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Technological approach to donkey milk cheesemaking

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BACKGROUND

Food products contribute to the income of equid farms

Milk still represents a «minor» product

Due to low allergenicity and functional properties it is attracting great interest

Pasteurized and fermented milk have been widely investigated and are available on the market

It has long been considered that is not possible to make cheese from mare or donkey milk

RECENT DEVELOPMENTS

- Calf chymosin was able to hydrolize equine K-cn cause forming a very weak gel. However, a curd did not form (Uniacke-Low and Fox, 2011)
- Fortification of equine milk with bovine k casein and Ca^{++} allowed rennet coagulation but the coagulum remained weak (Chang et al., 2006)
- Donkey milk has very small amounts of K-cn (Chianese et al., 2010) but probably it does not play the same role than in ruminant milk during coagulation
- Recently successful cheesemaking trials have been reported for donkey milk:

- a) a fresh cheese was obtained by using camel chymosin (Iannella et al, 2015)
- b) a semi-hard cheese was obtained by fortification with goat milk (Saric et al 2016)
- c) a fresh donkey cheese was prepared by using calf rennet under «extreme» cheesemaking conditions (Faccia et al, 2018)

FRESH DONKEY CHEESES

FRESH DONKEY CHEESE BY CAMEL CHYMOSIN (Iannella, 2015)

Raw milk

Processing time > 5 h

nr

Heating to 43°C

Addition of starter

Addition of camel chymosin

Hold for 5 h at 37°C

nr

nr

nr

nr

Removal of the whey

nr

nr

Cheese moulding

FRESH DONKEY CHEESE BY CALF RENNET (Faccia et al., 2018)

Processing time 3 h

Raw milk

Addition of 0.03 % $CaCl_2$

Heating to 40°C

Addition of starter (acidification to pH 6.50)

Addition of calf rennet

Hold for 40 min at 40°C

First cutting (5x5 cm)

Curd settling for 10 min

Second cutting (2x2 cm)

