. S. et al. Optimization of extraction time and temperature on antioxidant activity of Schizophyllum commune aqueous extract using response surface methodology. **Journal of Food Science and Technology**, v. 50, n. 2, p. 275–283, 2013.

ZORIĆ, Z. et al. Kinetics of the degradation of anthocyanins, phenolic acids and flavonols during heat treatments of freeze-dried sour cherry Marasca paste. **Food Technology and Biotechnology**, v. 52, n. 1, p. 101–108, 2014.

CHAPTER 2

3 YERBA MATE DRYING METHODS BEFORE TOASTING: BIOACTIVE COMPOUNDS, ANTIOXIDANT CAPACITY AND ACCEPTANCE OF MATE TEA

ABSTRACT

New drying alternatives for yerba mate leaves before toasting are necessary for the mate tea industry in order to reduce the time of processing and increase raw material quality. However, their effects in the acceptance by consumers and quantification of bioactive compounds are still unknown. Therefore, we tested seven drying methods for yerba mate leaves including traditional process and new alternatives: roasting + drying (rotary dryer); roasting + drying (conveyor dryer); microwave oven; oven dryer at 40, 60, 80 °C, and freeze drying. After drying, leaves were toasted, and teas were prepared. The acceptance of hot and iced tea was evaluated by consumers, and the bioactive compounds and antioxidant capacity was quantified. The iced and hot teas, elaborated with toasted mate dried in microwave oven, resulted in similar sensory acceptance compared to teas produced with traditional drying methods according to the Internal Preference Mapping. The toasted mate dried in freeze-drying had differential grouping for hot and iced teas. If aimed at specific consumer groups, segmentation can provide many yerba mate leaves drying options. However, we noticed that drying in oven is not recommended due to the low bioactive compounds content after these processes. Total phenolic compounds decreased with increasing temperature from oven dryers at 40, 60, and 80 °C, representing an average decrease of 66 % and lower antioxidant capacity, when compared to others. The contents of methylxanthines (caffeine and theobromine) were lower in traditional drying methods if compared to other methods, representing 39 % of the reduction. Therefore, the use of microwave oven for yerba-mate leaves drying is considered as a good alternative.

Key words: *Ilex paraguariensis*. Caffeine. Chlorogenic acid. Sensory analysis.

3.1 INTRODUCTION

Dried leaves and fine branches of the endemic South American plant commonly named as yerba mate or maté (*Ilex paraguariensis* A.St.-Hil) are used for preparing non-alcoholic beverages known as chimarrão and tererê. These beverages are consumed in Argentina, Paraguay, Uruguay, and Brazil (GRIGIONI et al., 2004; MACHADO et al., 2007). More recently, after its drying and toasting known as mate tea (DE GODOY et al., 2013) it has been consumed in other countries, including the United States, Germany, and Syria (CARDOZO JUNIOR, MORAND, 2016). The global market for tea is expected to grow by $7.9 billion between 2017 and 2022, with an average rate of 4 percent annually (higher than either coffee or other hot drinks) (BOLTON, 2019). Following the global trends, mate tea consumption in Brazil is also growing, helping the yerba mate production.

The reason for this growing market is due to the presence of beneficial bioactive compounds, such as phenolic and methylxanthines that are antioxidant, anti-inflammatory, antiatherogenic, anticarcinogenic, antiobesity, anti-glycogen, diuretic, and have energetic properties (HECK e DE MEJIA 2007; CARDOZO JUNIOR e MORAND 2016). These compounds may have their contents altered during the drying and toasting processes, result from degradation as well as new compounds formation, interfering directly in flavour and consumer acceptance of the final product (ZAIONS et al., 2014; CORSO; VIGNOLI; BENASSI, 2016; HIHAT; REMINI; MADANI, 2017; DIBANDA et al., 2020).

The postharvest processing of yerba mate consists of traditional drying methods, like pre-drying: roasting (“sapeco”), drying in rotary dryer or conveyor dryer, and milling (ISOLABELLA et al., 2010). However, these equipments are quite heavy, expensive, and require large spaces for allocation. They also operate in conditions to produce traditional drinks like “chimarrão and tererê” which demands specific process in order to acquire characteristic aroma and flavour. Thus, other drying methods need to be studied for different industrial segments such as tea.

Recent works have shown the advantages of using microwave oven to dry raw materials to food industry; it is efficient at maintaining bioactive compounds because of the fast heating that occurs within microwave technology; it is also very efficient at energy and easy to install (LIN; BREWER, 2005; DIBANDA et al., 2020). Studies comparing microwave technology with oven dryers are also the focus of researchers with the purpose of accomplishing not only an efficient process, but also, the overall quality after drying (HIHAT; REMINI; MADANI, 2017). Freeze-drying is another technology and, although the long processing time and an expensive drying method, it is preferred due its high final quality (BHATTA; JANEZIC; RATTI, 2020). Different drying methods have been studied for yerba-mate leaves, not only to obtain a better quality of the final product but also to the overall sensory aspect, and there are few information about the phytochemical effect on the plant after these processes.

Sensory analysis is seen as one of the most important characteristics in the final market- acceptance (YANG; LEE, 2019) thus, for development of new products are necessary to study efficient alternatives for drying the raw material. Nowadays, sensory studies with yerba mate teas, associated or not with phytochemical aspects, have been developed using oxidative processes (VALDUGA et al., 2016), spray dryer of mate extract (VALDUGA; BATTESTIN; FINZER, 2003), variation of percentage of sticks/leaves in tea composition (DE GODOY et al., 2013), with commercial yerba-mate teas (BARBOZA; CAZAL, 2018) and temperature of consumption with different sweeteners (CARDOSO; BATTOCHIO; CARDELLO, 2004). However, from our knowledge, no reports have been performed on teas acceptance and phytochemistry analysis of yerba mate toasted derived from various drying processes and compared with the traditional process.

Among the sensory attributes of yerba mate, taste and smell are critical factors that determines consumer preference and brand selection (DE GODOY et al., 2013). This area of research is widely supported by the food industry owing to the close relationship between product sensory characteristics and market success (ARES; JAEGER, 2013; GRIGIONI et al., 2004). Therefore, this study aimed to analyse the acceptance of hot and iced toasted mate tea and the content of bioactive compounds and antioxidant capacity of aqueous extract produced by raw material obtained from different drying processes.

3. 2 MATERIAL AND METHODS

3.2.1 Drying methods and toasted processes

The total of 15 kg of yerba mate leaves were used for each drying treatment (three repetitions with 5 kg for all treatments). Seven drying methods were applied to the leaves: (ROT) roasting for 3 min at 400 °C and drying in a rotary dryer for 30 min at 150 – 180 °C; (CON) roasting for 3 min at 400 °C and drying with conveyor dryer for 4h at 100 °C; (MIC) microwave oven for 5 min (1000 W, 2450 MHz); (D40) oven dryer at 40 °C; (D60) oven dryer at 60 °C; (D80) oven dryer at 80 °C; (FRE) freeze-drying for 72h at -40 °C and 200 mbar. After drying processes, leaves were milled (classified according to granulometry mesh 3.5) and homemade toasted at 110 °C for 10 min.

3.2.2 Mate tea formulation

For hot toasted tea, 16 g of toasted mate were added into 1 L of boiling water for 3 minutes. Afterwards, it was filtered and the volume was adjusted with hot water to reach 1 L.

For iced toasted tea, the only difference was the addition of 50 g of crystal sugar and 10 mL of Tahiti lime in this 1 L solution and further refrigeration at 6° C for 12 hours.

3.2.3 Consumer acceptance of yerba mate tea beverages

The Research Ethic Committee (REC) from the Pequeno Príncipe University, certified by the Process nº 17644719.2.0000.5580, approved the sensory analysis performed in the present work.

The research was conducted with one hundred consumers (men and women untrained tea consumers). We divided the sensory tests into two distinct sessions: hot and iced tea. On the first one, the consumers evaluated seven samples containing approximately 30 mL of hot mate tea at 60 °C; on the second session the consumers evaluated seven samples containing approximately 30 mL iced mate tea at 6 °C. For hot tea, we provided sugar and artificial sweeteners for the already sweetened mate, and the consumers were instructed to add the same amount of sugar, or sweeteners, in all samples, if they preferred it. The order of samples followed a completely balanced design (MACFIE; BRATCHELL, 1989) to avoid carry-over effects. Consumers ate crackers and drank mineral water to act as a palate cleanser in between samples (MCGOWAN; LEE, 2006).

Consumers rated each tea for overall degree of likeness using a 9-point structured hedonic scale ranging from (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly, 5 = neither dislike nor like, 6 = like slightly; 7 = like moderately; 8 = like very much, 9 = like extremely), according to ABNT (NBR 14141, 1998).

3.2.4 Aqueous extraction of toasted mate

The toasted mates, previously dried in each drying method, were processed in a coffee grinder (Cadence®) (classified as fine grinding – sieve 0.25 mm), after that, 10 mg of sample were weighted and diluted with 2 mL of MilliQ water. The material was agitated in vortex for 30 s and the extraction was carried out in Thermomixer Eppendorf® equipment at 450 rpm for 1 h at 60 ºC. The aqueous extracts were then filtered through a 0.22 µm filter which were used to determine the antioxidant capacity (DPPH and ABTS radicals); total phenolic compounds; and methylxanthines, caffeoylquinic acids (measured by HPLC-UV)

3.2.5 Content of total phenolic compounds

We determined the content of total phenolic compounds by the spectrophotometric method of Folin-Ciocalteau (SINGLETON; ROSSI, 1965) with minor modifications. In a volumetric flask, we added 0.1 mL of the extract, 6.0 mL of distilled water and 0.5 mL of the Folin-Ciocalteau reagent, followed by 1 min of stirring. Afterwards, 2 mL of 15 % aqueous Na_2CO_3 in water (w/v) solution was added, followed by stirring for 30 s. The final volume was adjusted with distilled water to reach 10 mL. After 2 h of reaction in the dark, the absorbance was measured at 760 nm in a Shimadzu®-1800 UV/VIS spectrophotometer. As reference, we established an analytical curve with gallic acid within the range of 0.25-13 $mg.L^{-1}$. The results were expressed in mg gallic acid equivalent (GAE) per gram of sample ($mg\ GAE.g^{-1}$) on a dry basis.

3.2.6 Antioxidant capacity (DPPH and ABTS radicals)

All antioxidant activities assays were performed in a spectrophotometer UV/VIS (Shimadzu, model 1800, Kioto, JPN). The antioxidant capacity of toasted mate extracts by free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined according to Brand-Willians et al. (1995) methodology with minor modifications. Firstly, we added 0.1 mL of sample to 3.9 mL of DPPH methanolic solution (0.06 $mmol.L^{-1}$), and kept it under dark for 30 min (reaction) and then the absorbance was measured at 515 nm. The results were compared with a Trolox standard curve (0-1000 $\mu mol.L^{-1}$) and expressed in µmol Trolox equivalent per gram of sample (TEAC $\mu mol.g^{-1}$) on dry basis.

The antioxidant capacity of extracts by free radicals ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was determined by reacting 10 mL ABTS (7 $mmol.L^{-1}$) with 176 µL potassium persulfate (140 $mmol.L^{-1}$) in the dark at room temperature for 16 h. An aliquot of 1 mL of this solution was added in 100 mL of sodium acetate buffer (20 $mmol.L^{-1}$) pH 4.5. The absorbance was adjusted to 0.7 ± 0.05 and 3 mL was added to 30 µL of the extract. After 2 h, the absorbance was determined (RE et al., 1999; YIM et al., 2013) with minor modifications. The results were compared with a Trolox standard curve (0–2500 $\mu mol.L^{-1}$) and expressed in µmol Trolox equivalent per gram of sample (TEAC $\mu mol.g^{-1}$) on dry basis.

3.2.7 Methyxanthines and caffeoylquinic acids by high performance liquid chromatography - HPLC-UV

For the chromatographic analyses, we used a Shimadzu® liquid chromatograph (UFLC), controlled by the LC solution software and equipped with automatic injector and UV detector (SPD-20A). Separation of compounds was performed using Shim-Pack CLC-ODS (M) C18 column (250 x 4.6 mm i.d., 5 μm particle size), protected by Shim-Pack CLC G-ODS pre-column (100 x 4.0 mm id) both from Shimadzu (Kyoto, Japan). The separation of compounds in the aqueous extract (20 μL of injection) was conducted at 30 °C using a flow of 0.5 mL.min^{-1}. The mobile phases consisted of a gradient elution of water with acetic acid Dinâmica® (99.9:0.1, v/v) (solvent A) and acetonitrile Merck® 100 % (solvent B). Compound detection was performed at the fixed wavelength of 280 nm. The gradient elution program was: 0-15 min (3-3 % B), 15-20 min (3-20 % B), 20-40min (20-20 % B), 40-45min (20-30 % B), 45-55 min (30-100 % B), 55-75 (100-100 % B), 75-80 (100-3 % B) and 80-95 (3-3 % B).

The identification and quantification of methylxanthines: 1,3,7-trimethylxanthine (caffeine) and 3,7-dimethylxanthine (theobromine) was performed by an analytical curve of caffeine and theobromine Sigma® standards at range of 0 to 1.0 mg.mL^{-1} and 0 to 0,5 mg.mL^{-1} respectively. The identification of caffeoylquinic acids (3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA) by Sigma® standards and semi-quantification was performed by an analytical curve at range of 0 to 10 mg.mL^{-1} of the 3-CQA Sigma®. Results were expressed as mg of compounds per g sample (mg.g^{-1}) on a dry basis.

3.2.8 Statistical analysis

For the analysis of consumer acceptance, we conducted data analysis using the Internal Preference Mapping (IPM) with principal component analysis (PCA). From the data of PCA, a Hierarchical Cluster Analysis (HCA) (Mcfie, 2007) was used to identify groups of consumers with different patterns of preference for each study (MÜLLER; HAMM, 2014). This analysis is based on standardized liking scores, using Euclidean distances and Ward's methodology for the agglomeration criterion (VARELA; BELTRÁN; FISZMAN, 2014). For each segment generated, we applied Tukey test at 5 % probability. The statistical software used was XLSTAT-Sensory (2014).

Bioactive compounds and antioxidant capacity data did not follow the assumptions for analysis of variance (normality and homogeneity of variances), thus we used the GLM (generalized linear models) with gamma distribution and means were compared with Tukey contrasts at 5 % probability. We used the statistical R software (R CORE TEAM, 2016) to perform the analysis.

3.3 RESULTS AND DISCUSSION

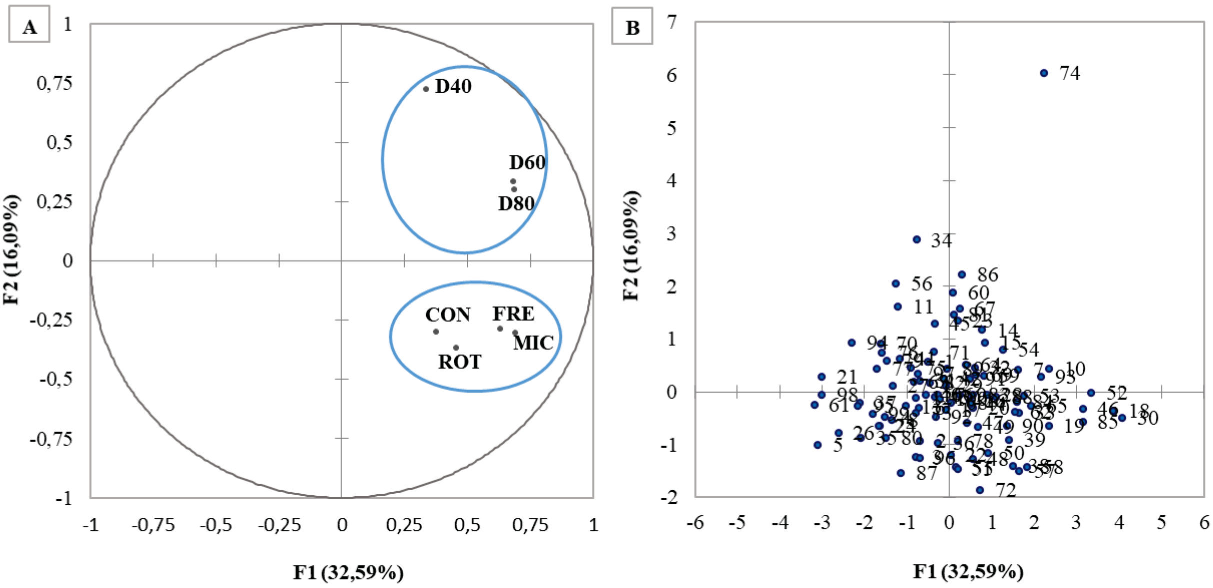
3.3.1 Internal preference mapping (IPM)

The first step in many postharvest operations is the removal of water from raw material (drying). However, this process can promote changes in appearance (color), aroma and taste of the subproduct - tea, which modifies the final quality (LEE; CHAMBERS, 2010; ROCHA; MELO; RADÜNZ, 2011).

For hot mate tea, the IPM represented by PCA graphic exhibited that the first two dimensions accounted for 48.68 % of the total variance, 32.59 % for the first component and 16.09 % for the second one, regarding their acceptance on mate tea pre-processed at different methods (FIGURE 1 – A and B). The major contribution in F1 and F2 of different drying processes are showed in the Table 1 with respective cosine square values. We did not use the third component in this study, as it would represent an increase of 14 % of data explanation (Table 1).

Due to the spatial separation of mate teas, it was possible to identify two groups with variation on their levels of acceptance (FIGURE 1-A) with their respective local point for consumers that contributed to the group (FIGURE 1-B). In the upper right quadrant, is the group formed with mate tea dried with dryers at 40, 60 and 80 ºC, while the second group, in the lower right quadrant, composes of mate teas dried with roasting + conveyor dryer, roasting + rotary dryer (both traditional processes), microwave oven and freeze drying. The lowest acceptance average (4.87 - slightly disliked) was for tea produced with leaves dried in an oven at 40 °C, representing an average rejection rate of 16.27 %.

FIGURE 1 - INTERNAL PREFERENCE MAPPING (IPM) FOR HOT MATE TEA



SOURCE: The author (2020).
SUBTITLE: (A) the position of mate tea processed with yerba mate dried in different conditions in relation to consumer acceptance and (B) consumers position in the space defined by the one and the second dimension. D40, D60 and D80 (over dryer at 40 °C, 60 °C and 80 °C respectively); CON (roasting + conveyor dryer); ROT (roasting + rotary dryer); FRE (freeze drying) and MIC (microwave oven).

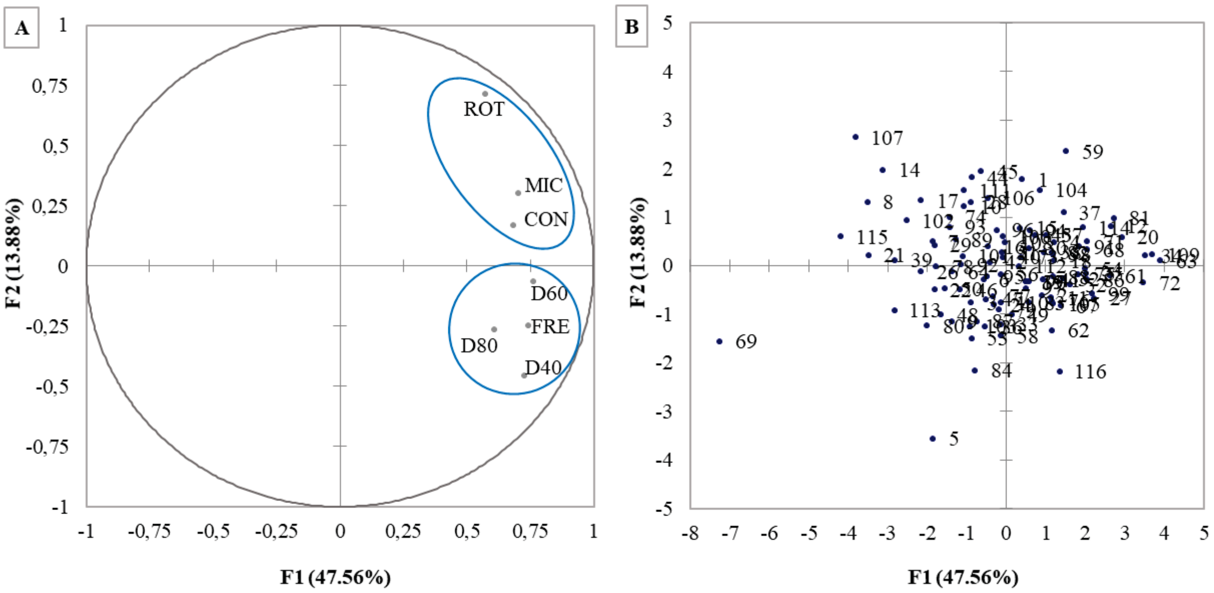
TABLE 1 – - PRINCIPAL COMPONENTS OBTAINED FROM THE HOT MATE TEA OF THE DRYING PROCESSES

Drying processes	Main component						
	F1	F2	F3	F4	F5	F6	F7
CON	0.142	0.091	**0.405**	0.299	0.002	0.058	0.004
ROT	0.208	0.136	**0.370**	0.184	0.008	0.000	0.093
MIC	**0.478**	0.094	0.004	0.051	0.199	0.046	0.127
FRE	0.401	0.083	0.006	0.030	**0.458**	0.000	0.022
D40	0.114	**0.522**	0.061	0.211	0.004	0.001	0.086
D60	**0.467**	0.111	0.140	0.004	0.000	0.161	0.117
D80	**0.471**	0.089	0.004	0.130	0.019	0.270	0.018
Eigenvalue	**2.281**	1.126	0.989	0.909	0.690	0.537	0.467
Variance (%)	**32.587**	16.092	14.129	12.991	9.858	7.667	6.675
Cumulative variance (%)	**32.587**	48.679	62.808	75.799	85.658	93.325	100.000

SOURCE: The author (2020).
SUBTITLE: CON (roasting + conveyor dryer); ROT (roasting + rotary dryer); MIC (microwave oven); FRE (freeze drying); D40, D60 and D80 (over dryer at 40 °C, 60 °C and 80 °C respectively).
NOTE: Values in bold correspond for each variable to the factor for which the square cosine is the largest.

In the case of iced mate tea, IMP represented by PCA exhibited the first two dimensions accounted for 61.44 % of the total variance, 47.569 % for the first component, and 13.88 % for the second (Figure 2-A and B). The major contribution in F1 and F2 of the different drying processes are showed in Table 2 with respective cosine square values. The addition of the third component would represent only 11.895 % of data explanation, so it was not used.

FIGURE 2 - INTERNAL PREFERENCE MAPPING (IPM) FOR ICED MATE TEA



SOURCE: The author (2020).

SUBTITLE: (A) the position of iced tea processed with yerba mate dried in different conditions in relation to consumer acceptance and (B) consumers position in the space defined by the one and the second dimension. D40, D60 and D80 (over dryer at 40 °C, 60 °C and 80 °C respectively); CON (roasting + conveyor dryer); ROT (roasting + rotary dryer); FRE (freeze drying) and MIC (microwave oven).

TABLE 2 – - PRINCIPAL COMPONENTS OBTAINED FROM THE ICED MATE THE OF THE DRYING PROCESSES

Drying processes	Main component						
	F1	F2	F3	F4	F5	F6	F7
CON	**0.469**	0.028	0.291	0.014	0.004	0.180	0.016
ROT	0.328	**0.506**	0.006	0.062	0.014	0.019	0.066
MIC	**0.496**	0.090	0.057	0.270	0.030	0.000	0.057
FRE	**0.553**	0.062	0.098	0.042	0.009	0.194	0.043
D40	**0.531**	0.210	0.013	0.051	0.059	0.005	0.130
D60	**0.580**	0.004	0.023	0.010	0.370	0.005	0.007
D80	**0.373**	0.071	0.345	0.142	0.013	0.043	0.012
Eigenvalue	**3.329**	0.972	0.833	0.591	0.498	0.445	0.332
Variance (%)	**47.564**	13.880	11.895	8.444	7.113	6.362	4.742
Cumulative variance (%)	**47.564**	61.444	73.339	81.783	88.896	95.258	100.000

SOURCE: The author (2020).

SUBTITLE: CON (roasting + conveyor dryer); ROT (roasting + rotary dryer); MIC (microwave oven); FRE (freeze drying); D40, D60 and D80 (over dryer at 40 °C, 60 °C and 80 °C respectively).

NOTE: Values in bold correspond for each variable to the factor for which the square cosine is the largest.

Consumer acceptance from iced tea IPM were represented into two groups (FIGURE 2-A); where in the upper right quadrant are samples dried in roasting + rotary, microwave oven and roasting + conveyor dryer; the lower right quadrant is the biggest group, from samples of iced tea with leaves dried using oven dryer and freeze drying. In Figure 2-B is the consumer distribution that contributed to each specific group condition. The lowest acceptance average (5.4 - neither like nor dislike) was observed in teas produced with leaves dried in oven at 40 °C and freeze drying, representing an average rejection rate of 10.61 %.

According to Teixeira (2009), taste is one of the most complex sensorial attributes and it can be affected by drink temperature. In this research, we infer that the acceptance of teas was influenced by the drying process and type of tea (hot and iced) because the internal preference map was explained 48 % for hot tea and 62 % for iced tea (Figures 1 and 2) with different contribution values in each components (Tables 1 and 2).

Specifically related to the process, dryers and traditional drying methods remove water by evaporation through hot air. The differences between the products generated will be a function of time, temperature and air speed drying (UDOMKUN et al., 2015; FIGIEL; MICHALSKA, 2017). Contrarily, freeze drying is a process in which water is removed from a product after it is frozen and placed under vacuum, allowing the iced to change directly from solid to vapour without going through a liquid phase (sublimation); however, volatile compounds could be removed by vacuum (NIREESHA et al., 2013). For microwave oven process, the drying occurs by microwave energy, and most of the moisture is instantly vaporized before leaving the material (PARIT; PRABHU, 2017); also, microwave energy can increase the availability of phenolics - binding of the plant matrix - increasing its antioxidant activity (HIHAT; REMINI; MADANI, 2017; DIBANDA et al., 2020).

The specific characteristics of each drying processes influenced the final product phytochemistry characteristics and consequently, varied its sensory characteristics and acceptance as observed in this work. This have been reported before for green tea, in which the process significantly affected the acceptance of the product, due to the fact that it altered the aroma and taste (LEE; CHAMBERS, 2010).

In general, it is noticed that regardless of the type of tea (hot or iced), mate teas from rotary drying, conveyor dryer and microwave oven are allocated into the same group by acceptance analysis as well as products dried in over dryers at 40, 60, and 80 °C. Therefore, it is possible to consider microwave oven drying as a new alternative method, providing mate tea products with similar acceptance as the products obtained from traditional yerba mate drying processes.

3.3.2 Consumer segmentation

Consumers may present variate preference patterns for some products due to their different hedonic responses, forming groups with shared hedonic patterns. This is known as consumer segmentation, and for some foods, considerable variations in taste, intensity of flavour or sensory profile can lead to a consumer segmentation (VARELA; BELTRÁN; FISZMAN, 2014).

The segmentation is very useful to identify new market opportunities, once the eating habits of men and women and their age can be quite different. (LI; JERVIS; DRAKE, 2015). Individual differences might not be simply random; tough, some of the contrasts must be due to differences in sensory physiology, and others seem to relate to eating habits.

