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in

Casasús I. (ed.), Lombardi G. (ed.).
Mountain pastures and livestock farming facing uncertainty: environmental, technical and socio-economic challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 116

2016
pages 137-141

Are the specific sensory properties of pasture cheeses linked to milk fat composition and bacterial dynamics?

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Abstract. The aim of this research was to determine the specific influence of milk fat composition on the sensory properties of cheeses derived from cows fed either pasture or a maize silage-based diet. Uncooked pressed Cantal cheeses were manufactured from skimmed milk with an identical chemical and microbial composition and two different pasteurized creams obtained from cows fed either pasture or maize silage. Cheeses produced from the pasture-derived cream were creamier, more elastic, more adhesive, and less firm than cheeses made from the maize silage-derived cream. The rind of pasture-derived cream cheeses was thinner and the relative abundance of *Staphylococcus, Brachybacterium* and *Yaniella* were higher than on the rind of the cheeses made with the cream derived from maize silage where *Lactococcus* was relatively abundant. These results confirm the direct role of fat composition, richer in unsaturated FA with low melting point when cows grazed pasture, on the cheese texture and suggest that the milk fat composition could change the microbiota of cheese rind, which in return can explain differences in cheese rind appearance. The flavour and the microbiota of the core of the cheeses made with the different creams were very similar, indicating that the milk fat composition plays a minor role on the flavour development of cheeses. Other diet-dependent aspects of milk biochemical or microbial composition may explain the stronger and more diverse flavour of cheeses that is frequently reported when cows are fed pasture.

Keywords. Pasture – Cheese fat composition – Cheese sensory quality – Cheese bacterial community.

Les propriétés sensorielles particulières des fromages fabriqués à partir de lait de pâturage sont-elles dues à la composition de la matière grasse laitière et la dynamique bactérienne?

Résumé. *Les objectifs de ce travail étaient de comprendre l'influence de la composition de la matière grasse laitière sur les propriétés sensorielles de fromages. Des fromages à pâte pressée non cuite de type Cantal ont été fabriqués avec des laits écrémés qui avaient une composition chimique et microbiologique identique mais qui étaient additionnés de crèmes pasteurisées produites soit par des vaches au pâturage, soit nourries avec de l'ensilage de maïs. Les fromages fabriqués avec la crème des vaches au pâturage avaient une texture plus crémeuse, plus élastique, plus adhésive et moins ferme que les fromages fabriqués avec la crème des vaches nourries avec l'ensilage de maïs. La croûte des fromages fabriqués avec une crème issue du pâturage était plus fine et* Staphylococcus, Brachybacterium *et* Yaniella *étaient relativement plus abondant que* Lactococcus. *Ces résultats confirment l'effet direct de la composition de la matière grasse du lait, plus riche en acides gras insaturés avec un point de fusion bas lorsque les vaches sont au pâturage, sur la texture des fromages. Ils suggèrent que la composition de la matière grasse est susceptible de modifier le microbiote de la croûte du fromage et serait à l'origine de fromages d'aspect différent. La flaveur et le microbiote des fromages fabriqués avec les deux crèmes étaient très voisins, montrant que la composition de la matière grasse joue un rôle mineur dans le développement de la flaveur des fromages. D'autres aspects de la composition du lait expliquerait la flaveur plus diversifiée et plus forte qui est souvent attribuée aux fromages issus du pâturage.*

Mots-clés. *Alimentation des animaux – Composition de la matière grasse – Qualité sensorielle – Communauté bactérienne.*

I – Introduction

Several studies have been conducted over the last years to understand the effect of animal diet on cheese sensory properties. The effects of forage type (e.g. pasture, hay, grass silage, maize silage) are well-known (Martin *et al*., 2005). Pasture-derived cheeses have a thinner rind, their colour is yellower, their texture is smoother and their flavour is more diverse and intense compared to maize silage-derived cheeses (Coppa *et al*., 2011; Coulon *et al*., 2004). These colour and texture features were attributed in particular to milk fat composition that is affected by animal diet. When cows grazed fresh grass, milk fat is richer in plant pigments (carotenoids) and in unsaturated fatty acids (FA) with a low melting point (Martin *et al*., 2005). It was suggested that the decrease in fat cohesion of these cheeses could cause a loss of butter-oil on cheese rind, creating a favourable environment for the development of a mould *Sporendonema casei* that gives its typical appearance to Cantal cheeses rind (Coppa *et al*., 2011; Lerch *et al*., 2015). The stronger and more diversified flavour of cheeses from pasture is still far from being understood. Current hypothesis is linked (i) to a possible higher oxidation of polyunsaturated FA, (ii) cheese microbiota which is also known to vary according to animal diet (Verdier-Metz *et al*., 2012); and (iii) to possible interactions between milk fat and microbiota. The aim of this study was to determine the specific influence of milk fat composition on sensory properties of Cantal cheeses, with a focus on the development of cheese microbiota.

