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Genetic Conservation and Medicinal Properties of Mate (*Ilex paraguariensis* St Hil.)

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ABSTRACT
This work provides an overview regarding genetic and botanical characteristics as well as chemical profile and potential utilization of mate (*Ilex paraguariensis* St Hil.) plant. The major components present in parts of mate plant are reviewed and possible health problems arising from customarily intake of hot infusions are addressed. Likewise, important applications of mate as drinks, cosmetics, and pharmaceuticals are commented along with relevant properties in human health treatment. Finally, it is discussed the mate world wide market in perspective with special emphasis on the path from the industry environment to costumer consumption.
KEY WORDS: Antioxidant activity, Breeding, Chemical profile, Genetic variability, *Ilex paraguariensis*

INTRODUCTION

Mate (*Ilex paraguariensis* St. Hill., Aquifoliaceae) a world-famous tea consumed in Brazil, Paraguay, Uruguay and Argentina, was used by the Indians of South America before European colonization. The raw matter, leaves and green stalks are processed as dye herb for the classical "Chimarrão", "Mate" or "Tereré", as fine or soluble powder for teas, or as essences used for different industrial purposes. "Chimarrão" or "mate" is prepared by steeping dry leaves and twigs of mate in hot water, where "tereré" is prepared with cold water (1). Although these are the most important applications of mate, new alternative products as drinks, cosmetics, and pharmaceuticals have been developed, and some of them are commercialized.

The annual production of mate in Argentina, Paraguay and South Brazil is about 1.4 million tons. Brazilian production represents 1/3 of the whole, and is one of the most important economical activities of many communities. As mate production is associated with small agricultural properties, it is considered a very important social activity, with approximately 750 small processing industries and more than 700 thousand direct and indirect employees (2).

In general, mate is considered as a stimulant drink that eliminates lassitude, increasing physical and mental activities. Biological and therapeutic activity on the cardiovascular, respiratory, muscular, gastrointestinal, renal and neurological systems have been attributed to the presence of xanthines, caffeine, theobromine, tannic substances, flavonoids, vitamins, and other substances present in mate extracts (3).

Caffeine has known effect on the central nervous system stimulating cerebral function. Vitamins C, E and those of the B complex present in mate are thought to stimulate defense mechanisms and muscular activity. Minerals and caffeine help blood circulation and cardiac work, reducing arterial pressure. Besides, mate is a potent diuretic (4).

Since its utilization in pharmaceutical formulations, mate has been incorporated in several official lists of medicinal herbs, such as the French pharmacopoeia in 1866 and 1884, Portuguese pharmacopoeia in 1876, Argentinean pharmacopoeia in 1898, and Brazilian Pharmacopoeia in 1929, among others (5).

The present review discusses botanical, geographic, genetic, chemical and pharmacological data of *Ilex paraguariensis*. The aim is to provide the readers an overview on the state of the art in the field of mate plant.

BOTANICAL ASPECTS OF *ILEX PARAGUARIENSIS*

The Aquifoliaceae family is formed by about 550 to 600 species, of which 400 belongs to the genus *Ilex*. This genus is formed by shrubs and trees from 2–25 m tall, with a wide distribution in Asia, Europe, North Africa, North and South America. The leaves are simple, and can be either deciduous or evergreen depending on the species, and may be entire, finely toothed, or with widely-spaced, spine-tipped serrations. In Brazil, 68 species of *Ilex* have been reported (6). Among Brazilian mate species, the most important are: *Ilex paraguariensis* (St. Hill., 1822), popularly known as "Erva mate" and whose leaves and stalks are commercialized in several South American countries; *Ilex dumosa* (Reisseck), popularly known as "caúna dos capões" or "congonha pequena", characterized by small leaves that are used to prepare teas with a delicate sweet-bitter taste with no caffeine; and *Ilex theezans* (Mar. ex Ressek) known as "caúna amargosa" or "congonha do mato". The last two species are considered as adulterants on mate production (7).

The mate plant (*Ilex paraguariensis*) is a shrub or small tree growing up to 15 meters tall. However, when pruned did not exceeds 7 meters, and under new agricultural and pruning systems they are maintained with 2 to 3 m. Its trunk has between 20 and 50 cm of diameter. The leaves are alternate and evergreen, 7–11 cm long and 3–5.5 cm wide, with a serrated margin. The flowers, located at axils of the terminal leaves, are small, hermaphrodites, pedunculated, greenish-white, with four petals. Though being hermaphrodites, flowers are unisexual by abortion (8). In this sense, mate can be considered as a dioic plant, with female plants exhibiting flowers with non functional stamini, and male plants showing residual pistil structures. Flowering occurs within september and december (spring and early summer), and fruiting takes place between january and march. The pollination is made by insects, although pollen transfer by wind is not discarded (9).

The fruits are tetralocular with succulent mesocarp and a hard endocarp. The fruits are red berry with 4–8 mm diameter (8). Mature fruits are eaten by birds that are responsible for the dissemination of *Ilex paraguariensis* (10).

As other species of the genus, *Ilex paraguariensis* is distributed in tropical and subtropical regions of Brazil, Argentina, and Paraguay, with a total area of 540.000 km^2. This area corresponds to 3% of South America, and 5% of Brazilian territory. Eighty percent of the area of distribution of *Ilex paraguariensis* is within Brazilian territory, including Rio Grande do Sul (RS), Santa Catarina (SC), Parana (PR), and Mato Grosso do Sul (MS) States (11).