Scalding to 46°C

Removal of 70% whey

Curd settling for 10 min

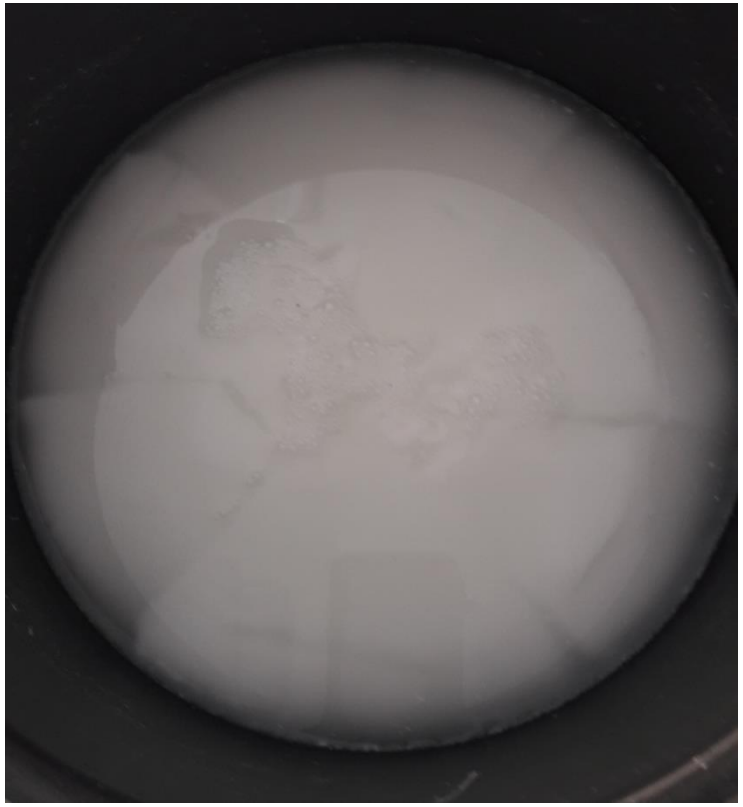
Removal of the remaining whey

Cheese moulding

Comparison between calf rennet and camel chymosin cheesemaking

	Calf rennet		Camel chymosin	
	Milk	Cheese	Milk	Cheese
pH	6.92 ± 0.01	6.12±0.06	7.06±0.06	5.34±0.08
Total solids %	nr	32.4± 2.35	nr	35.65 ± 4.35
Total protein %	1.73 ± 0.00	18.5±1.1	1.61±0.12	nr
Fat %	0·21±0·04	2.1±0.7	1.11±0.34	nr
Lactose %	6.72 ± 0.04	nr	6.08±0.28	nr
Cheese yield %	-	5.9±0.12	-	3.32±0.31

DONKEY CHEESE BY CALF RENNET



First cutting

Moulding

Cheese after 24 h

SENSORY CHARACTERISTICS (trained panel)

Attribute	Intensity (0-4)	Reference standard
TEXTURE		
Soluble	1	Meringue
Friable	3	Craker
ODOUR		
Egg yolk	2	Raw egg yolk
Animal	2	Raw donkey milk
Cooked milk	2	Boiled milk
TASTE		
Salty	2	NaCl solution 0.5%
Sweet	3	Saccarose solution 0.5 %

MOST RELEVANT ISSUES AND POSSIBLE SOLUTIONS

Soft texture, short shelf life → Improving texture for possible short ripening

Low yield → Improving milk quality
Improving retention of the whey proteins in the curd

Long processing time → Accelerating the secondary phase of coagulation

AIMS OF THE RESEARCH

A) Improving texture and yield
B) Reducing the processing time

by

- USE OF TRANSGLUTAMINASE (TGase)
- APPLYING A POST COAGULATION HEAT-TREATMENT (PGHT)

2 SEPARATED EXPERIMENTS (monofactorial design)

FIRST EXPERIMENT: TESTING TGase

TGase has the ability of strengthening the structure of protein gels by formation of covalent cross-links between glutamine and lysine residues of different protein molecules (Ozrenk, 2006). In the case of milk , it induces cross-linking of caseins and whey proteins (Jaros et al. 2010). Currently used to increase viscosity of yoghurt.

MATERIALS AND METHODS

Experimentation (4 replicates)

- Raw milk from Martina Franca jennies, milked twice a day (mechanical milker)
- Microbial TGase (Ajinomoto Foods Europe SAS)
- Cheesemaking trials (4 consecutive days) in 2 L pots under controlled temperature
- Cheese weighed after 24 h

Chemical analyses (in triplicate)

- Milk: pH, gross composition (MilkoScan), ash content (incineration at 530 °C)
- Cheese: viscosity from rennet addition to curd cutting (vibro viscometer), pH, moisture (oven-drying), fat (Soxhlet), total protein (Kjeldhal), ash content (incineration at 530 °C)

Electrophoresis: SDS-PAGE

EXPERIMENTAL DESIGN

CONTROL	TGase
Heating of milk to 40 °C	Heating of milk to 40 °C
↓	
Addition of: $CaCl_2$ + Starter	Addition of: $CaCl_2$ + Starter
↓	
Acidification to pH 6.3	Acidification to pH 6.3
↓	
Addition of Rennet	Addition of: **TGase** + Rennet
↓	
Coagulation	Coagulation
↓	
Curd cutting Curd setting for 10 min	Curd cutting Curd setting for 10 min
↓	
Scalding to 46 °C	Scalding to 46 °C
↓	
Whey removal Cheese moulding	Whey removal Cheese moulding