II – Materials and methods

Uncooked pressed Cantal cheeses were manufactured from skimmed milk with an identical chemical and microbial composition and two different pasteurized creams obtained from two groups of cows fed either pasture **(P)** or a maize silage-based diet **(M)**. The P cows (14 Montbéliarde and 14 Holstein cows) grazed on a mountain grassland in September and were supplemented with 2 kg of concentrate and the M cows (6 Montbéliarde and 6 Holstein cows) were kept indoors and fed a diet composed daily of 15 kg DM of maize silage, 2.5 kg DM of straw and 4 kg of concentrate. Twelve ten-kilogram Cantal cheeses were made during 6 days, using either raw (3 dates of cheesemaking) or pasteurized (3 dates of cheesemaking) P-derived skimmed milk, each added with either P- or M-derived pasteurized cream. The FA composition was analysed by gas-chromatography in milks and in five month-ripened cheeses (rind and core). The total bacterial DNA was extracted from the same samples, and then the variable region V3-V4 of the 16S rRNA gene was amplified prior to meta-barcoding sequencing on an Illumina MiSeq instrument. The sequence data were analysed using FROGS pipeline on Galaxy interface (Escudie *et al*., 2015). The cheese sensory properties were described by a panel of 10 assessors who gave an intensity score between 0 and 10 for 35 attributes (7 for appearance, 5 for texture, 9 for odour, 9 for aroma, 5 for taste). Data concerning FA composition were processed by Student's t-Test. Sensory data were analysed using a mixed model where the cow diet was the fixed factor, and the assessor was the random factor. A principal component analysis (PCA) was performed on the most abundant bacterial "species" (>0.05% of the total sequences) of cheese rind. The higher Shannon's diversity index, the higher bacterial diversity is important.

III – Results and discussion

Cheeses produced from P cream were creamier, more elastic, more adhesive, and less firm than cheeses made from M cream both added to raw or pasteurised skimmed milk (Table 1). The P cheese cores were richer in unsaturated FA (*cis*-MUFA, *trans*-MUFA, and PUFA), conjugated linoleic acids (CLA), C18:0, and cis9-C18:1 and poorer in short- and medium-chain saturated FA (C6:0+C8:0+C10:0 and C12:0+C14:0) and C16:0 (Table 2). These results confirm the direct role of P cream, richer in unsaturated FA with low melting point, on cheese texture (Martin *et al*., 2005).

The rind of P cheeses was thinner (lower spot salience and quantity) than that of M cheeses (Table 1) and the differences between P and M cheeses were more important in raw than in pasteurised milk. Similar results were obtained by Coppa *et al*. (2011) in a study comparing cheeses made with milk from grass- or hay-based diet. Metagenetic profiling showed that the bacterial balance of rind of raw milk cheeses was also different between cheeses produced from P or M cream (Fig. 1). From 3-month of ripening, the relative abundance of *Lactococcus* (Firmicutes) was higher on the M cheese rind while that of *Staphylococcus* (Firmicutes) and *Brachybacterium* and *Yaniella* (Actinobacteria) was higher on the P cheese rind. The Shannon's diversity index including all the "species" was higher on P cheese rind (1.319) than on M cheese rind (1.167).These results suggest that the milk fat composition could change the bacterial balance of cheese rind ecosystem, which in return may explain differences in cheese rind appearance.

Table 1. Sensory properties of Cantal cheese made with a same skimmed milk (raw or pasteurized) and pasteurized cream issued from cows fed pasture or a maize silage based diet

Parameters	Raw				Pasteurized			
	Pasture	Maize	SEM	p value	Pasture	Maize	SEM	p value
Texture (score 0-10)								
firm	5.25	6.59	0.215	**<0.001**	5.61	6.79	0.193	**<0.001**
elastic	3.98	3.04	0.232	**0.002**	4.09	3.12	0.245	**<0.001**
creamy	4.96	3.84	0.256	**0.021**	4.95	3.30	0.259	**<0.001**
adhesive	4.20	3.41	0.251	*0.055*	4.02	2.83	0.250	**0.009**
Appearance of cheese (score 0-10)								
spot salience	6.24	7.26	0.191	**0.002**	3.92	4.25	0.262	0.502
spot quantity	7.12	7.94	0.158	**0.006**	4.59	5.11	0.271	0.341

Table 2. Fatty acid composition of the core and the rind of cheese made with a same raw skimmed milk and pasteurized cream issued from cows fed pasture or a maize silage based diet

