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**Author:** Wilson, Erica Georgina  
**Title:** Contributions to the quality control of two crops of economic importance: hops and yerba mate  
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HPLC Determination of Arbutin in Ilex Species

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Introduction

The genus *Ilex* (Aquifoliaceae) comprises more than 400 species of dioecious trees and shrubs distributed throughout temperate and tropical regions of the world. The main areas of extant diversification are East Asia and South America. Most *Ilex* species are used for ornamental purposes, but a few of them have a reported medicinal use—*I. vomitoria* as an emetic and *I. paraguariensis*, as a tonic, diuretic and stimulant to reduce fatigue and aid gastric function (Gosmann et al., 1989; Commission E, 1999). Both these plants share a unique feature in this genus, which is the presence of caffeine and theobromine (Wilson et al., 1982; Clifford and Ramirez-Martinez, 1990; Reginatto et al., 1999; Coelho et al., 2001; Choi et al., 2005, Kim et al., 2010). The main use of *I. paraguariensis* lies in its leaves, which after drying and roasting are used to prepare a herbal tea, Yerba mate, an extremely popular beverage that is consumed in all the south of South America, especially Argentina, Uruguay, Paraguay and southern Brazil (Goldenberg D, 2002). In some of these countries this mate consumption can exceed coffee or tea consumption by far. Grown mostly in NE. Argentina, it constitutes one of the most important regional crops for internal consumption as well as being exported to neighbouring countries and increasingly to Syria, USA and EU countries due to its diuretic and stimulant activities. According to figures published by the INYM - National Institute of Yerba Mate, the total *I. paraguariensis* harvest in 2010 in Argentina, the main producer of the region, was 745 thousand tonnes, resulting in the production of above 250 thousand tonnes of Yerba Mate, while the total annual harvest in Brazil is in the order of 470 thousand tonnes (INYM, 2010). Even when most yerba mate companies grow their own *I. paraguariensis* a substantial part of their raw material is bought from a great number of smaller local producers and it is possible that some of this is adulterated or substituted with other cogenic species (Giberti, 1994) that grow wildly in the region. These are mainly *I. argentina*, *I. brasiliensis*, *I. brevicuspis*, *I. integerrima*, *I. microdonta*, *I. pseudobuxus*, *I. taubertiana*, *I.dumosa var dumosa*, *I.dumosa var guaranina* and *I. theezans* among others. Consequently, all issues concerning the chemical composition of these species acquire a high importance since they are unknowingly consumed by vast amounts of the population. Additionally this
information could contribute to the possibility of discerning them chemically from *I. paraguariensis*, providing a tool for their detection in quality control procedures.

The chemical composition of *I. paraguariensis* has been quite intensely studied in the last 20 years. Apart from its methylxanthine content mentioned above, it has a significant amount of polyphenols (Carini et al., 1998; Bravo et al. 2007; Jaiswal et al., 2009) and saponins (Gosmann et al., 1989, 1995; Kraemer et al., 1996). In the case of the other *Ilex* species mentioned above, there is a good knowledge of their saponin content (Taketa, 1994; Heinzmann et al., 1995; Constantine, 1995; Schenkel et al., 1995; Pires et al., 1997; Athayde et al., 1999, 2001; Taketa et al., 2000, Taketa et al., 2002) and a general consensus on the absence of methylxanthines (Reginatto et al., 1999; Clifford and Ramirez-Martínez, 1990). In the case of the polyphenolic content, until the moment of this investigation, the differences detected were quantitative rather than qualitative, since all the mentioned *Ilex* species contain caffeoylquinic acids (CQAs) mainly represented by chlorogenic acid isomers (CGAs) and isochlorogenic acid, apart from varying amounts of flavonoid glycosides rutin (Filip et al., 2001) and luteolin or kaempferol glycosides, over which there is no unanimous opinion. In the case of saponins, most *Ilex* species mentioned above have mono- or bidesmosidic derivatives of oleanolic acid, while *I. paraguariensis* is the only one to have ursolic acid derivatives (apart from some oleanolic acid derivatives). In general the saponin content is quite characteristic of each species, up to the point that some authors consider them to be possible candidates for biomarkers for the detection of substitutions or adulterations of *I. paraguariensis* (Pires et al., 1997).

