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Optimisation of the extraction and processing conditions of chamomile (*Matricaria chamomilla* L.) for incorporation into a beverage

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ABSTRACT

The total phenols, apigenin 7-glucoside, turbidity and colour of extracts from dried chamomile flowers were studied with a view to develop chamomile extracts with potential anti-inflammatory properties for incorporation into beverages. The extraction of all constituents followed pseudo first-order kinetics. In general, the rate constant (k) increased as the temperature increased from 57 to 100 °C. The turbidity only increased significantly between 90 and 100 °C. Therefore, aqueous chamomile extracts had maximum total phenol concentration and minimum turbidity when extracted at 90 °C for 20 min. The effect of drying conditions on chamomile extracted using these conditions was determined. A significant reduction in phenol concentration, from 19.7 ± 0.5 mg/g GAE in fresh chamomile to 13 ± 1 mg/g GAE, was found only in the plant material oven-dried at 80 °C ($p \leqslant 0.05$). The biggest colour change was between fresh chamomile and that oven-dried at 80 °C, followed by samples air-dried. There was no significant difference in colour of material freeze-dried and oven-dried at 40 °C.

1. Introduction

Chamomile (*Matricaria chamomilla* L.) is an annual herbaceous flowering plant native to Europe. It has been used traditionally as a medicinal and pharmaceutical preparation, due to its anti-inflammatory and antispasmodic properties. Recent research supports this use and shows these properties are partly due to its phenolic content (Maschi et al., 2008; McKay & Blumberg, 2006). The phenolic content consists of flavonoids, including flavone glycosides (e.g., apigenin 7-glucoside) and flavonols (e.g., quercetin glycosides and luteolin glucosides). Apigenin 7-glucoside is one of the main components of the flowers (Fonseca & Tavares, 2004; Mulinacci, Romani, Pinelli, Vincieri, & Prucher, 2000; Srivastava & Gupta, 2007) and as such has become the standard flavonoid to establish extract potency (European Pharmacopoeia, 2004). Phenolic acids including caffeic, chlorogenic acids and ferulic acid derivatives have also been found in the flowers of chamomile (Fonseca, Tavares, & Horvath, 2007; Mulinacci et al., 2000). Previous studies have shown that flavonoids, including apigenin, have anti-inflammatory properties (McKay & Blumberg, 2006; Kim, Son, Chang, & Kang, 2004); therefore, as well as the total phenolic content of chamomile extracts, the present study will also concentrate on the effect of extraction and processing on apigenin 7-glucoside.

Traditionally, chamomile flowers are prepared as an infusion with water, to make a tea. If these infusions can be optimised in terms of their phenolic content and, more specifically, apigenin 7-glucoside, they could have potential as beverages with anti-inflammatory properties. The effect of brewing temperature and time on phenols, specifically flavonoids, has been studied in green tea (Labbé, Tremblay, & Bazinet, 2006; Price & Spitzer, 1994), black tea (Price & Spiro, 1985; Spiro & Siddique, 1981) and rooibos tea (Jaganyi & Wheeler, 2003), but no data have yet been published on the extraction kinetics of phenols from chamomile tea. The quantity of the phenolic compounds (e.g., apigenin 7-glucoside) along with other factors influences the quality of the infusion. Colour and turbidity are also very important properties in beverages as one of the most important attributes of foods is their appearance (Hutchings, 1999), therefore, it is important to have information on the effect of extraction time and temperature on the content of phenols, colour and turbidity in chamomile extracts.

Another factor which will have an effect on the quality of the herb, and thus on the final beverage is post-harvest processing (e.g., drying). Drying is an important process for preserving plant material, as it inhibits enzymatic degradation and limits microbial growth. However, the drying process may result in quality loss in plants, such as colour changes or loss in active ingredients (Fennell, Light, Sparg, Stafford, & van Staden, 2004). Some research has been carried out on the effect of drying temperature on the essential oil content of chamomile (Borsato, Doni-Filho, & Ahrens, 2005; Knovalova, Rybalko, & Klimakhin, 1981), but there have been no data published on the effect of drying condition on the colour or total phenol content of chamomile infusions. Therefore, it is

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important to evaluate the effect of drying conditions on these parameters.