The area of occurrence of *Ilex paraguariensis* is within the parallels 21° and 30°S, and 48°30' and 56°10´W. This tree is generally found associated to the Araucaria forest in altitudes of 500 to 1500m, in a typical subtropical humid climate (approximately 1500mm/y) characterized by the absence of a dry season and average summer temperatures of 22°C. However, this species can occur in lower and higher altitudes and climates with a short dry season. Mate prefers deep soils with low level of nutrients, low pH and high concentrations of exchangeable aluminum (11). Figure 1 shows the area of mate distribution.

Figure 1: Occurrence area of mate (Ilex paraguariensis).

BREEDING OF *ILEX PARAGUARIENSIS*

Over the last decades, mate cropping has been stimulated to satisfy the increasing demand of raw mater. In general, planting is made using plantlets obtained from seeds collected from non-selected native plants. This practice originated heterogeneous populations with low productivity. Belingheri and Prat Kricun (12) concluded that less than 35% of the plants of a commercial planting are responsible for more than 50% of the overall production. Such fact alerts for the necessity of new strategies in mate production including the selection of high productive genotypes for the establishment of cultivated areas.

The existing high similarity of cultivation and utilization between tea (*Camellia sinensis*) and mate (*Ilex paraguariensis*) allowed using the breeding experience obtained in the last century in tea to delineate strategies for mate breeding. Among these experiences are the use of repeated measures of individual plants, the selection based on the production stability along several harvest seasons, the use of experimental designs with replications for clones recommendation, and the adoption of clonal experiments in at least two seasons before selection. However, other practices that were not adopted in tea breeding programs should be incorporated in mate breeding, like long term programs using progeny tests for clonal selection. In terms of tea quality, great advances have been obtained using taste proofs and biochemical tests, which together with the proper adaptations can be applied to mate (13).

Simeão et al. (14) estimated genetic and phenotypic parameters and predicted breeding values for parents and provenances of *Ilex paraguariensis*. Eight provenances with 164 progenies were evaluated on three locations (Ivaí, PR, Guarapuava, PR and Rio Azul, PR) for the trait leaf weight (LW). All the variance components, parameters and breeding values were obtained at the individual level, through multivariate analysis involving the three sites. The individual strict sense heritability for the trait LW were 0.15 in Ivaí, 0.62 in Guarapuava and 0.23 in Rio Azul, demanding more sophisticated selection methods in Ivaí and Rio Azul. The provenance effect was significant in Ivaí and Rio Azul, with intra-class phenotypic correlation of 0.13 in Ivaí and 0.09 in Rio Azul. The provenances were more stable across sites than progenies with genetic correlation of 0.95 between the sites Ivaí and Rio Azul. Genetic values for all provenances and parents in all sites were predicted for the trait LW. The best provenances are Barão de Cotegipe, Quedas do Iguaçu, Cascavel and Ivaí. Moreover, these authors showed a high correlation between plants surviving and leaf weight production, both within and among progenies.

In another experiment, Rosse and Fernandes (15) estimated genetic parameters and applied principal component techniques as a tool for the selection of several traits in mate. Heritability estimates were significant for most of the evaluated traits suggesting the possibility of great genetic gains with simple selection techniques. The shaft height trait showed high coefficient of variation, low heritability and low correlation with the other ones. Besides that, this trait showed to have the lowest contribution to the total variation of the data, as demonstrated by principal components, and could be discarded. Conversely, plant height and crown diameter accounted for the most part of the detected variation.

Sturion et al. (16) studied several populations and progenies to evaluate genetic parameters for leaf weight production, one of the most important characters in mate breeding. These experiments showed that the narrow sense heritability for this character range between 0.18 and 0.58, depending on the location in which the populations were evaluated. The mean narrow-sense heritability for leaf weight was estimated in 25%, a value that is considered moderate, and can reduce breeding gains if environmental variance is not reduced or controlled during the selection of mate progenies.

The selection of perennial plants involves the ranking of the candidates based on phenotypic values, which are more precise in very well controlled environments. In this sense, experiments made with Toledo population by Sturion et al. (17) allowed to estimate the genetic gains obtained by direct selection of leaf weight and crown volume in 59.5 and 58.4%, respectively, suggesting that crown volume can be used for indirect selection to improve leaf weight. Moreover, these authors concluded that the selection using simultaneously individual and family information is an efficient system for leaf weight production improvement in mate.

Based on several experiences, Sturion et al. (18) concluded that the most efficient method to increase the productivity of mate is the selection of parental plants based on progeny tests, or the identification of superior plants within the progeny to be used as parental plants. However, these methods are laborious and time consuming in perennial plants and represent an option for long term breeding programs. Conversely, the simple collection of seeds from selected plants in nature offers a rapid, although less efficient, method to improve mate productivity. The genetic progress obtained by this method depends on the control of environmental factors and the heritability of the characters under selection.

The knowledge about the genetic variability and the genetic control of important traits is of fundamental importance to establish the strategies to be adopted in a breeding program, as well as to estimate the genetic gains obtained by specific breeding methods. In this sense, it is important to develop progeny tests that can produce improved plants and allows the study of the heritability of important traits. Seeds produced in a progeny test can represent new material for a specific region. In other words, seeds genetically improved may not repeat their performance in other regions due to the strong genotype vs. environment interaction detected in mate. Two strategies may be adopted: a) select the superior genotypes for a given environment, or b) select the most stable genotypes in different environments. In the last case, we should remember that this approach implicates in loss of genetic gains in relation to those that can be obtained by the selection of genotypes specialized for a given environment. Among the methods adopted for mate improvement, the most efficient, in decrease importance order are: clonal propagation from selected plants, biparental progenies, and clonal progenies.

