Contributions to the quality control of two crops of economic importance : hops and yerba mate

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concluding remarks

Quality control of herbals or herbal substances is complex. This complexity is derived not only from the type of material that is being controlled but also from the objective of the method, i.e., what exactly is being controlled by the method.

In this thesis aspects involved in the quality of raw materials derived from two plants - *Humulus lupulus* and *Ilex paraguariensis* - used in two massive consumption products, beer and yerba mate, were investigated. The type of problem dictated the approach used in each case.

In the case of hops, we set out to isolate gram-scale amounts of pure compounds, six individual iso-α-acids, responsible for the bitter taste in beer. In view of the somewhat disappointing results obtained with preparative chromatographic methods, we attempted the separation using Centrifugal Partition Chromatography (CPC). We achieved the separation. On one hand, we explored the possibility of obtaining them, combining the CPC separation of bitter α-acids and subsequent isomerisation with the selective DCHA precipitation of the *trans*-iso-α-acid derivatives (Chapter 3). In view of the difficulties in scaling up this procedure, derived from its low yield, we continued working on the development of a CPC method that would allow us to separate all iso-acids. We developed a method that allowed us to obtain 5 of the 6 iso-α-acids (Chapter 4). But, simultaneously we worked on a third approach that turned out to be the most successful, since it allowed us to separate *cis* - from *trans* - iso-α-acids and obtain not only the pure isomers but also all -*cis* or all- *trans* isomerised hop extracts. This simple, cheap, fast method is described in Chapter 5. The selective β-cyclodextrin complexation of *trans*-iso-α-acids was achieved not only for isolated *cis*/*trans*-iso-α-acid pairs, but also for isomerised α-acid hop extracts used in the beer-brewing process. This was patented as a stable iso-α-acid extract, because though not supported by hard data, it had been evident for years, that the content of *trans*-iso-α-acids decreased rapidly in light-protected, closed beer containers, while the *cis*- iso-α-acid content remained stable. This of course conditioned shelf life of beer, since its typical bitter taste decreases and a disagreeable, unpalatable taste evolves.

During the last 2 years, a group of researchers from München, Germany published results of several studies of the analysis of stability of beer that throw a great deal of light on the mechanism and products of several iso-α-acid degradation reactions. Intelman et al. (2010) described conditions in which *trans*-iso-α-acids suffer

a proton-mediated cyclation that results in the appearance of degradation products that accumulate providing a disagreeable flavour, as described in Chapter 5.

Cis-iso-α-acids do not suffer this degradation process and though sensitive to light and oxygen, if kept in light and oxygen-depleted environments can remain in solution for more than 5 years with only a 10 % reduction in their original concentration.

Thus, our discovery of this simple and cheap method for the production of a *trans*-iso-α-acid free hop extract is important.

The possibility of counting on pure iso-α-acid reference compounds is also useful. These coumpounds are complexed with β-cyclodextrin and can be kept as solids with a very high stability. If dissolved in methanol (for analytical purposes) or sonicated in warm water and extracted with hexane, they easily release the pure iso-α-acids. After my work was finished, Alfi Khatib continued working and discovered that once *trans*-iso-α-acids were removed from the medium, it was possible to obtain *cis*-iso-α-acid complexes with β-cyclodextrin. These findings are published in Khatib et al. (2009, 2010).

In the case of Yerba mate, we decided to use a different approach to the classical approach used for identification purposes within quality control methods. When developing methods, all efforts are directed at choosing compounds or groups of compounds that could be active principles or when this is not possible, selecting one or two markers that will help in some way to either identify the plant material or detect adulterants.

For those of us who have worked with quality control methods for years, it has always been clear that the existing methods are unsatisfactory since we depend on chromatographical methods that are inevitably directed at particular groups of compounds and markers, leaving a wide, vulnerable gap in the information obtained, that is, the amount of compounds that are probably in the sample but are not detected because we are not targeting them. In this way, adulterations with other plants or even other parts of the plant (described in pharmacopoeial jargon as "Foreign organic matter" in a herb) are often undetected. Furthermore, there are probably components of the herbs themselves that have never been detected.

For *I.paraguariensis*, caffeine is always used as the compound to be detected for identification purposes. This is absolutely correct since with only one exception, most investigators have never detected this xanthine in any of the authochtonous *Ilex* species. In fact, it has only been inequivocally detected in another two of the more than four hundred existing *Ilex* species.

Adulteration of *I.paraguariensis* leaves with other *Ilex* species is very easy since their chromatographic profile of caffeoylquinic acid derivatives is qualitatively very similar. There is a qualitative difference in saponin content, but this is not detected readily, among other things, because saponins have a low detectability in HPLC-based methods.

We applied, thus, a very different approach. Using a ^{1}HNMR-based analysis of its metabolome, we were able to distinguish 10 cogeneric *Ilex* species. The major discriminating metabolites were found to be phenylpropanoids and arbutin (Chapter 8).

A major finding was the detection in cogeneric species of *I.paraguariensis*, but not in *I. paraguariensis* itself, of large amounts of arbutin, 4-O-glycoside of hydroquinone, a very active compound, which had never been detected in *Ilex* species. Some of them, contained between 5 and 10% (dw) of arbutin. This was discovered in the ^{1}HNMR spectrum due to the appearance of a large peak at δ=7.06. It was later confirmed by HPLC/DAD analysis against a reference compound (Chapter 9).

Arbutin, with its urinary antiseptic properties or skin whitening uses (external) is not inocuous on one hand and considering the amount of yerba mate that is consumed, should be taken into account if present, due to the adulteration of *I. paraguariensis* with other *Ilex* species.

But, above all, this exposes the main flaw, or at least, vulnerable point, of most quality controls methods. Using traditional methods we only find what we are looking for. For years, people have analysed *Ilex* extracts by HPLC, looking only at what appears from the first few minutes onward, i.e., theobromine or neo-chlorogenic acid. Arbutin, being extremely hydrophillic, elutes at the beginning of the chromatogram, together with most unretained compounds in a typical chromatographic profile method.

How can this be applied to quality control methods of Yerba mate? In the first place, it should be possible to detect adulterants, since their ^{1}HMMR profiles are very different. Secondly, having a characteristic metabolomic profile, material that has suffered the effects of microbial contamination, for example, another quality control issue, could be detected. The effect of changes in the processes used for manufacture of yerba mate could be easily monitored as well as the appearance or disappearance of compounds related to sensory attributes, aroma and colour, could be followed.

As to the detection of arbutin, this has been considered to be extremely valuable and a group of experts in the National Institute of Agricultural Technology (INTA) in Cerro Azul are eager to work in cooperation in order to develop a palatable Yerba mate

that could be considered as a functional food with a controlled content of the antioxidant and antiseptic arbutin.

A later publication furthered the metabolomic studies, achieving a clear metabolomic discrimination of the *Ilex* species and varieties used for our studies and making the chemotaxonomic classification of *Ilex* species possible (Choi et al., 2010).