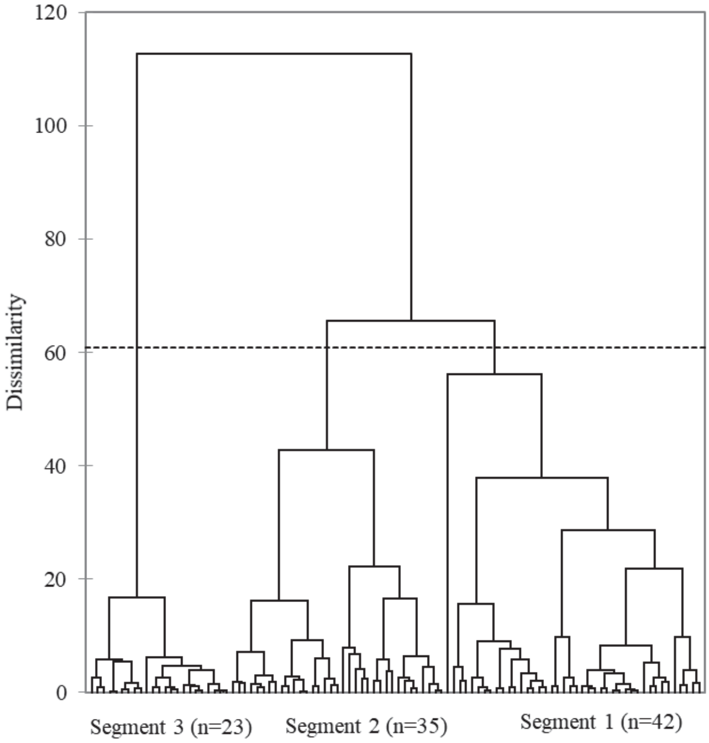
For hot and iced mate tea sensory data, we used Hierarchical Cluster Analysis (HCA), which generated three segments of consumers (composed by 42 - segment 1, 35 - segment 2 and 23 - segment 3 and composed by 44 – segment 1, 42 – segment 2 and 21 – segment 3). The HCA for hot (FIGURE 3) and for iced tea (FIGURE 4) exhibited pair-wise dissimilarity between different levels of teas acceptance, evaluated by consumers. To complement the analysis, we applied the Tukey test to identify the most accepted drying method by consumers in each segment for both teas (hot – Table 3, and iced – Table 4).

For hot mate tea (TABLE 3) within the segment 1, the only sample with average acceptance ≥ 7 (region of good acceptance in the hedonic scale) was the sample dried by oven dryer (80 ºC). The mate teas elaborated with yerba mate dried in microwave oven and conveyor dryer had the lowest acceptance, but without differences from others. Consumers of segment 1 comprises 42 consumers, most of them women aged from 18 to 45, with graduated level of education accustomed with drinking teas, mainly mate teas.

In segment 2 (35 individuals), the lowest acceptance was mate teas elaborated with yerba mate dried in dryer at 40 °C, with average 3.4 (disliked moderately on the hedonic scale). This segment was performed with older women with primary and secondary level of education, reported to consume less mate teas than individuals from segment 1.

The segment 3, the smallest one (23 individuals), comprised men (52 %), aged over 46 years old, having completed secondary school. For them, the majority of mate teas had good acceptance, scores varied from 6 to 8 (liked slightly to liked very much). They had lower acceptance by mate teas elaborated with yerba mate dried in oven dryer at 40 ºC, without differences from traditional processes. These consumers segmentation had the lowest discriminatory power.

FIGURE 3: HIERARCHICAL CLUSTER ANALYSIS FOR SEGMENTATION OF HOT MATE TEA



SOURCE: The author (2020).
NOTE: Dissimilarity: Euclidean distance; Agglomeration method: Ward.

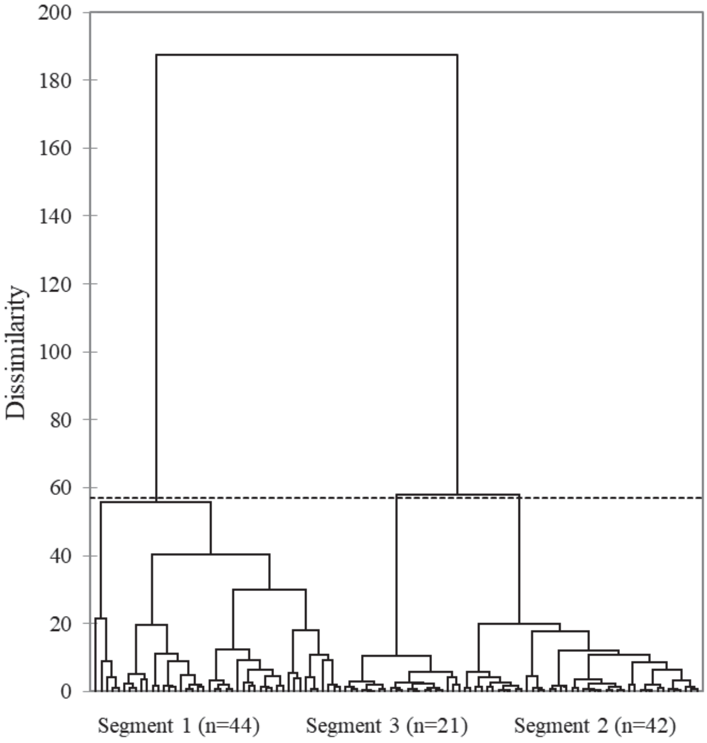
TABLE 3 - AVERAGE-LIKING SCORES OF HOT MATE TEAS ELABORATED WITH TOASTED YERBA MATE FROM DIFFERENT DRYING METHODS FOR THE THREE IDENTIFIED CONSUMERS SEGMENTS

Drying	Segment 1 (n=42)	Segment 2 (n=35)	Segment 3 (n=23)
Roasting + conveyor dryer	4,976b ± 0,26	5,829a ± 0,27	6,652ab ± 0,29
Roasting + rotary dryer	5,976ab ± 0,31	5,429ab ± 0,32	7,217ab ± 0,25
Microwave oven	4,929b ± 0,27	4,400bc ± 0,30	7,478a ± 0,20
Freeze drying	5,595ab ± 0,24	4,629abc ± 0,32	7,565 a± 0,25
Oven dryer at 40 ºC	6,143ab ± 0,73	3,400c ± 0,36	6,087b ± 0,39
Oven dryer at 60 ºC	6,262ab ± 0,27	4,914ab ± 0,29	7,522a ± 0,23
Oven dryer at 80 ºC	7,000a ± 0,20	4,286bc ± 0,33	7,391a ± 0,26

SOURCE: The author (2020).
NOTE: Values are reported as mean ± stantard error. Different letters in columns imply difference between samples ($p < 0.05$) by Tukey Test. Evaluated in 9-point hedonic scales, ranging from 1 (dislike extremely) to 9 (like extremely).

In the case of iced mate tea, FIGURE 4 represents all the consumers and segments; though, in this case, most part of the segment 1 included the opposite side of the samples (disliked), but the samples located at upper right quadrant in the IPM (conveyor dryer, rotary dryer and microwave oven) had better acceptance. Consumers of segment 2 are located in the central area of the graphic, meaning that fewer iced teas drying processes were accepted by sensory analysis with very similar scores. Nonetheless, the area of the segment 3 reaches graphic upper and the lower quadrant, and these individuals exhibited good acceptability values for all iced teas tested herein.

FIGURE 4: HIERARCHICAL CLUSTER ANALYSIS FOR SEGMENTATION OF ICED MATE TEA



SOURCE: The author (2020).
NOTE: Dissimilarity: Euclidean distance; Agglomeration method: Ward.

In segment 1 (44 individuals) the greatest scores (TABLE 4) were given to samples of iced tea elaborated with yerba mate dried in conveyor dryer, rotary dryer and microwave oven (scores between 5 to 6 on the hedonic scale). The worst products according to consumers from this segment were the iced teas from oven dryer at 40 °C and freeze-drying processes. Individuals from this segment were women (68 %) graduated and aged up to 36 years old.

The segment 2, the second major segment, comprised 42 individuals with the majority of men aged under 36-years-old and attended the university (70 %). For these individuals, all iced tea samples had no statistical differences and scores varied from liked slightly to liked moderately. The best scores were for mate dried in oven dryer at 60 ºC, 80 ºC, and rotary dryer.

In the third segment (21 individuals), the consumers had the highest acceptability compared to individuals from other segments with acceptance for all the samples with no significant differences, and this segment was composed by half women and half men aged up to 45-years-old with post-graduation level.

TABLE 4. AVERAGE LIKING SCORES OF ICED MATE TEAS ELABORATED WITH TOASTED YERBA MATE FROM DIFFERENT DRYING METHODS FOR THE THREE IDENTIFIED CONSUMERS SEGMENTS

Drying	Segment 1 (n=44)	Segment 2 (n=42)	Segment 3 (n=21)
Roasting + conveyor dryer	5,318a ± 0,28	5,929a ± 0,19	7,619a ± 0,18
Roasting + rotary dryer	5,955a ± 0,26	6.476a ± 0,16	7,762a ±0,18
Microwave oven	5,318a ± 0,29	5,952a ± 0,20	7,571a ± 0,21
Freeze drying	4,400bc ± 0,29	6,190a ± 0,16	7,429a ± 0,21
Oven dryer at 40 ºC	3,614c ± 0,27	5,905a ± 0,21	7,143a ± 0,17
Oven dryer at 60 ºC	4,909ab ± 0,29	6,452a ± 0,18	7,952a ± 0,18
Oven dryer at 80 ºC	4,795ab ± 0,28	6,548a ± 0,17	7,143a ± 0,30

SOURCE: The author (2020).
NOTE: Values are reported as mean ± stantard error. Different letters in columns imply difference between samples ($p < 0.05$) by Tukey Test. Evaluated in 9-point hedonic scales, ranging from 1 (dislike extremely) to 9 (like extremely).

Mate tea drink is widely known and consumed by different age and socioeconomic groups (CARDOSO; BATTOCHIO; CARDELLO, 2004). Females were predominant in this research, similar to a research by De godoy et al. (2013) that explored the acceptability of mate tea varying the percentage of leaves and sticks.

Evidence indicates that individuals differ greatly in flavour perception, in part due to genetic variations in chemoreceptor genes, since these differences in biology have been implicated as drivers of food choice, highlighting the need for behavioural scientists, sensory practitioners, and product developers to be aware of these differences (HAYES; FEENEY; ALLEN, 2013).

3.3.3 Bioactive compounds and antioxidant capacity of toasted mate from different drying methods.

Bioactive compounds analysis is very important to food market, since consumers are paying more attention to the health benefits of foods and drinks in their quest to maintain a healthy lifestyle (VALDUGA et al., 2019). It is crucial to design high-quality products in the processes of development (YANG; LEE, 2019), since the processes directly interferes with the final quality (LEE; CHAMBERS, 2010).

According to Table 5, microwave oven, freeze-drying, and traditional methods (roasting + rotary dryer and roasting + conveyor dryer) resulted in conservation of the highest contents of total phenolic compounds and antioxidant capacity by DPPH and ABTS radicals. The oven dryers promoted the lowest contents, representing an average decrease of 66 % in content of phenolic compounds when compared to others. Similar results have been shown in other plants, such as *Thunbergia laurifolia* (CHAN et al., 2012). Phenolic compounds have been reported for many physiological functions, such as antioxidant activity (ZHANG et al., 2020); in others words, acting as reducing agents as hydrogen donors, singlet oxygen quenchers, and metal chelating agents (HAMILTON et al., 1997). In this research, we found positive correlation ($p < 0.01$; $R^2 > 0.9$) among the total phenolic compounds, DPPH, and ABTS radicals. These results suggests that these phenolics are the main contributors to the antioxidant activity of mate beverages, as noted by Mateos et al. (2018); also, we confirm the importance of the drying method of raw material, since it directly interferes in the content of the bioactive compounds.

The caffeoylquinic acid (3-CQA, 4-CQA and 5-CQA) are the main phenolics compounds reported for yerba mate (MEINHART et al., 2017; MATEOS et al., 2018) and showed significant decrease in the dryers (TABLE 6). The beneficial effects of caffeoylquinic acids for human health includes antidiabetes, antiobesity, cardioprotection, neuroprotection, antitumor, hepatoprotection, and analgesic properties (NAVEED et al., 2018).

Reports with green tea (ZHUANG et al., 2020) suggests that caffeoylquinic acids are the main responsible for astringent taste, and the content variation of these, and other phenolic compounds, alters its astringency pattern; therefore, resulting in different conglomerate data of teas. This result may justify the internal preference mapping since teas (hot and iced) prepared with samples dried in oven dryers were grouped together (Figure 1 and 2). This is in accordance also with *Thunbergia laurifolia* leaves; whereas its tea produced by oven dryer at 50 °C was the least preferred by consumer (CHAN et al., 2012).

Methylxanthines (caffeine and theobromine), mainly caffeine, is a central nervous system stimulant (VALDUGA et al., 2019) which is important in the energy drink market.

Furthermore, caffeine is defined as responsible for the effects of yerba mate tea on suppress or reduce adipogenesis, lipogenesis, and body fat accumulation based on *in vitro*, *in silico*, and *in vivo* results (ZAPATA et al., 2019). Thus, yerba mate can be considered a modest source of these purine alkaloids compared with coffee (MATEOS et al., 2018). For Corso; Vignoli; Benassi (2016) caffeine is less affected by roasting processes and is more dependent on the raw material content; however, in this research, the contents of methylxanthines were reduced by traditional drying methods (TABLE 6), representing 39 % of reduction when compared to other methods. We attribute this effect to the roasting “sapeco” at 400 °C, step performed in the traditional system. Studies with coffee showed that caffeine is thermostable (WEI et al., 2012); however, its content may decrease with the reach of the sublimation temperature (185 ºC) (CASAL; OLIVEIRA; FERREIRA, 2000).

Our internal preference mapping showed that teas (hot and iced) produced with raw materials from traditional drying processes were grouped with the microwave oven, and it favoured a higher caffeine content (TABLE 6). Thus, it appears that although caffeine is a bitter substance, the set of other factors affects the separation of teas, and consequently, consumer acceptance. The impact of caffeine on central taste is not well understood, and although there has been considerable research on caffeine’s physiological and cognitive effects, there is a paucity of research investigating the effects of caffeine on taste sensibility (GRAMLING; KAPOULEA; MURPHY, 2019).

3.4 CONCLUSION AND RECOMMENDATIONS

In IMP analysis, the traditional drying methods were grouped with the microwave oven regardless of tea type (iced and hot). We recommend microwave oven as an alternative because it maintains similarity to traditional taste of mate tea, and it preserves bioactive compounds from antioxidant (total phenolic compounds and caffeoylquinic acids) and stimulant (caffeine and theobromine) characteristics. Nonetheless, if market goal is to provide products with lower levels of caffeine and theobromine then, traditional method is the most indicated.

The freeze-drying process had good results in maintaining caffeine and phenolic compounds; however it is a more expensive technology.

The results of consumer segmentation analysis identified different groups of consumers, which can give the possibilities of drying, according to different tastes. However, we do not recommend oven dryers since this technology provided raw material with low content of antioxidants compounds.

TABLE 5 - CONTENT (DRY BASIS) OF TOTAL PHENOLIC COMPOUNDS (CFT) AND THE ANTIOXIDANT CAPACITY BY THE DPPH AND ABTS RADICALS OF THE AQUEOUS EXTRACTS OF TOASTED YERBA MATE LEAVES FROM DIFFERENT DRYING METHODS.

Drying methods	mgEAG^{a}.g^{-1}	TEAC^{b} µm.g^{-1}	
	CFT^{C}	Radical DPPH	Radical ABTS
roasting + conveyor dryer	70,35^{bc} ±2,79	387,06^{a} ±1,30	1053,40^{a} ±192,91
roasting + rotary dryer	67,11^{c} ±5,52	368,04^{a} ±23,40	844,40^{ab} ±107,78
Microwave oven	77,26^{ab} ±12,94	372,37^{a} ±33,69	1042,00^{a} ±164,01
freeze drying	80,19^{a} ±2,68	380,83^{a} ±5,69	1082,09^{a} ±44,78
oven dryer 40°C	46,83^{d} ±2,02	262,99^{b} ±21,18	733,77^{b} ±63,43
oven dryer 60°C	20,76^{de} ±4,35	113,57^{b} ±2,30	408,43^{c} ±15,25
oven dryer 80°C	7,79^{e} ±0,58	62,91^{b} ±32,59	229,39^{d} ±26,38

SOURCE: The author (2020).
NOTE: Values are reported as mean ± stantard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.05$).
SUBTITLE: ^{a} gallic acid equivalent; ^{b} antioxidant capacity equivalent to Trolox; ^{c} total phenolic compounds.

TABLE 6 – CONTENT (DRY BASIS) OF CAFFEINE, THEOBROMINE, CAFFEOYLQUINIC ACIDS (3-CQA, 4-CQA, 5-CQA) IN AQUEOUS EXTRACTS OF TOASTED YERBA MATE LEAVES FROM DIFFERENT DRYING METODS

Drying methods	Content mg.g^{-1}				
	Methylxanthines		Caffeoylquinic acids		
	Caffeine	Theobromine	3-CQA	4-CQA	5CQA
roasting and rotary dryer	6,53^{c} ±1,24	0,51^{c} ±0,12	2,92^{a} ±0,54	2,27^{b} ±0,45	1,67^{a} ±0,36
roasting and conveyor dryer	8,76^{bc} ±2,26	0,72^{bc} ±0,18	3,40^{a} ±0,76	2,52^{b} ±0,62	1,96^{a} ±0,45
Microwave oven	12,29^{a} ±1,59	0,89^{ab} ±0,12	4,17^{a} ±0,56	3,31^{a} ±0,45	2,64^{a} ±0,39
freeze drying	14,21^{a} ±1,34	1,10^{a} ±0,13	4,08^{a} ±0,54	2,89^{b} ±0,39	2,29^{a} ±0,23
oven dryer 40°C	12,88^{a} ±1,18	0,98^{ab} ±0,09	1,85^{b} ±0,23	1,04^{c} ±0,11	0,86^{b} ±0,07
oven dryer 60°C	12,24^{a} ±1,32	0,84^{ab} ±0,09	0,45^{c} ±0,06	0,21^{cd} ±0,04	0,25^{c} ±0,04
oven dryer 80°C	10,94^{ab} ±1,35	0,78^{ab} ±0,11	0,21^{d} ±0,05	0,11^{d} ±0,05	0,13^{d} ±0,06
Retention Time (min)	31.0	28.0	30.0	30.2	28.6

SOURCE: The author (2020).
NOTE: Values are reported as mean ± stantard error (n = 3). Means followed by the same letters in column do not differ by Tukey's test ($p < 0.05$)

REFERENCES

ASSOCIAÇÃO BRASILEIRA DE NORMAS E TÉCNICAS (ABNT). **NBR 14141**. Escalas utilizadas em análise sensorial de alimentos e bebidas. Rio de Janeiro. 1998.

ARES, G.; JAEGER, S. R. Check-all-that-apply questions: influence of attribute order on sensory product characterization. **Food Quality and Preference**, v. 28, n. 1, p. 141–153, 2013.

BARBOZA, H. DE C.; CAZAL, M. DE M. Avaliação da influência de características sensoriais e do conhecimento nutricional na aceitação do chá-mate. **Brazilian Journal of Food Technology**, v. 21, n. 0, 2018.

BHATTA, S.; JANEZIC, T. S.; RATTI, C. Freeze-drying of plant-based foods. **Foods**, v. 87, n. 9, p. 1–22, 2020.

BRAND-WILLIANS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **Lebensmittel Wissenschaft und Technologie – Food Science and Technology**, v. 28, p. 25–30, 1995.

BOLTON D. **Market research reflects and predicts growth**. 2019. Available from: https://worldteanews.com/market-trends-data-andinsights/market-research-reflects-and-predicts-growth. Accessed 1 November .

CARDOSO, J. M. P.; BATTOCHIO, J. R.; CARDELLO, H. M. A. B. Equivalência de dulçor e poder edulcorante de edulcorantes em função da temperatura de consumo em bebida s preparadas. **Ciência e Tecnologia de Alimentos**, v. 24, n. 3, p. 448–452, 2004.

CARDOZO JUNIOR, E. L.; 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**, v. 21, p. 440–454, 2016.

CASAL, S.; OLIVEIRA, M. B.; FERREIRA, A. M. HPLC/diode-array applied to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. **Food Chemistry**, v. 68, p. 481-485, 2000.

CHAN, E. W. C. et al. Antioxidant and sensory properties of Thai herbal teas with emphasis on *Thunbergia laurifolia* Lindl. **Chiang Mai Journal of Science**, v. 39, n. 4, p. 599–609, 2012.

CORSO, M. P.; VIGNOLI, J. A.; BENASSI, M. DE T. Development of an instant coffee enriched with chlorogenic acids. **Journal of Food Science and Technology**, v. 53, n. 3, p. 1380–1388, 2016.

DE GODOY, R. C. B. et al. Consumer perceptions, attitudes and acceptance of new and traditional mate tea products. **Food Research International**, v. 53, n. 2, p. 801–807, 2013.

DIBANDA, F. R. et al. Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some fruit peelings. **Food Chemistry**, v. 302, 2020.

FIGIEL, A.; MICHALSKA, A. Overall quality of fruits and vegetables products affected by the drying processes with the assistance of vacuum-microwaves. **International Journal of Molecular Sciences**, v. 18, n. 1, 2017.

GRAMLING, L.; KAPOULEA, E.; MURPHY, C. Taste perception and caffeine consumption: an fMRI study. **Nutrients**, v. 11, n. 1, 2019.

GRIGIONI, G. et al. Flavour characteristics of *Ilex paraguariensis* infusion, a typical Argentine product, assessed by sensory evaluation and electronic nose. **Journal of the Science of Food and Agriculture**, v. 84, n. 5, p. 427–432, 2004.

HAMILTON, R. J. et al. Chemistry of free radicals in lipids. **Food Chemistry**, v. 60, n. 2, p. 193–199, 1997.

HAYES, J. E.; FEENEY, E. L.; ALLEN, A. L. Do polymorphisms in chemosensory genes matter for human ingestive behavior? **Food Quality and Preference**, v. 30, n. 2, p. 202–216, 2013.

HECK, C. I.; DE MEJIA, E. G. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**, v. 72, n. 9, 2007.

HIHAT, S.; REMINI, H.; MADANI, K. Effect of oven and microwave drying on phenolic compounds and antioxidant capacity of coriander leaves. **International Food Research Journal**, v. 24, n. 2, p. 503–509, 2017.

ISOLABELLA, S. et al. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. **Food Chemistry**, v. 122, n. 3, p. 695–699, 2010.

LEE, J.; CHAMBERS, D. H. Descriptive analysis and U.S. consumer acceptability of 6 green tea samples from China, Japan, and Korea. **Journal of Food Science**, v. 75, n. 2, 2010.

LI, X. E.; JERVIS, S. M.; DRAKE, M. A. Examining extrinsic factors that influence product acceptance: a review. **Journal of Food Science**, v. 80, n. 5, p. 901 - 909, 2015.

LIN, S.; BREWER, M. S. Effects of blanching method on the quality characteristics of frozen peas. **Journal of Food Quality**, v. 28, n. 4, p. 350–360, 2005.

MACFIE, H. J.; BRATCHELL, N. Designs to balance the effect of order of presentation. **Journal of Sensory Studies**, v. 4, p. 129–148, 1989.

MACHADO, C. C. B. et al. Determinação do perfil de compostos volateis e avaliação do sabor e aroma de bebidas produzidas a partir da erva-mate (*Ilex paraguariensis*). **Quimica Nova**, v. 30, n. 3, p. 513–518, 2007.

MATEOS, R. et al. Improved LC-MS characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry**, v. 241, p. 232–241, 2018.

MCGOWAN, B. A.; LEE, S. Y. Comparison of methods to analyze time-intensity curves in a corn zein chewing gum study. **Food Quality and Preference**, v. 17, n. 3–4, p. 296–306, 2006.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, n. March, p. 522–530, 2017.

MÜLLER, H.; HAMM, U. Stability of market segmentation with cluster analysis - A methodological approach. **Food Quality and Preference**, v. 34, p. 70–78, 2014.

NAVEED, M. et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. **Biomedicine and Pharmacotherapy**, v. 97, n. August 2017, p. 67–74, 2018.

NIREESHA, G. R. et al. Lyophilization / Freeze Drying - An Review. **International Journal of Novel Trends in Pharmaceutical Sciences**, v. 3, n. 4, p. 87–98, 2013.

PARIT, R. K.; PRABHU, M. C. S. Microwave fruit and vegetables drying. I**nternational Advanced Research Journal in Science, Engineering and Technology**, v. 4, n. 2, p. 82–84, 2017.

RE, R. et al. Antioxidant activity applying an improved abts radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9, p. 1231–1237, 1999.

ROCHA, R. P.; MELO, E. C.; RADÜNZ, L. L. Influence of drying process on the quality of medicinal plants: A review. **Journal of Medicinal Plant Research**, v. 5, n. 33, p. 7076–7084, 2011.

SINGLETON, V.; ROSSI, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v. 16, p. 144-158, 1965.

TEIXEIRA, L. V. Análise sensorial na indústria de alimentos. **Revista do Instituto de Latícinios Cândido Tostes**, v. 64, n. 366, p. 12–21, 2009.

UDOMKUN, P. et al. Influence of air drying properties on non-enzymatic browning, major bio-active compounds and antioxidant capacity of osmotically pretreated papaya. **LWT - Food Science and Technology**, v. 60, n. 2, p. 914–922, 2015.

VALDUGA, A. T. et al. Cytotoxic / antioxidant activity and sensorial acceptance of yerba-mate development by oxidation process. **Acta Scientiarum. Technology**, v. 38, n. 1, p. 115, 2016.

VALDUGA, A. T. et al. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. **Food Research International**, v. 120, n. November 2018, p. 478–503, 2019.

VALDUGA, A. T.; BATTESTIN, V.; FINZER, R. D. Secagem de extratos de erva-mate em secador por atomização. **Ciência e Tecnologia de Alimentos**, v. 23, n. 2, p. 184–189, 2003.

VARELA, P.; BELTRÁN, J.; FISZMAN, S. An alternative way to uncover drivers of coffee liking: Preference mapping based on consumers' preference ranking and open comments. **Food Quality and Preference**, v. 32, p. 152–159, 2014.

WEI, F. et al. Roasting Process of Coffee Beans as Studied by Nuclear Magnetic Resonance: Time Course of Changes in Composition. **Journal of Agricultural and Food Chemistry**, v. 60, p. 1005-1012, 2012.

YANG, J. E.; LEE, J. Consumer perception and liking, and sensory characteristics of blended teas. **Food Science and Biotechnology**, 2019.

YIM, H. S. et al. Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. **Journal of Food Science and Technology**, v. 50, n. 2, p. 275–283, 2013.

ZAIONS, I. et al. Physico-chemical characterization of *Ilex paraguariensis* St. Hil. during the maturation. **Brazilian Archives of Biology and Technology**, v. 57, n. 5, p. 663–667, 2014.

ZAPATA, F. J. et al. Caffeine, but not other phytochemicals , in mate tea (*Ilex paraguariensis* St . Hilaire) attenuates high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation. **Journal of Functional Foods**, p. 1036-1046, 2019.

ZHANG, D. et al. Antioxidant and antibacterial capabilities of phenolic compounds and organic acids from *Camellia oleifera* cake. **Food Science and Biotechnology**, v. 29, n. 1, p. 17–25, 2020.

ZHUANG, J. et al. Evaluation of astringent taste of green tea through mass spectrometry-based targeted metabolic profiling of polyphenols. **Food Chemistry**, v. 305, p. 125507, 2020.

