TYPICALP

Biochemical characterization of sheep milk YoAlp® and comparison with sheep fermented milk obtained with commercial starter cultures

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INTRODUCTION

Fermented milks are a source of bioactive peptides with different potential benefits on human health (mainly antioxidant, antihypertensive and hypoglycemic effects) and may be considered as functional foods. Furthermore, fermented milk made with sheep milk has higher nutritional value and higher concentrations of proteins, fats, minerals, and vitamins, if compared to milk of other species [1-3].
Aim of this study was to perform a biochemical characterization of sheep milk YoAlp®, a fermented milk obtained using local strains of lactic acid bacteria (LABs) (*Streptococcus thermophilus* and *Lactobacillus delbrueckii*) which have been isolated during years in Aosta Valley mountain dairy cattle farm. Moreover, this product has been compared with a sheep fermented milk (SFM) made with commercial starter cultures.

RESULTS

Chemical analysis

PEPTIDES

Peptides analysis have been carried out according to Sforza et al. and Rizzello et al. [4-5]. Comparing with SFM, YoAlp® has shown a higher number of peptides. Among these peptides, 20,78% are supposed to be potentially bioactive (DPP - dipeptidyl peptidase-IV - inhibitor, ACE - angiotensin converting enzyme – inhibitor and antioxidant) (Tab.1). Despite lower number of peptides, SFM seems to have more aminoacidic sequences with potential bioactive activity (29,87%). **Only 12,96% of peptides have been found in both samples** (Fig. 1).

Fig. 1 Number of peptides, probability to be bioactive and main possible effects in SFM and YoAlp®

$[M+H]^{+/3+*}$	m/z	Sequence	Position	Protein	Effect	Biological activity prediction	Samples
220		MA	24-25	β-LG	DPP inhib.	0,69	A, C
246		PM	124-125	β-LG	DPP inhib.	0,95	A, C
279		FN	151-152	β-LG	DPP inhib.	0,95	A, C
300*		VRGPFPIL	206-221	β-CN	ACE inhib.	0,83	C
328		FY	145-146	αS1-CN	unknown	0,98	C
375		PYP	57-59	k-CN	unknown	0,85	C
748		EMPFPK	108-113	β-CN	ACE inhib. - antimicrobial	0,77	C
757		DKIHPF	47-52	β-CN	ACE inhib.	0,69	C
815		LPYPYY	56-61	k-CN	unknown	0,8	A
907		LPQNILPL	70-77	β-CN	antimicrobial	0,69	A

Tab. 1 Most important pepdtides found in SFM (C) and YoAlp® (A) and their potential bioactivity

BIOLOGICAL ACTIVITY

As far as bioactivity is concerned, as expected by peptide profile analysis (Fig. 1), **ACE inhibitor activity is high for both samples** (97,00% and 97,95% wich correspond to 0,093 Captopril EQ µM and 0,096 Captopril EQ µM of ACE 0,1 U/mL) (Fig. 2).
Even in the case of antioxidant capacity, peptide profile bioactivity prediction has been confirmed by the assay, showing a **DPPH inhibition higher for SFM** (44,92% - 5,70 CEAC µg/mL of DPPH 0,2 mM) than for YoAlp® (35,02% - 4,43 CEAC µg/mL of DPPH 0,2 mM) [6] (Fig. 2).
Finally, in contrast with peptide profile bioactivity assumption, **DPP-IV inhibition seems to be higher in YoAlp®** than SFM (89,86% and 68,49% of inhibition) (Fig. 2). This result can be propabily due to some peptides which are present in YoAlp® and not yet identified. Indeed, a lower percentage of peptides has been identified in YoAlp® (38,46%) if compared to SFM (71,43%) as a consequence of a different enzimatic activity, on milk proteins during fermentation, of the two starter cultures considered in this study.

