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Note

Thistle (*Cynara cardunculus* L) flower as a coagulant agent for cheesemaking. Short characterization

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Summary – The use of *Cynara cardunculus* L as a coagulant for cheesemaking has been considered one of the most important factors for the quality of the Portuguese traditional ewe's milk cheeses, some of them benefiting of an '*Appellation d'Origine*'. However, the irregularity of the traditional utilization of this coagulant agent is claimed to contribute to the poor quality and homogeneity of the cheese. After some work on thistle flower production, the study of the best conditions for its use in cheesemaking as well as the influence on cheese quality started with the flower characterization. Thistle flower from different origins and of different kinds of plants was analyzed for moisture, ash and total nitrogen content, water activity and milk clotting activity (MCA). A short characterization of the flower showed great variability mainly related with drying conditions. MCA was similar to that of liquid commercial coagulants. Drying of the flower seemed to affect MCA.

cheese / clotting enzyme / thistle flower / *Cynara cardunculus*

Résumé – La fleur du chardon (*Cynara cardunculus* L) comme coagulant pour la fabrication de fromage. Caractérisation succinte. *L'utilisation du chardon comme coagulant a été considérée comme l'un des facteurs déterminants de la qualité des fromages typiques portugais au lait de brebis, dont certains sont protégés par une appellation d'origine contrôlée. Cependant, l'utilisation du chardon pose des problèmes causés par l'empirisme et le manque de contrôle de son activité. À la suite d'études antérieures sur la production de fleur de* Cynara cardunculus, *on cherche actuellement à étudier des conditions de production plus avantageuses et l'influence de l'extrait coagulant sur la technologie de fabrication et la qualité du fromage. Ce travail présente les résultats obtenus pour la caractérisation de la fleur de chardon. La fleur de* Cynara cardunculus *provenant de plusieurs sources a été analysée par rapport aux teneurs en matière sèche, cendre, azote total,* a_w *et activité enzymatique. Cette caractérisation succinte montre une grande variabilité de résultats, due surtout aux différents degrés de séchage des échantillons, dont les teneurs en eau variaient de 6 % à 47 %. L'activité coagulante (1:9 000–1:12 000) était très semblable à celle des coagulants liquides du commerce. La méthode traditionnelle de séchage de la fleur semble avoir une influence sur l'activité enzymatique qui, exprimée par rapport à la teneur en eau et à la teneur en azote total, présente, pour la fleur sèche, des diminutions significatives (32 % et 26 %).*

fromage / enzyme coagulante / fleur de chardon / Cynara cardunculus

INTRODUCTION

The traditional use of thistle flower (*Cynara cardunculus* L) as the coagulant agent of Portuguese ewe's milk traditional cheese is well known (Rasteiro, 1905; Vieira de Sá, 1974; Barbosa, 1983). It is even considered one of the most important factors for the high quality of those cheeses (Matos and Vieira de Sá, 1948). Some of them benefit of an '*Appellation d'Origine*' and the respective legislation imposes the use of this vegetal coagulant on the cheesemaking technology (Holstein, 1988; Vasconcelos, 1991).

Studies on thistle flower as a coagulant agent for cheesemaking come mainly from Portugal, Spain and South America. The concern enzyme characterization, technological factors and importance for cheese quality (Christen and Virasoro, 1935; Vieira de Sá and Barbosa, 1972; Tsouli, 1974; Marcos et al, 1978; Campos et al, 1990; Nuñez et al, 1991). Recently, vegetable coagulants of traditional cheesemaking technologies were investigated because its empirical use is claimed to be one of the most important factors for the highly variable cheese quality, reducing its acceptability, especially in urban areas (Otani et al, 1984; Aworh and Muller, 1987; O'Connor, 1990; Heimgartner et al, 1990; Macedo et al, 1993).

Thus, the possibility of the use of a controlled coagulant can contribute to the improvement and the preservation of the traditional cheesemaking activity, which for many years already is responsible for some high-quality and very typical products like Serra da Estrela, Serpa and Azeitão cheeses.

Following previous work on traditional Portuguese cheeses (Vasconcelos et al, 1989; Vasconcelos, 1991) and thistle flower production (Morbey, 1990), we intend to study the improvement of thistle flower production, conservation and utilization conditions, to achieve a better rationalization of cheesemaking and an improvement of cheese quality. In this paper we examine the first results obtained with a short characterization of thistle flower.

MATERIALS AND METHODS

Thistle flowers from different origins and different types of plants, harvested during flower season, were analyzed for moisture (drying at 101 °C ± 1 °C), total nitrogen (Kjeldhal), ash (incineration at 550–600 °C) and water activity (a_w; Rotronic Hygroskop DT, Rotronic AG). The different samples were taken from batches of non-dried and traditionally dried (30 days) flowers, and analyzed the day after the gathering of the flower. Also included were three samples of flower dried traditionally for a period of 1 day (referred to as medially dried flower).

For the determination of MCA we used thistle flower extracts prepared by maceration at 30 °C in a mortar, with 5% NaCl (1 g dry matter/50 mL). After 24 h, the mixture was filtered in a Whatman no 40 filter paper. The filtrate was analyzed for pH, total nitrogen (Kjeldhal) and MCA following the British Standard BS 3624 (BSI, 1963), which is based on work of Berridge (1952). Skim milk powder (Oxoid, L31) reconstituted to 12% with a 0.01mol/L $CaCl_2$ solution was used as the reference milk for MCA determination. Calf rennet standard (CHR. Hansen's Lab, Denmark) with MCA of 1:13250 (f-Soxhlet method), 90 RU/mL (Rennet Units, Berridge method) or 50 CHU/mL (CHR Hansen Units) (Alais, 1985; Sponcet et al, 1985; Prins, 1988) was used as the reference rennet.