CHAPTER 3

4 SEMI-HYDROPONIC SYSTEM AS A NEW METHOD FOR YERBA MATE PRODUCTION: EFFECT OF NITROGENIZED FERTIGATION AND GENOTYPE

ABSTRACT

Yerba mate leaves are used in several industrial segments, with a significative growing demand of this product. Mate leaves are produced in the field with harvesting at each 18 or 24 months and new production methods need to be developed. Therefore, we aimed to study the efficiency of semi-hidroponic cultivation as a new leaf production system evaluating leaf production of two genotypes of yerba mate in five nitrogen doses. Clonal plants were produced and transplanted into a semi-hidroponic system in gutters and converted into mini-stumps for the experiment onset. The effect of nitrogen (N) doses was evaluated in the nutritive solution (114, 206, 380, 760, and 1142 mg.L^{-1}), genotypes (EC40 e EC22), type of leaves (young and mature) and harvests (11 – representing two years). The results confirm the efficiency of the proposed system since it has made feasible the raw material production during the entire year (harvests on average every two months) with similar young and mature leaves proportion. There was little variation in productivity between both genotypes during harvests, which diminished seasonality effect. The average annual leaf production in second year was 4.2 ton ha^{-1} and 5.3 ton ha^{-1} for EC22 and EC40 respectively. The nitrogen effect was dependent on the genotype; for EC40 a smallest tested dose (114 mg.L^{-1}) is indicated, for EC22 the best dose was achieved in 206 mg.L^{-1} N; however in future experiments, smaller doses are ought to be tested for both genotypes. We recommend this system for yerba mate selected genotypes production to obtain young and mature leaves for niche markets with higher added value.

Key words: *Ilex paraguariensis*. Yield. Nitrate. Protected environment.

4.1 INTRODUCTION

Yerba mate or mate (*Ilex paraguariensis* A. St.-Hil.) is a native and cultivated plant from South American countries. Brazil is the world's largest producer (939.58 thousand tonnes per year) (IBGE 2018a, 2019b), followed by Argentina (291 thousand tonnes per year) and Paraguay (105 thousand tonnes per year) (RIACHI; DE MARIA, 2017; FAOSTAT, 2017). Of all species classified as permanent crop in the southern and central-western regions of Brazil, mate represents 21.8 % of the total harvested area, with average yield of 7.6 tonnes/ha (IBGE, 2018a).

Yerba mate dried leaves are used to prepare tereré and chimarrão (traditional South American drinks) or dried and roasted, known worldwide as mate tea (RIACHI; DE MARIA, 2017). Moreover, industrialized beverages, nutraceuticals and cosmetics have also been developed with this raw material (BARBOSA et al., 2018). Nowadays, in addition to drinks, *Ilex paraguariensis* is already part of some pharmacological and food formulation due to its bioactive compounds (BRACESCO, 2019). Considering the 100 most consumed plants in Brazil, mate leaves are one of the main sources of methylxanthines (caffeine and theobromine) (MEINHART et al., 2019), monocaffeoylquinic and dicaffeoylquinic acids (MEINHART et al., 2017), which are beneficial to human health (RIACHI; DE MARIA, 2017). Regarding species potential and its multiple uses, both national and international markets demand differentiated raw materials, with fixed phytochemical characteristics and with continuous production throughout the year.

For this purpose, the EMBRAPA (Brazilian Agricultural Research Corporation) started in 1997 a genetic breading program for yerba mate based on progeny selection criteria for high leaf production (RESENDE et al., 2000). More recently, plants have been phytochemically analyzed (CARDOZO JUNIOR et al., 2010) aiming to select potential genotypes for vegetative propagation to compose highly productive cultivation systems with desired chemical characteristics such as caffeine.

Currently, yerba mate is exploited in natural systems (extractivism with harvest every 24 to 48 months), intensive systems (monoculture with harvest every 12, 18 to 24 months) or in agroforestry systems (SANTIN et al., 2017a, 2017b, 2019; WESTPHALEN et al., 2019). Both in field production and in seedlings phase, nitrogen nutrition have been focus of several studies (PANDOLFO et al., 2003; SANTIN et al., 2008, 2013, 2019; NASCIMENTO et al., 2019; WESTPHALEN et al., 2019). Nitrogen is a primary nutrient for crop growth (XING et al., 2019) and is the macronutrient most exported by yerba mate leaves (33 to 37 g. kg^{-1}) (OLIVEIRA et al., 2014; SANTIN et al., 2019). Research has shown that the demand for nitrogen by yerba mate is high and it is necessary to supply this macronutrient to shorten the harvest interval (< 280 kg. ha^{-1} of N to harvest every 18 months and > 320 kg. ha^{-1} of N to harvest every 24 months) in field production (SANTIN et al., 2019).

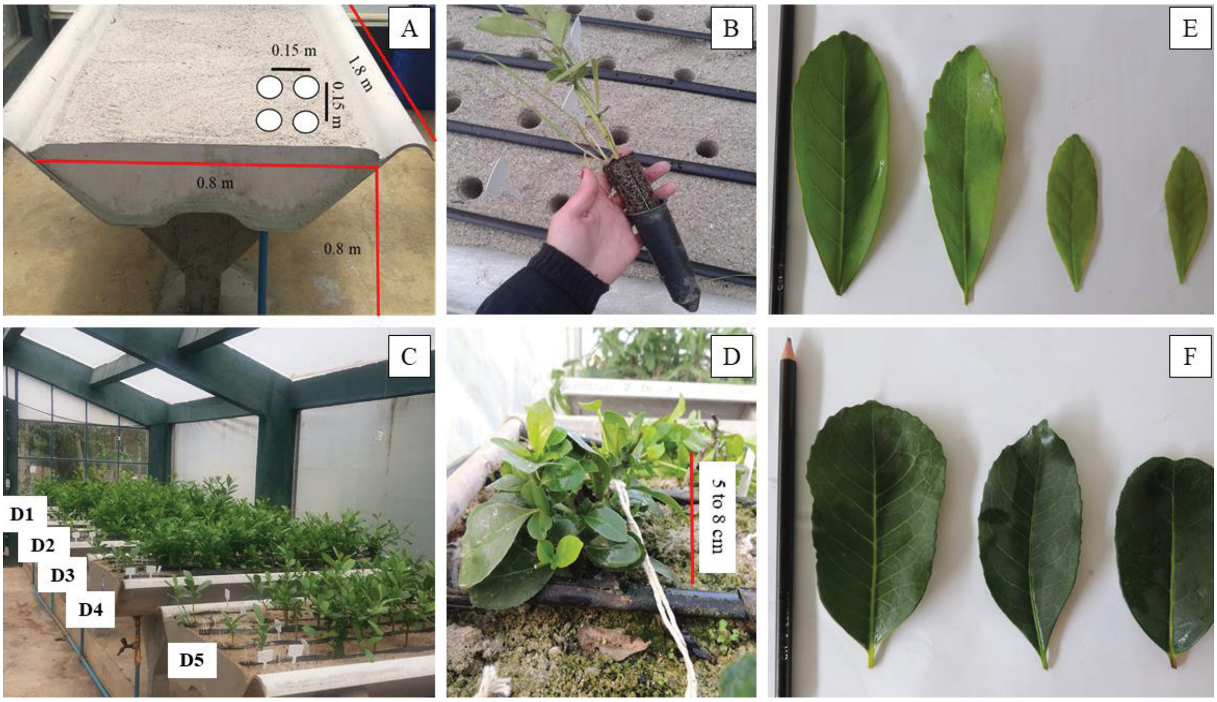
Several studies have been focused on the development of semi hydroponic systems for the production of ministumps using different N doses (e.g. 103.75, 124.1, 129, 206, 600 mg.L^{-1}) for different species, such as *Eucalytus dunni*, *Eucalyptus grandis* x *Eucalyptus urophylla*, *Araucaria angustifolia*, *Eucalyptus benthamii* x *Eucalytus dunni*, *Piptocarpha angustifolia*, *Ilex paraguariensis* (WENDLING; DUTRA; GROSSI, 2007; WENDLING; DUTRA, 2008; DA ROSA et al., 2009; ROCHA et al., 2015; PIRES et al., 2015; WENDLING; BRONDANI, 2015; PIRES et al., 2017; STUEPP et al., 2017; DE SÁ et al., 2018). However, no research has been conducted in order to establish the semi hydroponic system focusing on selected raw material production for various industrial sectors. Thus, the objective of this work was to evaluate leaf production of two yerba mate genotypes cultivated in a semi-hydroponic system with five nitrogen doses.

4.2 MATERIALS AND METHODS

Research was conducted between June 2016 and February 2019 in the Forest Species Propagation Laboratory of Embrapa Forestry, located in Colombo – PR / Brazil (25°20’ S and 49°14’ W, 950 m). According to Köppen, the climate of the region is temperate, type Cfb. The rooted cuttings obtained through the cuttings process described by Wendling and Brondani (2015) were produced from epicormic shoots of two 10-years-old mother plants (identified as genotypes EC22 and EC40) from a provenance and progeny trial in Ivaí – PR / Brazil (WENDLING et al., 2018) based on the genetic breeding program of EMBRAPA (RESENDE et al., 2000). This genetic material was selected by caffeine content according to previous study where the authors considered caffeinated (EC40) with 2.35 % caffeine content and decaffeinated (EC22) with 0.02 % caffeine content (HELM et al., 2015). According to ANVISA’s resolution n ° 277, a decaffeinated product has a maximum of 0.1 % (g/100g) caffeine (BRASIL, 2005).

EC22 and EC 40 rooted cuttings with approximately 120 days and 15 cm height were transferred to a semi-hydroponic gutter system filled with medium sand. Cuttings were planted at a 15 x 15 cm spacing (FIGURE 1-A and 1-B) inside a non-acclimatized greenhouse. To promote the development of side shoots, we carried out a pruning of apical buds from 5 to 8 cm (FIGURE 1-D) seven days after transplanting cuttings to the gutters. Between June 2016 (introduction in the system) and February 2017, we performed four conduction prunings (when plants reached about 50-60 cm height) to convert the cuttings into ministumps. We used the nutrient solution described by Wendling e Brondani (2015) as a standard; it comprises the following nutrients: N-NO_3^- (156.0 mg.L^{-1}), N-NO_4^+ (50.0 mg.L^{-1}), P^- (25.0 mg.L^{-1}), K^+ (200.0 mg.L^{-1}), Ca^{2+} (200.0 mg.L^{-1}), Mg^{2+} (45.0 mg.L^{-1}), S^- (76.9 mg.L^{-1}), B (1.5 mg.L^{-1}), Cu^{2+} (0.1 mg.L^{-1}), Fe^{2+} (5.0 mg.L^{-1}), Mn^{2+} (1.0 mg.L^{-1}), Zn^{2+} (0.7 mg.L^{-1}), Mo^{2-} (0.07 mg.L^{-1}). In this part of ministumps establishment, the nutrient solution described above was diluted in half, the electrical conductivity was maintained at 1.2 mS.m^{-2}, 25 °C, and the pH was adjusted to 5.5 (± 0.1).

FIGURE 1 - SEMI-HYDROPONIC GUTTER SYSTEM AND LEAF TYPE



SOURCE: The author (2020).
SUBTITLE: A- Gutters dimensions and plant spacing; B – Rooted cuttings with approximately 120 days of age and 15 cm height; C – Gutters with each nitrogen dose (D1, D2, D3, D4 and D5); D - Pruning of apical buds 5 to 8 cm;. E – Young leaves; F – Mature leaves.

From February 2017 to February 2019, we added the nutrient solution with the total nutrient concentration, varying only the nitrogen doses per gutter among treatments (FIGURE 1-C). N concentrations was established as follows (N-NO_3^-- N-NH_4^+; N-Total mg.L^{-1}; ratio NO_3^-/NH_4^+): D1(64 – 50; 114 mg.L^{-1}; 1.28); D2 (156 – 50; 206 mg.L^{-1}; 3.12) standard; D3(243 – 137; 380 mg.L^{-1}; 1.77); D4 (433 – 327; 760 mg.L^{-1}; 1.32); D5 (624 – 518; 1142 mg.L^{-1}; 1.20). The electrical conductivity of the solution was maintained at the following values: D1- 2.21, D2 - 2.47, D3 - 3.00, D4 - 3.76, and D5 - 4.74 mS.m^{-2}, 25 °C; and pH was adjusted at 5.5 (± 0.1) every week. The other nutrients were maintained as described in the standard solution. The ministumps received nutrient solution through a drip fertigation system three times a day in a total daily water flow of 5 L.m^{-2} (summer and spring) and 3.6 L m^{-2} (winter and autumn). In the heat season (when the temperature reaches 30º C), greenhouse sides were opened and a sprinkler system was turned on for 30 s every 60 min from 10 am to 7 pm, totalizing a daily flow of 3.75 L.m^{-2} water. We washed the sand after each harvest (± two months) with 40 L water per gutter.

The temperature (°C) was monitored twice a day using a maximum and minimum thermometer (Equitherm TM-38CAP). The Photosynthetically active radiation data (PAR) (µmol m^{-2} s^{-1}) was calculated from the global solar radiation (RG) measure (kJ m^{-2} h^{-1}), obtained at the SIMEPAR Weather Station (Paraná Meteorological System), approximately 5 kilometers to the greenhouse where the experiment was conducted. A reduction of 40 % in solar radiation input in the greenhouse due to the plastic used to cover it was discounted; the calculations considered the difference between the measuring by luxmeter with three reading periods (8 hours, 13 hours, 17 hours), inside and outside of the greenhouse, in an open area without shading. The conversion of RG into PAR was carried out following Reis (2019), where RG in kJ m^{-2} h^{-1} * 0.2778 = W m^{-2} and 1 W m^{-2} is about 2.02 µmol m^{-2} s^{-1} of PAR.

The harvesting point was determined when plants reached 50 - 60 cm height, always keeping 30 % of leaves in the ministumps. After harvests, the leaves were dried in a microwave oven for 5 min (Electrolux®1000 W, 2450 MHz) and weighed for dry mass determination. We evaluated leaf production per ministump and transformed it into leaves production per square meter per month.

Leaves type was determined by coloring and expansion. Young leaves are light green and more tender, while mature leaves are dark green and completely expanded (FIGURA 1-D and 1-E). The dates and the seasons of harvests are in Table 1.

The experiment was conducted in a randomized and factorial design (2 x 2 x 5 x 11) where the factors were two genotypes (EC 22 and EC 40), two leaves types (young and mature), five nitrogen doses (D1, D2, D3, D4, and D5) and eleven harvests (over two years). A total of five repetitions and five ministumps per repetitions were used, amounting to 25 ministumps per genotype at each dose of N.

The data did not show normality (Shapiro-Wilk teste $P < 0.05$) thus, we tested Generalized Linear Mixed Models (GLMM) ($P < 0.05$) to adjust the harvest effect over time, but it had no effect. Then we used the GLM (generalized linear models) ($P < 0.05$) with gamma distribution for leaves production. Leaves type was not significant in the overall model, so we tested the effects of the triple and double interactions among the eleven harvest, two genotype, and five N doses. The effect of the covariates maximum mean, minimum mean and medium mean temperature, and photosynthetically active radiation (PAR) mean were tested on the adjusted model. To compare factor means, we used Tukey's contrasts for generalized linear models. For the effect of doses, regression models were used using statistical R software (R CORE TEAM, 2016).

TABLE 1 - DESCRIPTION OF HARVEST TIMES

Harvest number	Seasons	Day/month/year	Growing year
1	summer	20/03/2017	1
2	autumn	12/05/2017	1
3	winter	08/08/2017	1
4	spring	10/10/2017	1
5	spring	04/12/2017	1
6	summer	15/02/2018	1
7	autumn	19/04/2018	2
8	winter	25/06/2018	2
9	winter	18/09/2018	2
10	spring	12/12/2018	2
11	summer	11/02/2019	2

SOURCE: The author (2020)

4.3 RESULTS

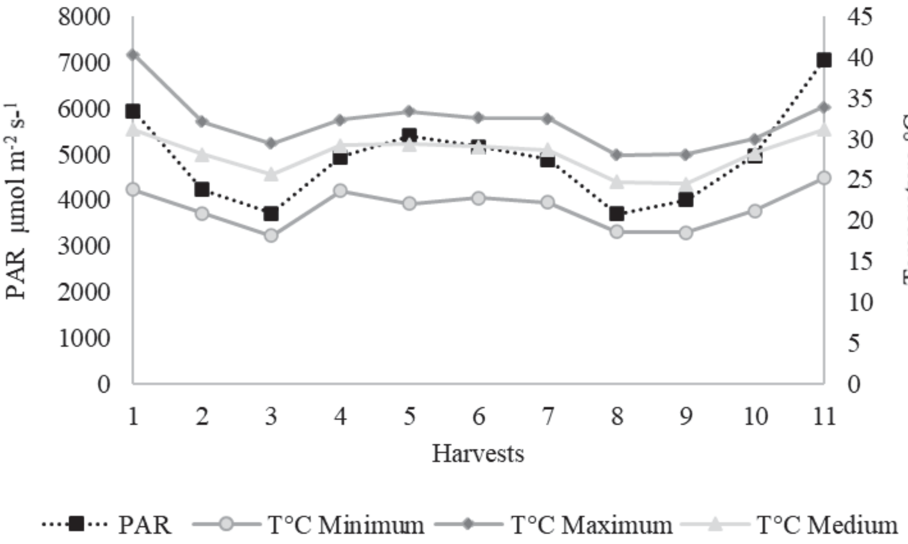
For leaves production (dry basis), the leaves type (young and mature) wasn't significant in the general mathematic model ($P \geq 0.5$). In other words, the management of semi-hydroponic system in the gutters established a similar leaves production at different development stages in a short period (about 2 months between each harvest).

There was significant effect of double interactions among factors: harvest:genotype ($P \leq 0.1$) and genotype:N dose ($P \leq 0.001$). The triple interaction was not significant ($P = 0.842$). The covariates medium-maximum temperature and medium-minimum temperature presented a significant effect ($P \leq 0.001$; $P \leq 0.1$, respectively) and were inserted in the model to explain the response of leaves production. There was no significant effect on medium photosynthetically active radiation (PAR) and medium temperature covariates ($P = 0.422$; $P = 0.102$, respectively).

The climatic data at harvest periods are in Figure 2. The highest medium-maximum temperature happened in harvest 1 (40.3 °C), corresponding to summer season and beginning of fertigation with the different nitrogen doses. It is also noticeable that, in a general way, there was a decreasing of production for both genotypes during the wintriest seasons

(FIGURES 2 and 3). To medium PAR, the higher radiation values occurred during the summer and spring seasons, and we observed the same tendency for leaves production (FIGURE 2 and 3) even though this covariate was not significant.

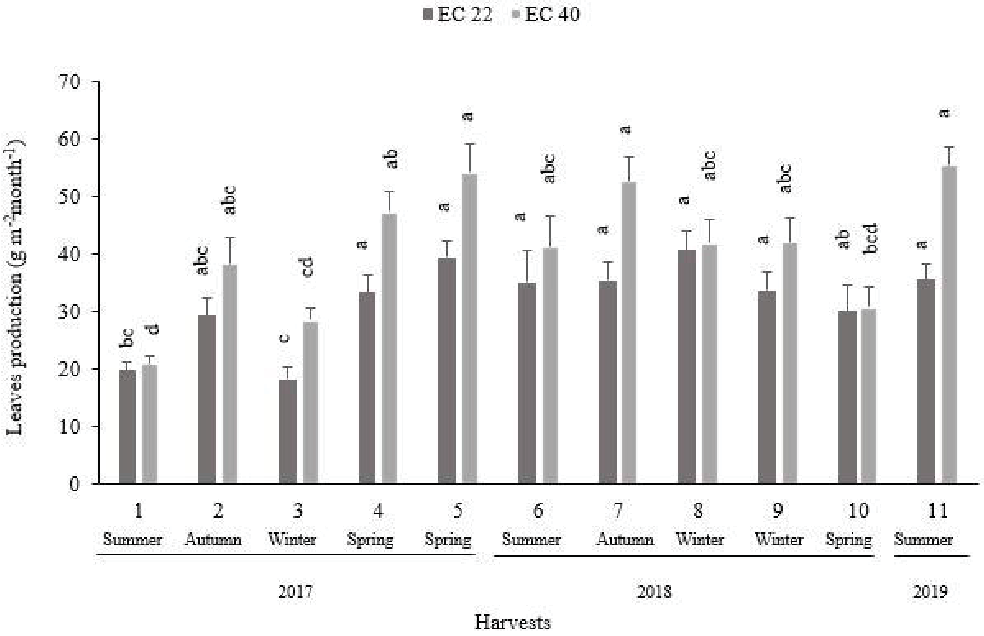
FIGURE 2 – CLIMATE DATA, MEAN TEMPERATURES (MAXIMUM, MINIMUM, AND MEDIUM), AND PHOTOSYNTHETICALLY ACTIVE RADIATION MEAN.



SOURCE: The author (2020).
NOTE: Additional information (harvest, season, year): (1, summer, 2017; 2, autumn, 2017; 3, winter, 2017; 4, spring, 2017; 5, spring, 2017; 6, summer, 2018; 7, autumn, 2018; 8, winter, 2018; 9, winter, 2018; 10, spring, 2018; 11, summer, 2019). The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

In the first year of growth (harvests 1 to 6) (TABLE 1) for both genotypes ministumps, leaves production was reduced in harvests 1 and 3. As for the second year (harvests 7 to 11) (TABLE 2), the production was more stable, with exception of harvest 10, which had a decrease in production for genotype EC40 (FIGURE 3). In general, the mean leaves production during the first year was 29.15 g.m^{-2}.month^{-1} (3.5 ton.ha^{-1}.ano^{-1}) for EC22 and 38.44 g.m^{-2}.month^{-1} (4.6 ton.ha^{-1}.ano^{-1}) for EC40 and during the second year it was 35.08 g.m^{-2}.month^{-1} (4.2 ton.ha^{-1}.ano^{-1}) for EC22 and 44.58 g.m^{-2}.month^{-1} (5.3 ton.ha^{-1}.ano^{-1}) for EC40. The EC40 production rates shows a medium increase of 13 % when compared to EC22.

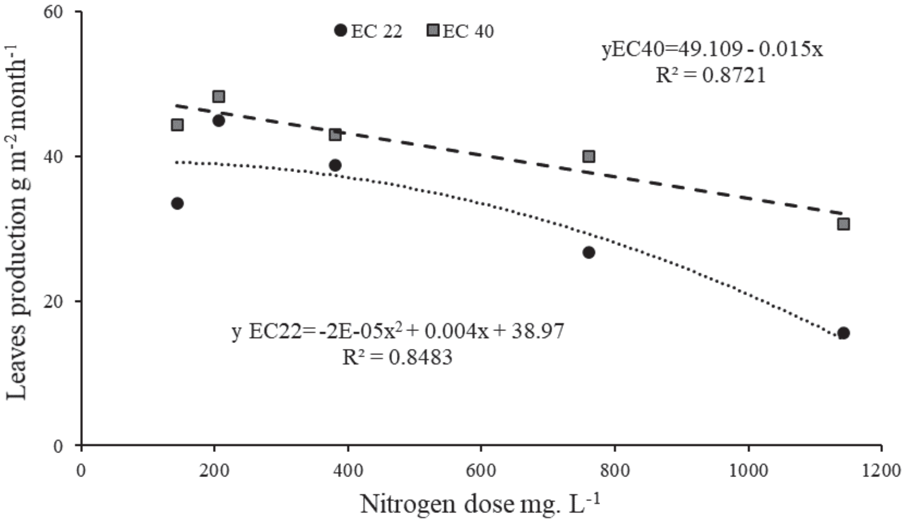
FIGURE 3 – LEAVES PRODUCTION OF GENOTYPES EC 22 AND EC 40 OVER TWO YEARS OF CULTIVATION IN SEMI-HYDROPONIC SYSTEM



SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error (n = 5). Means followed by the same letters do not differ by Tukey's contrasts for GLM ($p < 0.05$).
SUBTITLE: The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

The genotypes production was different as a function of nitrogen doses. For EC40, the adjusted regression model was linearly decreasing (FIGURE 4); in other words, with an increasing dose we observed a decrease in leaves production. For this genotype, the dose D1 (114 mg.L^{-1}) is recommended. For EC22, the response was quadratic, and D2 (206 mg.L^{-1}) was the better dose for leaves production. It was not possible to calculate the maximum technical efficiency dose, due to the lack of a maximum or minimum point in the range of values.

FIGURE 4 – ADJUSTED LEAVES PRODUCTION CURVE FOR NITROGEN DOSES FOR GENOTYPES EC22 AND EC40



SOURCE: The author (2020).
NOTE: Polinomial regression with quadratic effect for EC22 and linear regression for EC40. N total doses tested: D1 = 114 mg.L^{-1}; D2 = 206 mg.L^{-1}; D3 = 380 mg.L^{-1}; D4 = 760 mg.L^{-1}; D5 = 1142 mg.L^{-1}.

4.4 DISCUSSIONS

Yerba mate is traditionally harvested between May and September or December and February and only mature leaves and fine branches are provided. The results observed within this study allow us to assert that, through the proposed system, it is possible to achieve similar proportion of both young and mature leaves. Young leaves represent a different opportunity for yerba mate industry, as they make it feasible to obtain differentiated products (BASTOS et al., 2014). Blum-Silva et al. (2015) chemically analyzed yerba mate leaves with 1, 2, and 6 months age, and noted a decrease in methylxanthines percentage (caffeine and theobromine) and total phenolic compounds with leaves age maturation in field. Our results were satisfactory, because the semi-hydroponic system support leaves production at different development stages for a variety of industrial ends.

In terms of year production of processed leaves (dried), we obtained in the semi-hydroponic system during the second year an amount of 4.2 ton.ha^{-1} for EC22 genotype and 4.3 ton.ha^{-1} for EC40 genotype, with 0.15 x 0.15 spacing. Comparing with the conventional field cultivation fertilized with nitrogen and harvest over 12 months, Santin et al. (2019) obtained a production of 2.8 ton.ha^{-1} of green yerba mate, grown in 2 X 2 m spacing, considering leaves and fine branches (smaller than 7 mm of diameter) production. That confirms the efficiency of the system developed in this study regarding production, once it optimizes cultivation area, acquiring more plants per ha and continuous production throughout the year.

Analyzing genotypes leaves production throughout two years of growing, the lowest production was observed in harvests 1 and 3 (FIGURE 3) as consequence of the initial ministumps adaptation to the cultivation system. Similar results obtained by Pires et al. (2015) for *Araucaria angustifolia* ministumps reaffirm such assertion. On the second year, there was a production decrease mainly in the genotype EC40, in the harvest 10 (FIGURE 3). This production reduction was due to “ampoule” pest infection, mites and aphids. The “ampoule” is the second main pest of yerba mate, and, such as mites, damage them with deformation and leaves drops (PENTEADO JUNIOR; GOULART, 2019). Wendling; Dutra e Grossi (2007) have also noticed a massive aphids attack, causing a decrease in the medium number of minicuttings produced per ministump in that system.

With the exception of a lower leaves production in the aforementioned harvests, the production during the year has maintained itself stable, with only a mild variation similar to temperature variation and photosynthetically active radiation (FIGURE 1 and 2). Pimentel et al. (2019) observed corresponding results in a study with yerba mate minigarden cultivation; the authors evaluated four harvest periods, and this factor has not differed on production per ministump, with exception of a clone which reached higher productivity in the summer.

For Poletto et al. (2010), the best yerba mate growth results occurred in shaded environments, once it is a climax classified species, with good development light scarce environments. That study helps understanding our results for the semi-hydroponic system which is sheltered under a plastic cover, and it is known that global solar radiation is smaller inside the greenhouse due to plastic transmissibility. Such reduction may have favored the yerba mate plants growth. Additionally, the solar radiation may be compensated through diffuse radiation fraction, which is multidirectional and more efficient in forest canopy penetration, which is possibly favorable to plant growth (BURIOL et al., 1995).