In view of the similar qualitative chemical composition of these *Ilex* species it was rather surprising to find, in the course of the $^1$HNMR profiling of *Ilex* species, (Choi et al., 2005; Kim et al., 2010), intense signals at $\delta$ 7.06 (1H, d, $J$= 9.0 Hz), 6.88 (1H, d, $J$ = 9.0 Hz), 4.89 (1H, d, $J$ = 7.6 Hz) in the spectra of some of the analysed species, i.e., *I. argentina, I. brasiliensis, I. integerrima, I. microdonta, I. taubertiana*, and *I. theezans* (Table 9-1). These signals were assigned to H-2', H-3', and the anomeric proton of glucose in arbutin, respectively. This was confirmed by 2D-NMR spectra such as 1H-1H COSY, HMQC, and HMBC and comparison with the reference compound arbutin. The presence of arbutin had never been reported in these species, although Miura et al. (1985) reported the presence of arbutin in *I. latifolia*, an *Ilex* species autochthonous to Japan. In order to confirm this finding an HPLC method was developed and validated and applied to the identification and quantitation of arbutin in all the above-mentioned species.
**Arbutin** (4-hydroxyphenyl-β-D-glucopyranoside) is a p-glycosylated hydroquinone (Fig. 9-1) that was first isolated from natural sources and identified as a glucoside of hydroquinone in 1881 by Schiff and Michael in 1882 (Macbeth and Mackay, 1923). It has been found at very high concentrations in the leaves of several plant species, such as in *Arctostaphylos uva-ursi* (bearberry) leaves (7%) (Chukarena et al., 2007; Parejo et al. 2007) and in other plants such as *Vaccinium vitis-idaea* (cowberry) (Weizz, 2001, Pyka et al., 2007). Uva ursi has a long history of traditional medicinal use because of its diuretic and urinary antiseptic properties that are due to the presence of arbutin (Commission E, 2007; ESCOP, 2003; EP 7th Ed.). Its aglycone, hydroquinone is used industrially due to its powerful antioxidant properties in the manufacture of instant-drying glues.

Arbutin and its metabolite, hydroquinone, have been reported to inhibit the growth *in vitro* of *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Frohne D, 1970). After ingestion, arbutin is hydrolysed by intestinal flora to yield the aglycone, hydroquinone (Paper DH et al., 1993). It is then absorbed and its glucuronate and sulphate esters are excreted in the urine (Schindler et al., 2002, Kedzia B et al., 1975; Frohne D, 1970). These active hydroquinone derivatives exert an antiseptic and astringent effect on the urinary mucous membranes when the urine is alkaline (pH 8.0). Inhibitory growth activity against *Ureaplasma urealyticum* and *Mycoplasma hominis* has also been reported (Robertson et al., 1987).

Arbutin also has some antitussive activity: a dose of 50–100 mg/kg body weight given by mouth or intraperitoneally to unanesthised cats was able to decrease the number of efforts, intensity of cough attack, and cough frequency significantly (Strapkova et al., 1991).

Arbutin has also been used topically as a depigmenting agent (skin-whitening agent) as it inhibits melanin synthesis by inhibition of tyrosinase activity and is used in cosmetic preparations with this purpose (Hori et al., 2004; Maeda et al., 1996). This application is rather controversial, because though it is used especially in Asian countries in skin-whitening cosmetic products, its inclusion in cosmetics in the EU has not been approved, on the basis of the uncertainty of its long-term stability and consequent toxicity. When in contact with an acid medium, arbutin can be hydrolysed, releasing hydroquinone. It is on these grounds, and not due to any biological activity of arbutin itself, that its use in OTC products has been banned, on the basis of allegations of the possible occurrence of exogenous ochronosis (SCCP/1158/08), caused by hydroquinone. The FDA adopted a similar position to the EU in 2006. file://localhost/[/http://www.fda.gov/ohrms/dockets/98fr/E6-14263.htm].
There are some concerns regarding the toxicity of arbutin if consumed, very likely derived, again, from the release of hydroquinone, its aglucone, after ingestion. While there are few reports of adverse health effects associated with the production and use of hydroquinone, a great deal of research has been conducted with this phenol because it is a metabolite of benzene (Di Caprio, 1999). The results discussed by this author are rather alarming, though the pharmacodynamics of arbutin bioavailability itself play a very important role in its toxicity and have not been fully described.

