Antioxidants in milk and cheese: An insight along the dairy chain stakeholders

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70th Annual Meeting of the European Federation of Animal Science, City of Ghent (Belgium), 26-30 August 2019

Outline

1. Overview of milk antioxidants
2. Analytical methods
3. Animals and farm management
4. Dairy industry
5. Human health

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1. **Overview of milk antioxidants**
2. Analytical methods
3. Animals and farm management
4. Dairy industry
5. Human health

1. OVERVIEW

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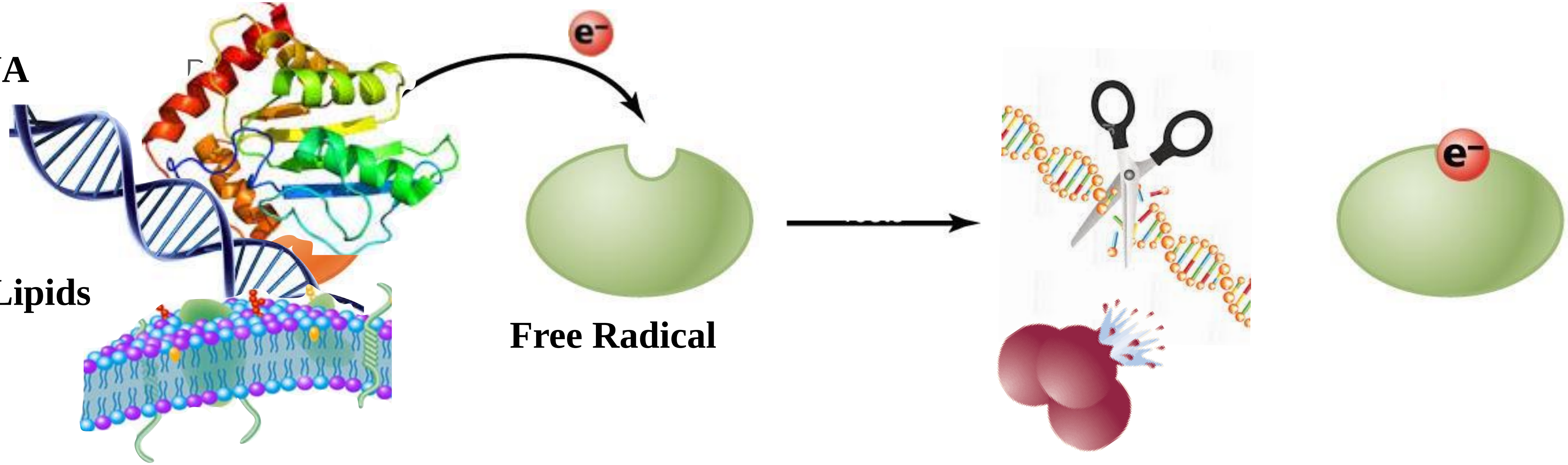
Red Ox Reactions