The objectives of this study were to optimise the extraction conditions of commercially bought chamomile flowers and to use the optimised extraction conditions to assess the effects of various drying conditions on the quality of chamomile extracts. Overall the study aims to develop chamomile extracts with potential anti-inflammatory properties for incorporation into beverages.

2. Materials and methods

2.1. Materials

Dried chamomile flowers were purchased from The Organic Herb Trading Company, Somerset, UK. Fresh chamomile flowers were organically grown in Co. Roscommon, Ireland. Laboratory grade gallic acid was purchased from Sigma–Aldrich (St. Louis, MO), and Folin–Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile and phosphoric acid were obtained from Sigma–Aldrich. Apigenin 7-glucoside standard was purchased from Extrasynthese (Lyon, France).

2.2. Extraction kinetics

Chamomile flowers (2.5 g) were steeped in 100 ml of distilled water at 57, 70, 80, 90 and 100 °C, for a number of independent time points ($n \geqslant 10$) at each temperature. After heating the extract was filtered using Whatman No. 1 filter paper under vacuum and was cooled immediately on ice.

2.3. Drying conditions

Fresh chamomile flowers were harvested in October 2006. The flowers were immediately separated into 250 g samples and dried using different treatments. The treatments were as follows: (1) air drying at ambient temperature (~21 °C), (2) drying in a forced-convection oven (Model No. FD 115/E2, Binder, Tuttlingen, Germany) at 40 °C, (3) drying in a forced-convection oven at 80 °C, (4) freeze-drying (Edwards Super Modulyo freeze-drier, Crawley, Sussex, UK) of flowers by immersion in liquid nitrogen and (5) extracting fresh flowers immediately. The dry weights of the chamomile flowers were calculated from samples oven-dried at 102 °C for 24 h or until constant weight was achieved. The moisture content of the fresh chamomile flowers was found to be 85% (wet basis).

2.4. Extraction of dried material

To analyse the colour and phenolic content of the chamomile flowers dried under various conditions, 2.5 g of dried samples was extracted in 100 ml of water at 90 °C for 20 min. For each treatment, extractions were completed in triplicate.

2.5. Total phenols

The total phenolic content of the extracts was determined by the Folin–Ciocalteu method, according to Singleton and Rossi (1965). Briefly, the extract (0.2 ml) was mixed with 0.5 ml of Folin–Ciocalteu reagent, 1.5 ml of 20% sodium carbonate solution and 7.8 ml of distilled water. The samples were left to stand for 2 h at room temperature and the absorbance at 760 nm was read on a UV–visible spectrophotometer (UV-1240, Shimadzu, Milton Keynes, UK). The results were expressed as gallic acid equivalent (mg GAE)/g dry weight of plant material.

2.6. Turbidity

Turbidity was measured using a UV–visible spectrophotometer (UV-1240, Shimadzu) at 800 nm. The percent transmittance (T%) was recorded and 100-T% was used as a measure of turbidity (Morton & Murray, 2001).

2.7. Colour

The Hunter L^* (lightness), a^* (red–green) and b^* (yellow–blue) values of the extracts were measured using a Minolta colorimeter (Model No. CR-300, Minolta Ltd., Milton Keynes, UK). The colorimeter was calibrated for internal light (D65), using a white calibration plate before carrying out colour measurements. Colour measurements were carried out in triplicate. Hue angle ($h°$) expresses the colour difference and is defined as red–purple: 0°, yellow: 90°, bluish-green: 180°, and blue: 270°. Chroma (C^*) is a measure of the purity or saturation of the colour. Hue angle and chroma were calculated using the following equations (McGuire, 1992):

$$\text{Hue angle } (h^\circ) = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$\text{Chroma } (C^*) = \left[(a^*)^2 + (b^*)^2\right]^{\frac{1}{2}} \quad (2)$$

2.8. Apigenin-7-glucoside analysis

Chamomile extracts were filtered through a 0.2 μm membrane filter, and 10 μl was injected directly onto ane HPLC column. HPLC separation was carried out using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA) in combination with an Agilent Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm i.d.; 5μm particle size) column with a C18 guard column (Phenomenex, Macclesfield, UK). The solvents used were 0.025 M phosphoric acid (**A**) and acetonitrile (**B**). The separations were performed at 30 °C by gradient elution at a flow rate of 1 ml/min. UV detection was at 335 nm. The following gradient was used: 0–10 min, from 20% to 40% **B**; 10–15 min, 40% **B**; 15–20 min, 20% **B**. Identification was established by spiking with a commercial standard.