A study carried out on reproductive toxicity was performed on rats. Arbutin was administered subcutaneously at 25, 100 or 400mg/kg body weight daily to male rats before mating, and to female rats during pregnancy and lactation. No effect on reproduction of male and female rats, or the development of the offspring was observed at doses of up to 100mg/kg body weight. Fetal toxicity was observed at doses of 400mg/kg body weight, which is an extremely high dose (Itabashi et al., 1988).

In view of the evident bioactivities of arbutin and our findings concerning the high concentration detected in some of the *Ilex* species, e.g., *I. brasiliensis* (above 10%), the possible substitution or adulteration of the massively consumed yerba mate with these herbs acquires a different dimension since it is not just a simple commercial issue or a question of sensory attributes but, eventually a major health concern.

### 9.2 Materials and Methods

#### 9.2.1 Materials

Dried plant material of 11 *Ilex* species was provided by the Estación Experimental Agraria de Cerro Azul (INTA) (Misiones, Argentina). The samples were harvested 2 months prior to their use, dried immediately for 3 min with a microwave (700 W),
ground, and preserved at -18 °C. Voucher specimens are deposited in the EEA Cerro Azul. Arbutin was purchased from Sigma - Aldrich (St. Louis, USA). Methanol HPLC grade and acetic acid (AR) were purchased from Merck Biosolve Ltd. (Valkenswaard, The Netherlands). Water was Milli-Q HPLC quality.

9.2.2 HPLC Analysis
A Waters HPLC system equipped with a 626 pump, a 2996 photodiode array detector and a 717 plus autosampler (Waters, Milford, MA) were used for arbutin determination. A volume of 20 µl of each sample was analysed using an Inertsil ODS-3 (4.6 x 250 mm, s-3 µm) (GL Sciences Inc., Tokyo, Japan) column and eluted with a 90:10 ratio of 1% AcOH/H₂O: 1% AcOH/MeOH (isocratic) for 4.5 min, followed by a linear gradient to 70:30 in 26 min. The flow rate was 1.0 mL/min. Arbutin was quantified at 282 nm.

9.2.3 Preparation of samples
Three different conditions were used to treat a sample of approximately 0.300 g of plant material: (I): *I. dumosa, I. dumosa* var. *guaranina, I. paraguariensis, I. taubertiana* samples were ground to a fine powder and sonicated for 10 minutes with 3x 25 ml of MeOH. The resulting extracts were pooled, filtered, taken to dryness with an rotary evaporator (Buchii, Switzerland) and redissolved in 10 ml of MeOH: H₂O (50:50). (II): Samples of *I. brevicepsis, I. microdonta, I. theezans* and *I. integerrima* were sonicated for 10 minutes with 2x 60 ml of MeOH and 2 x 40 ml of H₂O. The resulting extracts were pooled, filtered and taken to 200 ml in a volumetric flask with MeOH. (III): Samples of *I. argentina, I. pseudobuxus* *I. brasiliensis* were sonicated for 10 minutes with 2 x 75ml of MeOH and 2 x 50 ml of water. The resulting extracts were pooled, filtered and taken to 250 ml in a volumetric flask with MeOH. All samples were analysed by triplicate.

9.2.4 Preparation of standard solutions
A stock solution was prepared by accurately weighing approximately 25 mg (±0.01) of arbutin and diluting to 50.0 ml in a volumetric flask with MeOH: H₂O (60:40). From this solution (Stock 1) dilutions were made with the same solvent in order to build a calibration curve ranging between 0.5 mg/ml and 0.012 mg/ml.

9.2.5 Quantitation
Three replicates were prepared of each sample and injected twice; all areas with an RSD \( \leq 2\% \) were averaged.
9.2.6 Validation

9.2.6.1 Linearity: Dilutions of a stock solution of 5 mg (±0.01)/ml (MeOH: H₂O (60:40)) were prepared in order to obtain 5 points in a range of 0.5 to 0.012 mg/ml. This was done by triplicate.

