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Preliminary observations of flavonoid glycosides from the vegetable coagulant *Cynara* L. in protected designation of origin cheeses

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Abstract

Aqueous extracts of *Cynara cardunculus* L. (cardoon) flowers have been used for centuries as vegetable coagulants for traditional cheesemaking in the Iberian Peninsula. However, the full chemical composition of the extract that is added to the milk has never been studied. In this study we investigated the phenolic components of the aqueous extracts from cardoon flowers and their detection in cheese. Analysis by HPLC-diode array detection (HPLC-DAD) revealed the presence of one flavonoid-7-glycoside (isorhoifolin) and the aglycones apigenin and luteolin. Another major flavonoid-7-glycoside (apigenin-7-*O*-glucuronide) was also identified and its structure elucidated by ^{13}C-NMR. This represents the first report of these compounds in the aqueous extract of cardoon flowers. These compounds were also found in cheese curd made using this coagulant but were not present in the original milk, leading to the suggestion that these phenolic compounds could be used as authentication markers for cheeses made using cardoon extracts as the coagulant.

Keywords: Vegetable coagulant; *Cynara* spp; Authentication of cheese; PDO cheeses; Flavonoids

1. Introduction

The occurrence of phenolic compounds in milk and dairy products may be the consequence of several factors, such as the consumption of particular fodder crops by cows or their deliberate addition as specific flavouring or functional ingredients (O'Connell & Fox, 2001). However, for the manufacture of some traditional ewes' milk cheeses, such as some Spanish cheeses and the Portuguese varieties Serra da Estrela, Castelo Branco, Azeitão, Évora, Niza and Serpa [that have protected designations of origin (PDO)] the use of an aqueous extract of flowers from cardoon (*Cynara* spp.) as a coagulant is compulsory.

Aqueous extracts from cardoon flowers have been used for centuries as coagulants in traditional ewes' milk cheesemaking in the Iberian Peninsula. This extract is prepared in different ways by traditional cheesemakers (Roseiro, Barbosa, Ames, & Wilbey, 2003), but normally the cut, air-dried flowers are soaked in water at room temperature, then filtered through a cloth and the solution obtained is added to the milk (Fig. 1). Coagulation usually occurs within 30–60 min. This may therefore introduce into the milk, and hence into the cheese, a certain amount of phenolic compounds originating from the vegetable coagulant used.

Some studies on the chemical compositions of different parts of *Cynara* spp. have been reported (Roseiro et al., 2003). The driving force behind those

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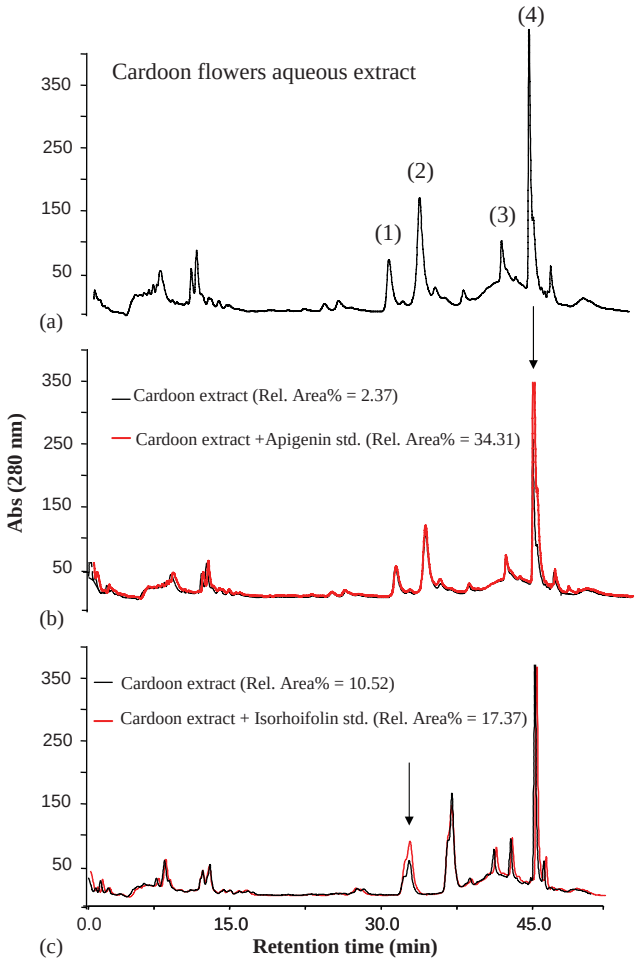