2.9. Data analysis

Extraction reaction rate constants (k) and equilibrium concentrations (C_∞) were calculated using nonlinear regression in Sigma Plot Version 10 (Systat Software, Inc., Chicago, IL) according to the first-order equation Eq. (3).

$$C = C_\infty(1 - \exp(-kt)) \quad (3)$$

The r^2 value was above 0.94 in all cases, indicating a good data fit to the model. Student's t-test (two-tailed) was used to compare the k and C_∞ values between the various temperatures for each parameter, results with $p \leqslant 0.05$ were considered significantly different.

A one-way analysis of variance and Tukey's pairwise comparisons were used to determine significant differences between the various drying treatments. All analyses were carried out in triplicate. SAS 9.1.3 was used for analyses (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Extraction

3.1.1. Extraction kinetics

The extraction of total phenols from chamomile flowers followed pseudo first-order kinetics (Fig. 1). This is in agreement with

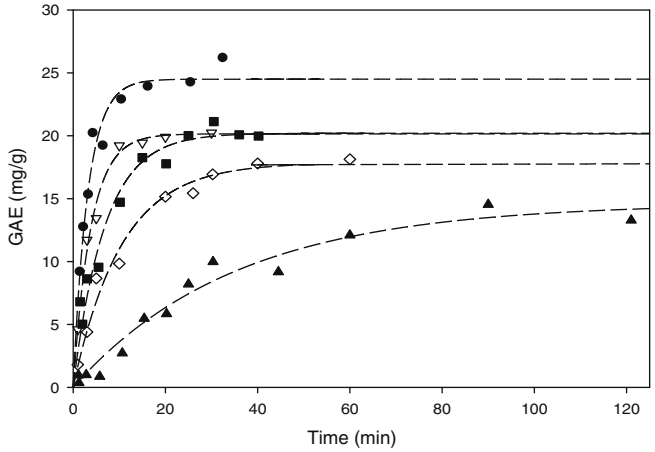

Fig. 1. Aqueous extraction of total phenols from chamomile flowers with time at temperatures of 57(▲), 70(◇), 80(■), 90(▽) and 100(●) °C (Note: broken lines represent the behaviour predicted by the pseudo first-order kinetic model).

other works, which have studied the extraction of phenolic compounds from black and green teas (Price & Spiro, 1985; Price & Spitzer, 1994). Also, an increase in the phenolic content of chamomile with increasing infusion time from 3 to 10 min after the addition of boiling water has been previously reported (De Lima, Melo, & Lima, 2004). The kinetic parameters of chamomile extraction are presented in Table 1. As the temperature increased from 57 to 100 °C the rate of extraction (k) of total phenols increased from 0.028 ± 0.004 to 0.31 ± 0.03 min^{-1}. The equilibrium concentration (C_∞) of total phenols increased with temperature from 14.6 ± 0.9 at 60 °C to 20.2 ± 0.7 mg/g dry weight (dw) at 80 °C; between 80 and 90 °C there was no significant difference in total phenols extracted ($p \geqslant 0.05$); at 100 °C C_∞ increased significantly to 24.5 ± 0.7 mg/g dw ($p \leqslant 0.05$). This is in agreement with Sharma, Gulati, and Ravindranath (2005), who found that green tea infused at 100 °C had a higher level of phenols (catechins) than tea infusions made at 80 °C.