GENETIC VARIABILITY IN *ILEX PARAGUARIENSIS*

Despite the increase in the mate cultivated area, production of mate is not enough to appease the demand of a growing market. In this context, the indiscriminate harvests from native plants are still a common practice in South and Southeast Brazil. The collection from native plants, associated with the enlargement of the agricultural frontier and the consequent reduction of natural forest units, represent a risk for the genetic variability of *Ilex paraguariensis*, and have led to the inclusion of this species in the official list of endangered plants. As could be expected, Brazil as the country with the largest area of mate distribution (80%) has the responsibility for the conservation of this species (19). However, until now just a small number of official initiatives have been proposed, and only isolated studies have been done.

Research on the variability of mate in Brazil has been carried out sporadically by different groups, using materials collected from specific regions (20-22). The lack of information about the overall genetic variability of mate prejudices the correct management of germplasm banks and the definition of politics of "*in situ*" and "*ex situ*" conservation.

Gauer and Cavalli-Molina (20) studied the genetic variability of four natural populations of *Ilex paraguariensis* using RAPD and seed proteins markers. Most of the bands analyzed for these markers (51.6% RAPD and 75% seed proteins) were present in the four populations, but in different frequencies. All the RAPD bands analyzed were polymorphic. The intra-population variability as measured by the Nei and Li (D) and Jaccard´s (J) distance were D=0.392 and J=0.343, respectively, indicating high intra-population variability.

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Conversely, the interpopulation variability was relatively low, with D= 0.422 and J= 0.320. A partition analysis (Shannon index) showed that 85% of the variability detected by RAPD markers occurs within populations, and just 15% between populations.

Cansian (23) evaluated the genetic variability within and among three natural populations of *Ilex paraguariensis* (Áurea, Erechim and Barão de Cotegipe) using RAPD markers. The polymorphism within populations varied from 24.9 to 36%. The similarity index (Jaccard) was 0.85 for Áurea, 0.88 for Erechim and 0.93 for Barão de Cotegipe, indicating that the genetic variability of mate populations may vary depending on the genetic background and gene flow. As reported by Gauer and Cavalli-Molina (20) the highest portion of the genetic variability occurs within population, with remarkable variation on the allelic frequencies. Cluster (UPGMA) and principal coordinates analysis allows clearly separating the three populations, showing that despite the geographic proximity, these populations represent different genetic entities. The genetic separation of mate populations (Figure 2) indicates that the "in situ" conservation strategies may favor the maintenance of the largest possible number of forest units to guarantee the genetic diversity of the species. These results are similar to those obtained by Vidor et al. (22) who evaluated the variability of the progenies of two populations of *I. paraguariensis*.

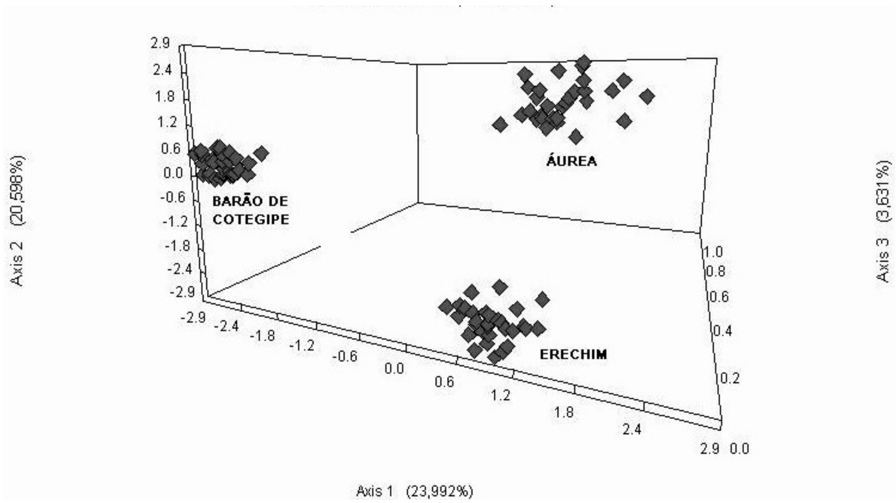
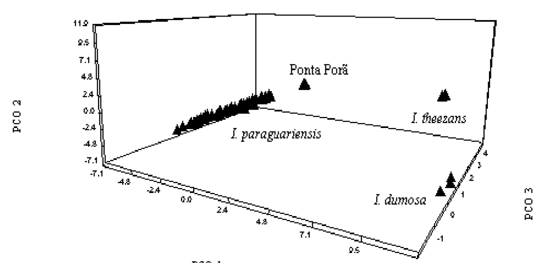


Figure 2: Principal Coordinates Analysis based on the Euclidian distances obtained from RAPD data of plants from three populations of I. paraguariensis, showing an evident genetic separation of the populations and a largest dispersion in Áurea population.

In another study, 20 populations collected along the distribution area of *Ilex paraguariensis* in Brazil, including samples from Mato Grosso do Sul, Paraná, Santa Catarina and Rio Grande do Sul States, were analyzed using RAPD markers. Two species of the genus *Ilex*, *I. dumosa* and *I. theezans*, were included in the analysis. The 24 primers selected for the analysis generated 291 fragments in *I. paraguariensis* with an average of 12.12 fragments per primer. A total of 76 fragments (26.11%) were polymorphic. The similarity index (Jaccard´s coefficient) varied in the range 0.845 - 0.972 with an average of 0.908. The cluster (UPGMA) and principal coordinates analysis allowed to separate the population of Ponta Porã collected in Mato Grosso do Sul from the other populations (Figure 3).

