

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 and a comparison between sheep fermented milk (SFM) made with commercial starter cultures and 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.

PEPTIDES

Peptides analysis have been carried out according to [4-5]. The comparison between SFM and YoAlp® peptide profile shows a higher number of peptides using autochthonous starter cultures (52) than commercial starter cultures (35) of which the 38,46% and the 71,43% have been identified respectively. Among these peptides 20,78% for YoAlp® and 29,87% for SFM are supposed to be potentially bioactive (DPP - dipeptidyl peptidase-IV - inhibitor, ACE - angiotensin converting enzyme – inhibitor and antioxidant) (Tab.1). **Only 12,96% of peptides have been found in both samples** (Fig. 1).

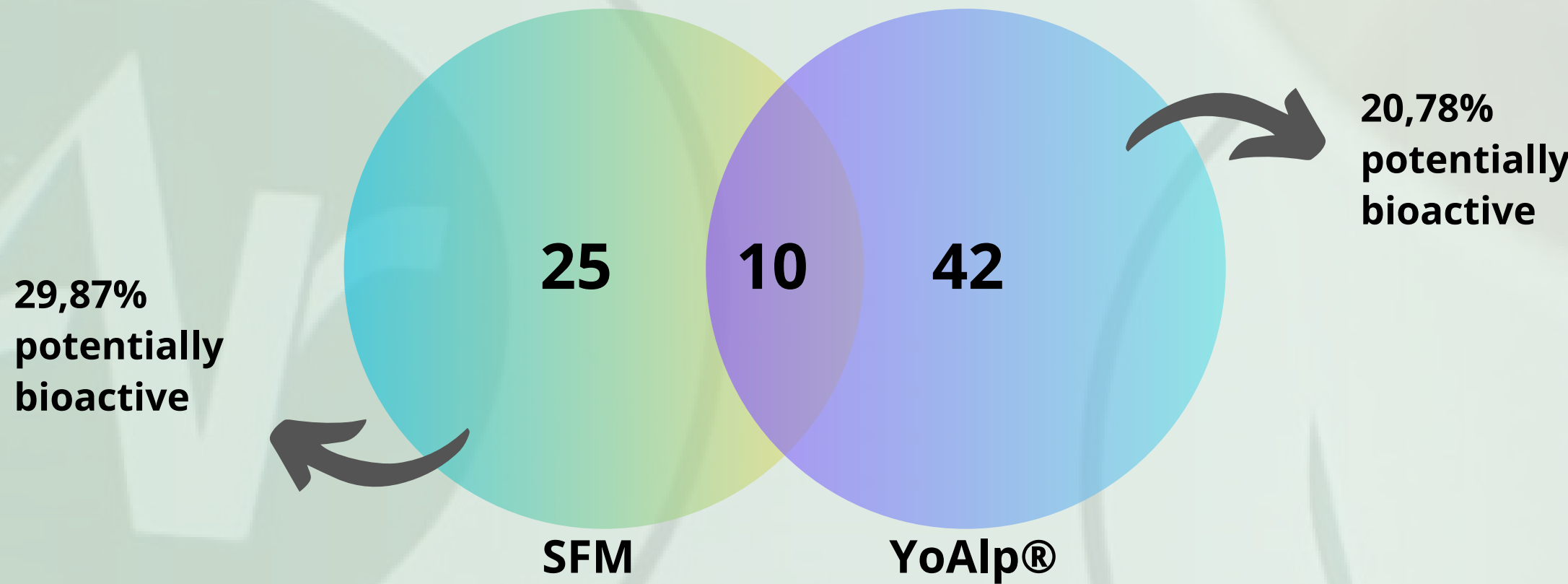


Fig. 1 Number of peptides and probability to exert a bioactive peptides in SFM (C) and YoAlp® (A)

BIOLOGICAL ACTIVITY

As far as bioactivity is concerned, **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). Similar values, as expected by peptide profile analysis, have been obtained using commercial and autochthonous starters. 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. 3). Finally, in contrast with peptide profile bioactivity assumption, **DPP-IV inhibition is higher using Aosta Valley lactic acid bacteria** if compared to commercial starter cultures (89,86% and 68,49% of inhibition) (Fig. 4).

FATTY ACIDS PROFILE

Analysis of fatty acid composition carried out in GC/MS and their quantification by GC/FID [7] showed that YoAlp® is an excellent source of desirable fatty acids such as **essential fatty acids** and it has a better **ω6/ω3 ratio** if compared 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. 5).

