



## Cottage cheeses functionalized with fennel and chamomile extracts: Comparative performance between free and microencapsulated forms



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### ABSTRACT

Globally, there is a trend for healthy food products, preferably incorporating natural bioactive ingredients, replacing synthetic additives. From previous screening studies, extracts of *Foeniculum vulgare* Mill. (fennel) and *Matricaria recutita* L. (chamomile) maintained nutritional properties and improved the antioxidant activity of cottage cheese. Nevertheless, this effect was limited to 7 days. Accordingly, aqueous extracts of these plants were microencapsulated in alginate and incorporated into cottage cheese to achieve an extended bioactivity. Plain cottage cheese, and cheese functionalized by direct addition of free decoctions, were prepared and compared. Independently of plant species, “functionalization type” factor did not show a significant effect on the nutritional parameters, as also confirmed in the linear discriminant analysis, where these parameters were not selected as discriminating variables. Furthermore, samples functionalized with microencapsulated extracts showed higher antioxidant activity after the 7th day, thereby demonstrating that the main purpose of this experimental work was achieved.

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### 1. Introduction

Plant-derived bioactive extracts and compounds are interesting ingredients used to functionalize foods (Carocho, Barreiro, Morales, & Ferreira, 2014). Aqueous extracts of *Foeniculum vulgare* Mill. (fennel) and *Matricaria recutita* L. (chamomile) are good sources of phenolic compounds, exhibiting different biological activities such as antioxidant and antimicrobial properties, as previously reported by our research group (Caleja, Barros, Antonio, Ciric, Barreira et al., 2015; Caleja, Barros, Antonio, Ciric, Soković et al., 2015). In these previous works, the preserving potential of fennel and chamomile extracts obtained from decoction was explored through their direct use as natural preservers. Their incorporation into cottage cheese maintained its nutritional characteristics and improved the antioxidant properties, namely the free radical's scavenging activity. However, after 7 days under storage the cheese samples showed an antioxidant capacity decrease which was associated with extract degradation (Caleja, Barros, Antonio, Ciric, Barreira et al., 2015; Caleja, Barros, Antonio, Ciric, Soković et al., 2015).

In fact, the use of natural bioactive extracts/compounds as food additives presents limitations because after extraction they can become susceptible to degradation. Therefore, microencapsulation may be considered as an appropriate process to overcome these limitations, since this technique can provide protection against the action of several environmental agents like oxygen, light, moisture or heat, ensuring an increase in their stability (Betz & Kulozik, 2011; Dias, Ferreira, & Barreiro, 2015). This process will preserve the bioactive compound by means of a surrounding coating shell around it (reservoir type particles) or by embedding it, homogeneously or heterogeneously, in a matrix (matrix type particles) (Çam, Içyer, & Erdogan, 2014). The controlled release along time or oriented to a specific site, can be achieved by means of different mechanisms, which depend on the used encapsulation materials, production process, and microcapsules' morphology and desired application (Martins et al., 2014). Alginate, a natural polymer obtained from bacteria and algae, is widely used for microencapsulation in several fields, namely in food industry (Goh, Heng, & Chan, 2012). This polymer is classified as non-toxic for oral administration, and is usually commercialized in its salt form (e.g. sodium alginate). In the presence of bivalent cations (e.g. Ca<sup>2+</sup>) it gels, giving rise to a material that resists acidic pH and dissolves at basic medium (disruption of the ionic network). In this way the release of the encapsulated compounds will occur in the intestinal tract

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(George & Abraham, 2006). Besides this, it presents good stability, biocompatibility, exudate-retaining ability and moderate antimicrobial activity (Goh et al., 2012). Its use in the food industry is permitted by the FDA – Food and Drug Administration (USA) and EFSA – European Food Safety Agency.

There are some documented examples dealing with the application of microencapsulation to natural extracts for use in functional foods (Dias, Ferreira et al., 2015). Our research group has successfully encapsulated *Fragaria vesca* L. (Dias, Barros et al., 2015) and *Rubus ulmifolius* Schott (Martins et al., 2014) extracts that were further incorporated into  $\kappa$ -carrageenan gelatin and yogurts, respectively.

In the present study, aqueous extracts of *F. vulgare* and *M. recutita* were prepared by decoction. Then, these extracts were used to functionalize cottage cheeses following two main strategies: (i) direct use (extracts in their free form), and (ii) use after stabilization through microencapsulation with alginate (extracts in their microencapsulated form). Microencapsulation was achieved by using an atomization/coagulation technique following a procedure developed in our research group (Dias, Barros et al., 2015; Martins et al., 2014). The incorporation of *F. vulgare* and *M. recutita* extracts into cottage cheeses was compared with samples without free or encapsulated extracts (control). Moreover the gain derived from the use of the microencapsulated form over the use of the free form was also evaluated (specifically color, nutritional value and antioxidant activity of the functionalized cottage cheese as a function of storage time). Besides studying individual changes induced by each of the defined factors (storage time and functionalization type) through a 2-way ANOVA, data were also analyzed by a linear discriminant analysis to determine which of the assayed independent variables (studied parameters) defined the majority of the differences in the average score profiles of the prepared cheese samples.

