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Effect of drying on the phenolic content and antioxidant activity of thistle flower

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Abstract

The thistle is a plant that is associated with the production of cheese, as it is responsible for the phenomenon of coagulation of milk. Lately there has been an increased the number of studies about this plant, related to the chemical composition in order to perceive the benefits for human health.
The aim of this study was to characterize the thistle flower in relation to its composition in phenolic compounds and also evaluate its antioxidant activity. For that, were studied samples of thistle flower from species *Cynara cardunculus* L. after freeze-drying and convective drying at different temperatures (40 ºC, 50 ºC and 60 ºC). After each treatment were made from each sample two successive extractions with solutions of methanol (98% v/v) and acetone (60% v/v). The extracts were then used for characterization of the samples in phenolic compounds and determination of antioxidant activity.
The results showed that the amount of phenolics and antioxidant activity in the flower thistle varies depending on the drying temperature decrease occurring as they use higher drying temperatures.

Key Words: Thistle, drying, phenolic compounds, antioxidant activity.

1. Introduction

The word thistle derives from Latin *cardùus*, which means "nod with the head", due to the ovoid shape resting on the oscillating flower stem. The thistle belongs to the family *Asteraceae*, with a variety of genres, including the genus *Cynara* L. consisting of eight species: *C. cardunculus* L., *C. syriaca* Boiss., *C. auranitica* Post, C. *cornigera* Lindley, *C. algarbiensis* Cosson, *C. baetica* (Spreng.) Pau, *C. cyrenaica* Maire et Weiller, and *C. humilis* L. [1].
In Portugal, the thistle flower of *C. cardunculus* L., *Cynara* gender, is widely used in the manufacture of various cheeses, including the Serra da Estrela cheese. This is used, after a drying process, as milk coagulant due to the presence of aspartic proteinases.
The drying is a very complex process, in which occur simultaneously mass and heat transfer phenomena [2] leading to structural changes at the physical and chemical levels in the products [3]. The drying of plants is a process designed to decrease the moisture content, allowing for an adequate preservation of the product, while maintaining the microbiological, physical and chemical quality for a longer time [4].
Drying is a common way to preserve foods, allowing to obtain stable products in the absence of any preservatives or harmful electromagnetic radiation exposure [5]. Traditionally, drying of the thistle flower for the production of cheese is made in the shade, in a cool and airy place, spread on trays that should be stirred periodically. When the flower is dry, keeping the violet color, is placed in cloth bags that keep hanging [6]. This process shows, however, some problems, either by remaining excessive time under oscillating weather conditions or due to the presence of fauna, in particular insects, which may result in significant damage to the final product [7]. The analysis of drying processes makes them understandable and eventually the establishment of operating conditions appropriate for each process and adequate to each food product [8].
Phenolic compounds are very much present in the plant kingdom, however the concentration of these compounds varies depending on the species and environmental conditions [9]. According to [10], thistle (*Cynara cardunculus* L.) presents a larger amount of phenolic

compounds in the seeds than in the leaves and flowers. Structurally, they can be divided into non-flavonoids, which have simple structures and flavonoids with the more complex molecular structure [11]. The non-flavonoids are numerous and variable in composition among different species and may occur esterified with sugars, alcohols, organic acids, or tartaric acid. Flavonoids include catechins, proanthocyanidins and anthocyanins. They may appear both in the free form or polymerized with other phenolic compounds or sugars. Phenolic compounds are essential in the growth and reproduction of plants, in addition to being responsible for defence mechanisms and contribute in pigmentation. To the phenolic compounds have been attributed beneficial health effects due to their antioxidant properties. [12] The genus *Cynara* L. is used for medical treatments [10, 13], including liver disease, diabetes, rheumatism, reducing blood glucose and cholesterol, and digestive, urinary, abdominal and intestinal disorders.
This study aimed to quantify phenolic compounds and antioxidant activity of the thistle flower of *C. cardunculus* L, subjected to different drying treatments.

