



## Effect of processing temperature on the stability of parthenolide in acidified feverfew infusions

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### ABSTRACT

The stability of parthenolide, the active ingredient in feverfew and a potential anti-inflammatory bioactive in beverages was evaluated at different pHs (2.9, 3.7, 4.6 and 6.0) during heat processing at 40–100 °C. The residual concentration of parthenolide was analysed by High Performance Liquid Chromatography and degradation kinetics determined using a non-isothermal method. Parthenolide degradation with thermal treatment followed pseudo-first order kinetics. The stability of parthenolide was significantly affected by pH and processing temperature. Feverfew infusions at near neutral pH levels exhibited good stability but a significant decrease in stability was observed at lower pHs. This model is likely to be a useful tool to predict the optimum pH and time-temperature profile required to retain parthenolide during heat processing.

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### 1. Introduction

Increasing consumer demand for nutritious and healthy foods has led to the development of new health promoting food and beverages in the global market (Pohjanheimo & Sandell, 2009). Over the past few years, secondary plant metabolites have attracted a lot of interest mainly due to their role in preventing diseases such as cardiovascular diseases, cancers, rheumatism and arthritis. These bioactive compounds may act as antioxidants, free radical scavenging or anti-inflammatory agents (Kahkonen et al., 1999; Kaur & Kapoor, 2001). As a result, several studies have focused on the use of phytochemicals and their possible application in food systems to promote health and overall wellbeing (Butt, Nazir, Sultan, & Schroen, 2008; Prabhasankar et al., 2009; Wang, Josdottir, & Olafsdottir, 2009).

Feverfew (*Tanacetum parthenium*) is a perennial medicinal herb that has been used traditionally for the treatment of various ailments including migraine headaches and relief of pain and inflammation from arthritis. Several studies have shown that a sesquiterpene lactone, parthenolide is responsible for the anti-inflammatory properties in feverfew (Bejar, 1996; Johnson, Kadam, Hylands, & Hylands, 1985; Williams, Harborne, Geiger, & Hoult, 1999). The medicinal properties of feverfew may provide opportunities for the development of functional beverages based on its aqueous extracts.

Previous studies in our laboratory have shown that freezing and subsequent freeze-drying of the aerial parts of feverfew was a good method for preserving the bioactive constituents (Marete, 2010).

Another study in our laboratory has shown that extraction temperatures  $\geq 75$  °C were suitable for preparing infusions rich in phenolic and parthenolide content. This extraction temperature was also found to minimise polyphenol oxidase (PPO) enzymatic activity thus producing infusions with a desirable colour for possible incorporation into a beverage (Marete, Jacquier, & O'Riordan, 2009). Recently, Marete, Jacquier, and O'Riordan (2011) have shown that refrigerated storage of feverfew infusions at mild acidic pH (around 4.6) was suitable for the preservation of their parthenolide, total phenol content and colour for approximately 4 months.

The application of feverfew infusions as functional food ingredients would require a study to establish the stability of active constituents to food processing treatments e.g. pasteurisation and sterilisation. Heat treatment is one of the most widely used methods of preserving and extending the shelf-life of processed foods and beverages mainly because of its ability to destroy micro-organisms and inactivate enzymes (Cruz, Viera, & Silva, 2008).

Thermal degradation of parthenolide in aqueous feverfew solutions at 40–80 °C has been reported by Fonseca, Rushing, Thomas, Riley, and Rajapakse (2006). However the authors have not examined the degradation kinetics during thermal processing or the effect of varying pH values commonly found in beverages. The degradation kinetics of parthenolide during heat processing would provide important information to the developers of functional food products by providing the optimum time-temperature profile to minimise parthenolide degradation or to predict the impact of heat treatments used to ensure microbiological safety on parthenolide degradation.

The use of a non-isothermal methodology based on the works of Dolan (2003) has been found to allow rapid access to all kinetic parameters of the degradation processes, while helping to mimic the heat processes encountered in the food industries (Dolan, 2003;

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Harbourne, Jacquier, Morgan, & Lyng, 2008). Therefore, the objectives of this study were first to determine the thermal degradation kinetics of parthenolide using a non-isothermal method in feverfew infusions, at pH levels representative of those that are commonly encountered in beverage products. Secondly to establish a model unifying the combined influence of time, temperature and pH on parthenolide degradation in order to predict the loss associated with heat processing in acidified beverages.

## 2. Materials and methods

### 2.1. Plant material

Organically grown feverfew was harvested in Roscommon, Ireland. The aerial parts were frozen at  $-20^{\circ}\text{C}$  and subsequently dried for 72 h using a freeze dryer (Edwards Super Modulyo, Davidson and Hardy Ltd., UK). The dried samples were then ground into a moderately fine powder as described in detail by Marete et al. (2009).

### 2.2. Infusion preparation

Feverfew powder (5.0 g) was extracted in 200 ml of distilled water at  $100^{\circ}\text{C}$  for 10 min with an aim of maximising the bioactive constituents and minimising polyphenol oxidase (PPO) activity (Marete et al., 2009). Extraction was carried out in triplicate. The pH of the infusions was then adjusted using various concentrations of citric acid (Reagent grade, Fisher Scientific, Leicestershire, UK) and sodium citrate (Reagent grade, Hopkin and Williams, Nottinghamshire, England) to achieve a pH of 2.9, 3.7, 4.6 and 6.0 with a final citric/citrate concentration of 0.06 M to mimic the citric content in fruit beverages. To minimise microbial degradation, 300 ppm of potassium sorbate (Reagent grade, Hoechst, Frankfurt, Germany) and 250 ppm of benzoic acid (Reagent grade, Sigma-Aldrich, Munich, Germany) were added as preservatives. Additional filtration using Acrodisc® 0.2  $\mu\text{m}$  GHP (hydrophilic polypropylene) membrane (Pall Life Sciences, Hampshire, UK) was carried out.