Allied to the management system with lateral drapes aperture and aspersion irrigation during the warm days, the high temperatures did not affect production for making it possible to maintain medium temperatures inside the greenhouse. Thus, the semi-hydroponic system in a protected environment could be considered of high efficiency whilst regarding a continuous production, being possible to obtain raw material throughout the year.

Basing on our results, the superiority of genotype EC40 is evident in the harvests and in the nitrogen doses (FIGURE 2 and 3), once it presented maximum productivity at the smallest tested dose. EC22 required more nutrient dose to achieve maximum productive potential.

The use of nitrogen by plants involves several steps, including absorption, assimilation and translocation (MASCLAUX-DAUBRESSE et al., 2010). The nitrogen use efficiency is very complex since each step is governed by multiple genetic and environmental factors in interaction. It is known that there is a lot of genetic variation in the different stages of N metabolism, even between varieties of the same species (XU; FAN; MILLER, 2012).

Several studies present evidence that there is a natural variation for nitrogen metabolism. Working with clones of *Salix* spp. the genetic variation in N use efficiency was confirmed (YANG et al., 2015). Chardon et al. (2010) in a study with 18 accessions of *Arabidopsis thaliana* concluded that nitrogen use efficiency was genetically determined, with an estimated heritability of 0.67, demonstrating great genetic control.

The cultivation of genotypes with high N use efficiency can help decreasing the surplus of fertilizers, reducing the environmental effects of discriminated use of N and production costs (McALLISTER; BEATTY; GOOD, 2012; YANG et al., 2015). More N use efficient genotypes would be able to maximize plant growth and yield with fewer fertilizers (CHARDON; NOËL; MASCLAUX-DAUBRESSE, 2012; McALLISTER; BEATTY; GOOD, 2012). These genotypes are highly targeted as they can be grown with a limited N supply (YANG et al., 2015), developing more sustainable agriculture (XU; FAN; MILLER, 2012).

Aside from the genetic factor observed in the aforementioned research, a decrease in leaves production is noted as the nitrogen doses increased, from the point of maximum productivity of the two genotypes (FIGURE 4). Bredemeier e Mundstock (2000) highlight that N absorption is modulated by: presence of specific carriers; affinity of these carriers in relation to nitrate or ammonium; and amount of N.

Two types of carriers guide the N absorption through plasmatic membrane. In the low concentration line, the high affinity transport systems (HATS) are capable of removing ions at very low rates and are passible of saturation, which means that its activity can be modulated. In the high concentration line, the low affinity transport systems (LATS) are activated. Albeit, unlike HATS, LATS-mediated uptake of NO_3^- or NH_4^+ is not saturable (BREDEMEIER; MUNDSTOCK, 2000; NACRY; BOUGUYON; GOJON, 2013). This way, the nutrients uptake shows a linear increase along with the external concentration augment, thus, LATS activity is generally not suppressed by the high NO_3^- or NH_4^+ supply, which may cause the exceeding of plant nutrient absorption (NACRY; BOUGUYON; GOJON, 2013) affecting metabolism and causing drop in leaves production.

Like in our study with yerba mate, reduced minicuttings production with increased N dose was observed in *Eucalyptus grandis* x *Eucalyptus urophylla*, that showed a quadratic response as a function of nitrogen doses, with reduced production in higher doses (more than 129 $mg.L^{-1}$) (ROCHA et al., 2015). On the other hand, for *Eucalyptus dunnii* the results as a function of N dose had an increasing linear response, with the highest dose (600 $mg.L^{-1}$) responsible for higher minicuttings production (ROSA et al., 2009). Those results also confirm the strong genetic effect in response to nitrogen use.

One of the possible causes for the production reduction with the increase of N doses is toxicity by ammonia. The toxic action of ammonia on plants can be caused by several mechanisms such as oxidative stress, reduction of carbon supply, damage to the structure of chloroplasts, inhibition of cation uptake and consequent changes in the plant's ionic balance, hormonal imbalance, reduction of photosynthesis, intracellular alkalization and extracellular acidification, inhibition of root breathing and stimulating photorespiration, high energy cost to maintain low levels of intercellular NH_4^+, among others (BITTSÁNSZKY et al., 2015; ESTEBAN et al., 2016). All these effects of ammonia excess can result in growth suppression and even plant death (ROOSTA; SCHJOERRING, 2008).

Normally, plants adapted to acidic soils use N preferably in ammoniacal form ($NH_4^{\ +}$), as verified for yerba mate seedlings (GAIAD; RAKOCEVIC; REISSMANN, 2006; ROSA et al., 2011). However, for adult yerba mate plants in the production phase, the N source preference is variable, without a general preference (SANTIN et al., 2014).

The high nitrate concentration may also have affected plant production. The concentration of external and internal nitrate affects the plant metabolism and alters the expression of specific plant genes (McALLISTER; BEATTY; GOOD, 2012). In a study with *Salix* spp. the high supply of nitrate reduced the biomass of 7 clones, suggesting that these clones were sensitive to high levels of N (Yang et al., 2015). In *Arabidopsis thaliana*, authors found a genotype that showed little tolerance to a high N supply, being inefficient under nutrition with high nitrate content; the increase in nitrate supply resulted mainly in N storage and not in biomass gain (CHARDON et al., 2010). In our study, the decrease in production (FIGURE 3) was a result of nutrients overdose instead of NO_3^- / NH_4^+ ratio imbalance, since the lowest (D1) and highest dose (D5) presented the same ratio between nitrate and ammonium (described in the methodology), and the lowest productivity was observed in D5.

Another point of discussion refers to the electrical conductivity of nutrient solutions, which increased with increasing N dose (2.21 to 4.74 $mS.m^{-2}$) (described in the methodology). Higher electrical conductivity at higher doses of N may also have contributed to lower leaf production. The greater the electrical conductivity of the solution, the bigger the amount of salts present in it. According to Flowers (2004), the inhibition of plant growth under high salinity can be explained by the decrease in osmotic potential. Thus, serious problems in water transport can be caused, leading to what is known as “salinity drought” (SILVEIRA et al., 2010).

According to Borges e Coelho (2009) a point to be observed in the use of nitrogen sources in fertigation is the cationic-anionic balance in the solution. If excess ammonium is applied, there may be a reduction in the absorption of other cations, as well as above-normal absorption of phosphates, sulphates and chlorides. The increase in nitrate causes a reduction in the absorption of phosphate and sulfates and an increase in the absorption of K^+, Ca^{2+} and Mg^{2+}. Thus, the increase in electrical conductivity can cause a nutritional imbalance (SILVEIRA et al., 2010).

In this way, salinity can cause major disturbances in plants metabolism, causing reduced growth and loss of production (SILVEIRA et al., 2010). In an experiment with *Eucalyptus* spp., Mendonça et al. (2010) found that the increase in electrical conductivity reduced the dry biomass and leaf area of seedlings of three species of eucalyptus. Thus, the increase in electrical conductivity of solutions with higher concentrations of N (FIGURA 3) may have partially contributed to a reduction in plant production.

4.5 CONCLUSION

The semi-hydroponic system results in a stable young and mature yerba mate leaf production at similar rates throughout the year with a harvest average interval of two months, and EC40 is 13 % more productive that EC22. In general, in second year the average leaf production for EC40 was 5.3 $ton.ha^{-1}$ and for EC22 it was 4.2 $ton.ha^{-1}$ (dry base).

For the EC40 genotype, we recommend the lowest dose (114 $mg.L^{-1}$) and for EC22 the best dose was 206 $mg.L^{-1}$. Future experiments with smaller doses are ought to be realized, once for EC40 the nitrogen dose response was decreasing linear and for EC22 second order polynomial; however, the absence of a minimum or maximum point within the range of values makes it impossible to calculate the maximum technical efficiency dose.

REFERENCES

BARBOSA, J. Z. et al. Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. **Anais da Academia Brasileira de Ciências**, v. 90, n. 1, p. 557–571, 2018.

BASTOS, M. C. et al. Mineral content of young leaves of yerba mate. **Pesquisa Florestal Brasileira**, v. 34, n. 77, p. 63–71, 2014.

BITTSÁNSZKY, A. et al. Overcoming ammonium toxicity. **Plant Science**, v. 231, p. 184–190, 2015.

BLUM-SILVA, C. H. et al. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. **Revista Brasileira de Farmacognosia**, v. 25, n. 1, p. 1–6, 2015.

BORGES, A. L.; COELHO, E. F. Aspectos básicos da fertirrigação. In: BORGES, A. L.; COELHO, E. F. (Eds.). **Fertirrigação em fruteiras tropicais**. 2. ed. Cruz das Almas - BA: Embrapa Mandioca e Fruticultura Tropical, 2009. p. 09–19.

BRACESCO, N. *Ilex paraguariensis* as a healthy food supplement for the future world. **Biomedical Journal of Scientific & Technical Research**, v. 16, n. 1, p. 15–18, 2019.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC n. 277, de 22 de setembro de 2005. **Regulamento técnico para café, cevada, chá, erva-mate e produtos solúveis. Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 22 set. de 2005.

BREDEMEIER, C.; MUNDSTOCK, C. M. Regulação da absorção e assimilação do nitrogênio nas plantas. **Ciência Rural**, v. 30, n. 1, p. 365–372, 2000.

BURIOL, G. A. et al. Trasmissividade a radiação solar do polietileno de baixa densidade utilizado em estufas. **Ciência Rural**, v. 25, n. 1, p. 1–4, 1995.

CARON, B. O. et al. Eficiência de conversão da radiação fotossinteticamente ativa interceptada em fitomassa de mudas de eucalipto. **Revista Árvore**, v. 36, n. 5, p. 833–842, 2012.

CARDOZO JUNIOR, E. L. et al. Quantitative genetic analysis of methylxanthines and phenolic compounds in mate progenies. **Pesquisa Agropecuária Brasileira**, v. 45, n. 2, p. 171–177, 2010.

CHARDON, F. et al. Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. **Journal of Experimental Botany**, v. 61, n. 9, p. 2293–2302, 2010.

CHARDON, F.; NOËL, V.; MASCLAUX-DAUBRESSE, C. Exploring NUE in crops and in *Arabidopsis* ideotypes to improve yield and seed quality. **Journal of Experimental Botany**, v. 63, n. 9, p. 3401–3412, 2012.

DA ROSA, L. S. et al. Efeito da dose de nitrogênio e de formulações de substratos na miniestaquia de *Eucalyptus dunnii* Maiden. **Revista Árvore**, v. 22, n. 6, p. 1025-1035, 2009.

DE SÁ, P. F. et al. Miniestaquia de erva-mate em quatro épocas do ano. **Ciência Florestal**, v. 28, n. 4, p. 1431-1442, 2018

ESTEBAN, R. et al. Review: Mechanisms of ammonium toxicity and the quest for tolerance. **Plant Science**, v. 248, p. 92–101, 2016.

FLOWERS, T. J. Improving crop salt tolerance. **Journal of Experimental Botany**, v. 55, n. 396, p. 307–319, 2004.

GAIAD, S.; RAKOCEVIC, M.; REISSMANN, C. B. N sources affect growth, nutrient content, and net photosynthesis in mate (*Ilex paraguariensis* St. Hil.). **Brazilian Archives of Biology and Technology**, v. 49, n. 5, p. 689–697, 2006.

HELM, V. C. et al. **Efeito do solvente na extração de teobromina e cafeína em progênies de erva-mate**. Colombo: Embrapa Florestas, 2015. 6 p. (Embrapa Florestas. Comunicado Técnico, 363).

IBGE. Produção Agrícola Municipal (PAM). 2018. Available from https:// https://sidra.ibge.gov.br/pesquisa/pam/tabelas. Accessed 5 November, 2019b.

IBGE. Produção da Extração Vegetal e da Silvicultura 2018 – PEVS 2018. Rio de Janeiro, v. 33, p. 1-8, 2019b.

MASCLAUX-DAUBRESSE, C. et al. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. **Annals of Botany**, v. 105, n. 7, p. 1141–1157, 2010.

MCALLISTER, C. H.; BEATTY, P. H.; GOOD, A. G. Engineering nitrogen use efficient crop plants: The current status. **Plant Biotechnology Journal**, v. 10, n. 9, p. 1011–1025, 2012.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, p. 522–530, 2017.

MEINHART, A. D. et al. Methylxanthines in 100 Brazilian herbs and infusions: Determination and consumption. **Emirates Journal of Food and Agriculture**, v. 31, n. 2, p. 125–133, 2019.

MENDONÇA, A. V. R. et al. Características fisiológicas de mudas de *Eucalyptus* spp submetidas a estresse salino. **Ciência Florestal**, v. 20, n. 2, p. 255–267, 2010.

NACRY, P.; BOUGUYON, E.; GOJON, A. Nitrogen acquisition by roots: Physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. **Plant and Soil**, v. 370, n. 1–2, p. 1–29, 2013.

NASCIMENTO, B. et al. Nitrogenated fertilization favors vegetative rescue and propagation of *Ilex paraguariensis*. **Cerne**, v. 25, n. 1, p. 76–83, 2019.

OLIVEIRA, E. V. et al. Composição nutricional de procedência e progênies de erva-mate (I*lex paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. **Ciência Florestal**, v. 24, n. 4, p. 793–805, 2014.

PANDOLFO, C. M. et al. Resposta da erva-mate (*Ilex paraguariensis* St. Hil.) a adubação mineral e orgânica em um latossolo vermelho aluminoférrico. **Ciência Florestal**, v. 13, n. 2, p. 37–45, 2003.

PENTEADO JUNIOR, J. F.; GOULART, I. C. G. DOS R. **Erva 20:** sistema de produção de erva-mate. Brasília - DF: Embrapa, 2019.

PIMENTEL, N. et al. Productivity of mini-stumps and rooting of mini-cuttings of erva mate (*Ilex paraguariensis* A. St.-Hil.) clones. **Ciência Florestal**, v. 29, n. 2, p. 559–570, 2019.

PIRES, P. et al. Sazonalidade e soluções nutritivas na miniestaquia de *Araucaria angustifolia* (Bertol.) Kuntze. **Revista Árvore**, v. 39, n. 2, p. 283–293, 2015.

PIRES, P. P. et al. Climatic oscillations in the production of *Eucalyptus benthamii* x *E. dunnii* shoots in mini-clonal hedge. **Bosque**, v. 28, n. 3, p. 487-494, 2017.

POLETTO, I. et al. Influência da inoculação de *Fusarium* spp. e níveis de sombreamento no crescimento e desenvolvimento da erva-mate. **Ciência Florestal**, v. 20, n. 3, p. 513–521, 2010.

REIS, M. G. **Practical guide: Conversion factors and general equations applied in agricultural and forest meteorology**. Available in: <www.researchgate.net/publication/3204568912019>. Access in 5 nov. 2019.

RESENDE, D. V. M. et al. **Programa de melhoramento da erva-mate coordenado pela Embrapa: resultados da avaliação genética de populações, progênies, indivíduos e clones.** Colombo: Embrapa Florestas, 2000, 60 p. (Embrapa Florestas. Circular Técnica, 43).

RIACHI, L. G.; DE MARIA, C. A. B. Yerba mate: An overview of physiological effects in humans. **Journal of Functional Foods**, v. 38, p. 308–320, 2017.

ROCHA, J. H. T. et al. Produtividade do minijardim e qualidade de miniestacas de um clone híbrido de *Eucalyptus grandis* x *Eucalyptus urophylla* (I-224) em função de doses de nitrogênio. **Ciência Florestal**, v. 25, n. 2, p. 273–279, 2015.

ROOSTA, H. R.; SCHJOERRING, J. K. Effects of nitrate and potassium on ammonium toxicity in cucumber plants. **Journal of Plant Nutrition**, v. 31, n. 7, p. 1270–1283, 2008.

ROSA, L. S. et al. **Adubação nitrogenada na fertirrigação de minicepas de** *Ilex paraguariensis* St. Hil. Anais do 5º Congreso Sudamericano De La Yerba Mate. **Anais**...Posadas: INYM/ INTA/INaM, 2011.

SANTIN, D. et al. Growth of mate tea tree seedlings fertilized with N, P and K. **Scientia Agraria**, v. 9, n. 1, p. 59–66, 2008.

SANTIN, D. et al. Crescimento e nutrição de erva-mate influenciados pela adubação nitrogenada, fosfatada e potássica. **Ciência Florestal**, v. 23, n. 2, p. 363–375, 2013.

SANTIN, D. et al. **Fontes de nitrogênio e técnicas de propagação de mudas atuam na produtividade de erva-mate**. 6º Congresso Sudamericano de Yerba Mate. **Anais**...Montevideo: 2014.

SANTIN, D. et al. Intervalos de colheita e adubação potássica influenciam a produtividade da erva-mate (*Ilex paraguariensis*) no Estado do Paraná. **Floresta**, v. 46, n. 4, p. 509–518, 2017a.

SANTIN, D. et al. Manejo de colheita e adubação fosfatada na cultura da erva-mate (*Ilex paraguariensis*) em fase de produção. **Ciência Florestal**, v. 27, n. 3, p. 783–797, 2017b.

SANTIN, D. et al. Adubação nitrogenada e intervalos de colheita na produtividade e nutrição da erva-mate e em frações de carbono e nitrogênio do solo. **Ciência Florestal**, v. 29, n. 3, p. 1199, 2019.

SILVEIRA, J. A. G. et al. Mecanismos biomoleculares envolvidos com a resistência ao estresse salino em plantas. In: GHEYI, H. R.; DIAS, N. DA S.; LACERDA, C. F. (Eds.). . **Manejo da salinidade na agricultura: estudos básicos e aplicados**. 1. ed. Fortaleza: INCTSal, 2010. p. 167–185.

STUEPP, A. C. et al. Successive mini-cuttings collection in *Piptocarpha angustifolia* ministumps: effects on maturation, adventitious root induction and root vigor. **Acta Scientiarum**, v. 39, n. 2, p. 245-253, 2017.

WENDLING, I. et al. Early selection and classification of yerba mate progenies. **Pesquisa Agropecuaria Brasileira**, v. 53, n. 3, p. 279–286, 2018.

WENDLING, I.; BRONDANI, E. Produção de mudas de erva-mate. In: WENDLING, I.; SANTIN, D. **Propagação e nutrição da erva-mate**. Brasília: Embrapa, 2015. p. 24-50.

WENDLING, I.; DUTRA, L. F.; GROSSI, F. Produção e sobrevivência de miniestacas e minicepas de erva-mate cultivadas em sistema semi-hidrônico. **Pesquisa Agropecuaria Brasileira**, v. 42, n. 2, p. 289–292, 2007.

WENDLING, I.; DUTRA, L. F. **Solução nutritiva para condução de minicepas de erva-mate (Ilex paraguariensis St. Hil.) em sistema semi-hidropônico**. Colombo: Embrapa Florestas, 2008. 4 p. (Embrapa Florestas. Circular técnica, 157).

WESTPHALEN, D. J. et al. Impact of different silvicultural techniques on the productive efficiency of *Ilex paraguariensis* A.St. Hill. **Agroforestry Systems**, 2019.

XING, Y. et al. A review of nitrogen translocation and nitrogen-use efficiency. **Journal of Plant Nutrition**, v. 42, n. 19, p. 2624–2641, 2019.

XU, G.; FAN, X.; MILLER, A. J. Plant nitrogen assimilation and use efficiency. **Annual Review of Plant Biology**, v. 63, n. 1, p. 153–182, 2012.

YANG, W. et al. Variations of growth, nitrogen accumulation and nitrogen use efficiency among 18 willow clones under two nitrogen regimes. **Agroforestry Systems**, v. 89, n. 1, p. 67–79, 2015.

CHAPTER 4

5 BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF YERBA MATE CULTIVATED IN A SEMI-HYDROPONIC SYSTEM: EFFECT OF NITROGEN FERTIGATION, SEASONALITY, LEAF TYPE AND GENOTYPE

ABSTRACT

Considering yerba mate phytochemical potential and its importance for several industrial uses, the understanding of factors that influence the fluctuation content of bioactive compounds is of primary relevance. Therefore, this study aimed to evaluate phytochemical content of yerba mate cultivated in semi-hydroponic system seeking to elucidate the seasonality, nitrogen fertigation, leaf type and genotype effect. We conducted cuttings in a semi-hydroponic system in gutters for two years. During this period, we evaluated the N fertigation (114, 206, 380, 760 and 1142 mg.L^{-1} doses), genotypes (EC40 - high caffeine and EC22 - decaffeinated), seasonality (11 harvests) and leaf type (young and mature leaves) in total phenolic compounds, methylxanthines (caffeine and theobromine), caffeoylquinic acids (3-caffeoylquinic, 4-caffeoylquinic e 5-caffeoylquinic acids) content, and antioxidant capacity by DPPH and ABTS radicals. We noted an inverse content amongst caffeine and theobromine for the genotypes EC40 (high caffeine) and EC22 (high theobromine). The compounds content and antioxidant capacity altered throughout the 11 harvests, confirming the seasonality effect. Young leaves presented a larger phytochemical potential than the mature leaves for both genotypes. Caffeoylquinic acids have demonstrated inversely proportional response to N doses meanwhile caffeine and theobromine had their content increased at the largest N dose. Thus, the semi-hydroponic system for production of selected yerba mate genotypes has come to muster great potential, once it promotes leaf production during all year with differentiated phytochemical profile in terms of content.

Key words: *Ilex paraguariensis*. Caffeine. Chlorogenic acid. Young leaves. Mature leaves.

5.1 INTRODUCTION

Ilex paraguariensis A. St.-Hil. is a native plant of South America and has a distribution area of 540.000 km², covering parts of Brazil territories, Argentina, and Paraguay (OLIVEIRA; ROTTA, 1983). The habit of consuming beverages was adopted centuries ago by native folks, the guarani, and has become traditional in its occurring region, consumed mainly as *chimarrão* or *tereré* (BRACESCO et al., 2011; BLUM-SILVA et al., 2015). More recently, there has been an increasing interest in the development of new forms of yerba mate consumption, such as capsules, tablets, pills, and other manufactured products (BECKER et al., 2019). There has been also an increasing interest in teas, energetics and cosmetics lines of products, due to phytochemical compounds that are beneficial to human health (BRACESCO, 2019).

Mateos et al. (2018) defined *Ilex paraguariensis* as an important source of polyphenols and with moderate rate of methylxanthines (caffeine + theobromine) among 8.21 and 10.25 mg.g^{-1}. Furthermore, it has high antioxidant capacity, associated with polyphenols composition, being caffeoyl derivatives (3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid) the main ones found in the specie (BLUM-SILVA et al., 2015; MEINHART et al., 2017). Flavonoids, saponins, and tannins are also present in the leaves (BRACESCO, 2019).

Yerba mate is a plant rich in bioactive compounds, considered as a functional food. The benefic nutritional and medicinal effects associated with yerba mate ingestion are attributed specially to polyphenol and methylxanthines (BLUM-SILVA et al., 2015; MATEOS et al., 2018). Researches held in previous years assert that yerba mate has antibiotic, antioxidant, and anti-diabetic properties. It also has a diuretic effect, it stimulates the central nervous system, has protective cardiovascular effects, also antimutagenic and lipids reduction (GAN et al., 2018; BRACESCO, 2019). The cancerous cell proliferation reduction was also noticed by Amigo-Benavent et al. (2017); Thus, yerba mate is a species that breeds a high potential for cancer prevention.

Methylxanthines and phenolic compounds contents are influenced by plant genetic (CARDOZO JUNIOR et al., 2007; CARDOZO JUNIOR et al., 2010). In a study with 51 yerba mate progenies, the caffeine and theobromine contents varied according to plants origin and presented elevated numbers of individual heritability, pointing out a high genetic control (CARDOZO JUNIOR et al., 2010). Frizon et al. (2015) studied 111 yerba mate samples coming from 3 regions of Paraná state / Brazil and found significative differences of total phenolic compounds for each region, which was justified by influences of agronomic practices, genetic lineage, weather, growing techniques, harvest time, and plant age, among other factors.

Plant cultivation conditions are decisive in phytochemicals production (BLUM-SILVA et al., 2015). According to a review by Gobbo-Neto e Lopes (2007), despite the existence of genetic control, secondary metabolites represent a chemical interface between plants and the environment; therefore, their synthesis is often affected by environmental conditions, such as water and nutrient availability, temperature, and radiation. In this context, Shubert et al. (2006) confirmed the mean seasonal fluctuations of methylxanthines (1.84 to 9.77 $mg.g^{-1}$) for *Ilex paraguariensis* populations throughout the year. The plant age, sex, as well as the different plant organs can also influence the number of metabolites produced and components relative proportions. For Blum-Silva et al. (2015) the age of yerba mate leaves directly influenced methylxanthines and total phenolic compounds levels, which was smaller in older leaves. In terms of nutrition, Palumbo, Putz e Talcott (2007) found that caffeine levels in *Ilex vomitoria* increased 5 - 10 times as a function of nitrogen fertilization whereas phenolic compounds were higher in the control.

Regarding the species potential, the semi-hydroponic cultivation system in a protected environment presents itself as an innovative option for yerba mate, specially for selected genetic material in phytochemical terms. This cultivation system provides yearlong raw material for a variety of industrial uses and, hence, a greater added value. The genotypes cultivated in semi-hydroponic system in this research came from a genetic breeding program developed by EMBRAPA Forests (Brazilian Agricultural Research Corporation) that started in 1997, where the green mass production is one of the main selection factors (RESENDE et al., 2000). More recently, progenies have been selected according to the content of bioactive compounds such as caffeine (CARDOZO JUNIOR et al., 2010). Thus, this study aimed to evaluate the phytochemical content of yerba mate cultivated in semi-hydroponic system seeking to elucidate the effect of seasonality, nitrogen fertigation leaf type and genotype.

5. 2 MATERIAL AND METHODS

5.2.1 Experimental conditions and treatments

Research was conducted between June 2016 and February 2019 in the Forest Species Propagation Laboratory of Embrapa Forestry, located in Colombo – PR / Brazil (25°20’ S and 49°14’ W, 950 m). According to Köppen, the climate of the region is temperate, type Cfb. The rooted cuttings obtained through the cuttings process described by Wendling and Brondani (2015) were produced from epicormic shoots of two 10-years-old mother plants (identified as genotypes EC22 and EC40) from a provenance and progeny trial installed in Ivaí – PR / Brazil (WENDLING et al., 2018) based on the genetic breeding program of EMBRAPA (RESENDE et al., 2000). This genetic material was selected by caffeine content according to previous study where the authors considered caffeinated (EC40) with 2.35 % caffeine content and decaffeinated (EC22) with 0.02 % caffeine content (HELM et al., 2015). According to ANVISA's resolution n ° 277 a decaffeinated product has a maximum of 0.1 % (g/100g) of the caffeine (BRASIL, 2005).