2. MATERIAL AND METHODS

2.1. Samples

The samples of thistle flower used in this study were of the species *Cynara cardunculus* L. harvested in the center region of Portugal, in Oliveira do Hospital, in ANCOSE (National Association of Breeders of Sheep Serra da Estrela) at the time of flowering in June.

2.2. Processing of the Thistle Flower

The thistle flowers were dried at different temperatures (40 °C, 50 °C and 60 °C) in a forced convection chamber set at the desired temperature. The chamber used was a WTB Binder, with an air speed of 0.5 m/s. Every 15 minutes a sample was taken in order to control the moisture content. Moisture was determined on a Halogen Moisture Analyser. Each drying process was completed when the sample reached 1% moisture. From these processes resulted 3 samples, designated as CS40, CS50 and CS60, for the thistle dried at 40, 50 and 60 ºC, respectively. In parallel, the thistle flower was frozen in a conventional freezer and then lyophilized at -50 ºC and 0,7 Pa. The sample resulting from lyophilization was designated CL.

2.3. Extraction of Phenolic compounds

The extraction of phenolic compounds from thistle was performed by adaptation of the procedure described by Ferreira et al. [14]. To 5g of each sample (CL, CS40, CS50 and CS60) was added 100 ml of a solution methanol:acetic acid (98:2). The extraction was kept under stirring for 1 hour, and the liquid fraction collected by filtration. This process was repeated again to give the methanol extract (ME). To the solid residue insoluble in methanol was added successively two times 100 ml solution acetone:water (60:40), kept under stirring for 1h. The resulting extract was designated by acetone extract (AE).

2.4. Determination of Phenolic compounds

The Folin-Ciocalteu method used for the determination of phenolic compounds, was according to what is described by Goncalves *et al.* [15]. The concentrations of phenolic compounds in the extracts were obtained by standard lines made with gallic acid concentrations between 0-0.5 g/l. The results were expressed in mg of gallic acid equivalents per gram of thistle.

2.5. Determination of Flavonoids

For determining the content of flavonoids the method used was an adaptation of the method described by Meda *et al.* [16]. The total flavonoids present in each sample were determined

by the standard line performed with solution concentrations of quercetin between 0.02 and 0.20 g/L. The results were expressed in mg of quercetin equivalents (QE) per gram of sample.

2.6. Antioxidant Activity

The determination of antioxidant activity was performed by the DPPH method [17] and by the ABTS method [18]. For obtaining the concentrations was drawn a straight line with the standard trolox in concentrations between 0.08 and 0.4 mmol. The results were expressed in mmole Trolox equivalents per gram of thistle sample.

3. RESULTS

3.1. Total Phenolic Compounds

Figure 1 shows the composition in phenolic compounds, expressed as gallic acid equivalents per gram of sample (mg GAE/g), obtained for the methanol extracts (ME) and acetone extracts (AE) of the samples of thistle lyophilized (CL) and dried at 40ºC (CS40), 50ºC (CS50) and 60ºC (CS60).