## 2. Materials and methods

### 2.1. Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany). Formic and acetic acids were purchased from Prolabo (VWR International, France). Sodium alginate was provided by Fluka Chemie (USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

### 2.2. Preparation of the bioactive extracts

Commercial samples of *F. vulgare* Mill. (fennel) and *M. recutita* L. (chamomile) were provided by Américo Duarte Paixão Lda. (Alcane, Portugal). The dried samples were powdered (~20 mesh) and submitted to decoction. Decoctions were performed by adding 5 g of plant material to 200 mL of distilled water, heated (heating plate, VLP scientific, Usmate, Italy), and allowed to boil for 5 min. The mixtures were left to stand for 5 min and filtered through Whatman No. 4 paper. The decoctions were then frozen and lyophilized in order to obtain the final extracts (FreeZone 4.5, Labconco, Kansas City, MO, USA).

### 2.3. Microencapsulation of the plant extracts and characterization

#### 2.3.1. Microencapsulation

Microspheres containing extracts of *F. vulgare* or *M. recutita* were prepared by using an atomization/coagulation technique as previously described in the literature (Dias, Barros et al., 2015; Martins et al., 2014).

Calcium alginate (matrix material) was obtained by combining sodium alginate with calcium chloride ( $\text{CaCl}_2$ ) (coagulation agent). Briefly, the atomization solution was prepared by dissolving firstly, 100 mg of the extract with 20 mL of distilled water under stirring at 250 rpm and room temperature, followed by filtration to remove remaining non-soluble trace residues. In the second step, 800 mg of sodium alginate was added and the solution was kept stirring, under the same conditions, until complete dissolution was achieved. The obtained alginate solution containing the extract was then atomized using a NISCO Var J30 system (Zurich, Switzerland) at a feed rate of 0.2 mL/min and a nitrogen pressure of 0.1 bar to produce the microspheres. The atomized microspheres underwent coagulation upon contact with a  $\text{CaCl}_2$  aqueous solution (500 mL at a concentration of 4% (w/v)) over a period of 4 h. The resulting microspheres were collected by filtration under reduced pressure, washed twice with distilled water, and further lyophilized and stored in the dark at 4 °C.

#### 2.3.2. Microcapsules characterization

Microspheres were analyzed by optical microscopy (OM) using a Nikon Eclipse 50i microscope (Tokyo, Japan) equipped with a Nikon Digital Sight camera and NIS Elements software for data acquisition. OM analysis was applied to assess the size and morphology of the microspheres after the atomization and coagulation stages. It was also possible to infer the presence/absence of extract inside the microspheres.

The effective extract incorporation into the alginate matrix was investigated by FTIR analysis. For that purpose, spectra of pure alginate, free extracts of *F. vulgare* or *M. recutita*, and the corresponding microspheres were collected on a FTIR Bomen (model MB 104) by preparing KBr pellets at a sample concentration of 1% (w/w). The spectra were recorded at a resolution of  $4\text{ cm}^{-1}$  in the spectral range between 650 and  $4000\text{ cm}^{-1}$  and by co-adding 48 scans. The encapsulation efficiency (EE) was also evaluated through the quantification of the non-encapsulated extract. For this purpose, the remaining extract in the coagulation and in the first washing solution were quantified by HPLC and added. The second washing solution presented no extract. The encapsulation efficiency was calculated according to the following expression:

$$EE = [(M_{e-t} - M_{e-ne}) / M_{e-t}] \times 100$$

in which  $M_{e-t}$  represents the theoretical amount of extract (the amount of extract used in the microencapsulation process),  $M_{e-ne}$  corresponds to the non-encapsulated extract remaining after the encapsulation process.

Since the extracts are complex mixtures, only the major phenolic compounds present in the extracts of fennel (quercetin-3-O-glucoside; Caleja, Barros, Antonio, Ciric, Soković et al., 2015) and chamomile (luteolin-O-glucuronide; Caleja, Barros, Antonio, Ciric, Barreira et al., 2015) were selected for EE evaluation.

### 2.4. Functionalization of cottage cheese with plant extracts

#### 2.4.1. Preparation of the cottage cheese samples

All of the cottage cheese samples were prepared by “Queijos Casa Matias Lda.” (Seia, Portugal), by using the milk serum obtained after the production of cheese. The remaining serum (liquid component) was pumped into a vat where it was mixed and heated to a temperature that ranged between 83 and 85 °C. After a few minutes at that temperature, the serum started to flocculate and rose to the surface where it was scooped into individual forms, left for a few minutes and packed with parchment paper. The incorporation of the extracts was carried out immediately before packaging, individually, into each one of the forms mentioned above, in order to guarantee a better distribution of the extract by the cottage cheese mass.

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