The extraction of apigenin 7-glucoside also followed pseudo first-order kinetics. Both the rate constant (k) and the equilibrium concentration (C_∞) of apigenin 7-glucoside extracted increased with temperature from 57 to 90 °C (Table 1); however, the k and C_∞ values at 100 °C were not significantly different from the values at 90 °C. Similar results were seen in a study which examined the extraction of soluble solids from oranges at 50–88 °C, where the rate constant increased with temperature up until 80 °C, after which the rate constant levelled off (Chambers, Exaudi-Larsen, & Price, 1996).

The evolution of colour (lightness and hue angle) with time at all temperatures was also fitted to the pseudo first-order kinetic model, and the results are presented in Table 1. The L_∞, equilibrium value for lightness, was not significantly different (55 ± 2) for extracts prepared at 57 to 90 °C (Table 2). However, at 100 °C the L_∞ decreased slightly to 49.6 ± 0.2, which indicates the extract became slightly darker at 100 °C. Overall, the rate of development of lightness and hue angle increased with an increase in temperature.

Table 2
The effect of drying treatment on the colour of chamomile extracts.

	Lightness (L^*)[a]	Hue angle (h°)[a]	Chroma (C^*)[a]
Fresh material	72 ± 3a	95 ± 2a	20 ± 1a
Freeze-dried	55 ± 2b	81 ± 2b	10 ± 3cd
Air-dried	56 ± 2b	66 ± 4c	13 ± 1cb
Oven-dried (40 °C)	61 ± 2b	78 ± 1b	16 ± 2ab
Oven-dried (80 °C)	48 ± 1c	40 ± 3d	6 ± 2d

[a] Each value represents the mean of 3 replicates. Means not sharing the same letter differ at $p \leqslant 0.05$.

From Table 1, it is clear that the rate constants for total phenols, apigenin 7-glucoside and hue angle seem to correlate well, indicating an increase in the extraction of phenols is accompanied by a decrease in hue angle. The k values for lightness were higher in comparison with k values for total phenols, apigenin 7-glucoside and hue angle, indicating the extract darkens more quickly, relative to the extraction of the phenolic compounds.

3.1.2. Formation of turbidity in the extract

The effect of temperature and time on the formation of turbidity is shown in Fig. 2. Turbidity increased with time at all temperatures up to ~10 min, after which time it stabilised. In the temperature range 57–90 °C the turbidity of the extracts at 800 nm did not go above 10%, indicating a low level of turbidity. However, at 100 °C the values increased to over 34%, indicating a higher level of turbidity in the extract. The increased turbidity at 100 °C was possibly due to tissue degradation of the flowers arising from turbulence during boiling. A high level of turbidity is undesirable in

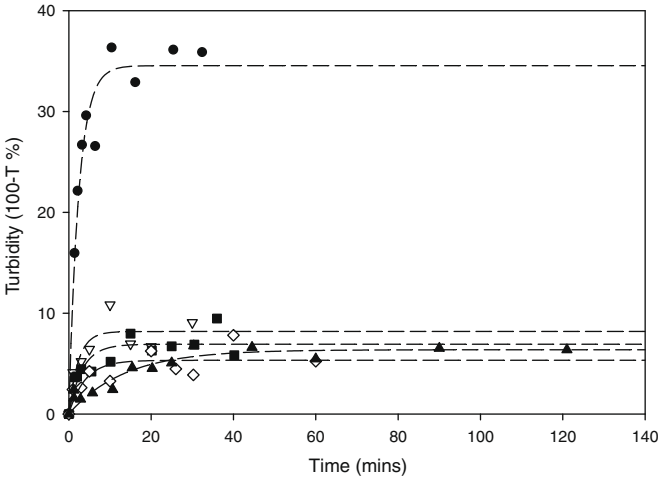

Fig. 2. Development of turbidity in chamomile extracts at temperatures of 57(▲), 70(◇), 80(■), 90(▽) and 100(●) °C (Note: broken lines represent the behaviour predicted by the pseudo first-order kinetic model).

Table 1
Extraction rate constants (k) and equilibrium concentrations (C_∞) of compounds in aqueous chamomile extracts.