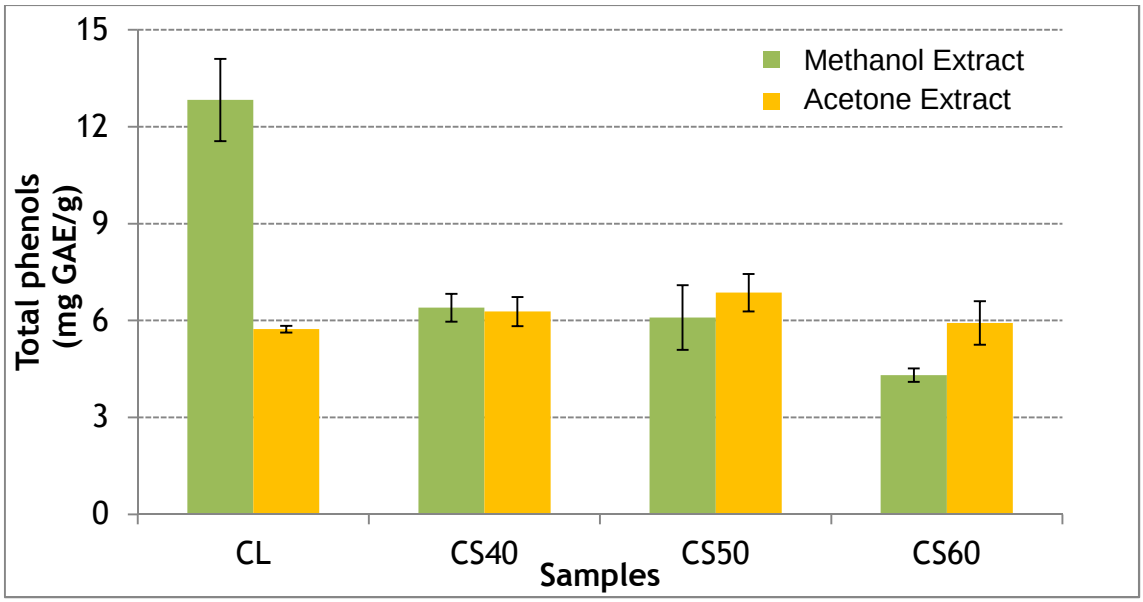

Figure 1: Amount of total phenolic compounds (mg GAE/g) present in extracts of methanol and acetone of the thistle samples studied. Legend: CL- lyophilized thistle, CS40- thistle dried at 40ºC, CS50 – dried at 50ºC, CS60 – dried at 60ºC.

The amount of phenolic compounds extracted with the different solvents (acetone and methanol) was generally similar to each other, except for LC sample in which 69% of the compounds were obtained from the methanol extract. However, in the dried samples there was a slight trend toward higher extraction with acetone, ranging between 50 and 58% of the total extracted.

From the observation of Figure 1 it was found that the lyophilized thistle (LC) had a total of 18.5 mg of gallic acid equivalents (GAE) per gram of thistle, resulting from the sum of the methanol and acetone extracts. The sample CS40 showed a much similar amount of phenolic compounds to the sample dried at 50 °C (CS50) with 12.7 and 13.0 mg (GAE) per gram of sample, respectively. The sample with the lowest amount of phenolic compounds was CS60 with 10.2 mg (GAE) per g of sample. These results may be due to the fact that the lyophilisation process takes place at low temperatures, while preserving as much as possible

the degradation of the phenolic compounds. In the processes of drying in the chamber, the higher the temperature the smaller the amount of phenolic compounds present.
Comparing the values obtained with the amount described in another study by Falleh *et al.* [10] carried out with thistle from the species *C. Cardunculus* L. grown in Tunisia, there was a big difference, because the amount reported was 6.96 mg GAE/g, a value inferior to those obtained in this work. This difference may be due to the drying process used because the amount reported in the literature comes from an artisanal drying, at direct sunlight, as in this work drying was by convective dryer/lyophilization, and hence under more controlled circumstances. It may also be due to the extraction process used, as in the study described by Falleh *et al.* [10] a single extraction was performed only during 30 min under agitation. Another factor was the different origins of the thistle flower used in the two studies, respectively, Portugal and Tunisia.

3.2. Flavonoids

The thistle lyophilized (Figure 2) showed, on average, in the methanol extract (23.40 mg QE/g sample) a quantity of flavonoid compounds 20% lower than the in acetone extract (29.08 mg QE/g sample).