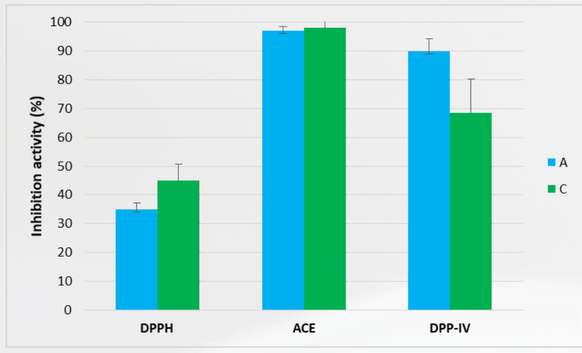
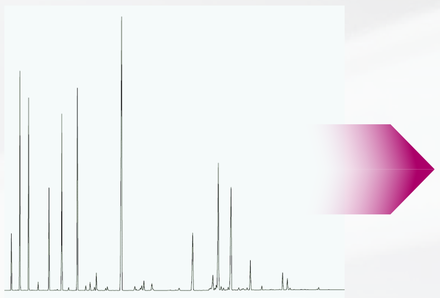


Fig. 2 DPPH, ACE and DPP-IV inhibition in SFM (C) and YoAlp® (A)
No statistical significant differences have been highlighted (p>0.05)

FATTY ACIDS PROFILE

Analysis of fatty acid composition carried out in GC/MS and their quantification by GC/FID [7] put in evidence that YoAlp® is an excellent source of **essential fatty acids** and it has a good **ω6/ω3 ratio**, similar to SFM. In addition, high levels of **CLA 9c, 11t** (up to 90% of total CLA), known to possess anticancer activities, have been found in both fermented milks (Fig. 3).



	%	
	YoAlp®	SFM
Σ satured	73,74	73,71
Σ unsaturated	26,26	26,29
Σ monounsaturated	20,80	20,83
Σ polyunsaturated	5,47	5,46
Σ CLA	1,13	1,13
Σ ω3	1,61	1,59
Σ ω6	2,63	2,63
Σ ω6/ω3	1,63	1,66

Fig. 3: GC/FID chromatogram and medium content (g/100 g FAT) for the identified fatty acid classes of YoAlp® and SFM . Mean values calculated on 2 samples, extracted in duplicate and analysed in triples.

AROMATIC PROFILE

The aromatic profile has been obtained through an SPME-GC/MS analysis, according to Jiang et al. [8]. YoAlp® has been shown to have a good content in volatile components, mainly ketones and acids, higher than SFM (Fig. 4). Considering the most important aromatic compounds that characterize the typical aroma of fermented milks, it has been shown that the autochthonous starters have a better aptitude than commercial ones in **diacetyl**, **acetoin**, **acetone** and **2-butanone** production (Fig. 5).

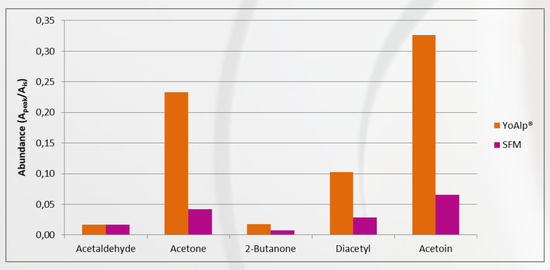


Fig. 4: Relative average abundance (Apeak/Ais) of identified aromatic compounds classes in YoAlp® and SFM. Analyses have been performed in triplicate.

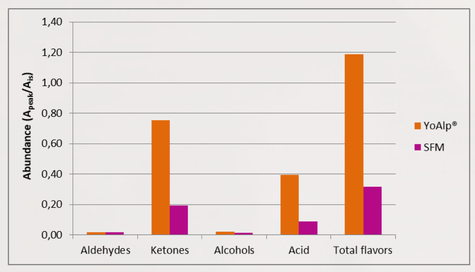


Fig. 5: Relative average abundance (Apeak/Ais) of aromatic compounds considered to have an impact on yogurt aroma in YoAlp® and SFM. Analyses have been performed in triplicate.

Product acceptance

Results obtained by the preliminary product acceptance test show a general high level of pleasantness. SFM and YoAlp® reached an evaluation higher than 6 (good), respectively 6,65±0,81 and 6,29±0,92, and no signifcant statistical diferences have been found (p=0.204) between these two fermented milks. In both cases, they have an adequate frmness and smooth surface without evidence of an excess of syneresis, and the typical fermented milk aroma has been observed as highlighted in VOCs profling. These results highlight that the use of local strains does not afect the overall consumer evaluation. Therefore, local strains have not negative impact on the fnal product acceptance and they are important to maintain biodiversity.

CONCLUSIONS

Local strains of lactic acid bacteria from Aosta Valley seems to work as well as commercial starters. Moreover, authocthonous starters contribute to preserve biodiversity and typicality. Further analyses are needed to identify more peptides generated by autochthonous LABs in order to understand microbial proteolytic activities, to confirm identified peptides biological effects and to investigate gastric digestion resistance of those which can have positive effects on human health.

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SCAN ME

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