Proteins and enzymes

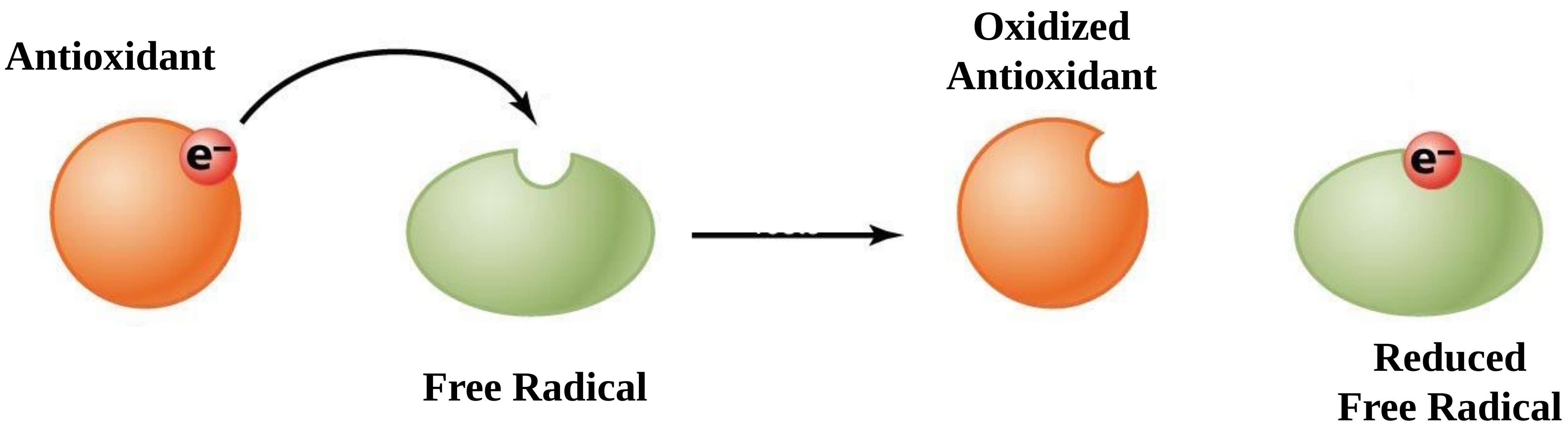
DNA

Lipids



Free Radical

Red Ox Reactions



Lipophilic antioxidants

Conjugated linoleic acids

The most active antioxidants in milk fat are conjugated linoleic acids (CLA) (Grażyna et al., 2017)

About 100-fold antioxidant activity than α-tocopherol (Badr El-Din and Omaye, 2007)

Sheep milk > cow milk > goat milk (Tsiplakou et al., 2009)

Dairy products: the most abundant sources in the human diet (Huth 2007)

Vitamin E (tocopherols)

Milk is not a rich source of vitamin E in human diet (Grażyna et al., 2017)

Vitamin E protects milk fat against autoxidation (Lindmark-Mansson and Akesson 2000)

Vitamin E content in milk is determined by tocopherols levels in animal feed (Grega et al., 2005) **and by technological treatments** (Niero et al., 2018b)

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Lipophilic antioxidants

Carotenoids and vitamin A (retinol)

Dairy products are rich in carotenoids and bioavailable vitamin A: the highest levels are found in ripened cheese and butter (Grażyna et al., 2017)

Milk vitamin A and carotenoids concentrations depend on by animal feed (Nozière et al., 2006)

Their anti-carcinogenic effects can be attributed to the presence of conjugated double bonds which absorb singlet oxygen

Hydrophilic antioxidants

Vitamin C (ascorbate)

Considered as the main water soluble vitamin present in milk (Khan et al., 2019)

Its free radical scavenging activity is due to low oxidation-reduction potential (Choe and Min, 2009)

Oxidation of ascorbic acid depends upon temperature, light and oxygen (Khan et al., 2019)

1. OVERVIEW

Caseins and whey proteins

Caseins

The antioxidant properties of caseins can be modulated by dephosphorilation of the protein chain

Proteolysis affects casein ability to inhibit peroxidation, due to exposition of amino acids with antioxidant properties (Philanto, 2006)

Whey proteins

Lactoferrin has been described as the most valuable protein in the human diet

Lactoferrin is an iron binding protein:

1. **Increases iron bioavailability for human cells**
2. **Decreases iron availability for micro organisms**
3. **Decreases iron pro-oxidation action**

β-lactoglobulin has protective effects on retinol and α-tocopherol (Grażyna et al., 2017)

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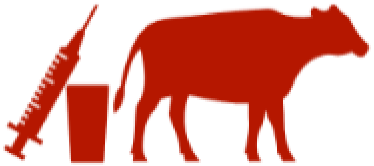
Different biological substrates

Feed level (fresh forages, hay, silages, concentrates, dietary supplements)



Animal level (blood, serum, milk, urine, faeces)

Product level (milk, cheese, butter, yogurt, cream, whey)

Gold standard methods

Analytical methods	Antioxidant compounds
High pressure liquid chromatography (HPLC)	Caseins, whey proteins, vitamin A, vitamin C, vitamin E, thiols, phenols, amino acids
Gas chromatography (GC)	Conjugated linoleic acids
Mass spectrometer (MS/MS)	Unknown antioxidant compounds Untargeted analyses
Spectrophotometer Spectrophotometric techniques	Vitamin C, total phenols, total thiols, total antioxidant activity
Enzyme linked immunosorbent assays ELISA	Enzymatic activity