The polymorphism index among the three species of *Ilex* under study was 75.51% of 392 fragments analyzed. Thirty seven specific amplification fragments were identified in *I. dumosa*, 21 in *I. theezans*, and 76 in *I. paraguariensis*. These markers may be used to control the quality of mate products. The mean Jaccard´s coefficient among *I. paraguariensis* samples and the other species of the genus was 0.396. These results indicate that the overall genetic diversity of mate-tree is relatively narrow, a fact that may be considered in conservation strategies. In fact, based on these results, germplasm banks ("ex situ") may be defined by genetic criteria selecting materials that represent the genetic variability of the species. On the other hand, to ensure the maintenance of rare alleles, *in situ* conservation may favored a large number small forest units rather than a small number of large units (23).Figure 3: Principal Coordinates Analysis based on the Euclidian distances obtained from RAPD data of 20 populations of *I. paraguariensis*, and representatives of *I. theezans* and *I. dumosa*.



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CHEMICAL COMPOSITION OF MATE

Several works in the literature show that mate composition is quite complex, comprising more than 250 volatile compounds such as methylxanthines (mainly caffeine, theophylline, and theobromine), tannins, saponnins, vitamins, a variety of hydrocarbons, alcohols, esters, aldheydes, fatty acids, carboxylic compounds and, in some cases, phenylpropanoids and mineral compounds, among others (23-28).

Gosmann et al. (29) and Kraemer (30) described the presence of 11 glycosides such as ursolic and oleanolic acids and also the sugar-based compounds arabinose, glycose and ramnose. Kraemer et al. (31) aiming at determining the chemical composition of other parts of mate besides leaves, performed TLC analysis of samples from root, barks and fruits. Athayde (25) investigated the presence of saponnins in leaves and fruits of Brazilian native *Ilex paraguariensis*.

Since the discovery of caffeine by Stenhouse in 1843 and theobromine by Oehrli in 1927 in *I. paraguariensis* (32), many works have been published regarding the content of these methylxanthines in leaves, barks and fruits of mate (33-39). Some researchers make reference to the presence of theophylline in leaves and in commercial products made from mate (35, 40).

The stimulating, antirheumatic, diuretic and digestive properties of infusion of green or dried mate leaves have been associated to the presence of the alkaloids caffeine, theophylline, and theobromine. For instance, it is well known that caffeine acts in the central nervous system, accelerates metabolism, the oxygen income in lungs, and increases heart rhythm (41). The chemical structure of these compounds is depicted in Figure 4.

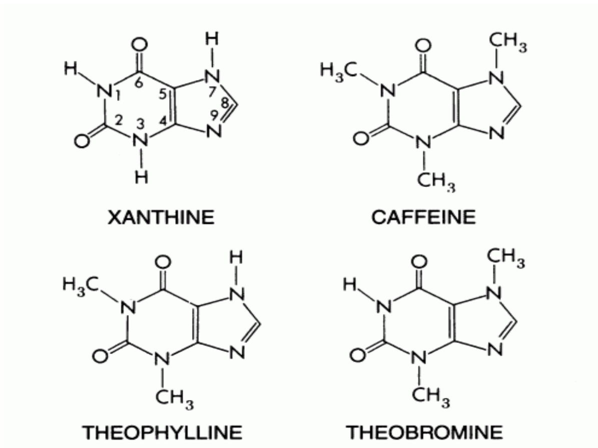

Figure 4: Molecular structures of methylxanthine compounds.

Kawakami and Kobayashi (28) used GC/MS to identify 196 compounds in the volatile oil of mate: 23 aliphatic alcohols, 24 aldheydes, 29 cetones, 15 acids, 8 lactones, 9 terpenes, 11 terpenic alcohols, 25 alicyclic, 11 phenols, 7 aromatics, 13 furanones, 6 pyrazines and 2 pyrroles.

The soluble solids content, total phenolic content (Folin-Ciocalteau method), antioxidant activity (ferric thiocyanate method) and essential oil composition of green mate and mate tea (roasted green mate) infusions were analyzed by Bastos et al. (42). Although the antioxidant activity of green mate and mate tea infusions were equivalent, the soluble solids content in green mate was higher (4.2%) than in mate tea infusions (3.2%). Important compounds that add flavor to plant infusions such as linalool, present in high concentrations in the green mate essential oil, were oxidized to linalool oxides after roasting. Limonene decreased from 19.5% to 7.3%, and furfural and methylfurfural were formed (42). According to Machado et al. (43) the main flavor compounds identified from green mate by gas chromatography/mass spectrometry were linalool, α-terpineol and trans-linalool oxide and in roasted mate were (E,Z)-2-4-heptadienal isomers and 5-methylfurfural.

Mate also contains hydrosoluble polyphenols such as isochlorogenic acid, caffeic acid and chlorogenic acid, which present antioxidant activity and may have hepatoprotective action (44; 45-47).

Filip et al. (48) determined the content of whole flavonoids and caffeoil-derived compounds present in the aqueous extracts of *I. paraguariensis* and observed that *I. paraguariensis* presented the highest concentration of phenolic compounds compared to the other *Ilex* species studied. The total content of caffeoil-derived compounds was 9.61 wt% for *I. paraguariensis* while a range of 0.12 - 1.90 wt% was found for other species.

Caffeic acid and caffeine concentrations of mate samples from south Brazil were in the range previously reported in samples from Argentina (0.023 wt% and 1.92 wt%, respectively) (48-49). Beverage from reforested plants had significantly higher caffeic acid and lower catechin, chlorogenic acid, caffeine and gallic acid content as compared to the beverage from native plants. Sensory tests revealed differences in the taste of beverages brewed from native plants and reforested plants and results may be explained by the levels of catechin, caffeic and chlorogenic acids found in the extracts, indicating the role of these substances in the taste properties of mate (50).

