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Effect of technological processing upon the antioxidant capacity of aromatic and medicinal plant infusions: From harvest to packaging

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ABSTRACT

Antioxidants are secondary metabolites in plants, designed to protect them from abiotic stress; however, they may also improve one's general health, following regular ingestion. Since most foods from plant origin are consumed only after processing and formulation, the final activity exhibited by their antioxidants may be rather different from that in the original plant.

Ten plants empirically used in Portugal in traditional medicine were accordingly studied – agrimony (*Agrimonia eupatoria*), eucalyptus (*Eucalyptus globulus*), walnut-tree (*Juglans regia*), myrtle (*Myrtus communis*), raspberry (*Rubus idaeus*), sage (*Salvia* sp.), savory (*Satureja montana*), sweet-amber (*Hypericum androsaemum*), thyme (*Thymus vulgaris*) and yarrow (*Achillea millefolium*), for total antioxidant capacity and total phenolic content. Significant variations were found between fresh and frozen forms: most plants decreased those features by 30–80 %. However, weather conditions prevailing during plant growth also had a significant impact, besides postharvest storage conditions – especially in the case of antioxidant capacity. Typically, a decrease occurred throughout processing and storage, which was maximum for myrtle and minimum for yarrow.

The results of this research are useful in attempts to preserve the antioxidant content of plant-derived foods, or of plant additives in foods, via rational manipulation of processing conditions after harvest and throughout storage.

1. Introduction

Besides classical preservation features imparted to foods, antioxidants have been increasingly sought for incorporation in human diets because of their claimed benefits as tools in preventive health. Antioxidants are indeed compounds able to protect cells against oxidative stress – which might otherwise lead to cell damage (Buhler and Miranda, 2000; Fennema, 1996; Rivero-Pérez et al., 2005; Valls-Bellés, Muñiz, González, González-Sanjosé, & Beltrán, 2002). Coronary heart diseases, ulcers, cancers and neurodegenerative diseases (e.g. Parkinson's and Alzheimer's) – further to overall ageing, are but a few examples of pathologies that can be prevented (or, at least, delayed) via regular and balanced inclusion of antioxidants in one's diet (Bamforth, 2002; Baxter, 2001).

Several higher plants, both in their leaves and their fruits, contain high levels of antioxidant compounds; their original physiological function is to provide protection against harmful Reactive Oxygen Species (ROS) formed e.g. during photosynthesis or respiration. Shiow and Zheng (2001) have shown that the environmental temperatures prevailing during plant growth affect the phenolic content and the corresponding antioxidant capacities of several fruits. In order to cope with heat stress, plants do resort to various alternative mechanisms – including maintenance of membrane stability, scavenging of ROS, and production of antioxidants concomitant with tannin oxidation. However, the antioxidant capacity after plant harvesting also changes as a function of technological processing – e.g. drying, freezing and storage.

The active roles of several plant infusions upon disease prevention, control or reduction have been attributed – at least in part, to antioxidant features of liposoluble constituents, e.g. vitamins A and E; water-soluble components, e.g. vitamin C; and several amphipathic molecules, broadly termed phenolic compounds (Ivanova, Gerova, Chervenkov, & Yankova, 2005).

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Phenolics include chiefly flavonoids, anthocyanins and lignins – and are probably the most important class of secondary metabolites in plants, in which they play a variety of roles in coping with unfavourable, externally imposed conditions leading to oxidative stress. In particular, flavonoids in plants are important actors in their physiology; besides their function as pigments in flowers and fruits aimed at attracting pollinator insects and seed disperser birds, flavonoids also participate in scavenging of UV-mediated radicals, while contributing to fertility and resistance to disease. Although some plants contain high levels of specific flavonoids, others possess only low concentrations thereof (Schijlen, Ric de Vos, van Tunen, & Bovy, 2004). Anthocyanins – which are a subclass within flavonoids, are greatly modulated by temperature in plant tissues: the higher the prevailing temperature, the lower their concentration in buds and fruits. One of the causes underlying this observation is their lower rate of synthesis and stability at higher temperatures (Wahid, Gelani, Ashraf, & Foolad, 2007).