9.2.6.2 Accuracy: Samples of *I. paraguariensis* were spiked with arbutin to obtain concentrations equivalent to 0.5, 1.0 and 1.5% for (I); 4.0, 4.5 and 5% (II) and 7.5, 10 and 12.5% (III) and treated according to procedures (I), (II) and (III) respectively. Three replicates of each concentration level were prepared and injected twice.

9.2.6.3 Limit of detection/limit of quantitation: based on an estimation of LOD: 3x Signal/Noise and LOQ= 10 x Signal/Noise, a solution of the corresponding concentration was prepared and injected.

9.2.6.4 Specificity: Baseline in the region of arbutin peak (Tr: 4.7± 0.5) in *I. paraguariensis* samples were evaluated at high sensitivity (1 mAU) for possible interference. The UV/Vis spectrum of the peak with Tr corresponding to arbutin was compared with that of the pure reference compound and the purity and peak profile confirmed with the tool provided by the instrument software.

9.2.7 NMR: Extraction and measurements were performed as described in Chapter 8 - Section Methods.

9.3 Results and discussion

9.3.1 HPLC method

Most methods used for HPLC analysis of phenolic acids and polyphenolic compounds consist in MeOH/water or ACN /water gradients with the addition of an acid. These methods usually begin with percentages of the organic solvent that do not allow the separation and/or detection of highly hydrophyllc compounds such as arbutin which elute at the begining of the chromatographic run together with other unretained compounds. This is, at least, a possible explanation for the failure to detect this bioactive metabolite that is present in such large quantities in many of the analysed *Ilex* species. In order to get an adequate *k'* for arbutin it was necessary to use methanol, since acetonitrile proved to be too strong, and run isocratic conditions (90:10 ratio of
1% AcOH/H2O: 1% AcOH/MeOH) until the elution of arbutin, after which a gradient to 70% AcOH/MeOH allowed the elution of the caffeoylquinic compounds and flavonoids that are present in all aqueous or hydroalcoholic extracts of *Ilex* species. Chromatograms of standard solutions and *I. paraguariensis*, *I. pseudobuxus* and *I. brevicaulis* can be observed in Fig.9-3.

Arbutin eluted with a retention time of 4.5 min and its identity was confirmed by comparison of its UV spectrum with a reference compound. The chromatogram in Fig. 9-4 shows the retention times of other significant polyphenolic compounds that can be observed in the sample chromatograms: chlorogenic acid, caffeic acid, rutin and the aglycones quercetin and kaempferol which were not detected in these samples.

Fig. 9-3 HPLC chromatograms of arbutin and methanol: water extracts of leaves of *Ilex* species. a) Arbutin 0.255 mg/ml; b) *I. pseudobuxus*; c) *I. paraguariensis* d) *I. brevicaulis*. Chromatographic conditions: C18 with isocratic elution of AcOH/H2O 1%: AcOH/MeOH 1% (90:10) 4 minutes, followed by a linear gradient to 30:70 in 26 minutes. Detection: 292 nm; flow rate: 1.0 ml/min. Tr arbutin: 4.83 ± 0.5 min.
9.3.2 Validation

The response of arbutin in the range of concentrations detected in the plant material was tested (0.5 mg and 0.0012 mg/ml) proving to be linear as can be observed in the graph below (Fig.9-5).

**Fig. 9-5.** Linearity evaluation for arbutin (0.5-0.0012 mg/ml) tested at 5 concentration points /3 replicates

9.3.2.1 Accuracy: Accuracy as determined by recovery rates is usually problematic when blank matrixes cannot be obtained, as is usually the case in plant material. In this case, *I. paraguariensis* was used as a blank since no peak was observed in the range of
retention times obtained with arbutin (4.83 ± 0.5) at high sensitivity (1.0 mA). Resolution ($R_s$) between arbutin and theobromine, the closest peak in I. paraguariensis chromatograms was 2.

