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Determination of water content in tea samples of *Cymbopogon citratus S.*, *Matricaria recutita L.*, *Mentha spp*. and *Pimpinella anisum L.* through Gravimetric and Thermogravimetric methods

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The excess of humidity in materials of plant origin allows the action of enzymes, which can cause degradation of chemical components and enable fungi and bacteria development. The humidity determination is one of the most important and most used measurements in food analysis, as it is tightly related to food quality, stability and composition. Teas are among humanity's most popular and most broadly diffused drinks, being the most common form of phytoterapeutical products and a natural source of chemical substances with activity against a broad variety of ailments. Humidity determination in teas of *Cymbopogon citratus* S., *Matricaria recutita* L., *Mentha* spp. and *Pimpinella anisum* L was performed through pharmacopoeia-standards gravimetric analysis, drying by infrared radiation and thermogravimetry, and the results obtained by each method were compared. All humidity indexes obtained were in accordance with the currently available official specifications. Water content values obtained by the pharmacopoeia-standards assay, infrared radiation assay and thermogravimetry presented statistical difference between them, due to the methods' technical features or the utilized materials' physical characteristics; still the thermogravimetry and the infrared radiation assay were the methods that presented the least statistical difference between their results. The pharmacopoeia-standards method presented itself as the more adequate method for the determination of water content in materials of plant origin, like teas.

Keywords: Humidity, Teas, Loss on Drying, Thermogravimetry, Infrared

Introduction

Water is the most abundant component in foods, and it has a controlling role in food microbiologic degradation and chemical stability (Frazier, 2009). Excess humidity in materials of plant origin allows the action of enzymes, which can lead to the degradation of their chemical components and enable fungi and bacteria development (Farias, 2002). The determination of water content is one of the most important and most utilized measurements in food analysis, as the humidity is tightly related to a product's stability, quality and composition (Bradley Jr., 2000; Cecchi, 2003).

The humidity represents the water contained within the food's composition, and this water can be categorized according to how strong it is bound to the food's components (Bradley Jr., 2000; Frazier, 2009): the Free Water, which is the water that is present at the outer surface of the food, being easily evaporated and measured by the majority of the drying methods; the Multilayer Water, which is the water that is more internally contained, locating itself around the food's macromolecules; and the Monolayer Water, which is the water that is chemically connected directly to the food's molecular structure, being unavailable for utilization by most microorganisms and harder to detect by the majority of the usual moisture analyzing methods (Cecchi, 2003; IAL, 2008; Frazier, 2009). The strength with which the water binds to the food is expressed by the measurement of Water Activity: the water will be freer and more reactive as its Water Activity value leans closer to 1 (Frazier, 2009). The drying process aims to reduce the Water Activity of a product, which justifies the fact that dry products are frequently more stable than humid products (Rankell et al., 1986).

Many water content determination methods are present in the literature, but none of them are able to be, at the same time, exact,

precise and of practical execution (Cecchi, 2003). Some problems encountered when performing these methods are, for example, the incomplete separation of water, the degradation of the analyzed product and the incorrect inclusion of the loss of volatile compounds as being loss of water, as well as the lengthy amount of time needed for performing each analysis (Cecchi, 2003; Isengard & Präger, 2003; IAL, 2008; Isengard & Breithaupt, 2009). The most frequently utilized water determination methods are based on mass loss occurred when the analyzed sample is submitted to heating, but this mass loss is just a result of the assay's chosen parameters and do not necessarily show the sample's real water content (Isengard & Breithaupt, 2009). Even the parameters established by official organizations are chosen by convention, and as these conditions are susceptible to change, the results obtained can also show variation themselves (Isengard & Breithaupt, 2009). Even with all these problems, oven dying methods are still the more usually utilized and officially recommended methods, since they require simple equipment making them widely available to almost every analytical laboratory (Isengard, 1995).