Two types of mate, with and without sticks and three brands for each type were evaluated for caffeine content and the time course of bitterness (51). Chemical analysis made by high performance liquid chromatography (HPLC) of the six mate infusions at 5 % wt/v showed that caffeine levels were higher for mate without sticks brands. The kinetic study of mate bitterness assessed by time-intensity (TI) curves revealed that the presence of sticks lowered maximum intensity, total duration, and area under the curve of bitter perception.

According to some published researches, flavonoids and lipophilic substances present in leaves of *I. paraguariensis* possess pharmacological effects, like appetite decrease (52). Potential properties like anticarcinogenic and antimutagenic have been attributed to these compounds (53-54). Among flavonoids in mate, rutine, quercetine and campferol-3-o-rutinoside merit special attention. The content of rutine, quercetine and campferol found in *I. paraguariensis* was 0.0060, 0.0031 and 0.0012 wt%, respectively (48). Yet, Matsubara (55) determined 2.0 - 3.3 mg/g for quercetine in leaves and 0.3 - 0.6 mg/g for this compound in infusions of mate (5g of leaves in 500 mL of water), a compound not detected in samples of commercial mate teas.

The vitamin E present in mate is beneficial for several parts of the human organism, while vitamin C helps in the blood circulation, acting as a natural defense to the organism. Among enzymes, it has been identified oxidase that together with caffeine exerts the function of organism nutrition (56). Some carbohydrates in leaves of mate were analyzed by Paredes et al. (57), and verified 2.8 wt%, 0.6 wt% and 0.3 wt% of saccarose, glucose and fructose, respectively.

Cansian et al. (58) investigated the semi-volatile compounds variation among Brazilian populations of *Ilex paraguariensis* in extracts obtained from supercritical carbon dioxide extraction; the following constituents were identified: caffeine, theobromine, phytol, eicosane, squalene, pentatriacontane, vitamin-E, and stigmasterol. Significant differences between populations were observed for all the analyzed compounds.

The low caffeine concentration observed in some populations indicated that it was possible to select populations with low caffeine content, which was particularly important considering that estimates of caffeine intake due to mate tea consumption in Brazil, Uruguay and Argentina exceeded by far intakes recorded for other beverages containing this alkaloid (59). This difference may be associated with climatic and physiological characteristics like photoperiod, seasonal temperature variations, light intensity, and leaves age, factors that influence the accumulation of methylxanthines, particularly caffeine (34, 60-61).

Similar results were found by Athayde and Schenkel (38) regarding the profile of methylxanthines in four Brazilian native populations of *I. paraguariensis*. Theobromine was detected at low concentrations in all the populations studied, while theophylline was not detected. Theobromine concentrations were within the range previously reported for mate leaves (35-36). The absence of theophylline might be associated with the low solubility of this specific methylxanthine in carbon dioxide. Phytol, a diterpenic acyclic alcohol used to synthesize vitamin E, K and widely employed in several fragrance products, presented concentrations in the range of 25.50 mg/g to 0.31 mg/g in the populations analyzed. As phytol is one of the chlorophyll precursors (62), variation in its content may be attributed mainly to the rate of sun exposure and age of leaves.

Squalene (a triterpene) is an intermediate in the biosynthesis of sterols (63) and has demonstrated proliferation activity in animal cancer studies, some radio protective effects, and may also have a cholesterol-lowering effect, though this has not been tested in humans (64). Great differences were found for the content of this compound, with concentrations ranging from 4.9 mg/g to 96.6mg/g.

It is well known that in all photosynthetic organisms, vitamin E (tocopherol) and other isoprenoids have important photoprotective role, either by dissipating excess excitation energy as heat or by scavenging reactive oxygen species (ROS) and suppressing lipid peroxidation. Compelling evidence indicates that these non-ubiquitous isoprenoids might be particularly relevant for adaptation capacity of plants to adverse climatic conditions by serving as additional and/or alternative protection mechanisms (65). The concentration of vitamin E did not present great variations within populations analyzed.

Stigmasterol is a commonly found sterol in vegetables and presented variations in the range of 43 mg/g to 14.49 mg/g in the populations investigated. Eicosane and pentatiacontane are alcanes present in the waxes of leaves; the greatest concentration was found in some populations, thus indicating differences in the age of leaves and some hydric stress in the collect period (58).

Saldaña et al. (36) reported the use of supercritical CO_2 for the extraction of purinic alkaloids where the solubility of caffeine and theobromine is discussed. Dariva et al. (66) investigated the effects of industrial processing variables of mate using the extracts from high-pressure carbon dioxide and observed that the drying step is major responsible for reduction of extraction yield. Also, it was determined the optimum extraction conditions as 35ºC, 200 bar and solvent flow rate of 2 g/min.

Malik et al. (67) reported high levels of B, Ca, Cu, Mg, Mn and Zn in mate, mainly green type. The authors mentioned that the intake of one liter of green mate provides one-third to half of the magnesium recommended daily allowance (RDA) (210-320 mg/day) and the entire RDA of Mn (2-5mg/day).

CHEMICAL VARIABILITY OF MATE

Mazzafera (34) verified that, when comparing leaves from the same plant, those coming from shadow parts presented higher theobromine contents. Under stress conditions some plants that produce alkaloids have the ability to increase the concentration of this compound (61). Additionally, for plants tolerant to shadow, it has been noted a raise in alkaloids concentration with shadow increase (68). The rise in defense substances against insects and fungi, such as caffeine and theobromine, can be seen as a warranty to prolong life of leaves in shadow habitats, thus compensating the biological investments needed to build these organs (69).