MILK COMPOSITION

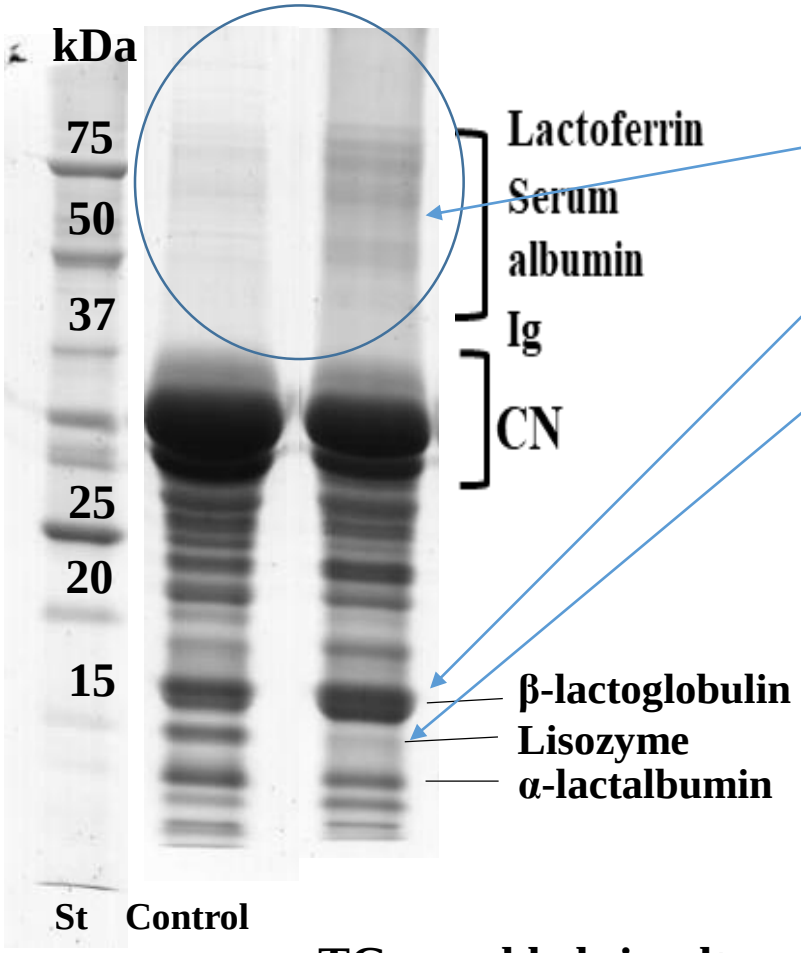
pH	7.02 ± 0.03
Protein %	2.29 ± 0.08
Fat %	0.25 ± 0.03
Lactose %	6.76 ± 0.07
Ash %	0.39 ± 0.04

RESULTS

	CONTROL	TGase
Milk acidification to pH 6.3, min	328.75 ± 16.63	318.75 ± 5.91
Viscosity*, mPA	25.9 ± 5.9^{B}	58.0 ± 2.7^{A}
Coagulation time, min	10.25 ± 3.42^{AB}	7.50 ± 1.44^{B}
Total time for moulding min	42.50 ± 6.61^{AB}	33.75 ± 5.54^{B}
pH after moulding	5.82 ± 0.06	5.77 ± 0.08
Cheese yield, %	7.39 ± 0.65	6.91 ± 0.42
Moisture, %	65.75 ± 2.73	63.71 ± 3.23
Ash, %	3.31 ± 0.31	3.20 ± 0.44
Protein, %	26.09 ± 2.97	28.19 ± 1.52
Fat, %	2,8 ± 0.06	2,6 ± 0.38

* At curd cutting

SDS PAGE ELECTROPHORESIS



New high molecular weight bands (not very intense)

β-lactoglobulin better recovered

Lisozyme poorly present (included in the new HMW aggregates ?)

Limited crosslinking action

Explanation: a) scarce presence of k-casein and high presence of whey proteins in donkey milk: the former is highly involved in crosslinking in milk, the latter are less (Cony et al., 2008).

b) low level of heat applied during cheesemaking.