The 5th edition of the Brazilian Pharmacopoeia lists 2 methods for the determination of water content in materials of plant origin, and a gravimetric method is recommended among them, the “Water Determination in Vegetable Drugs” assay. It consists on submitting the analyzed sample to cycles of oven heating followed by cooling and weighing, with the mass loss calculated at the end of each cycle. The "Water Determination in Vegetable Drugs" assay is but an adaptation of the "Loss on Drying" assay for materials of plant origin: the two methods only differ in parameters such as quantity of sample utilized (2 to 5 grams for materials of plant origin, versus 1 to 2 grams of samples in general), length of the drying step (5 hours for materials of plant origin, versus 2h for samples in general, except when specified otherwise) and stopping criteria (assays using materials of plant origin are stopped when the weighing difference does not exceed 5 milligrams, while the other assays in general are stopped when the sample keeps a constant weight across two weightings) (Farias, 2002; Borges et al., 2005).

Aiming to reduce the necessary amount of time to perform each analysis, methods with more efficient heat sources were introduced (Isengard & Präger, 2003; Isengard & Breithaupt, 2009). Moisture Analyzers or Moisture Determiners are appliances that consist on a scale attached to an infrared radiation heating system that provides faster liberation of the water molecules than the convective heat utilized in conventional drying ovens (Borges et al., 2005). However, this more intense heat can make the analyzed samples more susceptible to thermal degradation, causing them to release a greater amount of volatile compounds that can be incorrectly accounted in the mass loss calculation as water content (Isengard & Präger, 2003; Isengard & Breithaupt, 2009).

Thermal Analysis is the denomination reeived by a set of techniques applied for the measurement of physicochemical properties of a substance as a function of temperature or time (Thomas & Schmidt, 2000). Thermogravimetry is one of these techniques, and it is commonly used for the identification and characterization of substances through mass loss detection, being also able to detect, quantify and differentiate the different water types present in a substance (Komatsu et al., 1994; Thomas & Schmidt, 2000; Materazzi et al., 2005). One of Thermogravimetry's main advantages is the fact that it requires a very little quantity of sample to perform an analysis (Komatsu et al, 1994), but this technique however is a quantitative analysis of the mass loss, not being able to provide great resolution power as to the mass loss values and not being able to inform data about gases evolved during the heating process; for this information to be acquired, a Thermogravimetry assay must be coupled with another analytical method such as Mass Spectrometry or Infrared Spectrometry (Komatsu et al, 1994; Thomas & Schmidt, 2000; Materazzi et al, 2005).

The Thermal Analysis has been recently receiving attention from researchers for the

execution of studies regarding food characterization, since this technique's capacity on determining humidity and ash content can provide information about a food's storing conditions and material adulteration, respectively, which are very valuable data concerning the industrial food processing process (Araújo et al., 2006). Teas are among humanity's most popular and most broadly diffused drinks, are used both as food and as phytotherapeuticals, representing the most common form of administration of the latter (Awang, 2009) and, also, a natural source of chemical substances with activity against a broad variety of ailments (Trevisanato & Young-In, 2000). In some countries such as China and India, teas still currently stand as the "backbone" of the public health system (Trevisanato & Young-In, 2000; Barnes et al., 2007). According to data from the World Health Organization, 80% of the world's population uses plants or materials of plant origin as primary healthcare therapy (Barnes et al., 2007), and it is estimated that 25% of the modern pharmaceuticals have been originated, totally or partially, from chemical compounds found in plants (Fetrow & Avila, 2000). Plants possess a big history of usage in medicine, and are still being increasingly used today by the general population to complement, or even to substitute, the therapy with conventional medicine (Fetrow & Avila, 2000; Trevisanato & Young-In, 2000; Barnes et al., 2007; Awang, 2009). Phytoterapeuticals can offer an alternative to the conventional medicine for patients in non-life-threatening situations, as long as these products present quality and safety of use and are administered in an adequate manner (Barnes et al., 2007).