Different concentrations of caffeine, theobromine and theophylline were found in young and old leaves from plants with and without fruits in *I. paraguariensis*, thus showing variations in chemical composition in the same plant (34). Mazzafera (59) studied the concentration of caffeine and phenolic acids in mate infusions (chimarrão) of plants from different places of RS, SC and PR, and observed variations between 0.29 to 0.79 mg/mL for caffeine and 0.70 to 1.60 mg/mL for soluble phenols, whereas no correlation was found with places.

Higher contents of methylxanthines were found for plants cultivated in artificial shadow areas compared to their counterparts exposed to direct sun light (70). Athayde and Schenkel (38) investigated the presence of methylxanthines and saponnins in four populations (MS, PR, SC and RS) of *I. paraguariensis* samples collected in two seasons, spring and summer. These authors verified that summer samples coming from MS presented average caffeine contents higher than the others, without differences in theobromine concentration. In the spring samples, it was not observed significant differences in caffeine concentrations but a higher content of theobromine in populations of PR and RS. With regard to chemical analysis of saponnins, it was possible to identify all saponnins described *I. paraguariensis* only in the population of MS, with seasonal differences among them.

Cardozo Jr. (71) investigated the content of methylxanthines and phenolic compounds of 16 mate (*Ilex paraguariensis* St. Hil.) progenies from Brazilian regions (Ivaí, Quedas do Iguaçu, Cascavel and Barão de Cotegipe). Results revealed significant variations in total methylxanthines, caffeine and theobromine contents in progenies, according to plant origin. Total methylxanthines (0.560-0.734%) and caffeine (0.490-0.611%) contents followed the order: Ivaí>Quedas do Iguaçu>Barão de Cotegipe>Cascavel. Theobromine (0.132-0.068%) contents were inversely related to caffeine contents. Chlorogenic acid in the Cascavel progenie was lower (0.786%) than that found in the others (0.861-0.915%). No change occurred in total phenol and caffeic acid contents between progenies with regard to their origin.

ANTIOXIDANT ACTIVITY OF MATE

Recent findings about the oxidation mechanism of cellules responsible for a series of pathogenic diseases like arteriosclerosis, cancer and diabetes stimulated a great number of scientific researches on the oxidant action of substances naturally occurring in foods, capable of protecting live organs. Natural products containing phenolic compounds (72-77) as well as antioxidant activity of mate infusions (44-46, 78-79) have been the subject of growing research.

There are two different methods to evaluate the antioxidant activity of isolated substances: *in vitro*, using model systems in which the samples are tested regarding the ability of reducing the oxidation of polyunsaturated fatty acids, lipossomes or microssomes under standard conditions. In this case, the amount of oxidized products are measured as a result of added or not antioxidant substances, using as reference a system where a known antioxidant, e.g. BHT (Butylated Hydroxytoluole) or Trolox (hydrosoluble synthetic compound analogous to vitamin E) is used. It is well known that *in vitro* methods evaluate the effects of ingestion on cellular membranes. Several facts interfere in the *in vivo* antioxidant activity: reactivity of antioxidant substance relatively to free radicals, number of radicals that are susceptible to inactivation by this substance, interaction among antioxidant substances, its hydrophilic and hydrophobic nature, bioavailability, etc. In this regard, the reader may be interested in consulting the works of Mancini-Filho et al. (80), Mancini-Filho and Cintra (81), Silva et al. (82), Kahl and Hildebrandt (83) and Gray (84). Niki (85) has advised regarding careful interpretation of *in vitro* results.

Ingestion of mate infusions demonstrated to have important antioxidant activity *in vivo* and *in vitro*. The mechanism is related to the presence of substances capable of keeping free radicals formed at the beginning of the oxidation process. Mate infusions present high concentrations of chlorogenic acids and low contents of flavonoids. Several factors determine the concentration of these substances, such as time and temperature of infusion, mate to water wt/v ratio, material particle size, composition (percentage of leaves and barks) and the presence of adulterants.

Racanicci et al. (86) evaluated the use of aqueous extracts mate, prepared from dried leaves of *Ilex paraguariensis* St. Hilaire, to prevent lipids and vitamin E oxidation in precooked meat balls made from chicken breast added 0.5% salt and packed in atmospheric air. The authors stated that mate is an attractive alternative for protection of precooked meat products. The efficient protection was partially associated with a synergistic interaction with vitamin E. Such synergistic effects could involve radical scavenging by the vitamin E in the lipid phase followed by regeneration of vitamin E by water soluble phenolics from mate at the interphase between lipids and the aqueous phase of the meat. However, the development of new chicken meat products will depend on sensory evaluation of products with added mate.

Gugliucci and Stahl (78), in studying infusions and methanolic extracts towards evaluating the effects of these extracts on the start and propagation of LDL oxidation induced by Cu^{+2} and *in vitro* peroxide, concluded that both extracts inhibit the initial LDL oxidation and that the aqueous extract (50 mg of leaves/mL) is more efficient as antioxidant than ascorbic acid and BHT. A similar result was found by Gugliucci (45), who concluded that mate is capable of minimizing *in vivo* LDL oxidation effects, as confirmed by different analytical methods. Conversely, Campos et al. (44) evaluated two methods of antioxidant activity (inhibition by luminol chemoluminescence and decoloration of pre-formed cations derived from ABTS - 2,2'-azinobis -3-etylbenzotiazolin-6-sulfonate) observed that mate infusion possesses efficient and inefficient antioxidants, which determine the inhibition profile and were different from those obtained by trolox.