In all aforementioned cases, maximization of the antioxidant activity retained in plant extracts can be attempted via technological improvement of the extraction process; however, only a portion of the actual activity will be kept, so a major issue still remains pertaining to the inventory of antioxidants in the original plant material itself (prior to extraction and further processing). The main goal of this research effort was thus to assess the variation in total antioxidant capacity and total phenolic content, throughout processing and storage, of ten medicinal plants grown and collected in two consecutive years.

2. Materials and methods

2.1. Plant material, treatment and extraction

Agrimony (*Agrimonia eupatoria*, Rosaceae), eucalyptus (*Eucalyptus globulus*, Myrtaceae), walnut-tree (*Juglans regia*, Juglandaceae), myrtle (*Myrtus communis*, Myrtaceae), raspberry (*Rubus idaeus*, Rosaceae), sage (*Salvia* sp., Lamiaceae), savory (*Satureja montana*, Lamiaceae), sweet-amber (*Hypericum androsaemum*, Clusiaceae), thyme (*Thymus vulgaris*, Lamiaceae) and yarrow (*Achillea millefolium*, Asteraceae) were selected, following a preliminary screening for antioxidant capacity (Gião et al., 2007). These aromatic plants, frequently used in traditional medicine in Portugal, were provided by ERVITAL (Castro Daire, Portugal) – and were all produced via organic farming in the open air, under sufficiently standardized culturing conditions to allow market specifications to be met; the specific parts used of each such plant were described in detail elsewhere (Gião et al., 2007).

Plant samples were supplied at different stages of processing; fresh (1), frozen (2) and dehumidified/packed (3), in two consecutive years (2006 and 2007) – thus producing true experimental replicates; the assays were also obtained as duplicates or triplicates, so analytical replicates were provided as well – and all were used to estimate experimental variability. Furthermore, walnut-tree, sage, savory, sweet-amber and thyme were, after dehumidification under controlled relative humidity, maintained for one year in a dark room not above 25 °C – so as to assess the effect of storage (4). The whole manufacture process is depicted in detail in Fig. 1. Following harvest (1), plants were first cut using a guillotine – in order to increase their surface area, and so accelerate drying in the open air (at room temperature under good ventilation); eventually all plants were frozen to −18 °C by a pulse of cold air, and kept as such in bulk storage (2) before packaging. Upon thawing, dehumidification of condensates was by venting with clean air. At this stage, the plant material was either packaged in bags made of PVC and heat-sealed under vacuum (3), or else stored in a well-ventilated dark room not above 25 °C. In the latter situation, said plant material was again frozen and thawed (without any relevant storage interval in between) and dehumidified before being final packaging (4); this cascade of operations was aimed at decreasing