In view of this broad usage of phytotherapeuticals as a form of treatment, both complementary and even first choice, of many pathological conditions, and considering that teas are the most common form of presentation of these products, there is a necessity of a greater, stricter quality control for them. Therefore, the goal of this study is to perform water determination techniques according to 3 different methodologies: the gravimetric pharmacopoeia-standards assay, the desiccation through infrared radiation and the thermal analysis through Thermogravimetry, utilizing materials of plant origin presented as teas.

Experimental

Samples: Samples of teas of *Cymbopogon citratus* S. (lemongrass), *Matricaria recutita* L. (chamomile), *Mentha* spp. (peppermint) e *Pimpinella anisum* L. (anise) were made available by the laboratory "Laboratório Industrial Farmacêutico LIFAR", located in Porto Alegre, Rio Grande do Sul, in enough quantity for the execution of all required analysis. All lots of teas had a fabrication date dated from the same year of this study, and were stored in sealed amber glass flasks, protected from light and kept in environments with temperature and moisture control. These lots were named "internal", and will be identified from now on by the following acronyms: CAMLI (chamomile), CIDLI (lemongrass), EDLI (anise) and HLI (peppermint).

One lot of tea from each of the species already mentioned was also acquired commercially, from a different provider. These samples were utilized on the very same assays, following the very same analysis parameters, and kept stored in their original packaging. These lots were named "external", and will be identified from now on by the following acronyms: CAMLE (chamomile), CIDLE (lemongrass), EDLE (anise) e HLE (peppermint).

Water Content Determination following the pharmacopoeia-standards methodology: Water content determination for peppermint, lemongrass, anise and chamomile teas was performed following the "Water Determination in Vegetable Drugs" methodology by gravimetric assay, as described in the 5th edition of the Brazilian Pharmacopoeia. The assays were performed at the Quality Control Laboratory located inside the "Laboratório Industrial Farmacêutico LIFAR" complex, in Porto Alegre, Rio Grande do Sul. A mass of 2 to 5 grams of each sample was weighed on a

Shimadzu AY220 analytical balance. The samples underwent drying in a Quimis Q317M-22 oven, at a temperature of 105°C for 5 hours. The oven was pre-heated for 1h before the performing of the analysis. At the end of the drying process, the samples were allowed to cool in a glass desiccator with blue silica gel, and weighed. The glass weighing bottles utilized were also desiccated in the oven and allowed to cool previously to their usage in the assay, going through the same conditions as the samples. Mass loss percentage was calculated according to equation 1:

$$P\ (\%) \quad = \frac{P_1 - P_2}{P_a} \quad x\ 100\% \qquad (1)$$

where P_1 represents the weight of the bottle containing the sample before the desiccation, P_2 the weight of the bottle containing the sample after the desiccation, and P_a the mass of sample utilized.

Water Content Determination by desiccation through infrared radiation: Water content determination for peppermint, lemongrass, anise and chamomile teas was performed in a Quimis Q533M Humidity Analyzer apparatus. The assays were performed at the Quality Control Laboratory located inside the "Laboratório Industrial Farmacêutico LIFAR" complex, in Porto Alegre, Rio Grande do Sul. The Humidity Analyzer was turned on and allowed to rest for 30 minutes prior to the analysis, undergoing a 1-hour pre-heating step before the analysis of each tea sample. A mass of 2 to 5 grams of each sample was weighed on the appliance's scale, and the samples were desiccated for 1 hour at 105°C. The apparatus provided the Water Content value directly in percentage form at the end of the desiccation step.

Water Content Determination by Thermogravimetry: Water content determination for peppermint, lemongrass, anise and chamomile teas through thermogravimetric assay was performed in a TA Instruments SDT Q600 Thermogravimetric Analyzer. The assays were performed at the laboratory "Laboratório Multiusuário de Análise Térmica – LAMAT", at the Chemistry Institute inside the "Universidade Federal do Rio Grande do Sul" campus, in Porto Alegre, Rio Grande do Sul. The equipment underwent a pre-heating step prior to the analysis, that were executed with approximately 10 milligrams of each sample in an alumina crucible, utilizing a temperature range of 25ºC to 150ºC, a heating ramp of 10ºC/min and in a synthetic ultra-pure air atmosphere with a flow rate of 100 mL/min. The resulting mass loss curves were generated by the software TA Universal Analysis.

