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Influence of the ripening stage and the lyophilization of wild cardoon flowers on their chemical composition, enzymatic activities of extracts and technological properties of cheese curds

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Introduction

Milk-clotting proteases are the primary active agents in cheese manufacture. The use of plant proteases in cheese manufacturing promotes the great acceptability by the vegetarian population and may improve their nutritional intake. *Cynara cardunculus var. sylvestris*, which is commonly named 'wild cardoon', contains aspartic proteases (cardosins) mostly used in milk coagulation and cheese making.

The variability of these enzymes in cardoon flowers and their activity differences, due to the natural geographic localization, the flowering stage, and the seasonal climatic conditions, leads to variations in the sensory characteristics and yields of dairy products

Objectives

The aim of the present work was to select the appropriate *C. cardunculus* flowers batch, which produces the best clotting properties of enzymes, and presents the excellent applicability of its extract in cheese making process.

For this reason, chemical composition of wild cardoon flowers, collected in Tunisia at two ripening stages (A and B), was firstly studied. This could be relevant as a first step, to have a clear idea about the raw material quality, mainly the protein content. Then, enzymatic activities and protein profiles of the produced coagulant extracts, as well as technological properties of cheese curds (texture, yield and moisture), were compared. In addition to the ripening stage, the effect of flowers lyophilization on all these parameters was also evaluated, only in the case of flowers (A), due to their high moisture level.

Methodology

Cynara cardunculus L. flowers (petals and pistils)

2 ripening stages

Stage A — Flowers collected at the **end of June** — **Fresh and lyophilized flowers** → Extract I, Extract II

Stage B — Flowers collected at the **end of August** — **Fresh flowers (naturally dried)** → Extract III

1- Chemical composition of flowers

2- Enzymatic activities of extracts

Milk coagulation and curds production

3- Technological properties of curds (texture, moisture, yield)

Selection of the appropriate flowers batch

Results

Chemical composition of flowers

Table 1. Chemical composition of wild cardoon flowers (*C. cardunculus)* (% w/w of dry matter) collected in Tunisia, at two ripening stages.

	Flowers A	Flowers B
Dry matter (%)	91.83 ± 0.12 [a 2]	90.92 ± 0.26 [b]
Proteins	11.28 ± 0.02 [a]	9.13 ± 0.00
Lipids	18.56 ± 0.07 [a]	25.48 ± 1.65 [b]
Total sugars	16.80 ± 0.27 [a]	11.70 ± 0.68 [b]
Monosaccharides composition		
Rhamnose	1.19 ± 0.02 [a]	1.10 ± 0.16 [a]
Arabinose	6.03 ± 0.13 [a]	4.23 ± 0.23 [b]
Xylose	2.61 ± 0.07 [a]	2.12 ± 0.10 [b]
Mannose	0.44 ± 0.05 [a]	0.52 ± 0.01 [a]
Glucose	4.69 ± 0.05 [a]	2.47 ± 0.08 [b]
Galactose	1.83 ± 0.06 [a]	1.27 ± 0.10 [b]
Total dietary fibres	49.69 ± 0.91 [a]	60.12 ± 0.05 [b]
Insoluble dietary fibres	34.26 ± 1.97 [a]	45.63 ± 3.08 [b]
Hemicellulose	16.30 ± 1.74 [a]	17.16 ± 1.51 [a]
Cellulose	10.33 ± 0.25 [a]	17.73 ± 3.99 [a]
Lignin (ADL)	7.60 ± 0.10 [a]	10.73 ± 0.66 [b]
Ash	7.02 ± 0.02 [a]	7.96 ± 0.05 [b]
Mineral content (g/100g DM)		
Mg	0.15 ± 0.01 [a]	0.17 ± 0.00 [b]
Ca	0.58 ± 0.15 [a]	1.01 ± 0.07 [b]
K	1.71 ± 0.01 [a]	1.60 ± 0.03 [b]
Na	0.03 ± 0.01 [a]	0.02 ± 0.00 [a]

Protein profiles of coagulant extracts

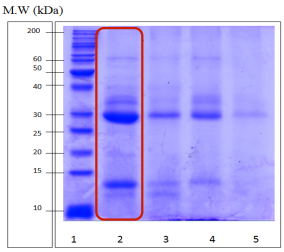
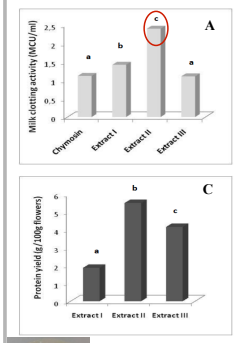


Figure 1. SDS PAGE electrophoretogram of crude extracts proteins from Tunisian cardoon flowers. Molecular weight markers **(lane1)**; Crude extract proteins of flowers (A) **(lane 2)**; Crude extract proteins of defatted flowers (A) **(lane 3)**; Crude extract proteins of flowers (B) **(lane 4)**; Crude extract proteins of defatted flowers (B) **(lane 5)**.

❖ **Flowers (A)** : Higher Protein content than that in **flowers (B)** with a rate of 23.5 %.
❖ **Extract II from lyophilized flowers (A)** : The highest number and protein bands intensity in gel electrophoresis

Enzymatic activities of coagulant extracts

Figure 2 (A, B, C & D). Influence of the ripening stage and lyophilization of wild cardoon flowers on milk-clotting activity **(A)**, proteolytic activity **(B)**, protein yield **(C)** and MCA/PA ratio **(D)** of crude extracts, as compared to calf rennet.



❖ **Lyophilized flowers A**
Extract II

❖ The highest protein extraction yield

❖ More extracted enzymes

❖ The highest Milk-clotting activity and MCA/PA ratio +++

Technological properties of curds

Rennet	Hardness (N)	Springiness	Gumminess (N)	Cohesiveness	Curd yield (%)	Moisture (%)
Chymosin	14.60 ± 0.65 [a]	0.86 ± 0.02 [a]	10.58 ± 0.21 [a]	0.72 ± 0.02 [a]	18.61 ± 0.53 [a]	68.21 ± 1.34 [a]
Extract (I)	4.87 ± 0.49 [b]	0.91 ± 0.01 [b]	3.30 ± 0.33 [b]	0.67 ± 0.01 [b]	17.34 ± 0.26 [b]	67.75 ± 0.58 [a]
Extract (II)	11.85 ± 1.03 [c]	1.12 ± 0.38 [a, b]	8.48 ± 0.87 [c]	0.71 ± 0.01 [a]	15.29 ± 0.05 [c]	62.80 ± 1.41 [b]
Extract (III)	11.91 ± 0.58 [c]	0.89 ± 0.03 [a, b]	8.63 ± 0.44 [c]	0.72 ± 0.02 [a]	13.63 ± 0.05 [d]	63.00 ± 0.56 [b]

❖ **Fresh flowers A (Extract I)** : Curd : crumbly texture – elevated moisture
↓ Lyophilization
❖ **Lyophilized flowers A (Extract II)** ⟶ **Better clotting properties**
↓
Curd texture improvement

Conclusion

Coagulant extract from fresh flowers (A), did not appear to be a good substitute for animal rennet, due to its low MCA, leading to poor curd texture. However, lyophilization of flowers (A) seems to be an efficient way to produce rennet with better clotting properties, leading to higher yield, moisture, and texture of fresh curd. These findings promoted the selection of lyophilized flowers (A), for subsequent coagulant extraction and *C. cardunculus* cheese production, in Tunisia.

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