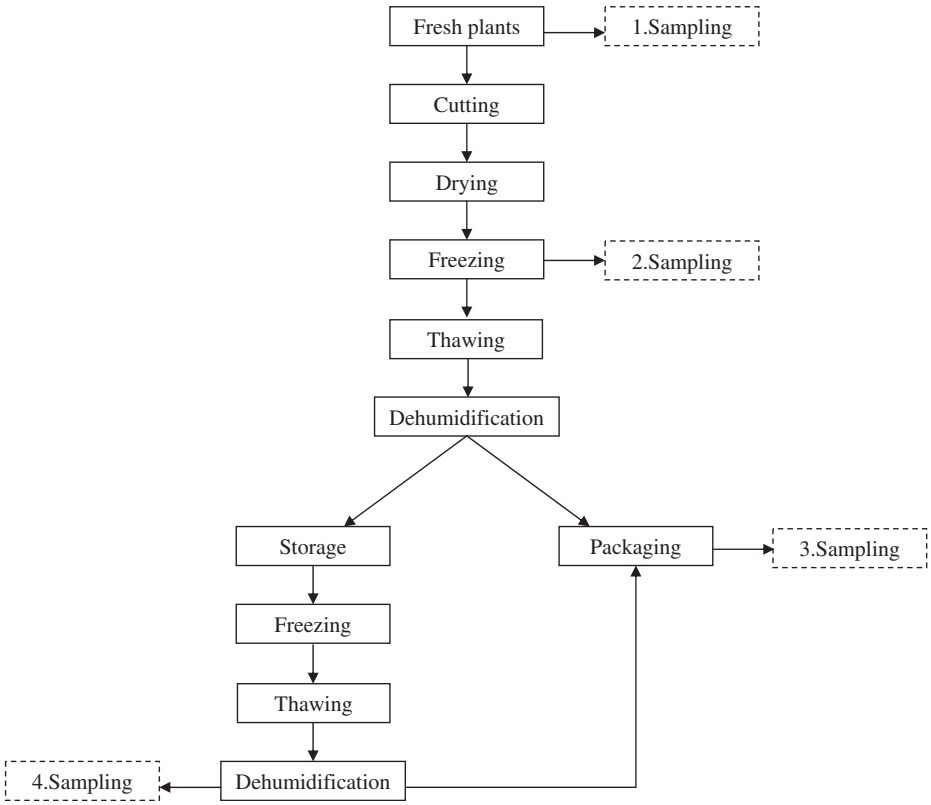

Fig. 1. Flowchart of typical plant processing, from harvest to packaging, with indication of points of sampling for experimental characterization.

moisture even further, as well as stabilizing it within the plant matrix.

For analysis, frozen plant samples were milled using a coffee mill; the corresponding powder was then added to boiling distilled water, at a ratio of 4 g of fresh powder per 110 mL of water; after drying, the ratio of addition was 1 g of dried powder to the same amount of water (as drying led to a weight loss of ca. 75%). After 5 min of contact, the infusion obtained was filtered through 0.45 μm-filters. This approach was intended to mimic the empiric mode of recovery and use of the active components as traditional medicines, rather than their use as food seasonings at large. Furthermore, different responses to the degree of extraction were expected as a function of the state of the material – fresh, frozen or dehumidified; however, a study of the traditional infusion process was once again meant, so the original feedstocks and extraction time were as close as possible to actual plant matrices and processing conditions at home, while accompanying the large scale manufacture of medicinal plants for the general market. After the extracts had been obtained, they were kept in ice.

2.2. Assay for total antioxidant capacity

As described previously (Gião et al., 2007), an $ABTS^{\bullet+}$ stock solution was prepared via addition, at 1:1 (v/v), of 7 mmol/L ABTS, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma–Aldrich, Steinheim, Germany) to 2.45 mmol/L potassium persulfate (Merk, Damstadt, Germany) solutions; the reaction took place for 16 h in the dark. The aforementioned $ABTS^{\bullet+}$ solution was duly diluted in ultra-pure water, in order to obtain an absorbance of 0.700 ± 0.020 at 734 nm (UV-VIS 1203 spectrophotometer, from Shimadzu, Tokyo, Japan). Aliquots of 10 μL were then accurately diluted, so as to produce an inhibition percentage between 20 and 80% after 6 min of reaction with 1 mL of $ABTS^{\bullet+}$ solution; analyses were run in triplicate. Using a calibration curve previously prepared with ascorbic acid as standard, the final result was expressed as equivalent concentration of this compound.

2.3. Assay for total phenolic content

The total concentration of phenolic compounds was determined following Gião et al. (2007). To 0.5 mL of sample, 0.5 mL of Folin–Ciocalteu reagent (Merk), 10 mL of 75 g/L sodium carbonate (Sigma–Aldrich) solution and water up to a final volume of 25 mL were added. Absorbance at 750 nm was measured (UV-VIS 1203 spectrophotometer), and analyses were run in duplicate. Gallic acid was used as standard, to prepare calibration curves in advance covering the ranges 4–80 and 20–400 mg/L; these two ranges were essentially linear, and produced absorbance values between 0.2 and 0.8 – as usual practice.

2.4. Statistical analyses

A statistical analysis, based on the assumption of general linear model-repeated measures, was applied to each set of experimental data using both analytical methods described above – for the two years considered together and separately, and throughout processing and storage. In order to assess the effect of prior storage for one year, a Wilcoxon test was performed – using again data encompassing both analytical methods. All analyses were run in SPSS, v. 16.0.0 (Chicago IL, USA).