All the assays previously cited were performed with all 8 samples in triplicate, in equipment calibrated previously to each analysis, and in environments with temperature and air humidity not greater than 30°C and 70%, respectively.

Data statistical analysis was performed through *t*-Student test, employed with a significance level of 5%. Each sample had its water content results compared to each other (pharmacopoeia-standards x infrared radiation, pharmacopoeia-standards x thermogravimetry, and infrared radiation x thermogravimetry).

Results and Discussion

Excess humidity in materials of plant origin is directly related to their physical, chemical and microbiologic degradation, making the water content measurement a very necessary procedure for the product's quality, stability and safety control. (Bradley Jr., 2000; Farias, 2002; Cecchi, 2003; Frazier, 2009). Table 1 lists the water content values obtained by the performed analysis. Figures 1, 2 and 3 present the comparison between the water content results obtained for the internal and external lots of each tea analyzed. Figures 4 and 5 show the mass loss x temperature curves generated by the thermal analysis assay.

Table 1 Water content in the teas of *Mentha* spp. peppermint – lots HLI and HLE), *Cymbopogon citratus* S. (lemongrass – lots CIDLI and CIDLE), *Pimpinella anisum* L. (anise, lots EDLI e EDLE) and *Matricaria recutita* L. (chamomile, lots CAMLI e CAMLE), through pharmacopoeia-standards desiccation, infrared radiation desiccation and Thermogravimetry.

	Water Content (%)		
	Pharmacopoeia-Standards Assay	**Infrared Radiation Assay**	**Thermogravimetry**
	average ± CV	average ± CV	average ± CV
CAMLI	8,60 ± 1,39%	10,01 ± 0,90%	8,24 ± 7,40%
CAMLE	10,48 ± 4,10%	11,73 ± 1,62%	7,24 ± 17,26%
CIDLI	5,55 ± 2,34%	7,45 ± 7,25%	7,80 ± 3,72%
CIDLE	9,03 ± 3,65%	10,46 ± 0,86%	6,68 ± 20,06%
EDLI	7,53 ± 1,85%	8,34 ± 1,56%	3,81 ± 2,36%
EDLE	9,00 ± 6,89%	9,18 ± 1,09%	3,56 ± 4,21%
HLI	8,75 ± 3,77%	10,61 ± 3,86%	9,01 ± 3,22%
HLE	10,57 ± 1,51%	11,90 ± 3,78%	8,53 ± 21,34%

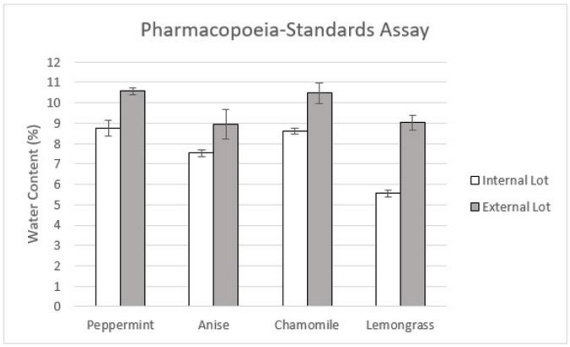

Figure 1 Comparison of water content results obtained from the pharmacopeia-standards assay for internal and external lot teas of Peppermint, Anise, Chamomile and Lemongrass.