Gold standard methods

Feed level (fresh forages, hay, silages, concentrates, dietary supplements)

Animal level (blood, serum, milk, urine, faeces)

Product level (milk, cheese, butter, yogurt, cream, whey)

Antioxidant compounds
Caseins, whey proteins, vitamin A, vitamin C, vitamin E, thiols, phenols, amino acids
Conjugated linoleic acids
Unknown antioxidant compounds Untargeted analyses
Vitamin C, total phenols, total thiols, total antioxidant activity
Enzymatic activity

≈ 200 reference analysis

≈ 200 sample preparation
+
≈ 200 analytical methods

2. ANALYTICAL METHODS

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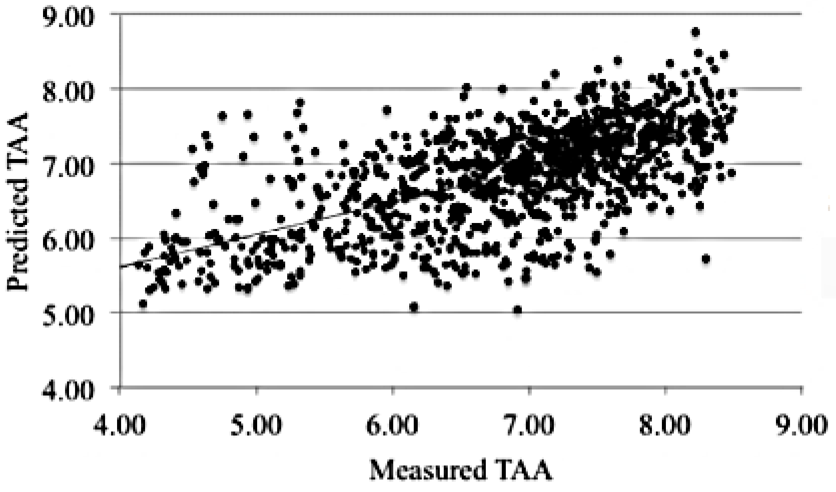
Prediction methods

Measured and MIRS-predicted total antioxidant activity (TAA) of Holstein Friesian cow milk

R^2_C = 0.46; R^2_{CV} = 0.41; RPD = 1.30 (Niero et al., 2019)



Near infrared calibration for the determination of antioxidant capacity in cheese (Revilla et al., 2016)

Item	N	SD	Estimate	R^2	SECV	RPD
Antioxidant capacity, µmol of Trolox/mg of cheese	154	1,341.3	3,005.7–11,053.5	0.63	939.1	1.43

2. ANALYTICAL METHODS

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3. ANIMALS & FARMING

Species effect

Vitamin content of cow, buffalo, sheep and goat milk

(Singh, 2004; Barlowska et al., 2011; Ren et al., 2015; Khan et al., 2019)

Vitamins	Cow Milk (mg/100 g)	Buffalo Milk (mg/100 g)	Goat Milk (mg/100 g)	Sheep Milk (mg/100 g)
Vitamin A[a]	46	69	185	146
Vitamin E[a]	0.21	0.19	0.03	–
Thiamine	0.05	0.05	0.068	0.08
Riboflavin	0.17	0.11	0.21	0.37
Niacin	0.09	0.17	0.27	0.416
Pantothenic acid	0.37	0.15	0.31	0.408
Vitamin B_6	0.04	0.33	0.046	0.08
Vitamin B_{12}	0.45	0.40	0.665	0.712
Biotin	2.0	13	1.5	0.93
Vitamin C[a]	0.09	2.5	1.29	4.16
Vitamin D	2.0	2.0	1.33	1.18