3. Results and discussion

In this study, the plants selected were only analysed at the stages of processing deemed to be critical points – i.e. those that would likely have a stronger impact upon the product final quality, and duly labelled as 1 (fresh), 2 (frozen), 3 (packaged) and 4 (stored). At each said point, the total antioxidant capacity (via $ABTS^{\bullet+}$ method) and the total phenolic content (via Folin–Ciocalteu method) were determined, and the results underwent statistically analysis.

The first approach encompassed data for the two years considered together; significant differences were found between years for the various plants, and for each plant throughout the process (at a significance level of 0.001). Therefore, the data encompassing those plants were analysed along the process, for each year independently. The average values, and corresponding standard deviations, obtained for the total antioxidant activity are depicted in Fig. 2i and iii, whereas their total phenolic content counterparts are plotted in Fig. 2ii and iv – for each plant and at each step within the process, in 2006 (Fig. 2i and ii) and 2007 (Fig. 2iii and iv).

Inspection of Fig. 2 indicates that, depending on the plant at stake in either year, there were significant differences throughout the process – both in terms of total antioxidant capacity and total phenolic content data; and that all steps along the process produced differences, despite a similar overall trend. In particular, some topical increases in antioxidant capacity and/or phenolic contents between harvest (1) and freezing (2) – e.g. sage, or between freezing and packaging (3) – e.g. myrtle and raspberry, were observed that might be a consequence of the intrinsic variability of the natural feedstocks, or of the limitations of the analytical methods selected. Furthermore, apparent inconsistencies between antioxidant capacity and phenolic content were likely attributable to the fact that compounds other than phenolics may also exhibit significant antioxidant capacity. Recall that the Folin–Ciocalteu method was originally introduced to assay proteins via determination of their phenolic groups – which are characteristic of Tyr residues. However, it is nowadays the most widespread method to quantitate phenolic compounds, following the detailed work by Singleton and Rossi (1965) for the Californian wine industry. Therefore, the results obtained by that method were assumed to assay for phenolic compounds specifically, rather than antioxidant compounds at large.

The differences between years are a consequence of substantial weather differences prevailing prior to plant harvest – as growth was on the same soil and followed similar cultural practices. The year 2006 was indeed warmer than 2007, but the latter was rainier than 2006. The warmer weather during 2006 was apparently led to a higher total antioxidant capacity, and a higher total phenolic content to a lesser extent, of the plants under scrutiny. Our results are consistent with those reported for strawberries (Shiow & Zheng, 2001) and several other plants (Wahid et al., 2007); the latter authors stated that high-temperature stress induces synthesis of phenolic compounds (e.g. flavonoids and phenylpropanoids), but also promotes lower anthocyanin levels. However, the effects of post-harvesting rather than those of growth conditions were at stake in this work, so differences between the weather prevailing in those consecutive years should eventually be taken as lurking variables that contributed to the intrinsic error of our (replicated) experiments.

Inspection of Fig. 2, one concludes that the majority of plants tested decreased both their total antioxidant capacity and their total phenolic content by ca. 30–80 %, between fresh (1) and frozen forms (2); however, from the plain frozen (2) to the packaged form (3), variations were not significant. These findings are in general agreement with those reported elsewhere (Adebooye, Vijayalakshmi, & Singh, 2008; Domínguez-López, Edgardo, & Navarro-Galindo, 2008; Viña & Chaves, 2008) pertaining to the negative effect of temperature upon the content of anthocyanins in