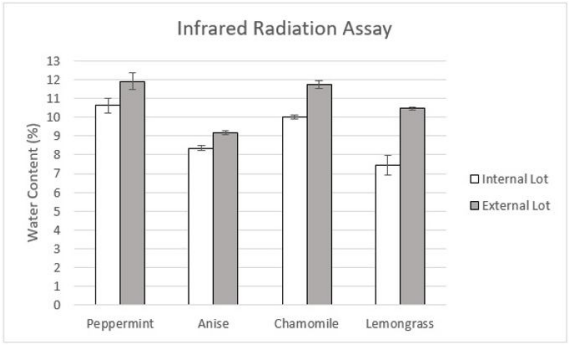

Figure 2 Comparison of water content results obtained from infrared radiation assay for internal and external lot teas of Peppermint, Anise, Chamomile and Lemongrass.

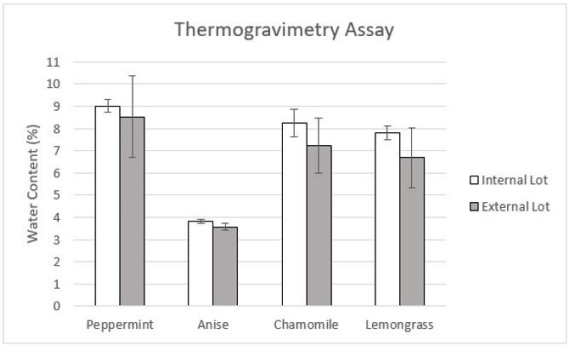

Figure 3 Comparison of water content results obtained from thermogravimetric assay for internal and external lot teas of Peppermint, Anise, Chamomile and Lemongrass.

Among the plant species utilized in this study, only lemongrass (*Cymbopogon citratus* S.) has a monograph included in the current edition of the Brazilian Pharmacopoeia, where the maximum water content value recommended is 11% (Brazil, 2010). As noted by table 1, all analysis performed with lemongrass tea provided results in accordance to the official standards. Chamomile (*Matricaria recutita* L.) has a monograph included in the 8th edition of the European Pharmacopoeia, where the maximum water content recommended is 12% (France, 2014). According to table 1, all analysis performed with chamomile tea provided results in accordance to these official standards. No maximum levels for the Loss on Drying assay were found for anise (*Pimpinella anisum* L.) and peppermint (*Mentha* spp.) on the official pharmacopoeias researched when making this study. For species that are not listed in a current pharmacopoeia, it is up to the industry that will utilize these materials to elaborate a monograph establishing its own quality standards (Farias, 2002).

In figures 1 and 2 it is possible to observe that the external lot teas presented a greater water content than the internal lot teas. That can be explained by the storage conditions of the teas: although all of them were stored protected from the sunlight and humidity, the internal lot samples (CAMLI, CIDLI, EDLI e HLI) were kept in sealed glass flasks, while the external lot teas (CAMLE, CIDLE, EDLE e HLE) were kept in their original commercial packaging,

that provides somewhat less protection against humidity.

In figures 4 and 5 it is possible to see a similar curve profile for all studied samples, as the mass decay between 25ºC and 105ºC, characteristic of the loss of water.

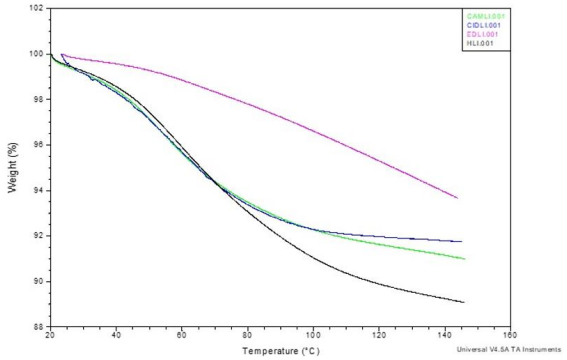

Figure 4 Mass loss curves obtained from the thermal analysis of the internal lot teas of Peppermint, Anise, Chamomile and Lemongrass.