The MCA of the extract, expressed as percentage of MCA of reference rennet, was calculated from the clotting time by the expression:

$$\frac{C1}{C2} \times \frac{t1}{t2} \times 100$$

where C1: concentration (mL/mL) of the reference rennet dilution; C2: concentration (mL/mL) of the extract dilution; t1: clotting time (s) for the

reference rennet; t2: clotting time (s) for the extract, considering a reference rennet MCA of 100. The results expressed as *f* or RU were obtained considering the MCA of the reference rennet expressed in those units.

All the determinations were made in triplicate. For result analysis we used descriptive statistics and analysis of variance (ANOVA), using the Scheffe test (Danzart, 1986) for comparisons of sample groups.

RESULTS AND DISCUSSION

From the global results of the short characterization of thistle flower we obtained a great variability of the different parameters, with a wide variation partially due to the drying degree of the flower. The moisture content varied from 6.15% to 47.32%, affecting the results of almost all considered parameters, and MCA varied from 47.64 to 78.71 RU/g flower. Barbosa (1983) noted that the chemical composition of thistle flower varies with the batch and with the year, but in this work the variations found can be explained by the moisture range (6% to 47%), which is due to the flower's different drying degrees. Under these conditions, the global results do not have great significance.

The results of the aggregation of samples according to the drying degree are shown in table I. The results obtained for the dried and medially dried flower were very similar, with moisture, total nitrogen and ash contents close to the results of Barbosa (1983) and Morbey (1990), and with low a_w which indicates a good keeping ability. In fact, traditionally dried flower is usually used during the whole traditional cheesemaking season (from October to May), although clotting activity decreases with flower keeping (Antunes and Santos, 1943).

Table I. Short characterization of thistle flower.
Caractéristiques de la fleur du chardon.

	Dried flower (n = 48)		*Non-dried flower (n = 21)*		*Medially dried flower (n = 3)*	
	Average	*SD*	*Average*	*SD*	*Average*	*SD*
Chemical composition						
Per 100 g flower						
Dry matter (g)	93.29[a]*	0.42	61.37[b]	5.89	89.93a	0.06
Total N (g)	1.958[a]	0.133	1.409[b]	0.196	2.148[a]	0.004
Ash (g)	4.36[a]	0.41	3.44[b]	0.28	4.32[b]	0.03
Per 100 g dry matter						
Total N (g)	2.009[b]	0.141	2.294[a]	0.204	2.392[ab]	0.004
Ash (g)	4.65[b]	0.40	5.60[a]	0.28	4.81[b]	0.03
Physical characteristics						
a_w	0.592[b]	0.047	0.866a	0.058	0.674[b]	0.004
Clotting activity						
RU/g flower	64.30[b]	7.01	62.02[b]	6.65	77.85[a]	0.75
RU/g DM	68.95[b]	7.73	102.03[a]	14.82	86.64[a]	0.83
RU/mgN flower	3.30[b]	0.45	4.47[a]	0.73	3.64[a, b]	0.04

* In the same row, different letters mean significant differences ($P < 0.05$).
* *Sur une même ligne, les valeurs suivies de lettres différentes sont significativement différentes (p < 0,05).*

The non-dried flower showed a moisture content of 30–47% and high a_w values that indicate difficulties in keeping. In fact, after a few days at room temperature or at 4 °C, the growth of molds and the sample degradation were evident. The MCA (RU/g flower) was higher for the medially dried flower, although this group had a significant lower number of samples than the others. Expressing MCA as a function of the dry matter (RU/g DM) and of the flower nitrogen content (RU/mg N flower), we can see that the higher MCA was obtained on the non-dried flower. Those results suggest that the traditional drying procedure can affect the MCA of thistle flower, with losses of 32% to 26% respectively, although the traditional drying procedure is not very drastic; the drying is achieved with low temperature (25–30 °C) with no direct incidence of sun light and during a variable time, frequently 30 to 60 days.

For all groups, MCA was close (1:10 151 to 1:15 022) to that of the liquid commercial clotting enzymes or rennets (1:10 000–1:15 000) and similar to the MCA described by Barbosa (1983) for the '*extracto impuro de cardo*', a vacuum-dried thistle flower extract for cheesemaking.

The flower drying degree influenced the characteristics of thistle flower.

Considering the composition, these results were expected and showed that the 1-day dried flower group was not different from the dried flower group.

The differences for MCA (RU/g DM and RU/mg N flower) suggest that the drying procedure result in an important loss of the flower MCA. Similar results were obtained for other traditionally dried vegetable sources of proteolytic enzymes. Baeza et al (1990) described that the sun drying of papaya (*Carica papaya* L) latex often results in a partial inactivation of the proteolytic activity and that the use of newer methods, with controlled time and drying temperatures, will allow to obtain products with higher activity and less contamination and with more constant biochemical properties.

CONCLUSION

The short characterization of thistle flower shows a great variability in the composition, mainly due to the drying degree of the samples. This was dependent on previous drying of flower after harvesting. Results obtained for the dried flower were similar to those obtained by other authors.

Traditional drying procedures seem to affect the flower MCA. In fact, the MCA (RU/g DM and RU/mg N flower) was significantly lower for dried flower when compared with the non-dried flower. In both groups the results for MCA showed a wide variation (CV of 11% and 16%) and this can mean that uncontrolled utilization of thistle flower may be an unfavorable factor for a stable cheesemaking technology as the extracts used are not standardized for milk clotting activity. Besides that, the high, non-specific proteolytic activity can also affect the technological steps and yield, as well as proteolysis rate during ripening (Vieira de Sá and Barbosa, 1972).

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