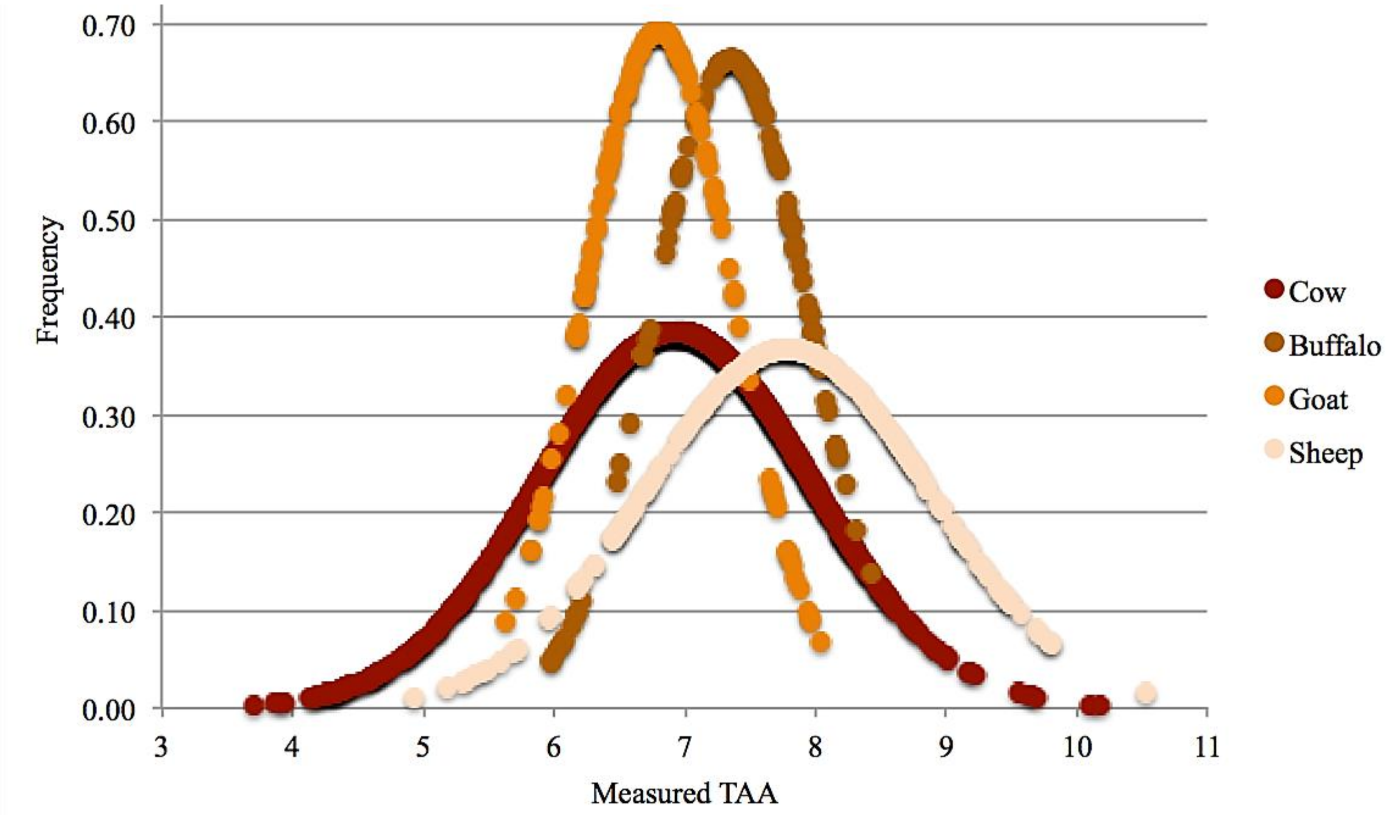
[a]Vitamin possesses antioxidant activity

3. ANIMALS & FARMING

Species effect

Distribution of total antioxidant activity (TAA, mmol/L of Trolox equivalents) in cow, buffalo, goat and sheep milk