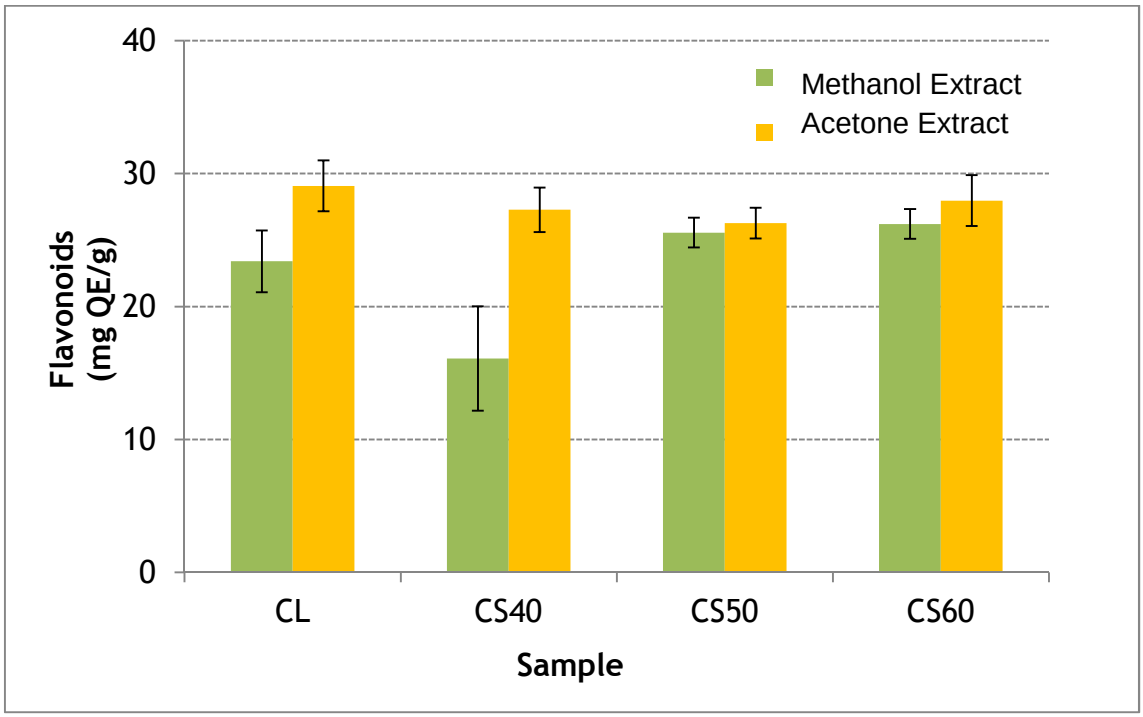

Figure 2: Amount of flavonoid compounds (mg QE/g) present in extracts of methanol and acetone of the thistle samples studied. Legend: CL- lyophilized thistle, CS40- thistle dried at 40ºC, CS50 – dried at 50ºC, CS60 – dried at 60ºC.

In the sample dried at 40 °C the methanol extract represents 37% (16.1 mg QE/g sample) of the total flavonoids, while with the acetone solvent was removed 27.3 mg quercetin equivalents per gram thistle. The methanol and acetone extracts relating to the samples dried at 50 °C and 60 °C had very similar percentages of extraction. In the methanol extracts of samples CS50 and CS60, were obtained 25.6 and 2.2 mg QE/g sample, respectively. In the acetone extract the flavonoid content was 26.3 mg quercetin equivalents per g in the case of sample CS50 and 27.9 mg QE/g thistle dried at 60 °C (CS60). The results led to infer that the sample CS60 contained a relative percentage of flavonoid compounds superior to the other samples.
Despite the different extraction conditions used, the results were similar to the values (1.9 to 3.3 g QE/100g of sample) for 8 described genera of the family *cynara* by other authors [19].

3.3. Antioxidant Activity

Figure 3 shows the antioxidant activity, expressed in mmol of equivalents of trolox per gram of sample (mmol TE/g), using the ABTS method, for samples of thistle lyophilized (CL) and dried at 40 °C (CS40), 50 °C (CS50) and 60 °C (CS60).