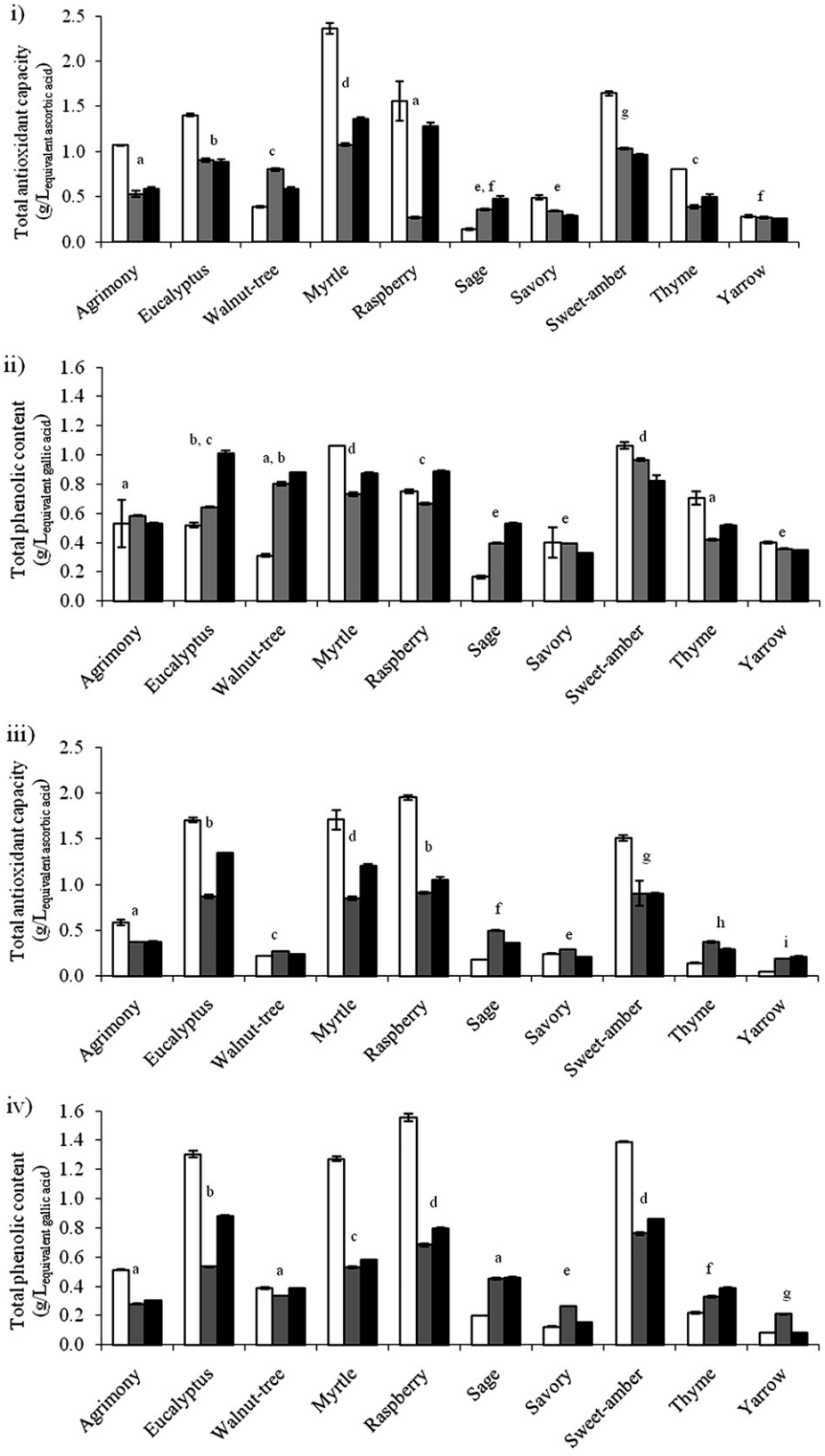

Fig. 2. Variation of total antioxidant capacity ($n = 3$) and total phenolic content ($n = 2$) throughout processing, expressed as mean ± standard deviation, as □ fresh plant, ▩ immediately after freezing, and ■ after packaging for 1 month: (i) total antioxidant capacity in 2006, (ii) total phenolic content in 2006, (iii) total antioxidant capacity in 2007 and (iv) total phenolic content in 2007. [a,b,c,d,e,f,g,h,i]Means for each plant encompassing the three conditions, without a common letter, are significantly different from each other ($P < 0.05$).