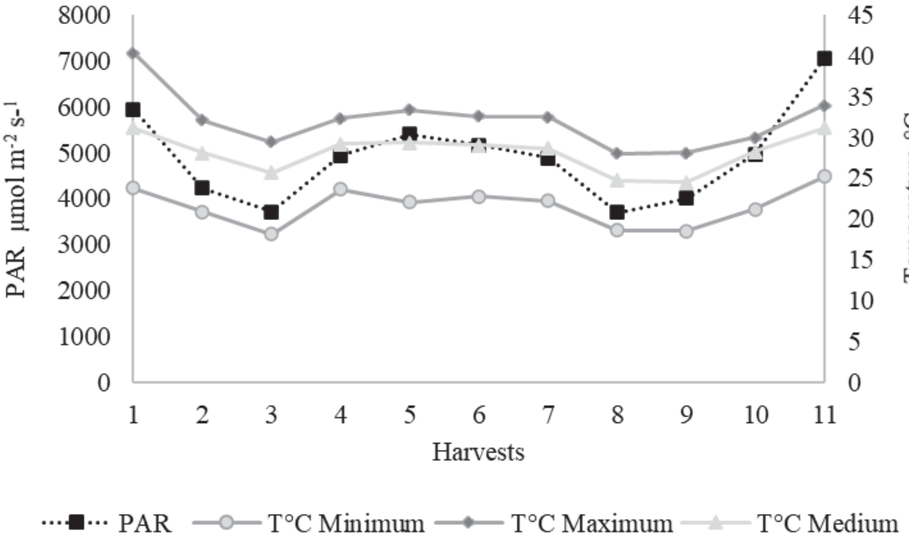
EC22 and EC 40 rooted cuttings with approximately 120 days and 15 cm height were transferred to a semi-hydroponic gutter system filled with medium sand. Cuttings were planted at a 15 x 15 cm spacing inside a non-acclimatized greenhouse. We used the nutrient solution described by Wendling e Brondani (2015) as a standard; it comprises the following nutrients: N-NO_3^- (156.0 $mg.L^{-1}$), N-NO_4^+ (50.0 $mg.L^{-1}$), P^- (25.0 $mg.L^{-1}$), K^+ (200.0 $mg.L^{-1}$), Ca^{2+} (200.0 $mg.L^{-1}$), Mg^{2+} (45.0 $mg.L^{-1}$), S^- (76.9 $mg.L^{-1}$), B (1.5 $mg.L^{-1}$), Cu^{2+} (0.1 $mg.L^{-1}$), Fe^{2+} (5.0 $mg.L^{-1}$), Mn^{2+} (1.0 $mg.L^{-1}$), Zn^{2+} (0.7 $mg.L^{-1}$), Mo^{2-} (0.07 $mg.L^{-1}$). In this part of ministumps establishment, the nutrient solution described above was diluted in half, the electrical conductivity was maintained at 1.2 $mS.m^{-2}$, 25 °C, and the pH was adjusted to 5.5 (± 0.1).

From February 2017 to February 2019, we added the nutrient solution with the total nutrient concentration, varying only the nitrogen doses per gutter among treatments (FIGURE 1-C). N concentrations was established as follows (N-NO_3^-- N-NH_4^+; N-Total $mg.L^{-1}$; ratio NO_3^-/NH_4^+): D1(64 – 50; 114 mg L^{-1}; 1.28); D2 (156 – 50; 206 mg L^{-1}; 3.12) standard; D3(243 – 137; 380 $mg.L^{-1}$; 1.77); D4 (433 – 327; 760 $mg.L^{-1}$; 1.32); D5 (624 – 518; 1142 $mg..L^{-1}$; 1.20). The electrical conductivity of the solution was maintained at the following values: D1- 2.21, D2 - 2.47, D3 - 3.00, D4 - 3.76, and D5 - 4.74 $mS.m^{-2}$, 25 °C; and pH was adjusted at 5.5 (± 0.1) every week. The other nutrients were maintained as described in the standard solution. The ministumps received nutrient solution through a drip fertigation system three times a day in a total daily water flow of 5 L m^{-2} (summer and spring) and 3.6 L m^{-2} (winter and autumn). In the heat season (when the temperature reaches 30º C), greenhouse sides were opened and a sprinkler system was turned on for 30 s every 60 min from 10 am to 7 pm, totalizing a daily flow of 3.75 $L.m^{-2}$ water. We washed the sand after each harvest (± two months) with 40 L water per gutter.

The clonal plants received a nutrient solution through a drip fertigation system three times a day in a total daily flow of 5 $L.m^{-2}$ (summer and spring) and 3.6 $L.m^{-2}$ (winter and autumn). In the heat season (when the temperature reaches 30º C), the sides of the greenhouse were opened and the sprinkler system is turned on for 30 s every 60 min from 10 am to 7 pm, totalizing 3.75 $L.m^{-2}$ of water per day.

The temperature (°C) was monitored twice a day using a maximum and minimum thermometer (Equitherm TM-38CAP). The Photosynthetically active radiation data (PAR) (μmol.m^{-2}.s^{-1}) was calculated from the global solar radiation (RG) measure (kJ.m^{-2}.h^{-1}), obtained at the SIMEPAR Weather Station (Paraná Meteorological System), approximately 5 kilometers to the greenhouse where the experiment was conducted. A reduction of 40 % in solar radiation input in the greenhouse due to the plastic used to cover it was discounted; the calculations considered the difference between the measuring by luxmeter with three reading periods (8 hours, 13 hours, 17 hours), inside and outside of the greenhouse, in an open area without shading. The conversion of RG into PAR was carried out following Reis (2019), where RG in kJ m^{-2} h^{-1} * 0.2778 = W m^{-2} and 1 W m^{-2} is about 2.02 μmol m^{-2} s^{-1} of PAR.

FIGURE 1 – CLIMATE DATA, MEAN TEMPERATURES (MAXIMUM MINIMUM AND MEDIUM) AND PHOTOSYNTHETICALLY ACTIVE RADIATION MEAN.



SOURCE: The author (2020).
NOTE: Additional information (harvest, season, year): (1, summer, 2017; 2, autumn, 2017; 3, winter, 2017; 4, spring, 2017; 5, spring, 2017; 6, summer, 2018; 7, autumn, 2018; 8, winter, 2018; 9, winter, 2018; 10, spring, 2018; 11, summer, 2019). The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

The harvesting point was determined when plants reached 50 - 60 cm height, always keeping 30 % of leaves in the ministumps. After harvests, the leaves were dried in a microwave oven for 5 min (Electrolux®1000 W, 2450 MHz) and weighed for dry mass determination. We evaluated leaf production per ministump and transformed it into leaf production per square meter per month.

The experiment was conducted in five gutters and each with one nitrogen dose. Five repetition with five clonal plants per repetition of each genotype (EC22 and EC40) were planted in the gutters. We did eleven harvestings during two years of cultivation, collecting the two types of leaves (young and mature). Leaf type was terminated by coloring and leaf expansion. Young leaves are light green and more tender and mature leaves are dark green and completely expanded. The table 1 showed the harvests with corresponding season and dates.

TABLE 1 - DESCRIPTION OF HARVEST TIMES

Harvest number	**Seasons**	**Day/month/year**	**Growing year**
1	summer	20/03/2017	1
2	autumn	12/05/2017	1
3	winter	08/08/2017	1
4	spring	10/10/2017	1
5	spring	04/12/2017	1
6	summer	15/02/2018	1
7	autumn	19/04/2018	2
8	winter	25/06/2018	2
9	winter	18/09/2018	2
10	spring	12/12/2018	2
11	summer	11/02/2019	2

SOURCE: The author (2020)

5.2.2 Aqueous extracts preparation

For the preparation of aqueous extracts, we diluted 10 mg of powder sample with 2 mL of MilliQ water. The material was mixed in a vortex Genie2® for 30 s and the extraction was carried out in a Thermomixer Eppendorf® equipment at 450 rpm for 1 h at 60 ºC. The extracts were then filtered through a 0.22 µm filter.

5.2.3 Content of phenolic compounds

We determined the content of total phenolic compounds by the spectrophotometric method of Folin-Ciocalteau (SINGLETON; ROSSI, 1965) with minor modifications. In a volumetric flask, we added 0.1 mL of the extract, 6.0 mL of distilled water and 0.5 mL of the Folin-Ciocalteau reagent, followed by 1 min of stirring. Afterwards, 2 mL of 15 % aqueous

Na_2CO_3 in water (w/v) solution was added, followed by stirring for 30 s. The final volume was adjusted with distilled water to reach 10 mL. After 2 h of reaction in the dark, the absorbance was measured at 760 nm in a Shimadzu®-1800 UV/VIS spectrophotometer. As reference, we established an analytical curve with gallic acid within the range of 0.25-13 mg.L^{-1}. The results were expressed in mg gallic acid equivalent (GAE) per gram of sample (mg GAE.g^{-1}) on a dry basis.

5.2.4 Antioxidant capacity (DPPH and ABTS radicals)

The antioxidant capacity of extracts by free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined according to Brand-Willians et al. (1995) methodology with minor modifications. First, we added 0.1 mL of sample to 3.9 mL of DPPH methanolic solution (0.06 mmol.L^{-1}); the reaction occurred in the dark at room temperature for 30 min, and the absorbance was then measured at 515 nm (BRAND-WILLIANS; CUVELLIER; BERSET, 1995).

The antioxidant capacity of extracts by free radicals ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was determined by reacting 10 mL ABTS (7 mmol.L^{-1}) with 176 µL potassium persulfate (140 mmol.L^{-1}) in the dark at room temperature for 16 h. An aliquot of 1 mL of this solution was added in 100 mL of sodium acetate buffer (20 mmol.L^{-1}) pH 4.5. The absorbance was adjusted to 0.7 ± 0.05. From this adjusted solution, 3 mL was added to 30 µL of the extract. After 2 h the absorbance was measured (RE et al., 1999; YIM et al., 2013) with minor modifications.

All antioxidant activities assays were performed in a spectrophotometer UV/VIS (Shimadzu, model 1800, Kioto, JPN). The results were compared with a standard curve (Trolox 0-1000 µmol.L^{-1} for DPPH and 0–2500 µmol.L^{-1} for ABTS) and expressed in µmol Trolox equivalent per gram of sample (TEAC µmol.g^{-1}) on dry basis.

5.2.5 LC-UV (liquid chromatography with ultraviolet detection)

For the chromatographic analyses, we used a Shimadzu® liquid chromatograph (UFLC), controlled by the LC solution software and equipped with automatic injector and UV detector (SPD-20A). Separation of compounds was performed using Shim-Pack CLC-ODS (M) C18 column (250 x 4.6 mm i.d., 5 µm particle size), protected by Shim-Pack CLC G-

ODS pre-column (100 x 4.0 mm id) both from Shimadzu (Kyoto, Japan). The separation of compounds in the aqueous extract (20 µL of injection) was conducted at 30°C with a flow rate of 0.5 mL.min^{-1}. The mobile phases consisted of a gradient elution of water with acetic acid (99.9:0.1, v/v) (solvent A) and acetonitrile Merck® 100 % (solvent B). Compound detection was performed at the fixed wavelength of 280 nm. The gradient elution program was: 0-15 min (3-3 % B), 15-20 min (3-20 % B), 20-40min (20-20 % B), 40-45min (20-30 % B), 45-55 min (30-100 % B), 55-75 (100-100 % B), 75-80 (100-3 % B) and 80-95 (3-3 % B).

The identification and quantification of 1,3,7-trimethylxanthine (caffeine) and 3,7-dimethylxanthine (theobromine) was performed by an analytical curve of caffeine and theobromine Sigma® standards at range of 0 to 1.0 mg.mL^{-1} and 0 to 0,5 mg.mL^{-1} respectively. The identification of caffeoylquinic acids (3-caffeoylquinic acid (3CQA), 4-caffeoylquinic acid (4CQA), 5-caffeoylquinic acid (5CQA)) by Sigma® standards and semi-quantification was performed by an analytical curve at range of 0 to 10 mg.mL^{-1} of the 3-CQA Sigma®. Results were expressed as mg of compound per g sample (mg.g^{-1}) on dry basis.

5.2.6 LC/MS (Liquid chromatography–mass spectrometry)

This analysis was performed to confirm the identity of compounds quantified and identified by LC/UV. The LC-MS conditions were optimized using standards of caffeoylquinic acid isomers (identified by 3-caffeoylquinic acid), caffeine (1,3,7-trimethylxanthine), and theobromine (3,7-dimethylxanthine). Positive ionization mode was the most efficient mechanism for ionizing caffeine (*m/z* 195.0896) and theobromine (*m/z* 181.0738), while the negative ionization mode was the most effective for ionizing 3-caffeoylquinic acid (*m/z* 353.0899).

LC-MS analyses was performed on a Prominence Ultra Fast Liquid Chromatograph (UFLC) Shimadzu®, with an autosampler maintained at 10 °C. The separation of compounds was performed using Synergi Fusion-RP 80A column (150 x 2.0 mm i.d, 4 µm particle size) Phenomenex® maintained at 40 °C. The mobile phase consisted of Solvent A (water) and Solvent B (acetonitrile), both containing 0.1 % formic acid. The gradient condition was as follows: 0–5 min (3-3 % B), 5–15 min (3-30 % B), 15–18 min (30-100 % B), 18–25 min (100-100 % B), 25-30 min (100-3 % B) and 30-35 (3-3 % B). The flow rate was 0.2 mL.min$^{−1}$ and the injection volume was 2 µL. The chromatographic method was ajusted using HPLC method transfer calculator (based on the methodology of LC-UV). The chromatograph was coupled to the mass spectrometer Bruker® MicroToF-QII equipped with a syringe pump and an electrospray (ESI) ion source. The ESI source operated in positive and negative ionization modes with a capillary voltage of 4500V or −4500 V, respectively. Source temperature was set at 180 °C for both ionization modes. The high purity nitrogen was produced using a high-purity nitrogen generator and used as desolvation gas with 6 L.min^{-1} flow and pressure at 2 bar, for both ionization modes. MS data were collected over a range of 100–800 m/z. All data were acquired using DataAnalysis 4.1 software.

5.2.7 Statistical analysis

The experiment was conducted in a randomized and factorial design (2 x 2 x 5 x 11) where the factors were two genotypes (EC 22 and EC 40), two leaf type (young and mature), five nitrogen doses (D1, D2, D3, D4 and D5), and eleven harvests (over two years). The data did not show normality (Shapiro-Wilk teste $P < 0.05$) and GLM (generalized linear models) did not adjust. Therefore, we used non-parametric analysis by Kruskal Wallis ($p < 0.05$). The statistical R software was used (R CORE TEAM, 2016).

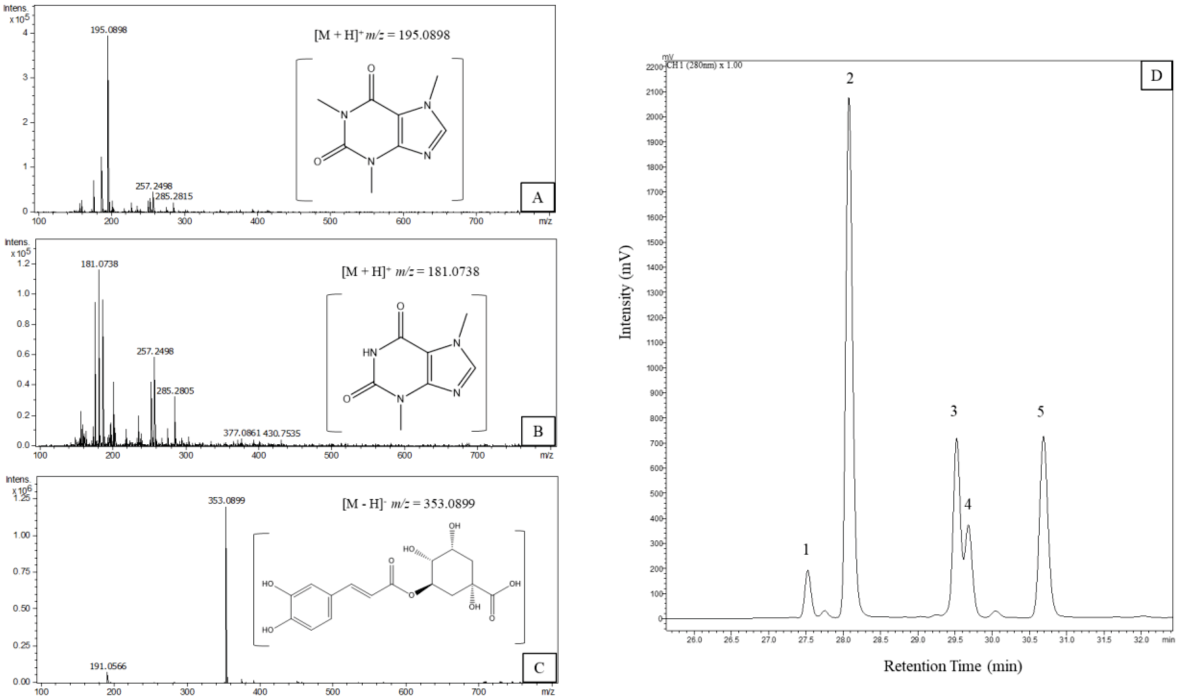
5.3 RESULTS

The compounds were identified at the LC/UV comparing to retention time (RT) of compounds in standard samples. The elution order was theobromine, 5-caffeyolquinic, 4-caffeoylquinic, 3- caffeoylquinic and caffeine with respective retention time (27.5, 28.1, 29.5, 29.7 e 30.7 minutes) (FIGURE 1D). Compounds were also confirmed by LC/MS through caffeine ionization $[M + H]^+$ (*m/z* 195.0896) (FIGURE 1A) ion fragment (*m/z* 138.0675*)*, theobromine $[M + H]^+$ (*m/z* 181.0738) (FIGURE 1B) ion fragment (*m/z* 163.0630*)* and 3-caffeoylquinic acid $[M - H]^-$ (*m/z* 353.0899) (FIGURE 1C) ion fragment (*m/z* 191.0577*)*.

We must highlight that caffeoylquinic acids (3, 4 and 5) are isomers; in other words, both composts present the same mass. Thus, the identification occured by elution order of LC/UV standards.

We observed a significative effect for harvest, nitrogen dose, leaf type, and genotype factors ($p < 0.05$) for caffeine, theobromine, total methylxanthines (sum of caffeine and theobromine); 3-caffeoylquinic acid, 4-caffeoylquinic acid, 5-acid caffeoylquinic totals (sum of 3-caffeoylquinic, 4-caffeoylquinic and 5-caffeoylquinic acids), total phenolic compounds, and antioxidant capacity (ABTS and DPPH radicals). This way, we decided to split the levels of genotype factor for the analysis of harvest, leaf type, and N dose effects. For the compound 3-caffeoylquinic acid and total caffeoylquinic acids, the genotype factor was not significant ($p > 0.05$); therefore, the mean of the two genotypes was considered for analysis of harvest, leaf type, and N dose.

FIGURE 2 –MOLECULAR IONS EXTRACTION OF COMPOUNDS BY LC / MS AND LC / UV CHROMATOGRAM WITH THE ELUATION ORDER OF COMPOUNDS.



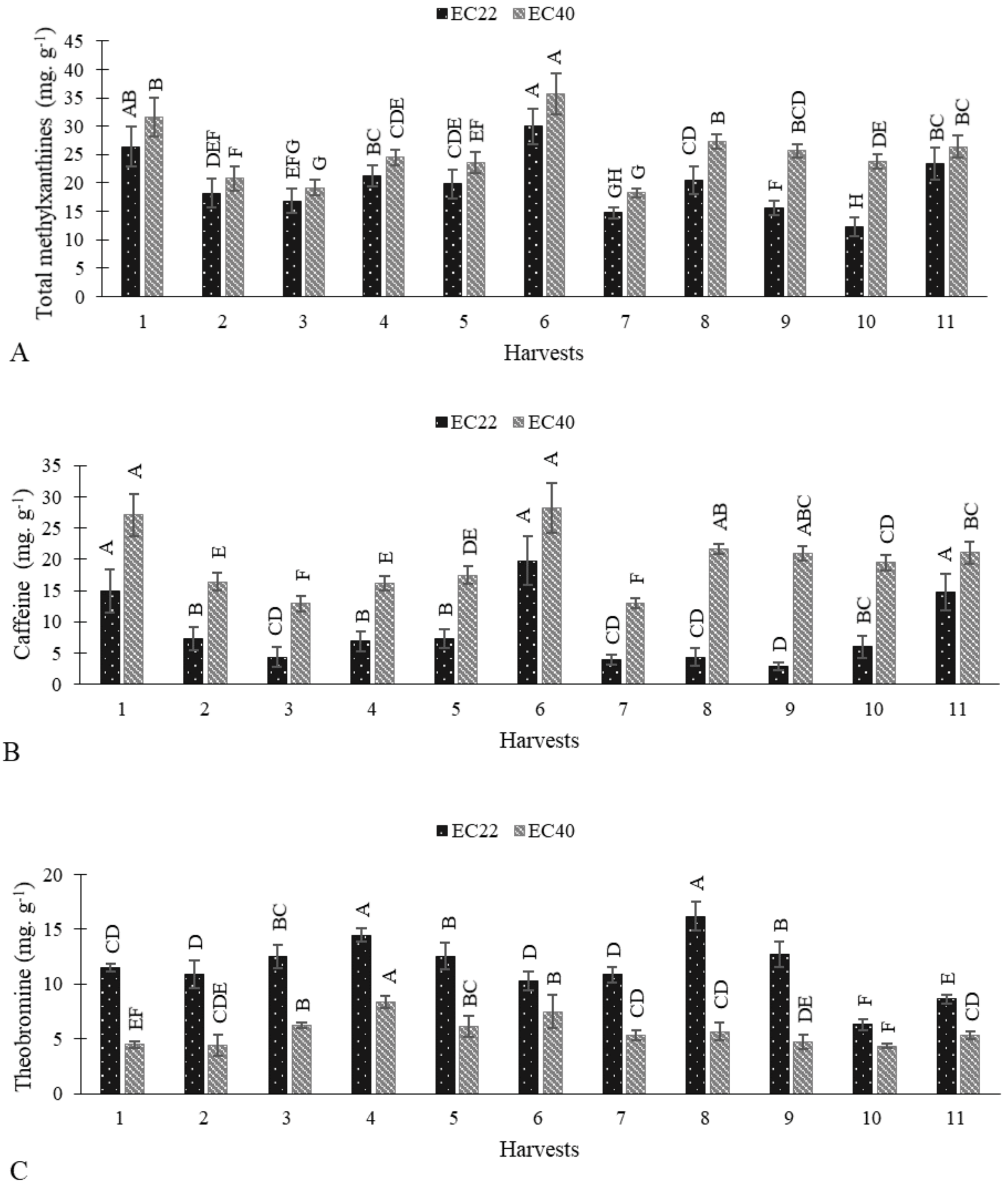
SOURCE: The author (2020).
SUBTITLE: A = Caffeíne $[M + H]^+$ (*m/z* 195.0896); B = Theobromine $[M + H]^+$ (*m/z* 181.0738); C = 3-caffeoylquinic acid $[M - H]^-$ (*m/z* 353.0899); D = LC / UV chromatogram being: 1: theobromine (RT 27.5 min), 2: 5-caffeoylquinic acid (RT 28.1 min), 3: 3-caffeoylquinic acid (RT 29.5 min), 4: 4-caffeoylquinic acid (RT 29.7 min) and 5: Caffeine (RT 30.7 min).

5.3.1 Effect of seasonality on the content of bioactive compounds and antioxidant capacity of the EC22 AND EC40 genotypes

Along two years of growth in semi-hydroponic system, 11 harvests were realized, the 1 to 6 harvests were in the first year and the 7 to 11 in the second year (Table 1). The year seasons were used to define the harvest date, when crops would reach the harvesting point (50 and 60 cm height). The aqueous extract content of total methylxanthines (sum of caffeine and theobromine), caffeine, and theobromine for yerba mate genotypes EC22 and EC40 are in Figure 3A, 3B, and 3C respectively. The EC22 genotype showed an average variation of total methylxanthines of 12.4 - 30.1 $mg.g^{-1}$ and EC40 of 18.3 - 35.7 $mg.g^{-1}$ over the 2 years cultivation (FIGURE 3A).

The genotypes showed differential profile in the production of theobromine and caffeine. Theobromine synthesis for EC22 was on average 50 % higher than EC40, whereas for caffeine the EC40 genotype produced an average of 56 % more than EC2 (FIGURE 3B and 3C). We noted that in summer season - for crops 1, 6 and 11 - caffeine synthesis for both genotypes was stimulated; however EC40 showed greater stabilization in caffeine content, as well as in theobromine and total methylxanthines during second year (from harvest 7). Variation in the content of compounds during the harvests, under the influence of temperature and radiation oscillation (FIGURE 1) was 6.3 - 14.6 $mg.g^{-1}$ and 4.4 - 8.4 $mg.g^{-1}$ for EC22 and EC40 respectively for theobromine, and 2.9 - 19.8 $mg.g^{-1}$ (EC22) and 12.9 - 28.3 $mg.g^{-1}$ (EC40) for caffeine.

FIGURE 3 – VARIATION OF THE TOTAL METHYLXANTHINES, CAFFEINE AND THEOBROMINE CONTENT FOR GENOTYPE EC22 AND EC40 THROUGHOUTS THE HARVESTS



SOURCE: The author (2020).

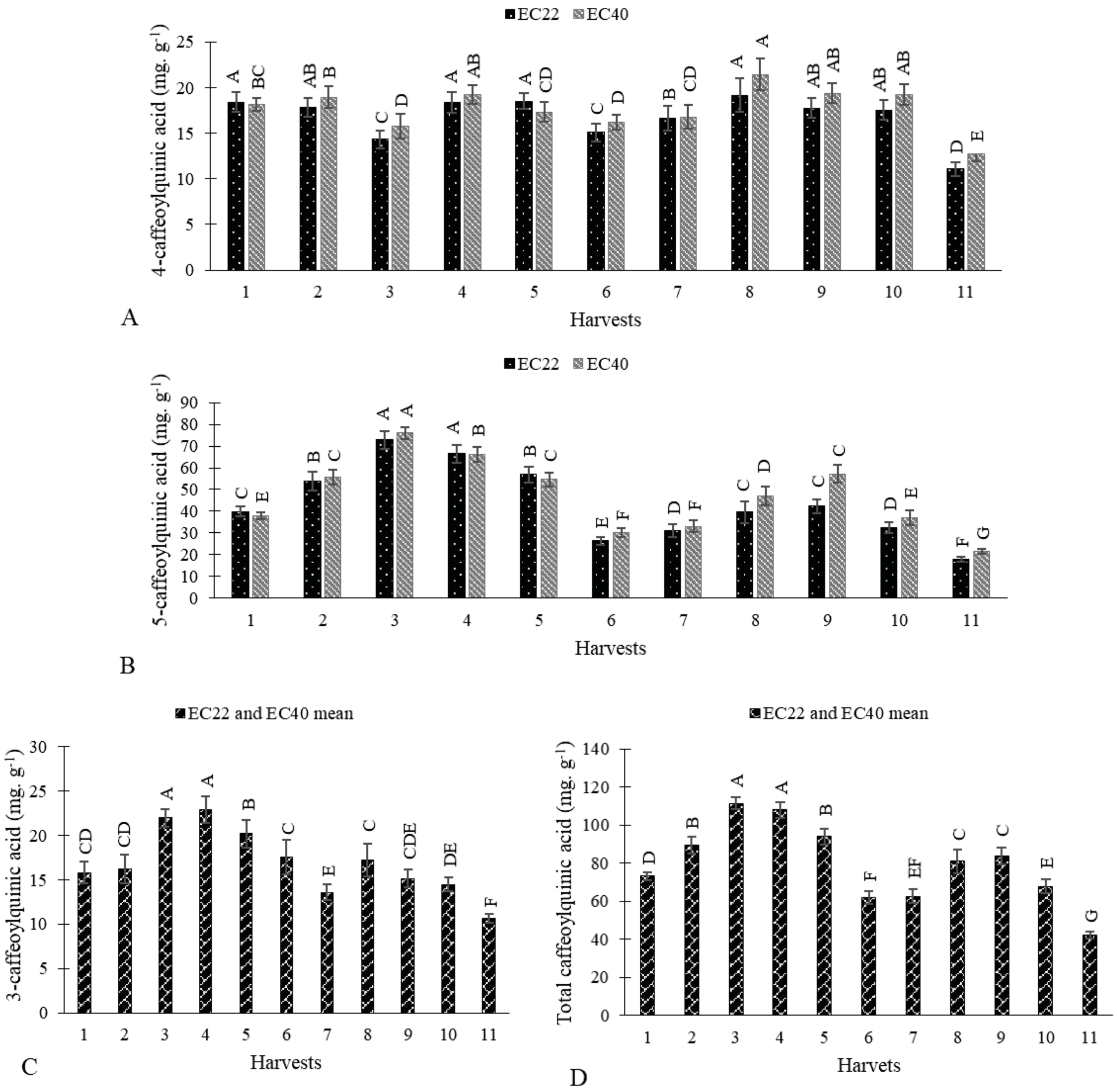
NOTE: Additional information (harvest, season, year): (1, summer, 2017; 2, autumn, 2017; 3, winter, 2017; 4, spring, 2017; 5, spring, 2017; 6, summer, 2018; 7, autumn, 2018; 8, winter, 2018; 9, winter, 2018; 10, spring, 2018; 11, summer, 2019). The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

NOTE: Values are reported as mean ± standard error. Means followed by the different letters between harvest for the same genotype differ by Kruskal Wallis ($p < 0.05$).