Schinella et al. (79) evaluated the effect of mate aqueous extracts on the enzymatic and non-enzymatic peroxidase in biological systems and in the formation of free radicals *in vitro*. They verified inhibition of enzymatic oxidation, even at low concentrations, possibly acting at the beginning of the process due to free radicals sequestration. The extracts demonstrated high activity to sequestrate superperoxide anions, non-related to the inhibition of xanthine oxidase. Filip et al. (46) studied the antioxidant activity of *Ilex* *paraguariensis* and other species of the *Ilex* gender and results showed that *Ilex paraguariensis* presented important antioxidant activity, a fact not observed for other species, and they related the results with the content of caffeic acid derived compounds, which are at higher concentrations than in other species.

Oxidation of human LDL and DNA denaturation of *Saccharomyces cerevisiae* are inhibited by mate infusions in a dosage-dependent faction. Other pharmacological actions of mate have been described. For example, Gugliucci and Menini (87) reported the use of mate infusion to the prevention of plasminogen and antitrombin III inhibition induced by methylglyoxal. This process inhibition is related to cardiovascular complications due to diabetes resulting from Amadori reactions with dicarboniles such as deoxyglucosone, methyl glycoxal and glycoxal by oxidative processes.

Miranda et al. (88) evaluated the antioxidant activity of mate tea as well as the ability to influence DNA repair in male Swiss mice. The data presented demonstrated that mate tea is not genotoxic in liver, kidney and bladder cells. The regular ingestion of mate tea increased the resistance of DNA to H_2O_2-induced DNA strand breaks and improved the DNA repair after H_2O_2 challenge in liver cells, irrespective of dose ingested. The authors suggested that mate tea could protect against DNA damage and enhance the DNA repair activity, which may be afforded by the antioxidant activity of the mate tea's bioactive compounds.

Gorzalczany et al. (89) studied the coleretic effect and intestinal propulsion after mate ingestion and results support the hepatoprotective and digestive activity attributed to this drinking. Baisch et al. (90) investigated the vaso-dilatatory effect of mate infusion in rats and suggest the presence of some compounds in mate drinking that can cause vasorelaxing endotely-dependent mediated by NO-cGMP or AMP.

The antibacterial activity of linalool, β-ionone, α-terpineol, geraniol, 1-octanol, octanoic acid, nerolidol and eugenol present in mate infusion was studied by Kubo and co-workers (91) against *Streptococus mutans*, which is one of the bacteria that cause dental carieses, and verified that all compounds exhibited some activity action. Muller et al. (92) tested the aqueous and organic extracts of South American plant extracts, including *Ilex paraguariensis,* for antiherpetic and antirabies activities. From the 18 extracts/fractions tested, six of them showed antiviral action with selectivity indexes greater than 7: *Ilex paraguariensis, Lafoensia pacari, Passiflora edulis, Rubus imperialis* and *Slonea guianenis*

Deladino et al. (93) obtained aqueous extracts of yerba mate and characterized by total polyphenols content and antiradical activity. Yerba mate lyophilized extracts were encapsulated with calcium alginate and calcium alginate-chitosan systems. The encapsulation of yerba mate extracts showed to be a promising technique for food supplementation with natural antioxidants, since the encapsulation procedure protected the antioxidant from the surrounding medium or the processing conditions during food production.

RELATIONSHIP BETWEEN CANCER INCIDENCE AND CHIMARRÃO CONSUMPTION - Inspection of literature reveals that while research is becoming progressively established for some plant infusions (e.g., *Camellia sinensis* and other plants rich in phenolic compounds), there is no corresponding development regarding drinking from mate (94-98). Just a few works have been devoted to check a possible relationship between mate infusions as chimarrão and the incidence of esophagus cancer, a commonly found illness in Brazil, Uruguay and Argentina. To take a glance at this serious disease, it may be opportune to cite that the standard decease coefficient due to esophagus cancer in Rio Grande do Sul (south Brazil) is around 14.3/100.000 inhabitants for men and 4.2/100.000 inhabitants for women, impressive figures according to WHO (World Health Organization).

According to Pinto et al. (99) consumption of chimarrão, regardless other risk factors like alcohol and tobacco, is responsible for around 20% of registered esophagus cancer in south and southeast of South America. Some studies suggest that the high temperature (in some cases above 60ºC) of chimarrão intake could be the main risk factor (100-102), while other authors suggest that substances present in chimarrão are potentially carcinogenic or could help increase possible problems caused by ingestion of great volumes, whose average consumption *per capita* is about 1.3 L/day, achieving in some places values as high as 6L/day (94).

Vassalo et al. (103) investigated the relationship between esophagus cancer and chimarrão intake in Uruguay in the period 1979-1984 keeping controlled the risk factors of alcohol and tobacco. The amount and frequency of chimarrão ingestion presented a negative independent effect. The authors suggested that tannins present in mate and/or processing products together with the high intake temperature would be responsible for disease advance. Later, it was confirmed the presence of high contents of carcinogenic compounds, polycyclic hydrocarbons that contaminate mate when wood is used in the processing steps (104). These substances and not the naturally occurring phenolic compounds could be acting as co-adjuvant in the disease development.

Dietz et al. (105) evaluated 55 patients in a hospital situated in Porto Alegre (Rio Grande do Sul, Brazil) during the period of march 1990 to december 1991, who suffered from esophagus cancer and compared with a population of 110 people that presented gastroenterologics illness but not tumor esophagic to the endoscopy. The control population was ordered in relation to sex and age range. Results demonstrated that tobacco, alcohol, mate, agriculturists and father antecedent of cancer were significantly more frequent in the cases of esophagus cancer in relation to control population. Consumption of chimarrão was also significant, independent on the intake amount.