Temperature (°C)	Total Phenols		Apigenin-7-glucoside		Hue angle		Lightness	
	k (min^{-1})	C_∞ (mg/g)	k (min^{-1})	C_∞ (mg/g)	k (min^{-1})	h_∞ (°)	k (min^{-1})	L_∞
57	0.028 ± 0.004	14.6 ± 0.9	0.023 ± 0.004	1.3 ± 0.1	0.029 ± 0.006	39 ± 5	0.051 ± 0.007	54 ± 2
70	0.096 ± 0.008	17.8 ± 0.6	0.032 ± 0.008	2.1 ± 0.3	0.053 ± 0.02	32 ± 2	0.24 ± 0.05	56 ± 2
80	0.15 ± 0.02	20.2 ± 0.7	0.13 ± 0.02	2.5 ± 0.1	0.09 ± 0.02	34 ± 3	0.36 ± 0.06	54 ± 1
90	0.26 ± 0.02	20.1 ± 0.4	0.29 ± 0.04	2.95 ± 0.08	0.26 ± 0.02	25 ± 1	1.0 ± 0.1	54 ± 1
100	0.31 ± 0.03	24.5 ± 0.7	0.28 ± 0.03	2.9 ± 0.1	0.4 ± 0.1	26 ± 1	1.15 ± 0.06	49.6 ± 0.2

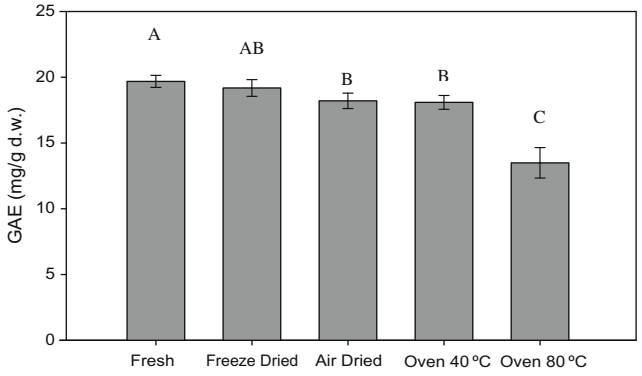

Fig. 3. The effect of drying treatment on the total phenol content of chamomile flowers.

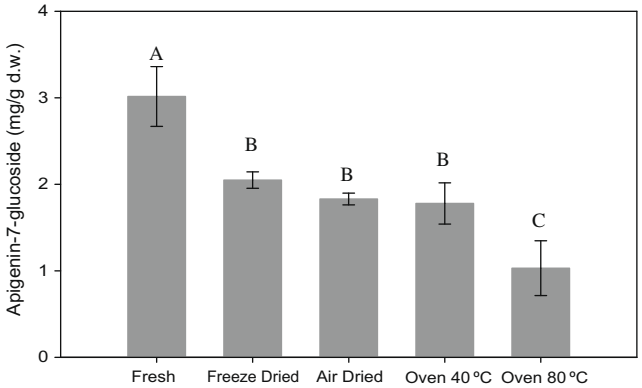

Fig. 4. The effect of drying treatment on the apigenin 7-glucoside content of chamomile flowers.

beverages such as tea, as consumers may perceive it as an indication of a lower quality beverage (Hutchings, 1999). For minimum turbidity a maximum temperature of 90 °C can be used and between 90 and 100 °C there is little improvement in total phenols or apigenin content. Therefore, for minimum turbidity and maximum phenolic content, chamomile flowers should be extracted at 90 °C for ~20 min.

3.2. Drying

3.2.1. Effect of drying on the phenolic content of aqueous chamomile extracts

The effect of drying on the total phenol content in chamomile extracts is shown in Fig. 3. The freshly extracted chamomile flowers had a higher content of phenols (19.7 ± 0.5 mg/g dw) than any of the dried samples, except those freeze-dried ($p \leqslant 0.05$). There was no significant difference between the total phenol content in samples freeze-dried, air-dried and oven-dried at 40 °C. Knovalova et al. (1981) reported that there was no difference in plant essential oil content between chamomile flowers air-dried and oven-dried at 40–45 °C; therefore, drying under these conditions does not seem to affect either the phenolic or essential oil content of chamomile flowers. However, there was a major decrease in the phenol concentration of chamomile flowers oven-dried at 80 °C ($p \leqslant 0.05$). A decrease in total phenols with increasing drying temperatures has been previously reported for other plants. Julkunen-Tiitto (1985) found that willow leaves dried below 50 °C had significantly higher total phenol content than those dried at 90 °C. The decrease in total phenols at high temperatures was possibly due to volatile phenols evaporating, decomposition of phenolic compounds and certain phenols combining with other plant components, so that they were not available for extraction (Julkunen-Tiitto, 1985).