The phenolic acids identified and quantified in this work were the isomers: 4-caffeoylquinic acid (FIGURE 4-A), 5-caffeoylquinic acid (FIGURE 4-B) and 3-caffeoylquinic acid (FIGURE 4-C). The sum of caffeoylquinic acids is represented in Figure 3-D. The major compound of aqueous extract for both genotypes in all harvests was 5-caffeoylquinic acid, which was, therefore, the most relevant isomer for yerba mate. The fluctuation in 5-CQA content was 17.7 - 72.7 $mg.g^{-1}$ for EC22 and 21.5 - 76.1 $mg.g^{-1}$ for EC40 depending on seasonality. 4-caffeoylquinic acid ranged from 11.1 to 19.2 $mg.g^{-1}$ and 12.7 to 21.1 $mg.g^{-1}$ for EC22 and EC40 respectively. For 3-caffeoylquinic acid and total caffeoylquinic acids (sum of the 3 isomers), no difference was found between genotypes, so results were expressed together (FIGURE 4C and 4D). In general, total content of isomers considering the average of two genotypes varied from 42.2 - 111.4 $mg.g^{-1}$, that is, this class of compounds at the peak of production represents 11 % of leaf dry weight.

The content of the 5-CQA isomer (majority) and the sum of three isomers were higher in the first year (TABLE 1), which refers to harvests 1 to 6; however, we noted that the content fluctuated based on temperature and radiation (see Figure 1) with an increased content in winter and a decrease in summer (FIGURE 4B and 4D).

FIGURE 4 -VARIATION OF TOTAL CONTENT 4-CAFFEOYLQUINIC ACID, 5-CAFFEOYLQUINIC ACID, 3-CAFFEOYLQUINIC, AND TOTAL CAFFEOYLQUINIC FOR GENOTYPE EC22 AND EC40 THROUGHOUT HARVESTS.



SOURCE: The author (2020).

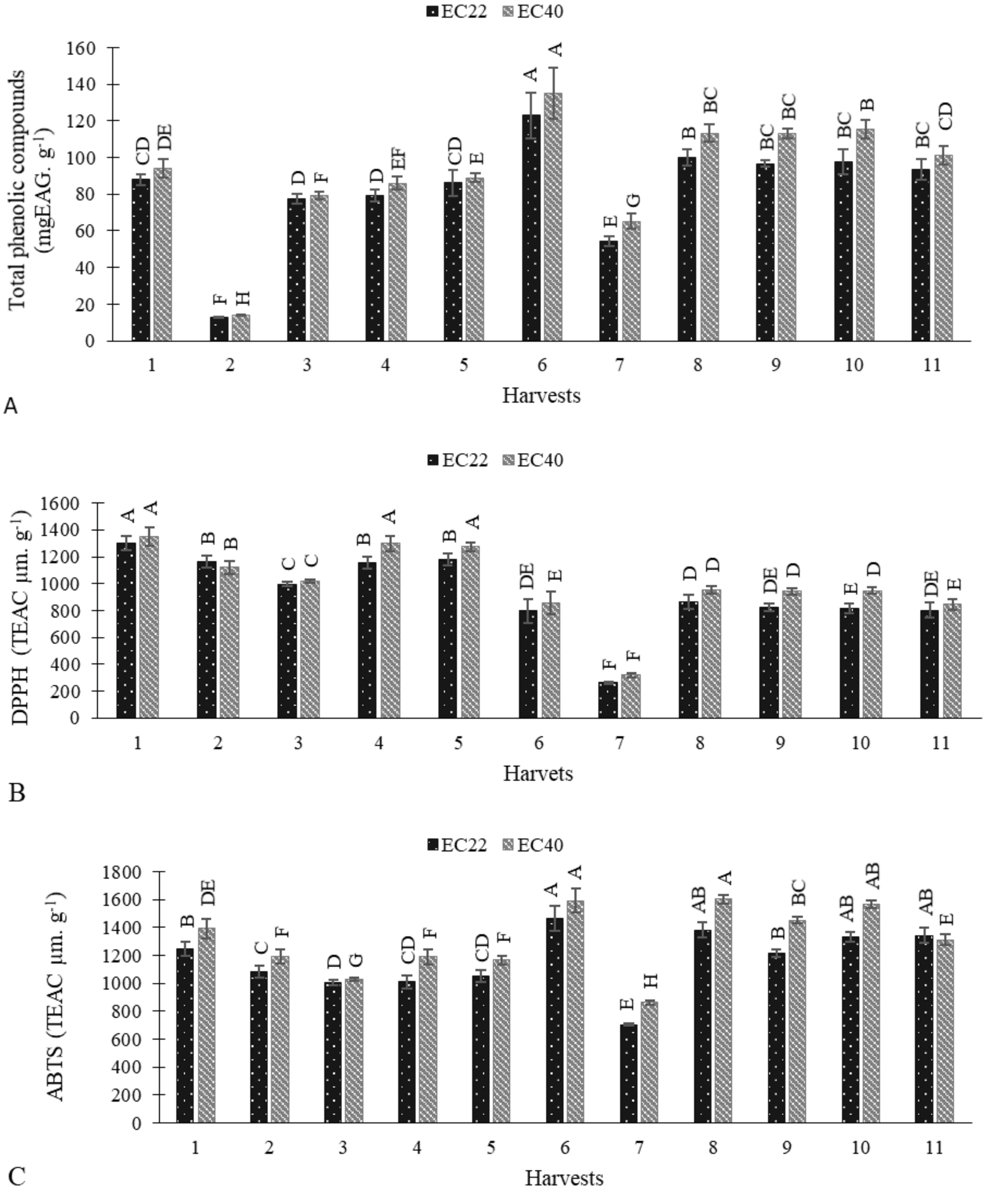
NOTE: Additional information (harvest, season, year): (1, summer, 2017; 2, autumn, 2017; 3, winter, 2017; 4, spring, 2017; 5, spring, 2017; 6, summer, 2018; 7, autumn, 2018; 8, winter, 2018; 9, winter, 2018; 10, spring, 2018; 11, summer, 2019). The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

NOTE: Values are reported as mean ± standard error. Means followed by the different letters between harvest for the same genotype differ by Kruskal Wallis ($p < 0.05$).

The content of total phenolic compounds and antioxidant capacity to capture DPPH and ABTS radicals are in Figure 5A, 5B and 5C, respectively. The lowest content of total phenolic compounds was observed in harvest 2 (year 1) and harvest 7 (year 2), during the autumn season (TABLE 1). All harvested EC40 genotype had a greater potential to produce total phenolic compounds than EC22, with an increase average content of 10 %. The variation over the entire study period was 12.9 - 122.9 mgEAG.g^{-1} and 13.8 - 135.1 mgEAG.g^{-1} for EC22 and EC40, respectively (FIGURE 5A).

The antioxidant profile of both genotypes extracts, mainly in the capture of ABTS radical (FIGURE 5C), followed profile variation of total phenolics. The genotype with greatest antioxidant potential for free radicals capture expressed in Trolox equivalent was EC40, corresponding to 7 % and 10 % higher capacity than EC22 for DPPH and ABTS respectively (FIGURE 5B and 5C).

FIGURE 5 – VARIATION OF TOTAL PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT CAPACITY BY DPPH AND ABTS RADICALS



SOURCE: The author (2020).

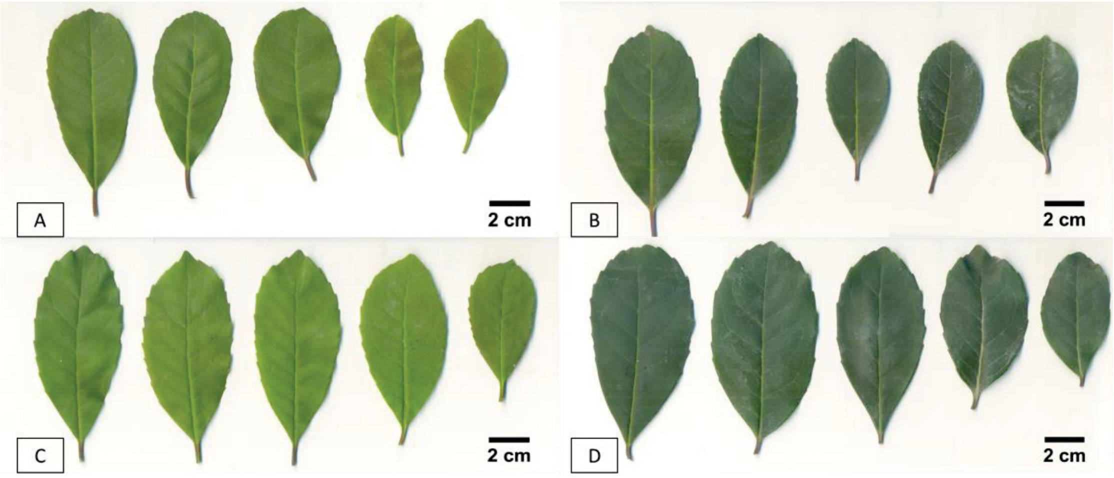
NOTE: Additional information (harvest, season, year): (1, summer, 2017; 2, autumn, 2017; 3, winter, 2017; 4, spring, 2017; 5, spring, 2017; 6, summer, 2018; 7, autumn, 2018; 8, winter, 2018; 9, winter, 2018; 10, spring, 2018; 11, summer, 2019). The harvest from 1 to 6 refers to first year and 7 to 11 refers to second year.

NOTE: Values are reported as mean ± standard error. Means followed by the different letters between harvest for the same genotype differ by Kruskal Wallis ($p < 0.05$).

5.3.2 Effect of leaf type on bioactive compounds content and antioxidant capacity of EC22 and EC40 genotypes

Several factors influence the content of bioactive compounds in plants, altering the qualitative and quantitative profile. The variation in phytochemicals content that occurs in leaves as a function of leaf type is a result of the different stages of development (young and mature leaves). The leaves of yerba mate in semi-hydroponic system did not show a size pattern, however we noted that young leaves were light green color and mature leaves were dark green (FIGURE 6A, 6B, 6C e 6D). The use of visual analysis of leaves color coupled with complete leaf expansion, has been described for a long time in literature (MILLS; SCOGGINS, 1998).

FIGURE 6 – DIFFERENTIATION BETWEEN YOUNG AND MATURE LEAVES OF THE YERBA MATE



SOURCE: The author (2020).
SUBTITLE: A: EC22 genotype young leaves; B: EC22 genotype mature leaves; C: EC40 genotype young leaves; D: EC40 genotype mature leaves;

The results of bioactive compounds contents and antioxidant capacity of the aqueous extracts produced with young and mature leaves from genotypes EC22 and EC40 of yerba mate are in Table 2. Table 3 shows 3-caffeylquinic acid and the total of caffeoylquinic acids (sum of 3, 4 and 5-caffeoylquinic acids) since there was no difference between the genotypes ($p > 0.05$).

We noted that, for both genotypes, young leaves corresponded to the highest content of bioactive compounds (except for theobromine - EC22) and antioxidant capacity by capturing the free radicals DPPH and ABTS (TABLE 2). In general, EC40 genotype was more efficient in compounds synthesis than EC22, except for 3-caffeoylquinic acid and total caffeoylquinic acids, for which genotypes showed no difference and response was expressed together (TABLE 3).

For total methylxanthines (sum of caffeine and theobromine), genotypes EC22 and EC40 corresponded to an average increase in the content of 22 % and 13 %, respectively, in young leaves compared to the mature leaves. For caffeine, there is a marked difference between genetic materials, with an average increase in content corresponding to 52 % for EC22 and 7 % for EC40 (TABLE 2).

The major compound 5-caffeoylquinic acid, of caffeoylquinic acids class, was the one that showed a high difference in leaf content as a function of leaf type for both genotypes, corresponding to an increase of 15 % and 8 % for EC22 and EC40, respectively, in young leaves. For total phenolic compounds, the increase in young leaves was similar between genotypes corresponding to an average of 10 %. (TABLE 1).

TABELA 2 – BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY BY DPPH AND ABTS RADICAL FOR DIFFERENT LEAF TYPE OF YERBA MATE GENOTYPES EC22 AND EC40

Bioactive compounds and antioxidant capacity		Leaf type			
		Genotype EC22		Genotype EC40	
		young	mature	young	mature
Methylxanthines (mg.g^{-1})	Caf	12.78 ± 1.32 A	4.04 ± 0.57 B	21.98 ± 1.28 A	16.98 ± 0.69 B
	Theo	11.57 ± 0.53 A	11.48 ± 0.57 A	6.46 ± 0.27 A	4.90 ± 0.41 B
	Total	24.35 ± 1.29 A	15.53 ± 0.76 B	28.44 ± 1.24 A	21.88 ± 0.75 B
Caffeoylquinic acids (mg.g^{-1})	4CQA	17.62 ± 0.57 A	15.93 ± 0.59 B	18.79 ± 0.61 A	16.67 ± 0.52 B
	5CQA	48.30 ± 2.89 A	38.91 ± 2.65 B	50.95 ± 2.81 A	43.01 ± 2.51 B
Total phenolic compounds (mgEAG.g^{-1})		90.42 ± 5.25 A	74.92 ± 3.92 B	100.85 ± 5.85 A	82.02 ± 4.09 B
Antioxidant capacity (TEAC µm.g^{-1})	DPPH	999.02 ± 46.35 A	849.68 ± 43.41 B	1070.53 ± 46.92 A	917.34 ± 41.54 B
	ABTS	1288.77 ± 50.36 A	1044.97 ± 49.51 B	1429.51 ± 49.83 A	1180.21 ± 48.92 B

SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error. Means followed by the different letters between leaf position of the stem for the same genotype differ by Kruskal Wallis ($p < 0.05$).
SUBTITLE: Caf: caffeine; Theo: theobromine; 4CQA: 4-caffeoylquinic acid; 5CQA: 5-caffeoylquinic acid

TABELA 3 – BIOACTIVE COMPOUNDS (3-CAFFEOYLQUINIC ACID AND TOTAL CAFFEOYLQUINIC ACIDS FOR DIFFERENT LEAF TYPE OF YERBA MATE

Caffeoylquinic acids	Leaf type	
	young	mature
3CQA (mg.g^{-1})	20.67 ± 0.63 A	13.07 ± 0.42 B
Total (mg.g^{-1})	88.51 ± 2.58 A	70.33 ± 2.44 B

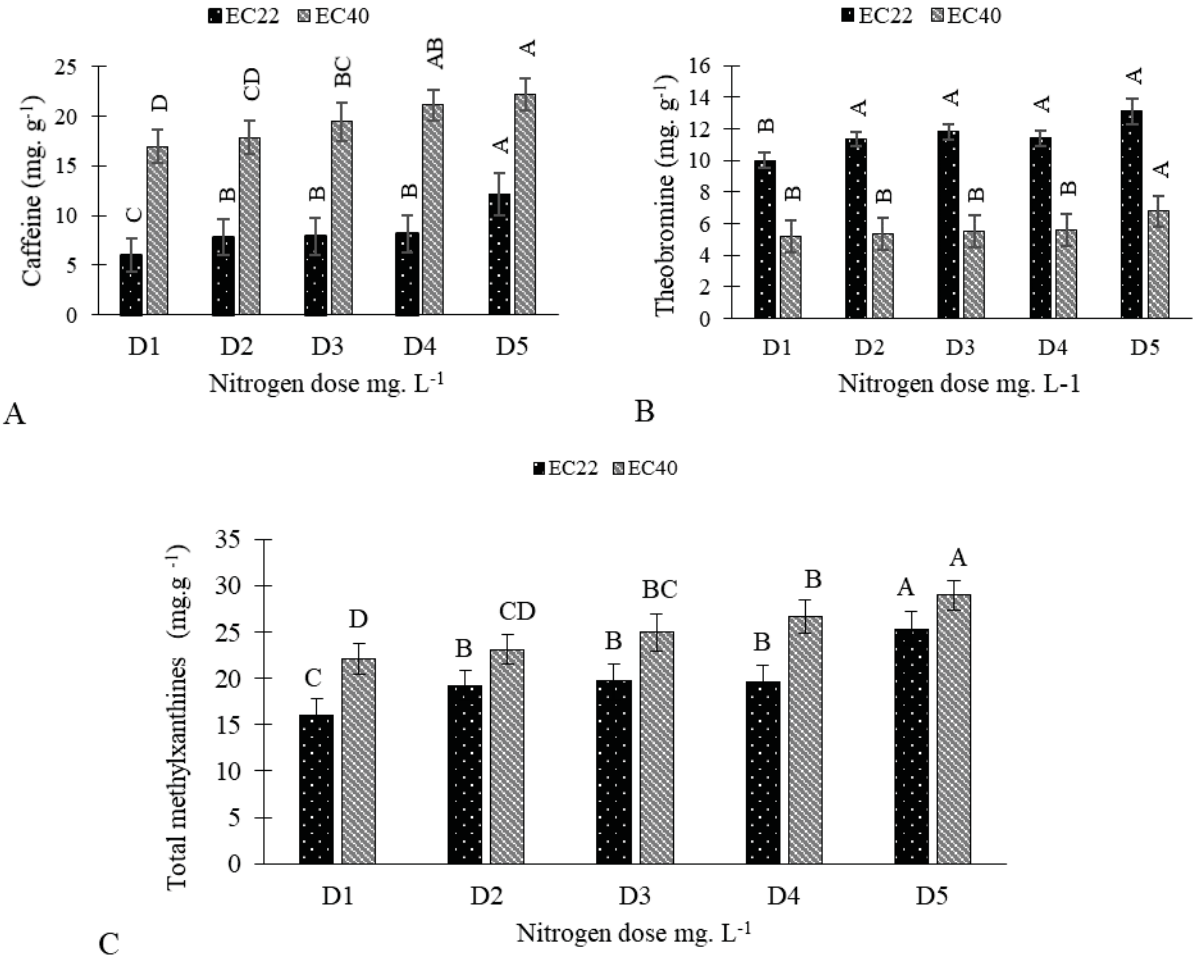
SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error. Means followed by the different letters between leaf position of the stem for the same genotype differ by Kruskal Wallis ($p < 0.05$).
SUBTITLE: 3CQA: 3-caffeoylquinic acid

5.3.3 Effect of nitrogen doses on the content of bioactive compounds and antioxidant capacity of EC22 and EC40 genotypes

The nitrogen influenced caffeine (FIGURE 7A), theobromine (FIGURE 7B), and total methylxanthines content in yerba mate leaves (FIGURE 7C). In terms of genotype, EC40 had a higher caffeine content, total methylxanthines and a lower theobromine content than EC22.

The caffeine content was higher for EC40 in doses D4 (760 mg.L^{-1}) and D5 (1142 mg.L^{-1}) and for EC22 in dose D5, representing a 45 % decrease when compared to EC40 in that dose (FIGURE 7A). For theobromine, we found that EC22 produced 51 % more than EC40 at the highest dose (D5) (FIGURE 6B). In terms of total methylxanthines (sum of caffeine and theobromine), the dose with higher N concentration resulted in the highest content for both genotypes (FIGURE 7C).

FIGURA 7 – EFFECT OF NITROGEN DOSE ON CAFFEINE, THEOBROMINE, AND TOTAL METHYLXANTHINES CONTENT FOR YERBA MATE GENOTYPE EC22 AND EC40.



SOURCE: The author (2020).

NOTE: Values are reported as mean ± standard error. Means followed by the different letters between nitrogen dose for the same genotype differ by Kruskal Wallis ($p < 0.05$).

SUBTITLE: N total doses tested: D1 = 114 mg.L^{-1}; D2 = 206 mg.L^{-1}; D3 = 380 mg.L^{-1}; D4 = 760 mg.L^{-1}; D5 = 1142 mg.L^{-1}.

In table 4 are the contents of 4-caffeoylquinic acid, 5-caffeoylquinic acid, total phenolic compounds, and antioxidant capacity by the radicals DPPH and ABTS for genotypes EC22 and EC40 as a function of N dose. In table 5 are the variables 3-caffeoylquinic acid and total caffeoylquinic acids (sum of 3, 4 and 5-caffeoylquinic acids) since there was no difference between genotypes (p> 0.05).

In general, unlike methylxanthines, caffeoylquinic acids (3,4,5 and total) and total phenolic compounds showed a decrease in content at the highest doses (D4 and D5). The antioxidant capacity by DPPH radical was not influenced by N doses, but the antioxidant capacity by ABTS radical was influenced by N doses for EC22 genotype. Nevertheless, the antioxidant capacity followed the variation of total phenolic compounds, and the non-statistical difference was certainly due to highest standard error of analysis (TABLE 4).

TABLE 4 - BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY BY DPPH AND ABTS RADICAL OF YERBA MATE GENOTYPES EC22 AND EC40 IN NITROGEN DOSES

G	N (mg.L$^{-1)}$	Caffeoylquinic acids (mg.g^{-1})		TPC (mgEAG.g^{-1})	Antioxidant capacity (TEAC μm.g^{-1})	
		4CQA	5CGA		DPPH	ABTS
EC22	D1	16.38±0.94BC	42.93±4.50ABC	78.42±7.01A	840.95±70.65A	1079.25±39.74C
	D2	18.07±0.97A	48.55±4.79A	85.92±7.73A	922.04±68.78A	1189.02±35.03AB
	D3	17.71±0.94AB	47.40±4.70AB	87.41±8.30A	973.95±70.31A	1257.07±42.55A
	D4	16.05±0.85C	40.74±4.31BC	80.26±7.33A	937.19±74.98A	1120.86±33.33BC
	D5	15.68±0.84C	38.41±3.91C	81.32±7.31A	947.60±78.70A	1188.13±36.98AB
EC40	D1	18.35±0.82AB	50.09±4.24A	92.65±8.11A	961.94±68.90A	1303.32±34.79A
	D2	18.07±1.07AB	50.88±4.61A	95.39±8.40A	998.68±68.88A	1341.11±35.15A
	D3	18.81±0.99A	48.23±4.22AB	94.45±8.97A	1030.99±75.0A	1344.99±42.32A
	D4	17.03±0.93BC	43.70±4.18B	88.78±7.86A	990.27±74.95A	1298.65±37.50A
	D5	16.41±0.71C	41.97±3.97B	85.92±8.08A	987.76±74.94A	1236.18±39.57A

SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error. Means followed by the different letters between nitrogen dose for the same genotype differ by Kruskal Wallis ($p < 0.05$).
SUBTITLE: N = total doses tested: D1 = 114 mg.L^{-1}; D2 = 206 mg.L^{-1}; D3 = 380 mg.L^{-1}; D4 = 760 mg.L^{-1}; D5 = 1142 mg.L^{-1}; G = genotype; 4CQA: 4-caffeoylquinic acid; 5CQA: 5-caffeoylquinic acid; TPC = total phenolic compounds.

TABELA 5 – BIOACTIVE COMPOUNDS (3-CAFEOYLQUINIC ACID AND TOTAL CAFFEOYLQUINIC ACIDS) FOR YERBA MATE IN NITROGEN DOSE

Caffeoylquinic acids (mg.g^{-1})	Nitrogen dose (mg.L^{-1})				
	D1	D2	D3	D4	D5
3CQA	17.112±1.074B	19.087±1.147A	17.941±1.09AB	15.46±0.961C	14.761±0.893C
Total	80.993±4.226A	86.879±4.467A	84.027±4.169A	74.23±4.118B	70.994±3.772B

SOURCE: The author (2020).
NOTE: Values are reported as mean ± standard error. Means followed by the different letters between nitrogen dose for the same genotype differ by Kruskal Wallis ($p < 0.05$).
SUBTITLE: N total doses tested: D1 = 114 mg.L^{-1}; D2 = 206 mg.L^{-1}; D3 = 380 mg.L^{-1}; D4 = 760 mg.L^{-1}; D5 = 1142 mg.L^{-1}; G = genotype; 3CQA: 3-caffeoylquinic acid.

5.4 DISCUSSIONS

5.4.1 Effect of seasonality on the content of bioactive compounds and antioxidant capacity of EC22 and EC40 genotypes

The seasonal oscillation of bioactive compounds described in item 5.3.1 of this study is expected since yerba mate genotypes were grown in a protected, uncontrolled environment. However, low temperatures in the coldest seasons are eased in this environment, just as in the warmer seasons proper management (sprinkling and opening the side curtains) tends to soften the heat. Even so, variation for the secondary metabolites studied is inevitable since environmental conditions can redirect metabolism and, hence, regulate active constituents production (Yang et al., 2018).

According to Ramakrishna and Ravishankar (2011); Bhandari, Tridib and Goswami (2019) plants tend to produce secondary metabolites to combat and adapt to environmental stressors (radiation, salinity, drought, high temperatures, and fluctuations), in addition to genetic, ontogenetic, and morphogenetic factors that also influence these variations (VERMA; SHUKLA, 2015). A study with *Camellia sinensis* leaves harvested at the beginning and end of spring, showed significant differences in several secondary metabolism compounds content (phenolic acids, flavonoids, catechins, among others), showing that the moment of harvesting is an important factor in terms of metabolism, which is intrinsically linked to green tea quality (ZENG et al., 2020).

We detected fluctuation due to seasonality in aqueous extract content, mainly 5-caffeoylquinic acid (majority), 3-caffeoylquinic acid, and total caffeoylquinic acids, with an increase in the winter season in both years of cultivation (FIGURE 4B, 4C and 4D). These compounds, together with 4CQA, are the main acids in the phenolic fraction of yerba mate (MATEOS et al., 2018) and its leaves are one of the main natural sources of these compounds (MEINHART et al., 2017). Thus, it is extremely important to understand the role of seasonality in bioactive compounds content, aiming to obtain standardized extracts or pure compounds for pharmacological purposes (BASTOS et al., 2011).

Heritability studies with yerba mate progenies have shown that 3-caffeoylquinic acid had a low to intermediate genetic effect in the restricted sense ($h2 < 40$ %) (CARDOZO JUNIOR et al., 2010b). Thus, it appears that in addition to the genetic factor, on average 50 % of the variation in this compound content is attributed to environmental factors.

The total phenolic compounds also fluctuated according to seasonality throughout the harvests (FIGURE 5A), with a reduced content in the autumn season for both genotypes; however, the variation was not similar to caffeoylquinic acids (FIGURE 4). It can be justified since there is a wide variety of compounds in terms of molecules classified as phenolic compounds (LIU et al., 2020), so seasonality can differentially influence the signaling for compounds synthesis in this group. The antioxidant capacity of extracts varied during harvests in the capture of free radicals ABTS and DPPH (FIGURE 5B, 5C), which was expected since the content of metabolites with antioxidant action, such as phenolic compounds (ZENG et al., 2020), fluctuated in the two years of cultivation. Several studies confirm the potential of these compounds with bioactive characteristics attributed to antioxidant capacity (BRAHMI et al., 2012; BLUM-SILVA et al., 2015; SOUZA et al., 2015).