Optimum extraction recovery was determined for the three ranges of concentrations found in the different species, which being very different resulted in the selection of the three slightly different conditions described (I, II and III) in order to optimise the efficiency (time/yield) and recovery rate.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>I. paraguariensis (mg ±0,1)</th>
<th>Added arbutin (mg ±0,1)</th>
<th>Determined arbutin (mg ±0,1)</th>
<th>Recovery (%)</th>
<th>Mean recovery (% ±SD)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1000.40</td>
<td>10.31</td>
<td>10.06</td>
<td>97.58</td>
<td>97.4±0.362</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>1000.10</td>
<td>10.52</td>
<td>10.25</td>
<td>97.43</td>
<td>96.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000.30</td>
<td>9.85</td>
<td>9.55</td>
<td>97.43</td>
<td>96.95</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>250.8</td>
<td>11.12</td>
<td>10.90</td>
<td>98.02</td>
<td>98.5±0.665</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>250.1</td>
<td>10.81</td>
<td>10.67</td>
<td>98.70</td>
<td>98.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250.4</td>
<td>9.78</td>
<td>9.65</td>
<td>98.67</td>
<td>98.8±0.118</td>
<td>0.12</td>
</tr>
<tr>
<td>III</td>
<td>100.5</td>
<td>10.81</td>
<td>10.49</td>
<td>97.04</td>
<td>97.4±0.362</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>100.8</td>
<td>10.75</td>
<td>10.70</td>
<td>99.53</td>
<td>99.1±0.362</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>100.4</td>
<td>10.53</td>
<td>10.50</td>
<td>99.72</td>
<td>99.1±0.362</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 9-I Recovery rates for arbutin added to samples of I. paraguariensis at three levels of concentrations i.e., 1, 4 and 10%, extracted with 3 different procedures (I, II and III) respectively as described in section 9.2.3.

Recovery rates of the three conditions were evaluated, obtaining a mean % recovery of 97.4% for (I); 98.5% for (II) and 98.8% for (III). In all cases no individual recovery value was below 97.0% and the RSD between replicates was ≤ 2.5%.

9.3.2.2 Limit of detection (LOD) calculated as: 2 x noise/signal, was found to be of 1 x 10^{-10} g and the limit of quantitation (LOQ) calculated as: 3 x limit of detection, was 3x 10^{-10} g. This was verified by the preparation and injection of solutions at these levels.

9.3.2.3 Specificity: The baseline of I. paraguariensis var paraguariensis chromatogram was evaluated at high sensitivity (0.1 mA) to detect the presence of compounds that could co-elute with arbutin. No peaks were detected at the retention time corresponding to arbutin.

In all chromatograms of samples in which arbutin was detected, the peak corresponding to arbutin was evaluated for purity using the software utility designed for this purpose.
The peak was found to have an index of 1.000, which corresponds to a pure peak. The spectra of the peak identified as arbutin by its retention time in samples and that of the reference compound were identical.

### 9.3.3 Content of arbutin

Results are shown in Table 9-2. All *Ilex* species samples analysed were found to contain arbutin with the exception of *I. paraguariensis*, *I. dumosa* and *I. dumosa var guaranina*. The species that contained most arbutin were *I. brasiliensis* (10.4%), *I. pseudobuxus* (10.0%) and *I. theezans* (9.8%). These were followed by *I. microdonta* (9.0%), *I. argentina* (6.79%), *I. integerrima* (5.23%), *I. brevicuspis* (4.74%) and *I. taubertiana* (2.23%). These results confirmed what had been detected in the $^1$H NMR spectra of the aqueous phase of *I. argentina*, *I. brasiliensis*, *I. integerrima*, *I. microdonta*, *I. taubertiana*, and *I. theezans* extracts. 