The effect of drying on the apigenin 7-glucoside content in chamomile shows a similar trend (Fig. 4). The extracts produced from fresh chamomile had an apigenin 7-glucoside content of 3.0 ± 0.4 mg/g dw, which was significantly higher than the content of any of the dried samples ($p \leqslant 0.05$). There was no significant difference in the apigenin 7-glucoside content between the chamomile flowers which were freeze-dried, air-dried or oven-dried at 40 °C (2.0 ± 0.4 mg/g dw). The biggest decrease in apigenin 7-glucoside to 1.0 ± 0.3 mg/g dw was seen in the samples oven-dried at 80 °C. The effect of drying treatment on the apigenin 7-glucoside content of willow leaves has been previously studied (Julkunen-Tiitto & Sorsa, 2001) but unlike chamomile flowers there was no significant difference between any drying treatments (fresh analysis, room drying at 22 °C, air drying at 60 or 90 °C) except for the freeze-dried material, which had a significantly lower content of apigenin 7-glucoside.

3.2.2. The effect of drying on the colour of chamomile extracts

The effect of drying on the colour of chamomile extracts is presented in Table 2. The extracts prepared from fresh chamomile flowers were lighter than the extracts made from the dried samples (i.e., higher *L*-value). There was no significant difference in lightness between the extracts from flowers freeze-dried, air-dried or oven-dried at 40 °C. The extract of the chamomile flowers oven-dried at 80 °C had the darkest colour ($L = 48 \pm 1$).

Chamomile extracts made from fresh flowers also had the highest hue angle ($H = 95 \pm 2°$), indicating a yellow–green colour (Table 2). The samples freeze-dried or oven-dried at 40 °C had a hue angle of ~80°, indicating a slightly redder colour in these extracts, compared to those from fresh chamomile. The hue angle of the air-dried chamomile was 50°, indicating a significant increase in the redness of the extract. This is possibly due to an increase in enzymatic browning of the chamomile flowers due to both the low temperature and slow nature of the drying treatment. The lowest hue angle of 40° ± 3° was found in chamomile flowers oven-dried at 80 °C, which also yielded the darkest extract. Other authors have noted a change in the colour of plant material at high drying temperatures, although few studies have determined the effect of drying temperature on the colour of plant extracts. Borsato et al. (2005) noticed that when chamomile flowers were dried at 95 °C they turned to an undesirable caramel colour. Also, willow leaves air-dried at 60 and 90 °C turned a 'brownish' colour, due to quinone formation and decomposition of the phenolics (Julkunen-Tiitto and Sorsa, 2001).

The chamomile extracts made from fresh flowers had the highest chroma (20 ± 1) and the samples oven-dried at 80 °C had the lowest chroma (6 ± 2) (Table 2). As mentioned above, chroma is a measure of the purity or saturation of the colour; therefore, a high chroma would be desirable in an extract. The chroma of the chamomile oven-dried at 40 °C was not significantly different from the fresh sample.

The extracts from chamomile flowers oven-dried at 40 °C were most similar to the chamomile extracted from fresh, in terms of colour. Also, the contents of total phenols and apigenin 7-glucoside in the sample oven-dried at 40 °C are not significantly different from those freeze-dried or air-dried. Therefore, oven-drying chamomile at low temperatures (i.e., 40 °C) was an effective means of preserving chamomile flowers.

4. Conclusion

Oven-drying chamomile at low temperatures (i.e., 40 °C) followed by aqueous extraction at 90 °C for 20 min appears to be an effective means of preparing an extract rich in phenols and low

in turbidity, which may have potential as an anti-inflammatory agent in beverages.

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