Seasonal variation was observed for total methylxanthines, as well as for caffeine and theobromine separately, and summer was the season that promoted the highest caffeine content (FIGURE 3B). In studies with *Camellia sinensis* varieties, Wakamatsu et al. (2019) found variation in caffeine content during four harvests, with a significant reduced content in the harvest corresponding to autumn-winter season; Bhandari, Tridib and Goswami (2019), studying the same species, observed a reduction in the content in autumn with an increase in spring. Schubert et al. (2006) followed the annual variation of two yerba mate populations and observed a reduction in total methylxanthines in late autumn and winter, with a gradual increase in early spring and higher production in summer. Similar results were obtained in our research with yerba mate genotypes; however, as the cultivation system in which the plants were grown was in a protected environment, in some harvests there was no marked reduction in the content of methylxanthines in colder seasons. Shen et al. (2015) verified in leaves of *Camellia sinensis* that the content of 105 metabolites was different in protected environments and in the field, due to differential signaling as a function of temperature.

Throughout the period of experiment, the EC40 genotype produced a greater amount of caffeine and a lower amount of theobromine, whereas for EC22 the inverse situation was observed. Cardozo Junior et al. (2010) observed the same profile in different yerba mate progenies, with a negative and significant correlation ($r^2 = -0.841$) between caffeine and theobromine. Heritability in the strict sense estimated for these compounds by the same authors was considered high ($h2> 50$ %), confirming high genetic control.

Our results showed that there are genotypes with a high caffeine content at the expense of theobromine, and others with a high theobromine content at the expense of caffeine. In the semi-hydroponic system, the EC22 genotype, previously selected as decaffeinated (maximum caffeine content 0.1 % - g / 100g or 10 mg.g^{-1}) (BRASIL, 2005), did not remain in this classification according to Brazilian legislation in the summer season harvests (harvests 1, 6 and 11). However, this genotype showed low caffeine compared to EC40 and decaffeinated in other seasons, corresponding to harvests 2, 3, 4, 5, 7, 8, 9 and 10 (Figure 3-B).

In general, considering compounds variation, according to seasonality, oscillations are probably regulated since the genes involved in the biosynthesis pathways are generally controlled at the level of transcription by various factors. These factors play an important role in regulating the concentration, accumulation and biosynthesis of different secondary metabolites (VERMA; SHUKLA, 2015).

5.4.2 Effect of leaf type on bioactive compounds content and antioxidant capacity of EC22 and EC40 genotypes

According to results described in item 5.3.2 (Table 2 and 3) young leaves, regardless of the genotype, presented higher content of bioactive compounds and antioxidant capacity than matures leaves. For methylxanthines, the results are in agreement with a research by Blum-Silva et al. (2015), who found a gradual reduction in total methylxanthines, caffeine, theobromine, and total phenolic compounds, as well as antioxidant capacity by the radical DPPH with the age of yerba mate leaves (1, 2 and 6 months). The same pattern was observed by Esmelindro et al. (2004) in the content of caffeine and theobromine in leaves aged 6, 12 and 18 months. These results are supported by the literature, since methylxanthines are produced in new leaves and, according to the “chemical defense theory”, high caffeine contents are found in young leaves for protection against pathogens and herbivores attack (ASHIHARA; SANO; CROZIER, 2008; RODZIEWICZ et al., 2014; YIN; KATAHIRA; ASHIHARA, 2015).

In a study by Liu et al. (2020), a reduction in the content of total phenolic compounds and phenolic acids was observed in mature leaves of *Camellia sinensis* when compared to young leaves, corroborating with our results for yerba mate. These quantitative differences are justified by metabolic changes due to leaf development, as verified for *Camellia sinensis* by Ryu et al. (2017). According to Wu et al. (2019) the protein profile of *Camellia sinensis* leaves was altered after maturation, with a decrease in the PAL enzyme (phenylalanine amonialiasis), which is involved in the metabolic pathway for phenolic compounds synthesis, as well as an abundance of proteins related to photosynthesis and carbon fixation, the opposite was found in young leaves.

In short, in this research we inferred again that genetic regulation at transcription level is the main justification for differences in bioactive compounds content depending on leaf type. According to a study by Deschamps et al. (2006) with *Ocimum basilicum*, young leaves accumulate high levels of methylchavicol (secondary metabolite), which gradually decreased as leaves matured, as well as levels of enzyme gene transcripts involved in the metabolic pathway and enzyme activity decreased with maturity.

5.4.3 Effect of nitrogen doses on bioactive compounds content and antioxidant capacity of EC22 and EC40 genotypes

Mineral nutrition, in addition to stimulating plant growth, influences secondary metabolites content and antioxidant activity (Yang et al., 2018).

In this study, the highest dose of N increased total and individual methylxanthines content (caffeine and theobromine) in both yerba mate genotypes (Figure 6A, 6B, 6C). It can be justified by the biosynthetic route of methylxanthines in yerba mate (ASHIHARA, 1993; ASHIHARA; SANO; CROZIER, 2008; YIN; KATAHIRA; ASHIHARA, 2015). According to these researches, the synthesis occurs from nucleotides that contain the nitrogenous bases adenine and guanine forming xanthosine, followed by methylxanthine formation and after, the synthesis of theobromine, which is a precursor of caffeine. The enzymes N-methyl transferases (7-methylxanthosine synthase, theobromine synthase and caffeine synthase) are reaction catalysts. Similar results were obtained by Palumbo, Putz, Talcott. (2007) for *Ilex vomitoria* where caffeine content and total methylxanthines increased with nitrogen supply.

The two genotypes had a similar profile in response to N doses; however, we verified an inversion in levels, with EC40 producing more caffeine than theobromine and EC22 showing an inverse response. There are no studies, as far as we know, for yerba mate with a focus on the expression of genes related to methylxanthine synthesis pathways; however, in a study by Zhu et al. (2019), the same relation was observed for *Camellia* plants. The authors confirmed that genotypes with high caffeine and low theobromine content had great expression of genes related to caffeine synthesis pathway, while materials with high theobromine content and low caffeine showed high expression of genes related to caffeine degradation pathway.

There was a reduction in 3-caffeoylquinic, 4-caffeoylquinic, 5-caffeoylquinic and total caffeylquinic acids content mainly in doses D4 and D5 of nitrogen (TABLE 4 and 5). It is justified since nitrogen controls the expression of many genes involved in several plant processes, including genes related to carbon metabolism. Plants have a complex regulatory mechanism that coordinates the capacity of nitrogen assimilation with the metabolism of C (VIDAL; GUTIÉRREZ, 2008; NUNES-NESI; FERNIE; STITT, 2010).

The aforementioned acids are derived from primary metabolism, specifically from respiration pentose phosphate pathway; therefore, the imbalance in C / N ratio may induce the phenolic compounds reduction, as verified by Palumbo, Putz, Talcott. (2007) in female plants of *Ilex vomitoria* fertilized with N. It must be noted that total phenolics did not show significative differences between doses; however, a reduction was observed in the higher doses of N.

For *Moringa oleifera*, the increase in N supply (0.7 - 294.7 mg.L^{-1}) corresponded to a reduction in leaves content of total phenolics, 4-caffeoylquinic acid, as well as less activity of the enzyme PAL (phenylalanine amonialiasis). This enzyme is responsible for primary to secondary metabolism connection (it is the main enzyme involved in phenolic compounds synthesis) (GUILLÉN-ROMÁN et al., 2018).

5.5 CONCLUSIONS

Our results show that for both genotypes, young leaves had higher levels of bioactive compounds and antioxidant capacity than mature leaves. Bioactive compounds, as well as antioxidant capacity, showed seasonal fluctuations for both genotypes. Caffeine presented a high content in summer and 5-caffeoylquinic acid presented a higher content in winter.

The EC40 genotype showed greater antioxidant potential, total phenolic compounds, and caffeoylquinic acids content than EC22 throughout the harvests. In terms of total methylxanthines, we observed the same standard. However, we verify that there is a genotype with high caffeine content at the expense of theobromine (EC40) and a genotype with a high theobromine content at the expense of caffeine (EC22).

Total methylxanthines, caffeine and theobromine levels were influenced by nitrogen for both genotypes, resulting in the highest levels at the greater dose (1142 mg.L^{-1}). The isomers (3-caffeoylquinic acids, 4-caffeoylquinic acids, and 5-caffeoylquinic acids) had an inversely proportional response to N doses.

The EC40 genotype is considered caffeinated (caffeine content > 10mg.g^{-1}) in all seasons, regardless of nitrogen dose and leaf type; the EC22 genotype is considered decaffeinated (caffeine content ≤ 10mg.g^{-1}), except in summer season, young leaves, and in the highest nitrogen dose (1142 mg.L^{-1}).

The cultivation of selected yerba mate genotypes in a semi-hydroponic system is an innovative and potential option. It allows the production of raw material with a distinguished phytochemical profile throughout the year, as well as in different leaf type in the same plant. Depending on the class of compounds, the nitrogen dose can also change its content.

REFERENCES

AMIGO-BENAVENT, M. et al. 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**, v. 106, p. 125–138, 2017.

ASHIHARA, H. Purine metabolism and biosynthesis of caffeine in mate leaves. **Phytochemistry**, v. 33, n. 6, p. 1427–1430, 1993.

ASHIHARA, H.; SANO, H.; CROZIER, A. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. **Phytochemistry**, v. 69, n. 4, p. 841–856, 2008.

BASTOS, J. K. et al. Seasonality role on the phenolics from cultivated *Baccharis dracunculifolia*. **Evidence-based Complementary and Alternative Medicine**, 2011.

BECKER, A. M. et al. Spray-dried yerba mate extract capsules: clinical evaluation and antioxidant potential in healthy individuals. **Plant Foods for Human Nutrition**, v. 74, n. 4, p. 495–500, 2019.

BHANDARI, K.; TRIDIB, B. DE; GOSWAMI, K. Evidence based seasonal variances in catechin and caffeine content of tea. **SN Applied Sciences**, v. 1, p. 1740, 2019.

BLUM-SILVA, C. H. et al. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. **Revista Brasileira de Farmacognosia**, v. 25, n. 1, p. 1–6, 2015.

BRACESCO, N. et al. Recent advances on *Ilex paraguariensis* research: Minireview. **Journal of Ethnopharmacology**, v. 136, n. 3, p. 378–384, 2011.

BRACESCO, N. *Ilex Paraguariensis* as a healthy food supplement for the future world. **Biomedical Journal of Scientific & Technical Research**, v. 16, n. 1, p. 15–18, 2019.

BRAHMI, F. et al. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. **Industrial Crops and Products**, v. 38, n. 1, p. 146–152, 2012.

BRAND-WILLIANS, W. CUVELLIER, M. E. BERSET. C. Use of a free radical method to evaluate antioxidant activity. **Food Science and Technology**, v. 28, p. 25–30, 1995.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC n. 277, de 22 de setembro de 2005. **Regulamento técnico para café, cevada, chá, erva-mate e produtos solúveis. Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 22 set. de 2005.

CARDOZO JUNIOR E. L. et al. Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. **Journal of Food Composition and Analysis**, v. 20, n. 7, p. 553–558, 2007.

CARDOZO JUNIOR, E. L. et al. Quantitative genetic analysis of methylxanthines and phenolic compounds in mate progenies. **Pesquisa Agropecuária Brasileira**, v. 45, n. 2, p. 171–177, 2010.

DESCHAMPS, C. et al. Developmental regulation of phenylpropanoid biosynthesis in leaves. **International Journal of Plant Sciences**, v. 3, n. 167, p. 447–454, 2006.

ESMELINDRO, A. Â. et al. Influence of agronomic variables on the composition of mate tea leaves (*Ilex paraguariensis*) extracts obtained from co2 extraction at 30 °C and 175 bar. **Journal of Agricultural and Food Chemistry**, v. 52, n. 7, p. 1990–1995, 2004.

FRIZON, C. N. T. et al. Determination of total phenolic compounds in yerba mate (*Ilex paraguariensis*) combining near infrared spectroscopy (NIR) and multivariate analysis. **LWT - Food Science and Technology**, v. 60, n. 2, p. 795–801, 2015.

GAN, R. Y. et al. Health benefits of bioactive compounds from the genus *ilex*, a source of traditional caffeinated beverages. **Nutrients**, v. 10, n. 11, 2018.

GUILLÉN-ROMÁN, C. J. et al. Effect of nitrogen privation on the phenolics contents, antioxidant and antibacterial activities in *Moringa oleifera* leaves. **Industrial Crops and Products**, v. 114, p. 45–51, 2018.

GOBBO-NETO, L.; LOPES, N. P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Quimica Nova**, v. 30, n. 2, p. 374–381, 2007.

HELM, V. C. et al. **Efeito do solvente na extração de teobromina e cafeína em progênies de erva-mate**. Colombo: Embrapa Florestas, 2015. 6 p. (Embrapa Florestas. Comunicado Técnico, 363).

LIU, Z. et al. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. **Journal of Food Composition and Analysis**, v. 86, p. 103385, 2020.

MATEOS, R. et al. Improved LC-MS characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry**, v. 241, p. 232–241, 2018.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, n. March, p. 522–530, 2017.

MILLS, H. A.; SCOGGINS, H. L. Nutritional levels for anthurium: young versus mature leaves. **Journal of Plant Nutrition**, v. 21, n. 1, p. 199-203, 1998.

NAKAMURA, K. L. et al. Genetic variation of phytochemical compounds in progenies of *Ilex paraguariensis* St . Hil . **Biotechnology**, p. 116–123, 2009.

NUNES-NESI, A.; FERNIE, A. R.; STITT, M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. **Molecular Plant**, v. 3, n. 6, p. 973–996, 2010.

OLIVEIRA, Y. M. M.; ROTTA, E. **Área de distribuição natural de erva-mate (Ilex paraguariensis St. Híl.)**. Seminário sobre atualidade e perspectivas florestais. **Anais**...Curitiba: Embrapa - CNPF, 1983

PALUMBO, M. J.; PUTZ, F. E.; TALCOTT, S. T. Nitrogen fertilizer and gender effects on the secondary metabolism of yaupon, a caffeine-containing north American holly. **Oecologia**, v. 151, n. 1, p. 1–9, 2007.

RAMAKRISHNA, A.; RAVISHANKAR, G. A. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signaling and Behavior**, v. 6, n. 11, p. 1720–1731, 2011.

RESENDE, D. V. M. et al. **Programa de melhoramento da erva-mate coordenado pela Embrapa: resultados da avaliação genética de populações, progênies, indivíduos e clones.** Colombo: Embrapa Florestas, 2000, 60 p. (Embrapa Florestas. Circular Técnica, 43).

RE, R. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9–10, p. 1231–1237, 1999.

RODZIEWICZ, P. et al. Influence of abiotic stresses on plant proteome and metabolome changes. **Acta Physiologiae Plantarum**, v. 36, n. 1, p. 1–19, 2014.

RYU, H. W. et al. Comparison of secondary metabolite changes in *Camellia sinensis* leaves depending on the growth stage. **Food Control**, v. 73, p. 916–921, 2017.

SCHUBERT, A. et al. Variação anual de metilxantinas totais em amostras de *Ilex paraguariensis* A. St.-Hil.(erva-mate) em Ijui e Santa Maria, Estado do Rio Grande do Sul. **Química Nova**, v. 29, n. 6, p. 1233–1236, 2006.

SINGLETON, V.; ROSSI, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v. 16, p. 144-158, 1965.

SHEN, J. et al. Metabolite profiling of tea (*Camellia sinensis* L.) leaves in winter**. Scientia Horticulturae**, 2015.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, n. 4, p. 105–113, 2015.

VIDAL, E. A.; GUTIÉRREZ, R. A. A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis*. **Current Opinion in Plant Biology**, v. 11, n. 5, p. 521–529, 2008.

WAKAMATSU, M. et al. Catechin and caffeine contents in green tea at different harvest periods and their metabolism in miniature swine. **Food Science and Nutrition**, v. 7, n. 8, p. 2769–2778, 2019.

WENDLING, I. et al. Early selection and classification of yerba mate progenies. **Pesquisa Agropecuaria Brasileira**, v. 53, n. 3, p. 279–286, 2018.

WU, L. Y. et al. Complementary iTRAQ proteomic and transcriptomic analyses of leaves in tea plant (*Camellia sinensis* L.) with different maturity and regulatory network of flavonoid biosynthesis. **Journal of Proteome Research**, v. 18, n. 1, p. 252–264, 2019.

YANG, L. et al. Response of plant secondary metabolites to environmental factors. **Molecules**, v. 23, n. 4, p. 1–26, 2018.

YIM, H. S. et al. Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. **Journal of Food Science and Technology**, v. 50, n. 2, p. 275–283, 2013.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of maté (*Ilex paraguariensis*). **Natural Product Communications**, v. 10, n. 5, p. 707–712, 2015.

ZENG, C. et al. Metabolomics analysis of *Camellia sinensis* with respect to harvesting time. **Food Research International**, v. 128, p. 108814, 2020.

ZHU, B. et al. Caffeine content and related gene expression: novel insight into caffeine metabolism in *Camellia* plants containing low, normal, and high caffeine concentrations. **Journal of Agricultural and Food Chemistry**, v. 67, n. 12, p. 3400–3411, 2019.

GENERAL CONCLUSIONS

With the results of chapter 1, we can conclude that the microwave is a technically viable alternative for drying yerba mate leaves as it contributed to the maintenance of green color, the content of bioactive compounds of interest (such as caffeine, theobromine, caffeoylquinic acids and total phenolic compounds), antioxidant capacity, as well as ash, proteins, lipids, and fibers higher or similar to traditional drying processes.

In chapter 2, the internal preference map of hot and iced toasted mate tea produced with different drying methods showed that microwave oven can be recommended as an alternative to maintain similarity to the traditional taste of mate tea and also to maintain the bioactive compounds level with antioxidant (total phenolic compounds and caffeoylquinic acids) and stimulant (caffeine and theobromine) characteristics. However, the traditional drying methods are most indicated to produce tea with lower caffeine levels.

In chapters 3 and 4 we concluded that the semi-hydroponic system for the grown of selected yerba mate genotypes can be considered a technically viable and potential alternative, as it allows the continuous production and similar proportion of young and mature leaves throughout the year, with an average harvest period of two months. The EC40 genotype showed higher leaf production than the EC22 genotype, with an increase in leaf production average (dry basis) of 1.1 ton ha^{-1}. Leaf production can supply different industrial segments since the young leaves present higher content of stimulating compounds such as caffeine and antioxidant compounds such as caffeoylquinic acids.

Future experiments with lower doses of nitrogen should be performed, since in this study, the lowest dose (114 mg.L^{-1}) is recommended for the EC40 genotype that presented linear response and the dose 206 mg.L^{-1} is recommended for EC22 that presented quadratic response. However, we were unable to calculate the maximum technical efficiency dose for this genotype due to the absence of a minimum or maximum point in the value range. The above ideal doses for leaf yield also favored the high content of caffeoylquinic acids, which are the main phenolic compounds of yerba mate.

The genotypes used in this research were selected according to the caffeine content where EC40 is considered caffeinated (content > 0.1 % caffeine) and EC22 decaffeinated (caffeine content ≤ 0.1 %), according to Brazilian legislation. An increase in caffeine content for both materials is associated with an increase in nitrogen dose, and the EC22 genotype became caffeinated at the highest nitrogen dose (1142 mg.L^{-1}). In addition, for to maintain the reduced level of caffeine content and, consequently, the classification as decaffeinated for the EC22 genotype, it is not recommended to use young leaves and harvests in the summer. The EC40 genotype showed variation in caffeine content but remained caffeinated in all evaluated treatments.

Another important point concerning the evaluated genotypes is the inversion of caffeine and theobromine contents. We observed that the EC40 presented in all leaf harvests high caffeine content and lower theobromine content, being the opposite for EC22 genotype.

In summary, yerba mate is a species with high potential in phytochemical and nutrition components, and in this work, we verified that many factors influence those contents, including nutrition, leaf development, genotypes, seasonality and the drying process (post-harvest).

FINAL CONSIDERATIONS

Efficiency of the microwave oven in the maintenance of mate leaves bioactive compounds was observed in chapter 1. Therefore, this process represents a potential alternative to undertake new products based on yerba mate. Future researches, including the analysis of the economic viability of this technology would be extremely valuable to confirm if the addition of the phytochemical potential would provide an the economic return when compared to traditional drying processes.

In chapter 2, we evaluated the consumer acceptance of hot and iced teas produced with toasted mate previously dried with different methods. Future works, including a trained panel for sensory analysis, would be relevant to elucidate which compounds would contribute to taste (astringency, bitterness, sweetness) and aroma of the tea.

In chapters 3 and 4, we confirmed the possibility to produce selected yerba mate genotypes in a semi-hydroponic system, allowing the the continuous production of raw material (all year) with two leaf types (young and mature) in different harvestings with different content of bioactive compounds. Also, we elucidated the effect of nitrogen in terms of leaves yield and compounds content. Future works would compare the cultivation system proposed in this research with others already consolidated for yerba mate and clarify the preference of yerba mate in the absorption of NO_3^- and/or NH_4^+, since there is no literature on this topic.

In terms of secondary metabolism, the analysis of transcriptome for yerba mate will be the future, since most of the secondary metabolites are regulated at the level of transcription. As a complement, an extremely consistent work would be the joint analysis of the transcriptome, proteomics, and metabolomics to elucidate the fluctuation of the compounds in response to seasonal, nutritional, and genetic variations.

In general, based on this work, many possibilities for new researches and products based on a new way of cultivating yerba mate can be explored.

GENERAL REFERENCES

AMIGO-BENAVENT, M. et al. 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**, v. 106, p. 125–138, 2017.

AOAC. Official Methods of analysis. **The Association of Official Analytical Chemists International**, v. 38, n. 8, p. 431, 2016.

ARES, G.; JAEGER, S. R. Check-all-that-apply questions: influence of attribute order on sensory product characterization. **Food Quality and Preference**, v. 28, n. 1, p. 141–153, 2013.

ASHIHARA, H. Purine metabolism and biosynthesis of caffeine in mate leaves. **Phytochemistry**, v. 33, n. 6, p. 1427–1430, 1993.

ASHIHARA, H.; CROZIER, A. Biosynthesis and metabolism of caffeine and related purine alkaloids in plants. **Advances in Botanical Research**, v. 30, p. 117-205, 1999.

ASHIHARA, H.; SANO, H.; CROZIER, A. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. **Phytochemistry**, v. 69, n. 4, p. 841–856, 2008.

ASSOCIAÇÃO BRASILEIRA DE NORMAS E TÉCNICAS (ABNT). NBR 14141. Escalas utilizadas em análise sensorial de alimentos e bebidas. Rio de Janeiro. 1998.

BARBOSA, J. Z. et al. Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. **Anais da Academia Brasileira de Ciências**, v. 90, n. 1, p. 557–571, 2018.

BARRIUSO, B.; ASTIASARÁN, I.; ANSORENA, D. A review of analytical methods measuring lipid oxidation status in foods: a challenging task. **European Food Research and Technology**, v. 236, n. 1, p. 1–15, 2013.

BASTOS, J. K. et al. Seasonality role on the phenolics from cultivated *Baccharis dracunculifolia*. **Evidence-based Complementary and Alternative Medicine**, 2011.

BASTOS, M. C. et al. Mineral content of young leaves of yerba mate. **Pesquisa Florestal Brasileira**, v. 34, n. 77, p. 63–71, 2014.

BECKER, A. M. et al. Spray-dried yerba mate extract capsules: clinical evaluation and antioxidant potential in healthy individuals. **Plant Foods for Human Nutrition**, v. 74, n. 4, p. 495–500, 2019.

BERNAUD, F. S. R.; RODRIGUES, T. C. Fibra alimentar - ingestão adequada e efeitos sobre a saúde do metabolismo. **Arquivos Brasileiros de Endocrinologia e Metabologia**, v. 57, n. 6, p. 397–405, 2013.

BERTÉ, K. A. S. et al. Chemical composition and antioxidant activity of yerba-mate (*Ilex paraguariensis* A . St . -Hil ., Aquifoliaceae) extract as obtained by spray drying. **Journal of Agricultural and Food Chemistry**, v. 59, p. 5523–5527, 2011.

BHANDARI, K.; TRIDIB, B. DE; GOSWAMI, K. Evidence based seasonal variances in catechin and caffeine content of tea. **SN Applied Sciences**, v. 1, p. 1740, 2019.

BHATTA, S.; JANEZIC, T. S.; RATTI, C. Freeze-drying of plant-based foods. **Foods**, v. 87, n. 9, p. 1–22, 2020.

BITTSÁNSZKY, A. et al. Overcoming ammonium toxicity. **Plant Science**, v. 231, p. 184–190, 2015.

BLUM-SILVA, C. H. et al. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. **Revista Brasileira de Farmacognosia**, v. 25, n. 1, p. 1–6, 2015.

BOLTON D. Market research reflects and predicts growth. 2019. Available from: https://worldteanews.com/market-trends-data-andinsights/market-research-reflects-and-predicts-growth. Accessed 1 November .

BORGES, A. L.; COELHO, E. F. Aspectos básicos da fertirrigação. In: BORGES, A. L.; COELHO, E. F. (Eds.). **Fertirrigação em fruteiras tropicais**. 2. ed. Cruz das Almas - BA: Embrapa Mandioca e Fruticultura Tropical, 2009. p. 09–19.

BRACESCO, N. et al. Recent advances on *Ilex paraguariensis* research: Minireview. **Journal of Ethnopharmacology**, v. 136, n. 3, p. 378–384, 2011.

BRACESCO, N. *Ilex Paraguariensis* as a healthy food supplement for the future world. **Biomedical Journal of Scientific & Technical Research**, v. 16, n. 1, p. 15–18, 2019.

BRAGHINI, F. et al. Physico-chemical composition of mate , before and after simulation of mate. **Pesquisa Agropecuária Gaúcha**, v. 20, p. 7–15, 2014.

BRAHMI, F. et al. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. **Industrial Crops and Products**, v. 38, n. 1, p. 146–152, 2012.

BRAND-WILLIANS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **Lebensmittel Wissenschaft und Technologie – Food Science and Technology**, v. 28, p. 25–30, 1995.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC n. 277, de 22 de setembro de 2005. **Regulamento técnico para café, cevada, chá, erva-mate e produtos solúveis. Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 22 set. de 2005.

BREDEMEIER, C.; MUNDSTOCK, C. M. Regulação da absorção e assimilação do nitrogênio nas plantas. **Ciência Rural**, v. 30, n. 1, p. 365–372, 2000.

BURIOL, G. A. et al. Trasmissividade a radiação solar do polietileno de baixa densidade utilizado em estufas. **Ciência Rural**, v. 25, n. 1, p. 1–4, 1995.

BUTIUK, A. P. et al. Study of the chlorogenic acid content in yerba mate (*Ilex paraguariensis* St. Hil.): effect of plant fraction, processing step and harvesting season. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 3, n. 1, p. 27–33, 2016.

CARDOSO, J. M. P.; BATTOCHIO, J. R.; CARDELLO, H. M. A. B. Equivalência de dulçor e poder edulcorante de edulcorantes em função da temperatura de consumo em bebida s preparadas. **Ciência e Tecnologia de Alimentos**, v. 24, n. 3, p. 448–452, 2004.

CARDOZO JUNIOR E. L. et al. Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. **Journal of Food Composition and Analysis**, v. 20, n. 7, p. 553–558, 2007.