Fatty acids (g/100gFA)	Core			Rind		
	Pasture	Maize	p value	Pasture	Maize	p value
C6:0+C8:0+C10:0	4.93 ± 0.06	5.67 ± 0.17	**0.002**	3.75 ± 0.15	4.57 ± 0.55	*0.067*
C12:0+C14:0	11.6 ± 0.19	13.9 ± 0.30	**<0.001**	10.0 ± 90.27	13.3 ± 0.16	**<0.001**
C16:0	24.2 ± 0.48	34.0 ± 0.98	**<0.001**	26.8 ± 0.60	37.0 ± 2.69	**0.003**
cis9-C18:1	22.5 ± 0.44	19.1 ± 1.03	**0.006**	21.0 ± 0.49	17.9 ± 1.68	**0.037**
Σ CLA	2.25 ± 0.11	0.69 ± 0.06	**<0.001**	1.86 ± 0.08	0.60 ± 0.06	**<0.001**
Σ SFA	60.2 ± 0.24	69.1 ± 1.06	**<0.001**	62.1 ± 0.30	70.6 ± 1.95	**0.002**
Σ *cis*-MUFA	27.1 ± 0.35	24.6 ± 1.06	**0.018**	24.9 ± 0.51	22.5 ± 2.08	0.127
Σ *trans*-MUFA	5.30 ± 0.15	2.08 ± 0.07	**<0.001**	5.72 ± 0.23	2.20 ± 0.08	**<0.001**
Σ PUFA	5.86 ± 0.04	3.31 ± 0.05	**<0.001**	5.91 ± 0.04	3.78 ± 0.15	**<0.001**
cis9-C18:1/C16:0	0.93 ± 0.04	0.56 ± 0.05	**<0.001**	0.78 ± 0.04	0.49 ± 0.08	**0.004**

Mean values (± standard deviation: n = 3); FA: fatty acids; CLA: conjugated linoleic acids; SFA: saturated FA, PUFA: poly-unsaturated FA; LCPUFA: long-chain PUFA; MUFA: mono-unsaturated FA.

The flavour of P and M cheeses was very similar (results not shown) both in raw and pasteurised skimmed milk cheeses while previous results (Martin *et al*., 2005) reported that pasture-derived cheeses have a more intense and diversified flavour by comparison to maize silage cheeses. This discrepancy may link to the fact that in the present study, only the cream composition changed, whereas skimmed milk biochemical and microbial composition remained the same between the

two types of cheeses. In addition, during ripening, the composition of the dominant bacterial taxa in cheese core was the same whatever the added cream (results not shown). These results suggest that the milk fat composition plays a minor role on flavour. Other diet-dependent factors linked to milk biochemical or microbial composition may be involved in the specific flavour development of cheeses made with milk from cows fed pasture.

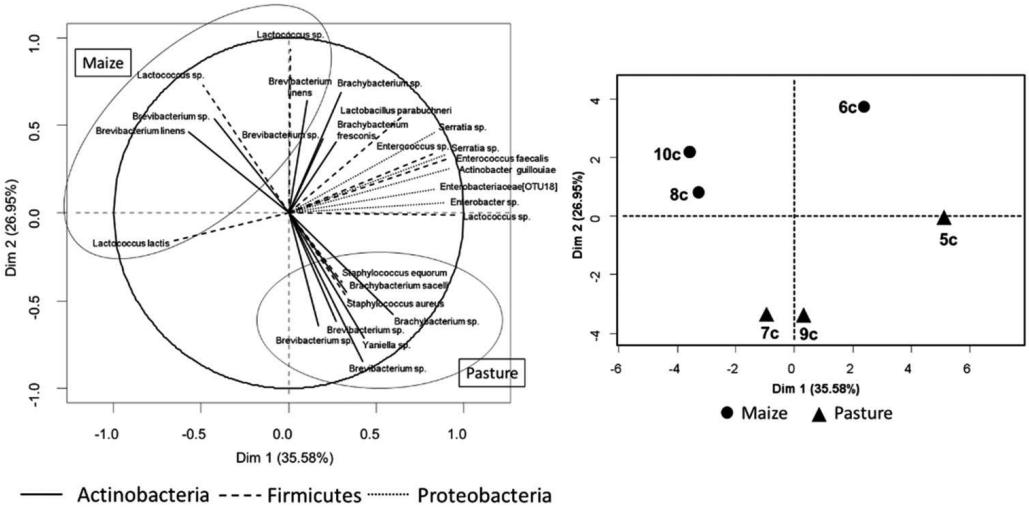

Fig. 1. Principal component analysis (PCA) performed on the most abundant bacterial "species" of cheese rind. Plot of rind samples of milk raw cheeses and variable distribution according to milk fat composition.

IV – Conclusions

The results of this experiment allow to go a step further in the understanding of the role of cow diet in the development of the sensory properties of cheeses. The direct role of the milk fat composition derived from pasture on the less firm cheese texture is confirmed and an interaction between the composition of milk fat and the development of surface microbiota on raw milk cheese is shown for the first time. The flavour is not different between the two types of cheeses in our study, in contrast with other data that frequently reported differences between cheeses made from pasture and maize silage, indicating that milk fat composition is not always implicated in the cheese flavour. Other diet-dependent factors linked to milk biochemical or microbial composition may be involved in flavour differences.

Acknowledgments

We thank the Région Auvergne (France) for its contribution to the funding of this experiment.

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