AROMATIC PROFILE

The aromatic profile has been obtained through an SPME-GC/MS analysis [8]. YoAlp® has been shown to have a good content in volatile components, higher than SFM, mainly represented by ketones and acids (Fig. 6). Considering the most important aromatic compounds for the aroma of a fermented milk, it has been shown that the autochthonous ferment has a better aptitude than SFM for the production of **diacetyl, acetoin, acetone** and **2-butanone** (Fig. 7).

RESULTS

[M+H] ^{+/3+}	m/z	sequence	position	protein	effect	Biological activity probability	Matrix
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*	VRGPFPII	206-221	β-CN	ACE inhib.		0,83	C
328	FY	145-146	αS1-CN			0,98	C
375	PYP	57-59	k-CN			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			0,8	A
907	LPQNILPL	70-77	β-CN	antimicrobial		0,69	A

Tab. 1 Most important peptptides found in SFM (C) and YoAlp® (A)

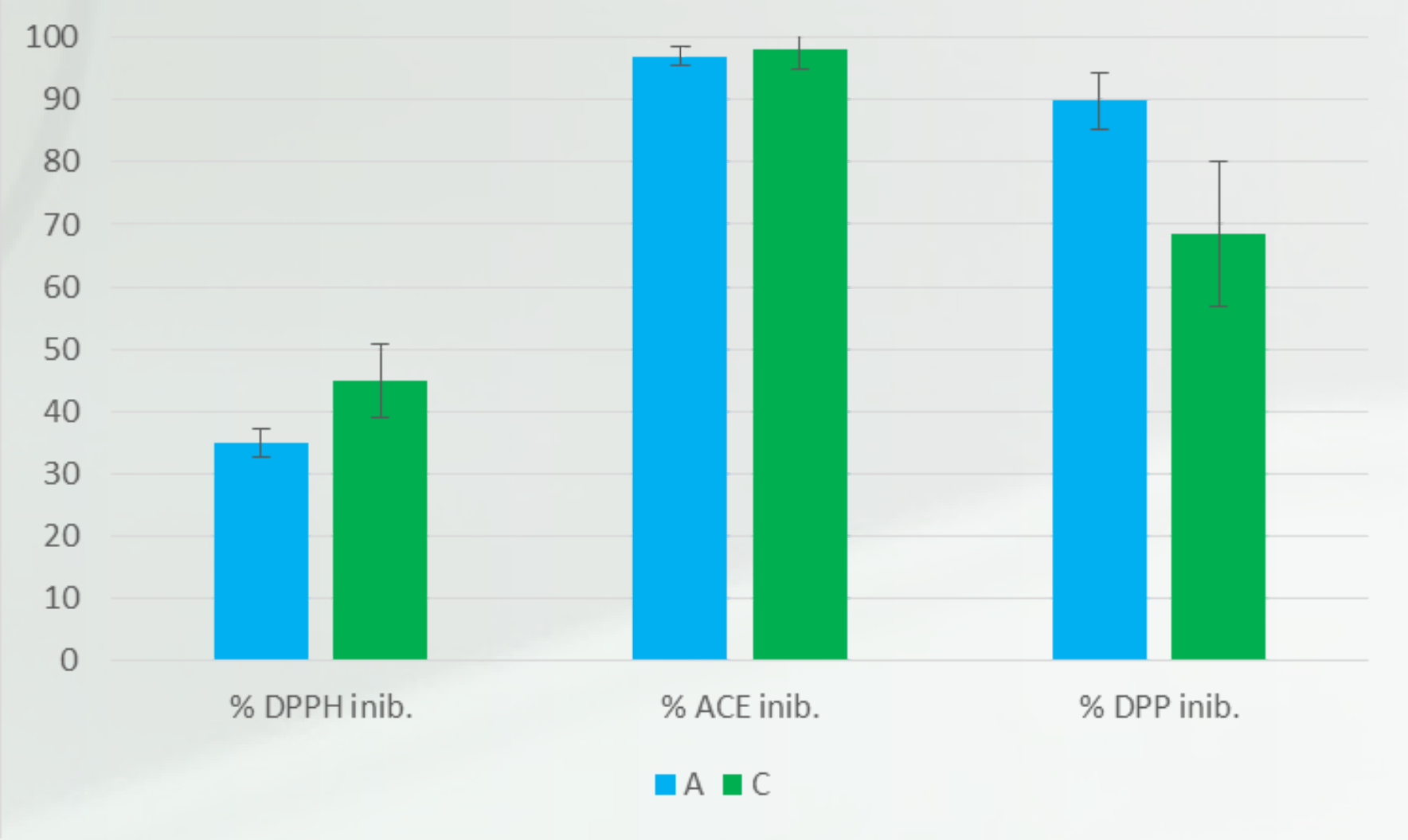
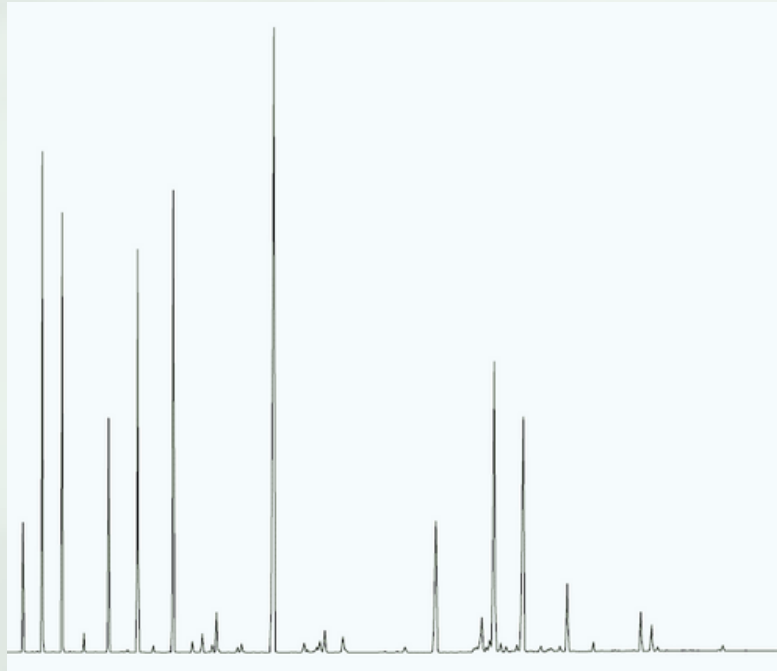