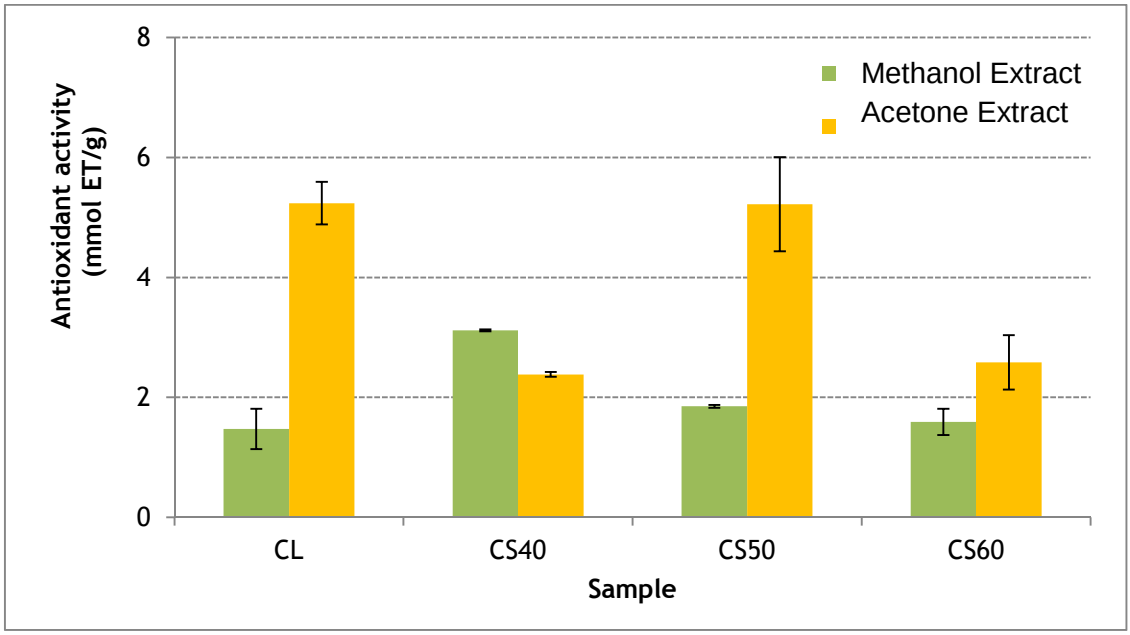

Figure 3: Antioxidant activity (mmol Trolox equivalent/g) by ABTS method in the methanol and acetone extracts of the thistle samples studied. Legend: CL- lyophilized thistle, CS40- thistle dried at 40ºC, CS50 – dried at 50ºC, CS60 – dried at 60ºC.

The samples of lyophilized thistle (CL) and thistle dried at 50 °C (CS50) showed a higher antioxidant activity than that of samples dried at 40 °C (CS40) and 60 ºC (CS60). The value was 6.7 mmol TE/g sample, of which 78% was due to the contribution of the acetone extract. The values obtained for samples CS40 and CS60 were 5.5 and 4.2 mmol TE/g sample, respectively. As to sample the CS60 the methanol extract contributed with 38% of the antioxidant activity, similarly to the LC and CS50 samples (22% and 26%). On the contrary, in the sample CS40 the methanol extract was responsible for 57% of the antioxidant activity.
According to Gouveia and Castilho [20] the antioxidant activity determined by the ABTS method for thistle flower from Madeira was 4.2 mmol TE/g sample, slightly lower than the values obtained in this work.
The antioxidant activity values obtained by the analytical method DPPH are disclosed in Figure 4. The values quoted for methanol and acetone extracts are expressed in mmol TE/g sample.
By observing the Figure 4 one can see that the antioxidant activity determined by the DPPH method was very similar in the thistle flower samples lyophilized and dried at 40 and 50 ºC. The total antioxidant activity of the two extracts was, respectively, 32.8, 33.1, 30.9 mmol TE/g for samples CL, CS40, CS50. The sample of thistle dried at 60 °C showed the lowest antioxidant activity, 71% less compared to the antioxidant power of the sample of thistle lyophilized. This lower value is in agreement with the least amount of phenolic compounds present in this sample. Several authors [21, 22] described a positive correlation between the content of phenolic compounds and antioxidant activity of different samples. Similar to what was found for the ABTS method, the values obtained for the thistle flower from Madeira [20] were slightly lower than those presented in this study.