TGase added simultaneously with rennet improved curd firmness and shortened the coagulation time. The protocol needs to be improved in order to increase the effect of the enzyme (higher temperature and dose ?)

SECOND EXPERIMENT: TESTING PGHT (preliminary results)

Heating the milk causes denaturation of the whey proteins and their binding to caseins. Heating immediately after enzymic coagulation has began should help to better retain the whey proteins in the casein network.

MATERIALS AND METHODS

Experimentation (2 replicates)

- Raw milk from Martina Franca jennies, milked twice a day (mechanical milker)
- Cheesemaking trials (2 on the same day) in 0,500 L beker under controlled temperature
- Moulding without cutting (very soft coagulum)
- Cheese weighed after 24 h

Chemical analyses (in triplicate)

- Milk: pH, gross composition (MilkoScan), ash content (incineration at 530 °C)
- Cheese: pH, moisture (oven-drying)

Electrophoresis: SDS-PAGE

EXPERIMENTAL DESIGN

CONTROL	PCHT
Heating of milk to 40 °C	Heating of milk to 40 °C
Addition of: $CaCl_2$ + Starter	Addition of: $CaCl_2$ + Starter
Acidification to pH 6.3	Acidification to pH 6.3
Addition of Rennet	Addition of Rennet
Coagulation	Coagulation
Curd cutting Curd setting for 10 min	Heating in water bath to 80°C Curd setting for 10 min
Scalding to 46 °C	Whey removal
Whey removal Cheese moulding	Cheese moulding

MILK COMPOSITION

pH	7.04 ± 0.01
Protein %	1.89 ± 0.02
Fat %	0.22 ± 0.03
Lactose %	6.66 ± 0.02
Ash %	0.31 ± 0.02

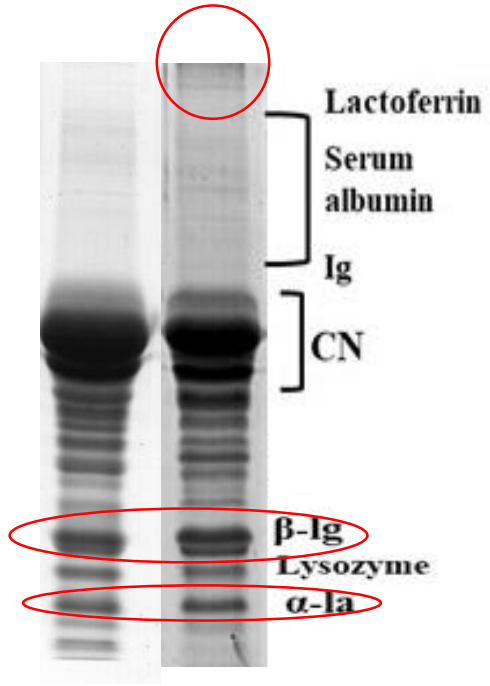
RESULTS

	Control	PCHT
pH	5.88 ± 0.07^{B}	6.22 ± 0.03^{A}
Moisture %	63.14 ± 0.33^{B}	73.84 ± 0.13^{A}
Yield %	5.31 ± 0.19^{B}	7.89 ± 0.24^{A}

PCHT causes immediately the precipitation of the coagulum. It was softer curd than the control (texture was not improved)

Yield was highly improved (better retention of whey proteins + higher moisture)

The experimentation is in progress



Control PGHT

SDS PAGE Electrophoresis

OVERALL CONCLUSIONS

Making cheese from donkey milk is technically possible

Milk coagulation can be done by several enzymic coagulants (the problem is not the primary phase of coagulation)

The sensory characteristics of donkey cheese are unique

Difficult curd formation is probably due to poor concentration of casein: any technologies that are able to concentrate the milk should be have a positive effect on yield and quality

The 2 variables tested (TGA and PGHT) should be combined


THANK YOU FOR ATTENTION