Fig. 2-3-4 DPP, DPPH and ACE inhibition in SFM (C) and YoAlp® (A)



	Sheep YoAlp®	Sheep yogurt
Σ saturated	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. 5: GC/FID chromatogram and medium content (g/100 g FAT) for the identified fatty acid classes of Sheep YoAlp® and Sheep yogurt . Mean values calculated on 2 samples, extracted in duplicate and analysed in triples.

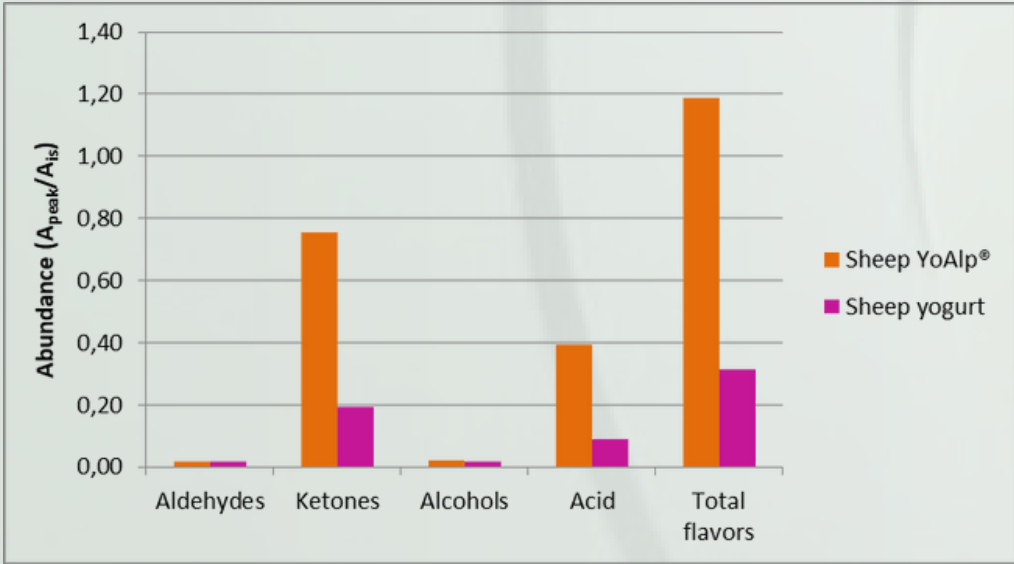


Fig. 6: Relative average abundance (Apeak/Ais) of identified aromatic compounds classes obtained from the triple analysis of two repetitions of sheep YoAlp® and sheep yogurt.