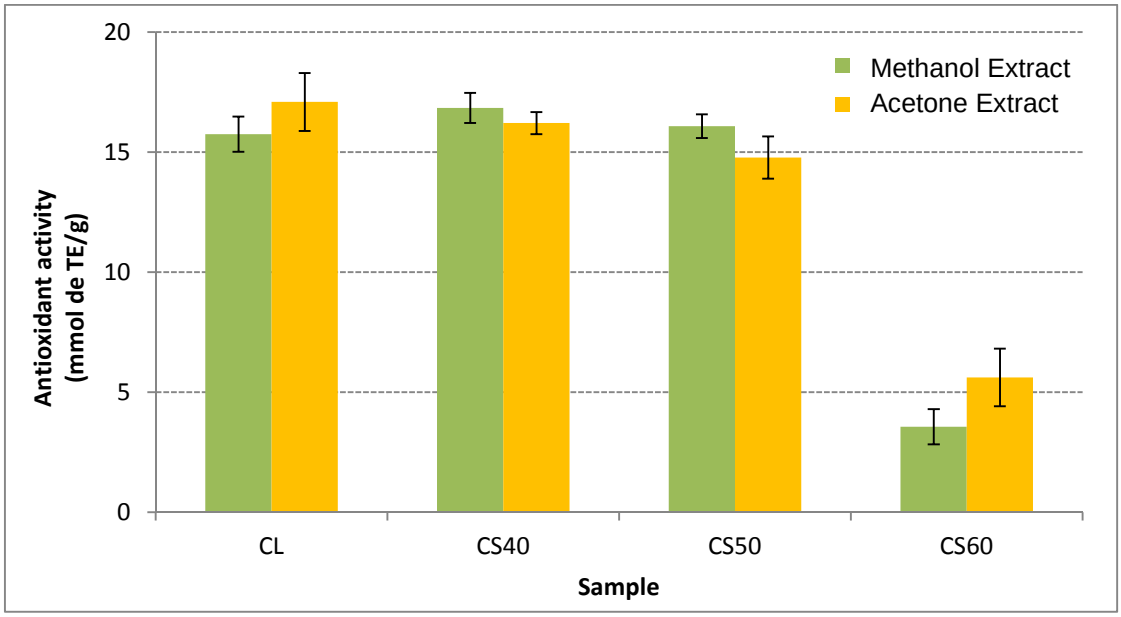

Figure 4: Antioxidant activity (mmol Trolox equivalent/g) by the DPPH method in the methanol and acetone extracts of the thistle samples studied. Legend: CL- lyophilized thistle, CS40- thistle dried at 40ºC, CS50 - dried at 50ºC, CS60 - dried at 60ºC.

Comparing with the results obtained by the ABTS method, there was a relatively lower contribution of the compounds present in the extracts of acetone to the total antioxidant activity as determined by the DPPH method.
The percentage of phenolic compounds obtained in different extracts was similar to the contribution of the extracts for antioxidant activity determined by the DPPH method, not occurring the same relationship in the case of ABTS. In this method, the acetone extract was responsible for the higher antioxidant activity. This fact may be related to the higher solubility of ABTS in aqueous solvents, allowing quantification of higher values of antioxidant activity.

3. Conclusions

The lyophilized thistle flower showed 18.5 mg GAE/g of total phenolic compounds. The drying in the oven resulted in a decrease of their quantity, being this effect most visible when the temperature was higher. In general, the amount of phenolic compounds extracted with methanol was similar to that extracted with acetone. The amount of flavonoid compounds in flowers of the thistle was similar regardless of the drying conditions of the thistle.
The values of the antioxidant activity of the samples were slightly different in accordance with the method used. Despite these differences, the lyophilized thistle showed the highest antioxidant activity, while the sample dried at 60 °C showed the lowest value.
The temperature at which the drying was done was important to preserve the antioxidant activity of the phenolic compounds on the thistle flower.

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