CARDOZO JUNIOR, E. L. et al. Quantitative genetic analysis of methylxanthines and phenolic compounds in mate progenies. **Pesquisa Agropecuária Brasileira**, v. 45, n. 2, p. 171–177, 2010.

CARDOZO JUNIOR, E. L.; 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**, v. 21, p. 440–454, 2016.

CARON, B. O. et al. Eficiência de conversão da radiação fotossinteticamente ativa interceptada em fitomassa de mudas de eucalipto. **Revista Árvore**, v. 36, n. 5, p. 833–842, 2012.

CENI, G. C. et al. Influence of application of microwave energy on quality parameters of mate tea leaves (*Ilex paraguariensis* St. Hil.). **Food Technology and Biotechnology**, v. 47, n. 2, p. 221–226, 2009.

CENI, G. C. et al. Oxidases from mate tea leaves (*Ilex paraguariensis*): Extraction optimization and stability at low and high temperatures. **Bioprocess and Biosystems Engineering**, v. 31, n. 6, p. 541–550, 2008.

CHAN, E. W. C. et al. Antioxidant and sensory properties of Thai herbal teas with emphasis on Thunbergia laurifolia Lindl. **Chiang Mai Journal of Science**, v. 39, n. 4, p. 599–609, 2012.

CHARDON, F. et al. Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. **Journal of Experimental Botany**, v. 61, n. 9, p. 2293–2302, 2010.

CHARDON, F.; NOËL, V.; MASCLAUX-DAUBRESSE, C. Exploring NUE in crops and in *Arabidopsis* ideotypes to improve yield and seed quality. **Journal of Experimental Botany**, v. 63, n. 9, p. 3401–3412, 2012.

CHEN, Y.; MARTYNENKO, A. Combination of hydrothermodynamic (HTD) processing and different drying methods for natural blueberry leather. **LWT - Food Science and Technology**, v. 87, p. 470–477, 2018.

CIEMNIAK, A. et al. Assessing the contamination levels of dried teas and their infusions by polycyclic aromatic hydrocarbons (PAHs). **Journal fur Verbraucherschutz und Lebensmittelsicherheit**, v. 14, n. 3, p. 263–274, 2019.

CITTADINI, M. C. et al. Neuroprotective Effect of *Ilex Paraguariensis* Intake on Brain Myelin of Lung Adenocarcinoma-Bearing Male Balb/c Mice. **Nutrition and Cancer**, v. 71, n. 4, p. 629–633, 2019.

COLPO, A. C. et al. 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**, v. 209, p. 185–195, 2016.

COMINO, C. et al. Isolation and functional characterization of a cDNA coding a hydroxycinnamoyl transferase involved in phenylpropanoid biosynthesis in *Cynara cardunculus* L. **BCD Plant Biology**, n. 14, 2007.

CORSO, M. P.; VIGNOLI, J. A.; BENASSI, M. DE T. Development of an instant coffee enriched with chlorogenic acids. **Journal of Food Science and Technology**, v. 53, n. 3, p. 1380–1388, 2016.

DE GODOY, R. C. B. et al. Consumer perceptions, attitudes and acceptance of new and traditional mate tea products. **Food Research International**, v. 53, n. 2, p. 801–807, 2013.

DE MEJÍA, E. G. et al. Yerba mate tea (*Ilex paraguariensis*): phenolics, antioxidant capacity and in vitro inhibition of colon cancer cell proliferation. **Journal of Functional Foods**, v. 2, n. 1, p. 23–34, 2010.

DESCHAMPS, C. et al. Developmental regulation of phenylpropanoid biosynthesis in leaves. **International Journal of Plant Sciences**, v. 3, n. 167, p. 447–454, 2006.

DIBANDA, F. R. et al. Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some fruit peelings. **Food Chemistry**, v. 302, 2020.

DUTTA, A.; SEN, J.; DESWAL, R. Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L). G. Don. **Plant Cell Reports**, v. 26, n. 10, p. 1869–1878, 2007.

EFING, L. C. et al. Caracterização química e capacidade antioxidante da erva-mate**. Boletim do Centro de Pesquisa de Processamento de Alimentos**, v. 27, p. 241–246, 2009.

ESMELINDRO, A. Â. et al. Influence of agronomic variables on the composition of mate tea leaves (*Ilex paraguariensis*) extracts obtained from co2 extraction at 30 °C and 175 bar. **Journal of Agricultural and Food Chemistry**, v. 52, n. 7, p. 1990–1995, 2004.

ESMELINDRO, M. C. et al. Caracterização físico-química da erva-mate: influência das etapas do processamento industrial. **Ciência e Tecnologia de Alimentos**, v. 22, n. 2, p. 193–204, 2002

ESTEBAN, R. et al. Review: Mechanisms of ammonium toxicity and the quest for tolerance. **Plant Science**, v. 248, p. 92–101, 2016.

FAOSTAT. **CROPS**. 2017. Available from: http://www.fao.org/faostat/en/#data/QC. Accessed 5 November, 2019.

FIGIEL, A.; MICHALSKA, A. Overall quality of fruits and vegetables products affected by the drying processes with the assistance of vacuum-microwaves. **International Journal of Molecular Sciences**, v. 18, n. 1, 2017.

FLOWERS, T. J. Improving crop salt tolerance. **Journal of Experimental Botany**, v. 55, n. 396, p. 307–319, 2004.

FRIZON, C. N. T. et al. Determination of total phenolic compounds in yerba mate (*Ilex paraguariensis*) combining near infrared spectroscopy (NIR) and multivariate analysis. **LWT - Food Science and Technology**, v. 60, n. 2, p. 795–801, 2015.

GAIAD, S.; RAKOCEVIC, M.; REISSMANN, C. B. N sources affect growth, nutrient content, and net photosynthesis in mate (*Ilex paraguariensis* St. Hil.). **Brazilian Archives of Biology and Technology**, v. 49, n. 5, p. 689–697, 2006.

GAN, R. Y. et al. Health benefits of bioactive compounds from the genus *ilex*, a source of traditional caffeinated beverages. **Nutrients**, v. 10, n. 11, 2018.

GERHARDT, M. Colonos ervateiros: história ambiental e imigração no Rio Grande do Sul. **Revista Esboços**, v. 18, n. 25, p. 73–95, 2011.

GOBBO-NETO, L.; LOPES, N. P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Quimica Nova**, v. 30, n. 2, p. 374–381, 2007.

GRAMLING, L.; KAPOULEA, E.; MURPHY, C. Taste perception and caffeine consumption: an fMRI study. **Nutrients**, v. 11, n. 1, 2019.

GRIGIONI, G. et al. Flavour characteristics of *Ilex paraguariensis* infusion, a typical Argentine product, assessed by sensory evaluation and electronic nose. **Journal of the Science of Food and Agriculture**, v. 84, n. 5, p. 427–432, 2004.

GUILLÉN-ROMÁN, C. J. et al. Effect of nitrogen privation on the phenolics contents, antioxidant and antibacterial activities in *Moringa oleifera* leaves. **Industrial Crops and Products**, v. 114, p. 45–51, 2018.

GUPTA, R. K. et al. Maillard reaction in food allergy: Pros and cons. **Critical Reviews in Food Science and Nutrition**, v. 58, n. 2, p. 208–226, 2018.

HAMILTON, R. J. et al. Chemistry of free radicals in lipids. F**ood Chemistry**, v. 60, n. 2, p. 193–199, 1997.

HAWKESFORD, M. et al. Function of macronutrients. In: Marschner, H. **Mineral nutrition of higher plants.** 3nd ed**.** Academic Press, 2011. p. 135-189.

HAYES, J. E.; FEENEY, E. L.; ALLEN, A. L. Do polymorphisms in chemosensory genes matter for human ingestive behavior? **Food Quality and Preference**, v. 30, n. 2, p. 202–216, 2013.

HECK, C. I.; DE MEJIA, E. G. Yerba mate tea (*Ilex paraguariensis*): A comprehensive review on chemistry, health implications, and technological considerations. **Journal of Food Science**, v. 72, n. 9, 2007.

HIHAT, S.; REMINI, H.; MADANI, K. Effect of oven and microwave drying on phenolic compounds and antioxidant capacity of coriander leaves. **International Food Research Journal**, v. 24, n. 2, p. 503–509, 2017.

HOSSAIN, M.; BARRY-RYAN, C.; MARTIN-DIANA, A. B. Effect of drying method on the antioxidant capacity of six larniaceae herbs. **Food Chemistry**, v. 123, n. 1, p. 85–91, 2010.

IBGE. **Produção Agrícola Municipal (PAM)**. 2018. Available from https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9117 producao-agricola-municipal-culturas-temporarias-e. Accessed 5 November, 2019

.

ISOLABELLA, S. et al. Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. **Food Chemistry**, v. 122, n. 3, p. 695–699, 2010.

KOVÁCIK, J. et al. Variation of antioxidants and secondary metabolites in nitrogen-deficient barley plants. **Journal of Plant Physiology**, v. 171, p. 260–268, 2014.

LEE, J.; CHAMBERS, D. H. Descriptive analysis and U.S. consumer acceptability of 6 green tea samples from China, Japan, and Korea. **Journal of Food Science**, v. 75, n. 2, 2010.

LI, X. E.; JERVIS, S. M.; DRAKE, M. A. Examining extrinsic factors that influence product acceptance: a review. **Journal of Food Science**, v. 80, n. 5, p. 901 - 909, 2015.

LIN, L. et al. Thermal inactivation kinetics of *Rabdosia serra* (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. **Food Chemistry**, v. 134, n. 4, p. 2021–2029, 2012.

LIN, S.; BREWER, M. S. Effects of blanching method on the quality characteristics of frozen peas. **Journal of Food Quality**, v. 28, n. 4, p. 350–360, 2005.

LIU, Z. et al. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. **Journal of Food Composition and Analysis**, v. 86, p. 103385, 2020.

MACFIE, H. J.; BRATCHELL, N. Designs to balance the effect of order of presentation. **Journal of Sensory Studies**, v. 4, p. 129–148, 1989.

MACHADO, C. C. B. et al. Determinação do perfil de compostos volateis e avaliação do sabor e aroma de bebidas produzidas a partir da erva-mate (*Ilex paraguariensis*). **Quimica Nova**, v. 30, n. 3, p. 513–518, 2007.

MASCLAUX-DAUBRESSE, C. et al. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. **Annals of Botany**, v. 105, n. 7, p. 1141–1157, 2010.

MATEOS, R. et al. Improved LC-MS characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry**, v. 241, p. 232–241, 2018.

MAYER, A. M. Polyphenol oxidases in plants and fungi: going places? A review. **Phytochemistry**, v. 67, n. 21, p. 2318–2331, 2006.

MCALLISTER, C. H.; BEATTY, P. H.; GOOD, A. G. Engineering nitrogen use efficient crop plants: The current status. **Plant Biotechnology Journal**, v. 10, n. 9, p. 1011–1025, 2012.

MCGOWAN, B. A.; LEE, S. Y. Comparison of methods to analyze time-intensity curves in a corn zein chewing gum study. **Food Quality and Preference**, v. 17, n. 3–4, p. 296–306, 2006.

MEINHART, A. D. et al. Chlorogenic acid isomer contents in 100 plants commercialized in Brazil. **Food Research International**, v. 99, n. March, p. 522–530, 2017.

MEINHART, A. D. et al. Methylxanthines in 100 Brazilian herbs and infusions: Determination and consumption. **Emirates Journal of Food and Agriculture**, v. 31, n. 2, p. 125–133, 2019.

MENDONÇA, A. V. R. et al. Características fisiológicas de mudas de *Eucalyptus* spp submetidas a estresse salino. **Ciência Florestal**, v. 20, n. 2, p. 255–267, 2010.

MILLS, H. A.; SCOGGINS, H. L. Nutritional levels for anthurium: young versus mature leaves. **Journal of Plant Nutrition**, v. 21, n. 1, p. 199-203, 1998.

MÜLLER, H.; HAMM, U. Stability of market segmentation with cluster analysis - A methodological approach. **Food Quality and Preference**, v. 34, p. 70–78, 2014.

NACRY, P.; BOUGUYON, E.; GOJON, A. Nitrogen acquisition by roots: Physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. **Plant and Soil**, v. 370, n. 1–2, p. 1–29, 2013.

NAKAMURA, K. L. et al. Genetic variation of phytochemical compounds in progenies of *Ilex paraguariensis* St . Hil . **Biotechnology**, p. 116–123, 2009.

NASCIMENTO, B. et al. Nitrogenated fertilization favors vegetative rescue and propagation of *Ilex paraguariensis*. **Cerne**, v. 25, n. 1, p. 76–83, 2019.

NAVEED, M. et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. **Biomedicine and Pharmacotherapy**, v. 97, p. 67–74, 2018.

NIREESHA, G. R. et al. Lyophilization / Freeze Drying - An Review. **International Journal of Novel Trends in Pharmaceutical Sciences**, v. 3, n. 4, p. 87–98, 2013.

NUNES-NESI, A.; FERNIE, A. R.; STITT, M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. **Molecular Plant**, v. 3, n. 6, p. 973–996, 2010.

OLIVEIRA, E. V. et al. Composição nutricional de procedência e progênies de erva-mate (I*lex paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. **Ciência Florestal**, v. 24, n. 4, p. 793–805, 2014

OLIVEIRA, Y. M. M.; ROTTA, E. **Área de distribuição natural de erva-mate (Ilex paraguariensis St. Híl.)**. Seminário sobre atualidade e perspectivas florestais. **Anais**...Curitiba: Embrapa - CNPF, 1983

OZKAN, I. A.; AKBUDAK, B.; AKBUDAK, N. Microwave drying characteristics of spinach. **Journal of Food Engineering**, v. 78, n. 2, p. 577–583, 2007.

PALUMBO, M. J.; PUTZ, F. E.; TALCOTT, S. T. Nitrogen fertilizer and gender effects on the secondary metabolism of yaupon, a caffeine-containing north American holly. **Oecologia**, v. 151, n. 1, p. 1–9, 2007.

PANDOLFO, C. M. et al. Resposta da erva-mate (*Ilex paraguariensis* St. Hil.) a adubação mineral e orgânica em um latossolo vermelho aluminoférrico. **Ciência Florestal**, v. 13, n. 2, p. 37–45, 2003.

PARIT, R. K.; PRABHU, M. C. S. Microwave fruit and vegetables drying. **International Advanced Research Journal in Science, Engineering and Technology**, v. 4, n. 2, p. 82–84, 2017.

PASSARDI, R. L. et al. Drying of *Ilex paraguariensis* Saint Hilaire by microwave radiation. **Drying Technology**, v. 24, n. 11, p. 1437–1442, 2006.

PENTEADO JUNIOR, J. F.; GOULART, I. C. G. DOS R. **Erva 20:** sistema de produção de erva-mate. Brasília - DF: Embrapa, 2019.

PERONE, C. A. S.; CAPOBIANCO, M. PETROLINI; PAPARELI, J. S. Determination of polyphenols (tanines) in foods products (teas) using a biosensor of polyphenol oxidase, obtained of crude extract of banana nanica peel (*Musa acuminata*) and characterization this biosensor. **Revista do Instituto de Ciência da Saúde**, v. 27, n. 1, p. 28–34, 2009.

PIMENTEL, N. et al. Productivity of mini-stumps and rooting of mini-cuttings of erva mate (*Ilex paraguariensis* A. St.-Hil.) clones. **Ciência Florestal**, v. 29, n. 2, p. 559–570, 2019.

PIRES, P. et al. Sazonalidade e soluções nutritivas na miniestaquia de *Araucaria angustifolia* (Bertol.) Kuntze. **Revista Árvore**, v. 39, n. 2, p. 283–293, 2015.

POLETTO, I. et al. Influência da inoculação de *Fusarium* spp. e níveis de sombreamento no crescimento e desenvolvimento da erva-mate. **Ciência Florestal**, v. 20, n. 3, p. 513–521, 2010.

R CORE TEAM. A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. 2016. ISBN 3-900051-07-0. Available in: <http: //www.R-project.org>.

RAJA, K. S. et al. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. **Journal of the Saudi Society of Agricultural Sciences**, p. 1–7, 2017.

RAMAKRISHNA, A.; RAVISHANKAR, G. A. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signaling and Behavior**, v. 6, n. 11, p. 1720–1731, 2011.

RE, R. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9–10, p. 1231–1237, 1999.

REIS, M. G. **Practical guide: Conversion factors and general equations applied in agricultural and forest meteorology**. Available in: <www.researchgate.net/publication/3204568912019>. Access in 5 nov. 2019.

RESENDE, D. V. M.; STURION, A. J.; CARVALHO, P. A.; SIMEÃO, M. R.; FERNANDES, S. C. J. **Programa de melhoramento da erva-mate coordenado pela Embrapa: resultados da avaliação genética de populações, progênies, indivíduos e clones.** Colombo: Embrapa Florestas, 2000, 60 p. (Embrapa Florestas. Circular Técnica, 43).

RIACHI, L. G.; DE MARIA, C. A. B. Yerba mate: An overview of physiological effects in humans. **Journal of Functional Foods**, v. 38, p. 308–320, 2017.

RICHARD-FORGET, F. C.; GAUILLARD, F. A. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. **Journal of Agricultural and Food Chemistry**, v. 45, n. 7, p. 2472–2476, 1997.

ROCHA, J. H. T. et al. Produtividade do minijardim e qualidade de miniestacas de um clone híbrido de *Eucalyptus grandis* x *Eucalyptus urophylla* (I-224) em função de doses de nitrogênio. **Ciência Florestal**, v. 25, n. 2, p. 273–279, 2015.

ROCHA, R. P.; MELO, E. C.; RADÜNZ, L. L. Influence of drying process on the quality of medicinal plants: A review. **Journal of Medicinal Plant Research**, v. 5, n. 33, p. 7076–7084, 2011.

RODZIEWICZ, P. et al. Influence of abiotic stresses on plant proteome and metabolome changes. **Acta Physiologiae Plantarum**, v. 36, n. 1, p. 1–19, 2014.

ROOSTA, H. R.; SCHJOERRING, J. K. Effects of nitrate and potassium on ammonium toxicity in cucumber plants. **Journal of Plant Nutrition**, v. 31, n. 7, p. 1270–1283, 2008.

ROSA, L. S. et al. **Adubação nitrogenada na fertirrigação de minicepas de** *Ilex paraguariensis* St. Hil. Anais do 5º Congreso Sudamericano De La Yerba Mate. **Anais**...Posadas: INYM/ INTA/INaM, 2011.

RUDRA, S. G. et al. Enthalpy entropy compensation during thermal degradation of chlorophyll in mint and coriander puree. **Journal of Food Engineering**, v. 86, n. 3, p. 379–387, 2008.

RYU, H. W. et al. Comparison of secondary metabolite changes in *Camellia sinensis* leaves depending on the growth stage. **Food Control**, v. 73, p. 916–921, 2017.

SANTIN, D. et al. Adubação nitrogenada e intervalos de colheita na produtividade e nutrição da erva-mate e em frações de carbono e nitrogênio do solo. **Ciência Florestal**, v. 29, n. 3, p. 1199, 2019.

SANTIN, D. et al. Crescimento e nutrição de erva-mate influenciados pela adubação nitrogenada, fosfatada e potássica. **Ciência Florestal**, v. 23, n. 2, p. 363–375, 2013.

SANTIN, D. et al. **Fontes de nitrogênio e técnicas de propagação de mudas atuam na produtividade de erva-mate**. 6º Congresso Sudamericano de Yerba Mate. **Anais**...Montevideo: 2014.

SANTIN, D. et al. Growth of mate tea tree seedlings fertilized with N, P and K. **Scientia Agraria**, v. 9, n. 1, p. 59–66, 2008.

SANTIN, D. et al. Intervalos de colheita e adubação potássica influenciam a produtividade da erva-mate (*Ilex paraguariensis*) no Estado do Paraná. **Floresta**, v. 46, n. 4, p. 509–518, 2017.

SANTIN, D. et al. Manejo de colheita e adubação fosfatada na cultura da erva-mate (*Ilex paraguariensis*) em fase de produção. **Ciência Florestal**, v. 27, n. 3, p. 783–797, 2017.

SCHUBERT, A. et al. Variação anual de metilxantinas totais em amostras de *Ilex paraguariensis* A. St.-Hil.(erva-mate) em Ijui e Santa Maria, Estado do Rio Grande do Sul. **Química Nova**, v. 29, n. 6, p. 1233–1236, 2006.

SHEN, J. et al. Metabolite profiling of tea (*Camellia sinensis* L.) leaves in winter**. Scientia Horticulturae**, 2015.

SILVA, F. DE A. S. E; AZEVEDO, C. A. V. DE. The Assistat Software Version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, n. 39, p. 3733–3740, 2016.

SILVEIRA, J. A. G. et al. Mecanismos biomoleculares envolvidos com a resistência ao estresse salino em plantas. In: GHEYI, H. R.; DIAS, N. DA S.; LACERDA, C. F. (Eds.). . **Manejo da salinidade na agricultura: estudos básicos e aplicados**. 1. ed. Fortaleza: INCTSal, 2010. p. 167–185.

SINGLETON, V.; ROSSI, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **American Journal of Enology and Viticulture**, v. 16, p. 144-158, 1965.

ŚLEDŹ, M. et al. Selected chemical and physico-chemical properties of microwave-convective dried herbs. **Food and Bioproducts Processing**, v. 91, n. 4, p. 421–428, 2013.

SOUZA, A. H. P. et al. Phytochemicals and bioactive properties of *Ilex paraguariensis*: An in-vitro comparative study between the whole plant, leaves and stems. **Food Research International**, v. 78, p. 286–294, 2015.

SOYSAL, Y. Microwave drying characteristics of parsley. **Biosystems Engineering**, v. 89, n. 2, p. 167–173, 2004.

TAIZ, L.; ZEIGER, E. **Plant Physiology**, Sunderland: Sinauer Associates Inc, 2013.

TEIXEIRA, L. V. Análise sensorial na indústria de alimentos. **Revista do Instituto de Latícinios Cândido Tostes**, v. 64, n. 366, p. 12–21, 2009.

UDOMKUN, P. et al. Influence of air drying properties on non-enzymatic browning, major bio-active compounds and antioxidant capacity of osmotically pretreated papaya. **LWT - Food Science and Technology**, v. 60, n. 2, p. 914–922, 2015.

VALDUGA, A. T. et al. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. **Food Research International**, v. 120, n. November 2018, p. 478–503, 2019.

VALDUGA, A. T. et al. Cytotoxic / antioxidant activity and sensorial acceptance of yerba-mate development by oxidation process. **Acta Scientiarum. Technology**, v. 38, n. 1, p. 115, 2016.

VALDUGA, A. T.; BATTESTIN, V.; FINZER, R. D. Secagem de extratos de erva-mate em secador por atomização. **Ciência e Tecnologia de Alimentos**, v. 23, n. 2, p. 184–189, 2003.

VARELA, P.; BELTRÁN, J.; FISZMAN, S. An alternative way to uncover drivers of coffee liking: Preference mapping based on consumers' preference ranking and open comments. **Food Quality and Preference**, v. 32, p. 152–159, 2014.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, n. 4, p. 105–113, 2015.

VIDAL, E. A.; GUTIÉRREZ, R. A. A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis*. **Current Opinion in Plant Biology**, v. 11, n. 5, p. 521–529, 2008.

WAKAMATSU, M. et al. Catechin and caffeine contents in green tea at different harvest periods and their metabolism in miniature swine. **Food Science and Nutrition**, v. 7, n. 8, p. 2769–2778, 2019.

WENDLING, I. et al. Early selection and classification of yerba mate progenies. **Pesquisa Agropecuaria Brasileira**, v. 53, n. 3, p. 279–286, 2018.

WENDLING, I.; BRONDANI, E. Produção de mudas de erva-mate. In: WENDLING, I.; SANTIN, D. **Propagação e nutrição da erva-mate**. Brasília: Embrapa, 2015. p. 24-50.

WENDLING, I.; DUTRA, L. F.; GROSSI, F. Produção e sobrevivência de miniestacas e minicepas de erva-mate cultivadas em sistema semi-hidrônico. **Pesquisa Agropecuária Brasileira**, v. 42, n. 2, p. 289–292, 2007.

WESTPHALEN, D. J. et al. Impact of different silvicultural techniques on the productive efficiency of *Ilex paraguariensis* A.St.Hill. **Agroforestry Systems**, 2019.

.

WOŹNIAK, D. et al. Caffeoylquinic acids. **Handbook of Dietary Phytochemicals**, p. 1–40,2020.

WU, L. Y. et al. Complementary iTRAQ proteomic and transcriptomic analyses of leaves in tea plant (*Camellia sinensis* L.) with different maturity and regulatory network of flavonoid biosynthesis. **Journal of Proteome Research**, v. 18, n. 1, p. 252–264, 2019.

XING, Y. et al. A review of nitrogen translocation and nitrogen-use efficiency. **Journal of Plant Nutrition**, v. 42, n. 19, p. 2624–2641, 2019.

XING, Y. et al. A review of nitrogen translocation and nitrogen-use efficiency. **Journal of Plant Nutrition**, v. 42, n. 19, p. 2624–2641, 2019.

XU, G.; FAN, X.; MILLER, A. J. Plant nitrogen assimilation and use efficiency. **Annual Review of Plant Biology**, v. 63, n. 1, p. 153–182, 2012.

XU, G.; FAN, X.; MILLER, A. J. Plant nitrogen assimilation and use efficiency. **Annual Review of Plant Biology**, v. 63, n. 1, p. 153–182, 2012.

YANG, J.-E.; LEE, J. Consumer perception and liking, and sensory characteristics of blended teas. **Food Science and Biotechnology**, 2019.

YANG, L. et al. Response of plant secondary metabolites to environmental factors. **Molecules**, v. 23, n. 4, p. 1–26, 2018.

YANG, W. et al. Variations of growth, nitrogen accumulation and nitrogen use efficiency among 18 willow clones under two nitrogen regimes. **Agroforestry Systems**, v. 89, n. 1, p. 67–79, 2015.

YIM, H. S. et al. Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. **Journal of Food Science and Technology**, v. 50, n. 2, p. 275–283, 2013.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of mate (*Ilex paraguariensis*). **Natural Product Communications**, v. 10, n. 5, p. 707–712, 2015.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of maté (*Ilex paraguariensis*). **Natural Product Communications**, v. 10, n. 5, p. 707–712, 2015.

ZAIONS, I. et al. Physico-chemical characterization of *Ilex paraguariensis* St. Hil. during the maturation. **Brazilian Archives of Biology and Technology**, v. 57, n. 5, p. 663–667, 2014.

ZAPATA, F. J. et al. Caffeine, but not other phytochemicals , in mate tea (*Ilex paraguariensis* St . Hilaire) attenuates high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation. **Journal of Functional Foods**, p. 1036-1046, 2019.

ZENG, C. et al. Metabolomics analysis of *Camellia sinensis* with respect to harvesting time. **Food Research International**, v. 128, p. 108814, 2020.

ZHANG, D. et al. Antioxidant and antibacterial capabilities of phenolic compounds and organic acids from *Camellia oleifera* cake. **Food Science and Biotechnology**, v. 29, n. 1, p. 17–25, 2020.

ZHU, B. et al. Caffeine content and related gene expression: novel insight into caffeine metabolism in *Camellia* plants containing low, normal, and high caffeine concentrations. **Journal of Agricultural and Food Chemistry**, v. 67, n. 12, p. 3400–3411, 2019.

ZHUANG, J. et al. Evaluation of astringent taste of green tea through mass spectrometry-based targeted metabolic profiling of polyphenols. **Food Chemistry**, v. 305, p. 125507, 2020.