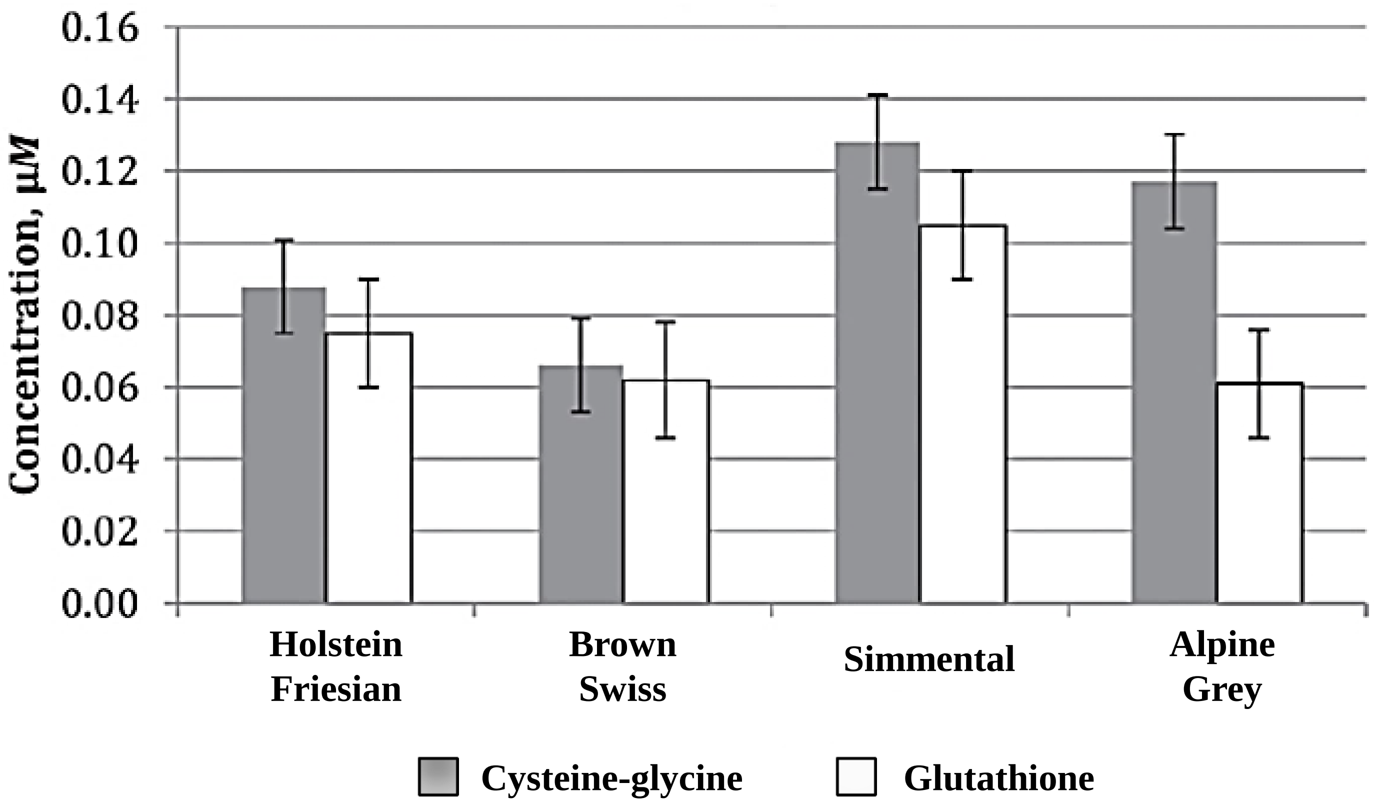
(Niero et al., 2018a)



Breed effect

Least squares means (SE) of cysteine-glycine and glutathione across breeds

(Niero et al., 2015)



3. ANIMALS & FARMING

Breed effect

Vitamin content (μg/g of fat) of milk from the Minhota and Holstein-Friesian breeds