Fig. 1. HPLC separations of aqueous extract from cardoon flowers: (a) peaks (1) isorhoifolin, (2) unidentified flavonoid glycoside, (3) luteolin, (4) apigenin. Co-injections of cardoon extract with (b) apigenin and (c) isorhoifolin.

studies was the use of *Cynara* spp. for their medicinal properties and as food, particularly in the case of the common artichoke (*Cynara cardunculus* var. *scolymus*), due to their phenolic compounds, namely flavonoids. Flavonoids are a group of polyphenolic compounds widely distributed in plants that occur naturally in fruit, vegetables, nuts, seeds, flowers and bark, and are therefore part of the human diet (Cook & Samman, 1996). They have structures based on 2-phenylbenzopyrazone and differ from each other in the pattern of hydroxylation, degree of unsaturation and type and position of any sugar substituents (Pietta & Mauri, 1991). Flavonoids usually occur in plants as O-glycosides, in which one or more of the flavonoid hydroxyl groups is bound to a sugar or sugars by an acid–labile hemiacetal bond (Markham, 1982). The carbohydrate moieties mainly comprise D-glucose, L-rhamnose, glucorhamnose, galactose and arabinose (Havsteen, 1983).

Flavonoids have been reported to exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory and antiallergenic actions (Havsteen, 1983; Hanasaki, Ogawa, & Fukui, 1994). A great number of epidemiological studies associate the intake of these compounds with a reduced risk of cardiovascular disease and cancer (Scalbert & Williamson, 2000). Artichoke (*Cynara cardunculus* var. *scolymus*, formerly *Cynara scolymus*) leaves are traditionally used for the treatment of dyspepsia, but other beneficial effects, including a pronounced antioxidative capacity, have also been reported (Gebhardt, 1997). One study has also revealed that aqueous extracts of artichoke leaves, given at high-dose, may inhibit cholesterol biosynthesis (Gebhardt, 1998), and that luteolin was the flavonoid responsible for this effect. Recently, dicaffeoylquinic acids from *Cynara* spp. were established as a leading class of HIV-integrase inhibitors for the development of highly selective anti-HIV agents (Slanina et al., 2001).

Although the chemical components of *Cynara* spp. leaves have been extensively studied, studies of the composition of their flowers are less common (El-Negoumy, El Sayed, & Saleh, 1986; El-Ansari, El-Negoumy, & Saleh, 1988) and, apart from the studies on the specific enzymes and the work of Barbosa (1983), no references were found relating to the chemical composition of aqueous extracts of the flowers from *Cynara* spp.

Both El-Negoumy et al. (1986) and El-Ansari et al. (1988) have reported the presence of flavonoids, namely 7-*O*-glycosides of apigenin and luteolin, in the flowers of *Cynara* spp. Considering that most of the flavonoid-*O*-glycosides are water soluble (Markham, 1982), the primary aim of the present study was to detect and characterise the flavonoid-7-*O*-glycoside composition of the aqueous extracts of the flowers from *Cynara* spp. used as coagulant. Together with the specific coagulating enzymes (cardosins) other compounds from the aqueous extracts of *Cynara* spp. are likely to be present in the cheese matrix. Hence, a secondary aim was to detect the presence of components of *Cynara* spp. in the cheese, as a potential means of establishing the use of vegetable coagulant from this species in its manufacture and thus confirming the authenticity of the cheese.

2. Materials and methods

2.1. Materials

Dried flowers of *Cynara cardunculus* L. were obtained from a cheesemaker in the demarcated region for Serpa cheese in Alentejo, Portugal, and the aqueous extract was prepared by soaking 30 g dried flowers in 500 mL cold water and macerating with a mortar and pestle. This extract was filtered through a cloth, freeze-dried to