### 2.3. Heat treatment

Following pH adjustments, a preliminary experiment was set up to determine the heat processing time which would cause an unacceptable degradation of parthenolide at each of the pH levels. Aliquots (1.5 ml) of the pH adjusted feverfew infusions were placed in capped HPLC vials. The heating medium used was water. The infusions at pH 6.0 were heated at  $80^{\circ}\text{C}$  and the lower pH infusions (4.6, 3.7 and 2.9) were heated at  $100^{\circ}\text{C}$ . The sample vials were placed in a saucepan containing water which was placed on a hot plate (Ika® WERKE GmbH and Co., Germany). The temperatures of the samples were recorded at 1 min intervals during cooking with a temperature logger (Model No. MMS3000-GP4, ISE, Inc., Cleveland, OH) and a fast response flexible Type K thermocouple with a diameter of 0.64 mm, Teflon coating and a welded tip (Industrial Temperature Sensors, Naas, Ireland), which was placed into one sample test tube to represent each lot of tubes. The tubes used were small HPLC vials ( $32 \times 11.6$  mm, 1.5 ml) closed with a Teflon septum through which the thermocouple was inserted, thus preventing any liquid loss even at high temperature. Because of the small volumes of these vials, the temperature was deemed constant without gradient throughout.

The set up of this preliminary experiment was applied to all the later experiments. After determining the processing time which would cause an unacceptable degradation, the infusions were then processed at temperatures of 40, 50, 60, 70, 75, 80, 85, 90 and  $100 (\pm 2)^{\circ}\text{C}$  for different holding times. Feverfew infusions at a pH of 2.9, 3.7, 4.6 and 6.0 were heated for 7, 50, 120 and 240 min respectively.

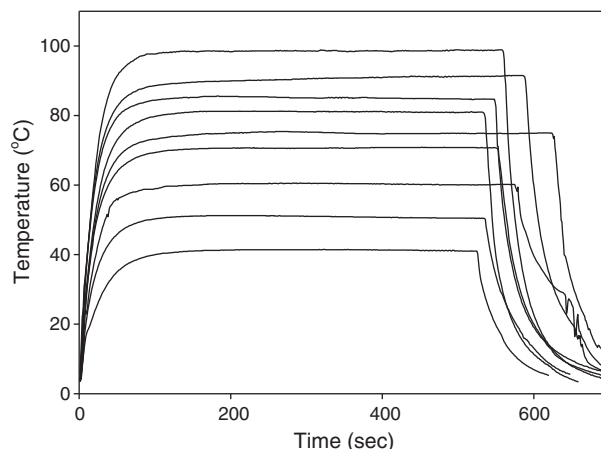


Fig. 1. Temperature profiles recorded during thermal treatment ( $40$ – $100^{\circ}\text{C}$ ) of feverfew infusions at pH 2.9 as a function of heating time.

An example of the temperature profiles of the infusions at pH 2.9 processed at  $40$ – $100^{\circ}\text{C}$  is shown Fig. 1. All the infusions were then cooled in iced water to circa  $5^{\circ}\text{C}$  prior to HPLC analysis.

### 2.4. Parthenolide analysis

HPLC analysis of parthenolide content in the feverfew infusions was carried out as described in detail in Marete et al. (2009) using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA). Reversed phase chromatography was performed using an Agilent ZORBAX Eclipse XDB-C18 ( $150 \text{ mm} \times 4.6 \text{ mm i.d.}$ ;  $5 \mu\text{m}$ ), mobile phase of acetonitrile (HPLC grade, Sigma-Aldrich): water (55:45 v/v), flow rate of 1 ml/min and UV detection at 210 nm. The injection volume was 20  $\mu\text{l}$ .

### 2.5. Determination of the kinetic parameters

The kinetic parameters at reference temperature of  $100^{\circ}\text{C}$  were assessed using a non-isothermal model based on Dolan (2003). Briefly, the independent variables time ( $t$ ) and the processing temperature ( $T$ ) were combined into one variable, the thermal history ( $\beta$ ) according to Eq. (1).

$$\beta = \int_0^t \exp \left[ \frac{-E_a}{R} \left( \frac{1}{T(t)} - \frac{1}{T_r} \right) \right] dt \quad (1)$$

Where  $T_r$  is the arbitrary reference temperature ( $100^{\circ}\text{C}$  here) and  $T(t)$  is the temperature ( $T$ ) at time ( $t$ ).

The measured  $C/C_0$  values were then plotted as a function of  $\beta$ . The reaction rate  $k_r$  at  $T_r$  was then determined and a set of  $C/C_0$  values was calculated according to Eq. (2) (Harbourne et al., 2008).

$$\left( \frac{C}{C_0} \right)_{\text{calculated}} = \exp(-\beta \times k_r) \quad (2)$$

Where  $C_0$  is the initial concentration and  $C$  is the final concentration. Parameters of the kinetic model, reaction rate constant ( $k_r$ ) and activation energy ( $E_a$ ) were calculated by minimising the sum of squares error between experimental and calculated values for  $C/C_0$  by using Solver in Excel (Harbourne et al. 2008).

The time required for 10% degradation ( $t_{0.1}$ ) of the total parthenolide content was calculated according to Eq. (3).

$$t_{0.1} = \frac{-\ln(0.9)}{k} \quad (3)$$

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