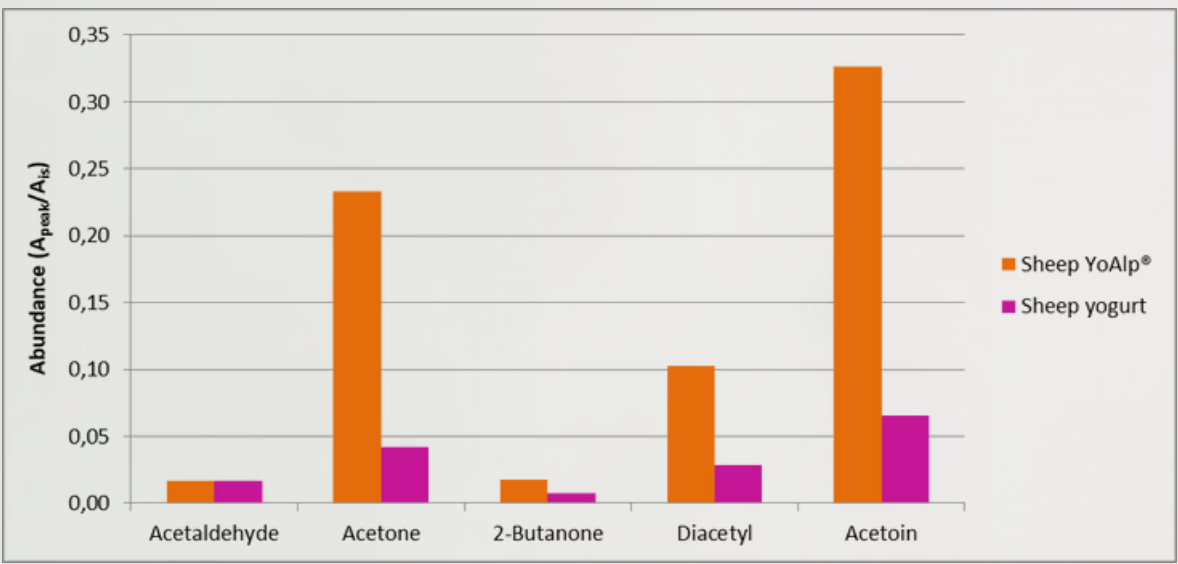


Fig. 7: Relative average abundance (Apeak/Ais) of aromatic compounds considered to have an impact on yogurt aroma, obtained from the triple analysis of two repetitions of sheep YoAlp® and sheep yogurt.

CONCLUSIONS

Local strains of lactic acid bacteria from Aosta Valley seems to work as well as the commercial, preserving biodiversity and typicity. However, further analyses are needed to identify peptides generated by autochthonous LABs in order to understand microbial proteolytic activities and to investigate gastric digestion resistance of bioactive peptides which can have positive effects on human health.

REFERENCES

- Balthazar C.F., Pimentel T.C., Ferrão L.L., Almada C.N., Santillo A., Albenzio M., Mollakhalili N., Mortazavia A.M., Nascimento J.S., Silva M.C., Freitas M.Q., Sant’Ana A.S., Granato D. and Cruz A.G., 2017. Sheep Milk: Physicochemical Characteristics and Relevance for Functional Food Development. Comprehensive Reviews in Food Science and Food Safety. Vol. 00, 2017. DOI: 10.1111/1541-4337.12250
- Park Y.W., Juárez M., Ramos M. and Hainlein G.W.F., 2007. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research 68 (2007) 88-113. DOI: 10.1016/j.smallrumres.2006.09.013
- Mohapatra A., Shinde A.K. and Singh R., 2019. Sheep milk: a pertinent functional food. Small Ruminant Research. DOI: 10.1016/j.smallrumres.2019.10.002
- Sforza, S.; Cavatorta, V.; Lambertini, F.; Galaverna, G.; Dossena, A.; Marchelli, R. Cheese peptidomics: A detailed study on the evolution of the oligopeptide fraction in Parmigiano-Reggiano cheese from curd to 24 months of aging. J. Dairy Sci. 2012, 95, 3514-3526
- Rizzello, C.G.; Losito, I.; Gobetti, M.; Carbonara, T.; De Bari, M.D.; Zamboni, P.G. Antibacterial Activities of Peptides from the Water-Soluble Extracts of Italian Cheese Varieties. J. Dairy Sci. 2005, 88, 2348-2360
- Prieto, J.M. Procedure: Preparation of DPPH Radical, and antioxidant scavenging assay. 2012.
- Bergamaschi M. and Bittante G., 2017. Detailed fatty acid profile of milk, cheese, ricotta and by products, from cows grazing summer highland pastures. Journal of Dairy Research. 84(3): 329-338. DOI: 10.1017/S0022029917000450
- Jiang J., Bjoerck L., Fondén R. and Emanuelson M., 1996. Occurrence of Conjugated Cis-9, Trans-11-Octadecadienoic Acid in Bovine Milk: Effects of Feed and Dietary Regimen. Journal of Dairy Science. Vol. 79, Issue 3, pp. 438-445. DOI: 10.3168/jds.S0022-0302(96)76383-X

ACKNOWLEDGEMENTS

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