Castellsagué et al. (106) studied the relationship between hot drinking intake, including chimarrão, and the ingestion of other food with high esophagus cancer risk. In that research it was employed 830 patients having the sickness and 1779 control people in 5 hospitals in South America. After statistical elimination of the risk factors of tobacco and alcohol, it was confirmed the association of esophagus cancer with consumers of hot chimarrão in great amounts, for both sexes. It is worth mentioning that the coupled effect of temperature and amount of chimarrão intake resulted in double potential risk, especially in the case of great volumes of hot chimarrão intake (>1.5 L/day). Ingestion of other hot drinkings, like tea and coffee, also corroborate the association risk, though this fact was not observed for pure coffee intake. The authors verified the significant protective effect of fruits, cereals and vegetables, and a moderate risk increase in the case of fats, salt and meat ingestion.

As verified by De Stefani et al. (107), ingestion of chimarrão increases 1.6 times of lung cancer risk among men that consume hot chimarrão. De Stefani et al. (108) reported a case study relating diet and risk of kidneys cancer in Uruguay from 1988 to 1995. After normal adjust of risk factors variables, it was observed that red meat ingestion presented a risk factor as high as 3.4, while mate intake was associated with a risk factor of 3.0 for high volumes ingestion. A review on this subject was provided by Goldenberg (109), in which it is shown that chimarrão intake must be considered a risk factor for the development of oral and oropharyngeal cancer.

De Stefani et al. (110) investigated the relationship between bladder cancers with mate drinking in Uruguay. In the period of 1996-2000, 255 incident cases transitional cell carcinoma of the bladder and 501 patients treated in the same hospital and in the same time period were frequency matched on age, sex, and residence. Both cases and control were face-to-face interviewed on occupation, tobacco smoking, alcohol drinking and intake of mate, coffee, tea, and soft drinks. Ever mate drinking was positively associated with bladder cancer risk and the risk increased for increasing duration and amount of mate drinking. In a similar work in Argentina involving 114 case-control pairs, Bates et al. (111) concluded that the consumption of mate was associated with bladder cancer in ever-smokes, but not in never-smokers.

Kamangar et al. (112) conducted a study to determine whether drinking mate could lead to substantial exposure to polycyclic aromatic hydrocarbons (PAH), including known carcinogens, such as bezo[α]pyrene. The concentrations of 21 individual PAHs were measured in dry leaves of eight commercial brands of yerba mate and in infusions made with hot (80°C) or cold (5°C) water. The total concentrations of the 21 PAHs in different brands of yerba mate ranged from 536 to 2,906 ng/g dry leaves. Bezo[α]pyrene concentrations ranged from 8.03 to 53.3 ng/g dry leaves. For the mate prepared using hot water 1.37% of the total measured PAHs and 50% of the bezo[α]pyrene content were released into the 12 infusions, which is indicating very high concentrations of carcinogenic PAHs in yerba mate leaves and in ht and cold mate infusions. These results support the hypothesis that the carcinogenicity of mate may be related to its PAH content.

CONCLUSIONS AND RESEARCH DIRECTIONS

The industrial processing of mate has not significantly changed along the years and consists basically of three steps: *sapeco*, drying and *cancheamento*. Mechanic sapeco is basically a gyratory bent metallic cylinder, from which mate is submitted to direct fire resulting in burnt leaves. This step must be carried out as fast as possible after mate harvesting. This practice removes superficial plant humidity, inactivate oxidase enzymes (peroxidase and polyphenoloxidase) and prevent leaves to become dark and with unpleasant taste. The drying step is accomplished in proper places, long enough to provide crisp leaves. "Cancheamento" means mate triturating or fragmentation, just after drying step, and is generally performed using a metallic or woody device, where the plant is sieved, thus becoming a raw material to the preparation of commercial teas. Then, mate with selected particle size is homogenized in mixing apparatus having helical parts.

The development of new products depends of course on fundamental and industrial environment research. Mate product despite increasing participation in national and international market, is still limited from a commercial and industrial point of view. Besides the commonly use of mate for infusions (chimarrão), reaching 80% of the whole consumption, chemical composition of mate leaves have huge potential to other applications, as shown in Table 1, below. Analysis of Brazilian exports demonstrate that in the three states Paraná, Santa Catarina and Rio Grande do Sul, almost all mate exportations were directed to Uruguay. After 1988, exportations for other countries such as Germany, Syria, Japan and United States of America start becoming relevant, as some of them recognized mate as an important food and medicinal renewable source. Moreover, the possibility of expanding mate exportations to MERCOSUL countries has motivated improvements in product industrialization and commercialization.

Table 1: Summary of possible mate applications from industrial to consumer consumption.

Industrial Segment	Commercial Products	Consumption type
Drinking	• Chimarrão • Tererê • Mate infusion: toasted, green/cooked, soluble	Hot or cold infusion
	• soft drinking • juices • beer • wine	Diluted leaves extracts
Food stuffs	• natural colorant • food preservatives	Chlorophyll and essential oil

	• ice cream • candies, chocolates and caramels • gums	
Remedy	• nervous stimulant • compounds for the treatment of hypertension and pneumonia	Caffeine and theobromine extracts Flavonoids extracts
General Hygiene	• domestic and hospital bactericidal and antioxidant • sterilizing • emulsificant • waste water treatment • urban garbage recycle	Saponnins and essential oil extracts
Personal care products	• perfumes • deodorants • cosmetics • soaps	Leaves extracts and chlorophyll

Production of mate without agro-chemical agents (pesticides, fungicides, herbicides) together with bio-dynamics agriculture parameters (e.g., organic fertilization, correct field handling), may confer to mate important quality certifications. For this purpose, important components must be incorporate to mate chain, like International Demeter standards from IFOAM (International Federation of Organic Agriculture Movements).

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