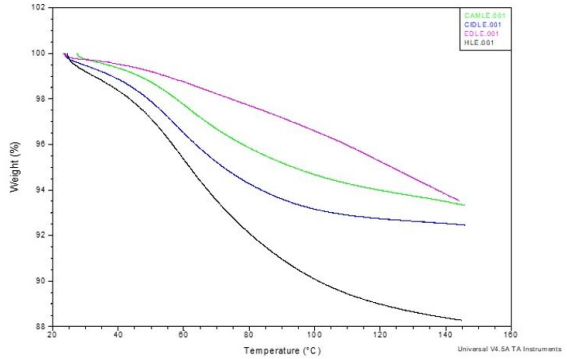

Figure 5 Mass loss curves obtained from the thermal analysis of the external lot teas of Peppermint, Anise, Chamomile and Lemongrass.

When compared with each other, the pharmacopoeia-standards method and the infrared radiation method provided results with statistical difference for all but one sample of tea (anise EDLE, which provided a *t*-test value of 0,702, indicating statistical similarity between the results obtained from both methods). That can be explained due to the difference of the type of heat utilized on each technique: infrared-generated heat is more penetrating than the convective heat generated by the regular drying ovens, and in materials of plant origin that can lead to a greater thermal degradation of the samples, making them release their volatile compounds in a greater quantity and causing a bigger mass difference at the end of the process.

The comparison between the results obtained from the pharmacopoeia-standards assay and the thermal analysis through Thermogravimetry also showed statistical difference between the two techniques, with 3 of the 8 teas analyzed presenting *t*-test values higher than 0,05: peppermint teas HLI e HLE provided *t*-test values of 0,483 and 0,257 respectively, and chamomile tea CAMLI provided a *t*-test value equal to 0,462. As seen in table 1, some of the teas analyzed by thermogravimetry presented results with high variability, as shown by the high Variation Coefficient values. This variation could have been caused due to differences of particle size of each material: teas can be made of leaves (as in the peppermint and lemongrass teas), inflorescences (as in the chamomile tea) and fruits (as in the anise tea), and the size difference of each material was more noticeable in the thermal analysis because the crucible utilized holds a very little volume of material, around 10 milligrams in comparison with 2 to 5 grams on the pharmacopoeia-standards assay. The glass weighing bottles utilized in the pharmacopoeia-standards desiccation hold a much larger amount of material, allowing a better, more homogeneous distribution of the material and thus resulting in a smaller variability of the obtained results. The high data variability observed in the Thermogravimetry analysis could have contributed to the fact that more samples showed *t*-test values greater than 0,05. For a better comparison between these two techniques, a crunching and/or sieving step of each sample could be introduced prior to the execution of the assay; however, depending on the method of crushing utilized, water and volatile compound content loss can occur, compromising the final mass loss result.

The comparison between the results obtained by desiccation through infrared radiation and thermal analysis through Thermogravimetry again showed statistic difference between the two techniques, with 4 out of the 8 analyzed teas providing *t*-test values greater than 0,05: the lemongrass teas CIDLI e

CIDLE showed *t*-test values of 0,470 e 0,06 respectively, as well as the chamomile tea CAMLI (*t*-test value of 0,06) and the peppermint tea HLE (*t*-test value of 0,06). The increased number of samples showing *t*-test values greater than 0,05 can once again be explained by the increased variability showed by the Thermogravimetry results.

Conclusions

Between the techniques addressed by this study, the thermal analysis is surely the most sophisticated one, but its greater sensitivity turned out to be a problem when the utilized material had variation on its particle size, besides having a much higher operation cost in an analytical laboratory. The Humidity Analyzers, for utilizing heat from infrared radiation, ended up causing greater thermal degradation of the samples, resulting in higher mass loss values than the ones obtained from the other techniques; furthermore, the greater amount of time needed to the execution of each analysis should be considered, as the appliances can only hold one sample at a time. Therefore, considering its greater simplicity and smaller cost of operation (and consequent greater availability to the analytical laboratories everywhere), and its capacity to hold more than one sample at the same time (which makes up for the long duration of each analysis), the pharmacopoeia-standards desiccation method showed itself as being the more adequate method for water content determination in materials of plant origin, presented in the form of teas.

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