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arbutin (%) DW± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. dumosa var dumosa</em></td>
<td>nd</td>
</tr>
<tr>
<td><em>I. dumosa var guaranina</em></td>
<td>nd</td>
</tr>
<tr>
<td><em>I. paraguariensis var paraguarensis</em></td>
<td>nd</td>
</tr>
<tr>
<td><em>I. taubertiana</em></td>
<td>2.25±0.48</td>
</tr>
<tr>
<td><em>I. brevicuspis</em></td>
<td>4.75±1.72</td>
</tr>
<tr>
<td><em>I. integerrima</em></td>
<td>5.23±1.08</td>
</tr>
<tr>
<td><em>I. argentina</em></td>
<td>6.68±9.77</td>
</tr>
<tr>
<td><em>I. microdonta</em></td>
<td>9.04±6.81</td>
</tr>
<tr>
<td><em>I. theezans</em></td>
<td>9.88±3.89</td>
</tr>
<tr>
<td><em>I. brasiliensis</em></td>
<td>10.44±4.93</td>
</tr>
<tr>
<td><em>I. pseudobuxus</em></td>
<td>10.60±3.38</td>
</tr>
</tbody>
</table>

As can be observed in Fig 9-10, intense signals at δ 7.06. (1H, d, J = 9.0 Hz), 6.88 (1H, d, J = 9.0 Hz), and 4.89 (1H, d, J= 7.6 Hz) appeared in these spectra and were assigned to H-2’, H-3’, and the anomeric proton of glucose in arbutin, respectively. This was confirmed by 2D-NMR spectra such as 1H-1H COSY, HMQC, and HMBC and comparison with the reference compound arbutin (Choi et al., 2005).
After this investigation had ended, a report on the identification of arbutin in *Ilex theezans* appeared in which the authors described the isolation and identification by MS, $^1$H and $^{13}$CNMR of a sulphate derivative of arbutin, arbutin-2'-sulphonyl (Andrade *et al.*, 2004) at a concentration of 0.09%.

Considering that it could be possible for this compound to have a similar retention time in an HPLC method with an acidic mobile phase, special attention was given to this issue in a further metabolomic analysis of these species and diverse 2D-NMR spectra such as COSY, HSQC AND HMBC were applied (Kim *et al*.; 2010). The H-2' of the sulfonyl gives shifts from $\delta$ 3.49 to $\delta$ 4.30 (Andrade *et al*., 2004). In the case of our *Ilex theezans* extract, in the COSY spectrum, H-1 correlates only with $\delta$ 3.49.
Additionally, the $^{13}$C chemical shift of C-2 of arbutin-2-sulfonyle should be shifted downfield from $\delta$ 75.9 to $\delta$ 82.0. In this study, the HMBC spectrum of I. theezans extract showed the correlation of H-1 only with $\delta$ 75.9 (C-2 of arbutin) and $\delta$ 152.2 (C-1' of arbutin). Based on these results, it was concluded that major form of arbutin is not as a sulfonyle but rather its free form.
9.4 Conclusions

The presence of arbutin in these *Ilex* species had never been reported when we finished our study. The amount of arbutin in species such as *I. brasiliensis*, *I. pseudobuxus* and *I. theezans* and the fact that this compound has very interesting bioactivities as described in the introduction is a very important finding. It is to be noted that all these species contain considerable amounts of other bioactive compounds with interesting antioxidant properties such as a great number of caffeoylquinic esters, rutin and other quercetin and luteolin analogues, all of which are assumed to be responsible for the various biological activities detected in these species.

The significance of the finding is probably greater when referred to quality control issues. The fact that arbutin can be easily detected in most *I. paraguariensis* adulterants, clearly indicates this compound as a marker. Some authors have proposed the use of saponins as markers owing to the fact that most of these *Ilex* species have at least one and often more than one saponin that cannot be detected in yerba mate (Pires et al., 1998). That is certainly an interesting contribution but not so easily transferred to routine quality control analyses. The detection of arbutin is very simple and the concentration we detected in the leaves of these species (between 10.5 and 4.2 % (dried weight) added to the extremely low LOD means that arbutin is very conspicuous.

Lastly, the presence of large amounts of this bioactive and relatively atoxic compound in *Ilex* species has awoken the interest of yerba mate experts at INTA (National Institute of Agricultural Technology) since it might be possible to create a variety of *I. paraguariensis* that could produce arbutin. Most of the above mentioned *Ilex* species are rather bitter and cannot be used to replace *I. paraguariensis* in the production of yerba mate. But if *I. paraguariensis* could be made to produce arbutin, this would convert it into a "super-functional food", providing not only caffeine and the large amount of CQAs and saponins typical of this herb, but also the interesting bioactivities of arbutin.
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