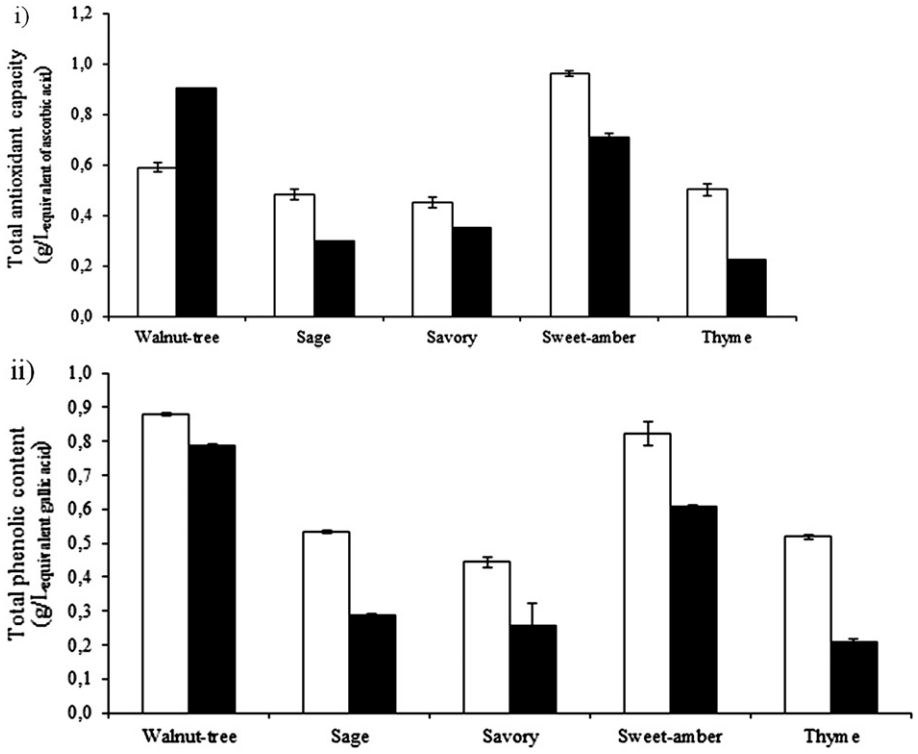

Fig. 3. Variation of total antioxidant capacity ($n = 3$) and total phenolic content ($n = 2$) throughout processing, expressed as mean ± standard deviation, □ after packaging in 2006, and ■ after storage for 1 year in 2007: (i) total antioxidant capacity and (ii) total phenolic content. (The samples used were similar to those referred to in Fig. 2.)

roselle, and upon the antioxidant power in celery; and to the negative effect of mechanical processing and aqueous extraction upon the antioxidant profile of two leaf vegetables, *Solanum nigrum* L. and *Amaranthus cruentus* L. Furthermore, there are plants for which both the total antioxidant capacity and the total phenolic content increased throughout the process: in 2006, the exceptions were walnut-tree and sage; in 2007, the same was observed for those plants, and additionally for yarrow, thyme and savory. For the remaining plants that decreased their antioxidant capacity during processing in 2007, the main difference was again observed between fresh (1) and frozen states (2).

The effect of storage (4) was treated separately, as the experimental conditions made available permitted evaluation of only five plants (at most). The average values, and corresponding standard deviations, for total antioxidant capacity and total phenolic content are represented in Fig. 3. All plants displayed significant differences in total antioxidant content, between the packaged and stored states, in 2006: walnut-tree, sage and sweet amber, at a significance level of 0.05; savory, at a significance level of 0.01; and thyme, at a significance level of 0.001. Concerning total phenolic content, savory showed no significant differences within the same period – whereas sage, sweet amber and thyme underwent differences at a significance level of 0.05, and walnut-tree at a significance level of 0.01.

Finally, one may argue about the stability of the extracts – yet those done with water (data not shown) were actually found to be more stable than when using methanol or ethanol as solvents.

4. Conclusions

The processing applied to plant material, from harvest to packaging, influences the antioxidant capacity and the total phenolic content of its infusions: typically, a decrease is observed, the magnitude of which depends on the plant (being maximum for myrtle and minimum for yarrow). However, the prevailing weather conditions prior to harvest also affect those parameters to some degree, and hence should not be uncoupled from processing. In a similar fashion, storage affects negatively those two parameters. Plant infusions with better performance will accordingly be expected if processing extent is minimized and long storage is avoided. The foregoing results will be useful mainly to manufacturers of plant extracts for aqueous infusions, and also in their interaction with plant growers and suppliers.

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