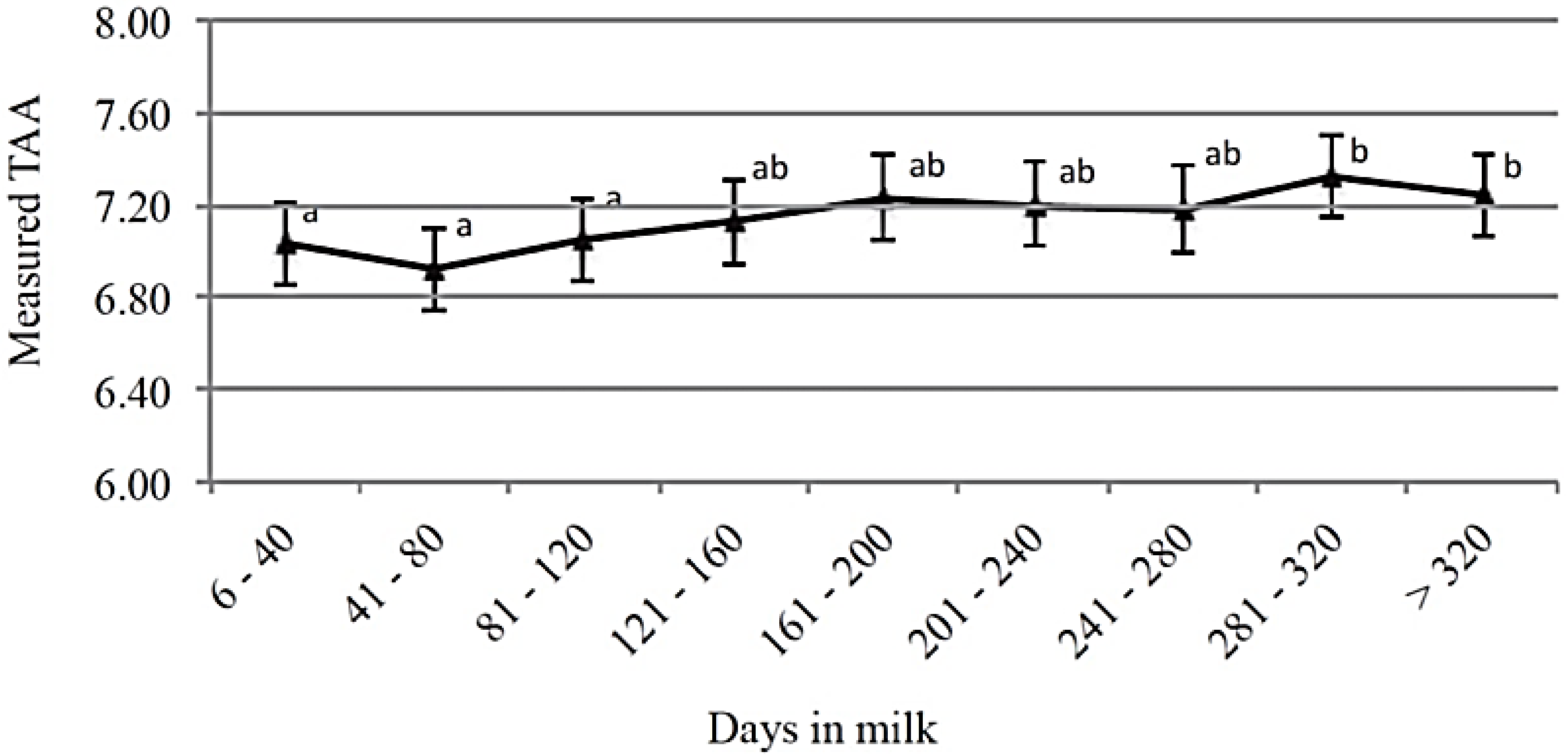
(Ramalho et al., 2012)

	Minhota		Holstein-Friesian		
Vitamin	Mean	SD	Mean	SD	*P*-value
Retinol	0.22	0.15	0.13	0.08	***
Retinyl palmitate	17.89	8.54	15.12	9.93	*
Total vitamin A	9.98	4.70	8.39	5.45	*
α-Tocopherol	32.79	19.46	15.52	6.68	***
Vitamin D_3	0.11	0.07	0.10	0.07	NS
Provitamin D_3	0.46	0.39	0.77	0.69	*
β-Carotene	3.60	2.13	1.45	1.15	***

$*P < 0.05$; $***P < 0.001$.

Lactation stage

Least squares means of total antioxidant activity (TAA, mmol/L of Trolox equivalents) in Holstein Friesian cow milk during lactation (Niero et al., 2019)



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Milk yield and quality traits

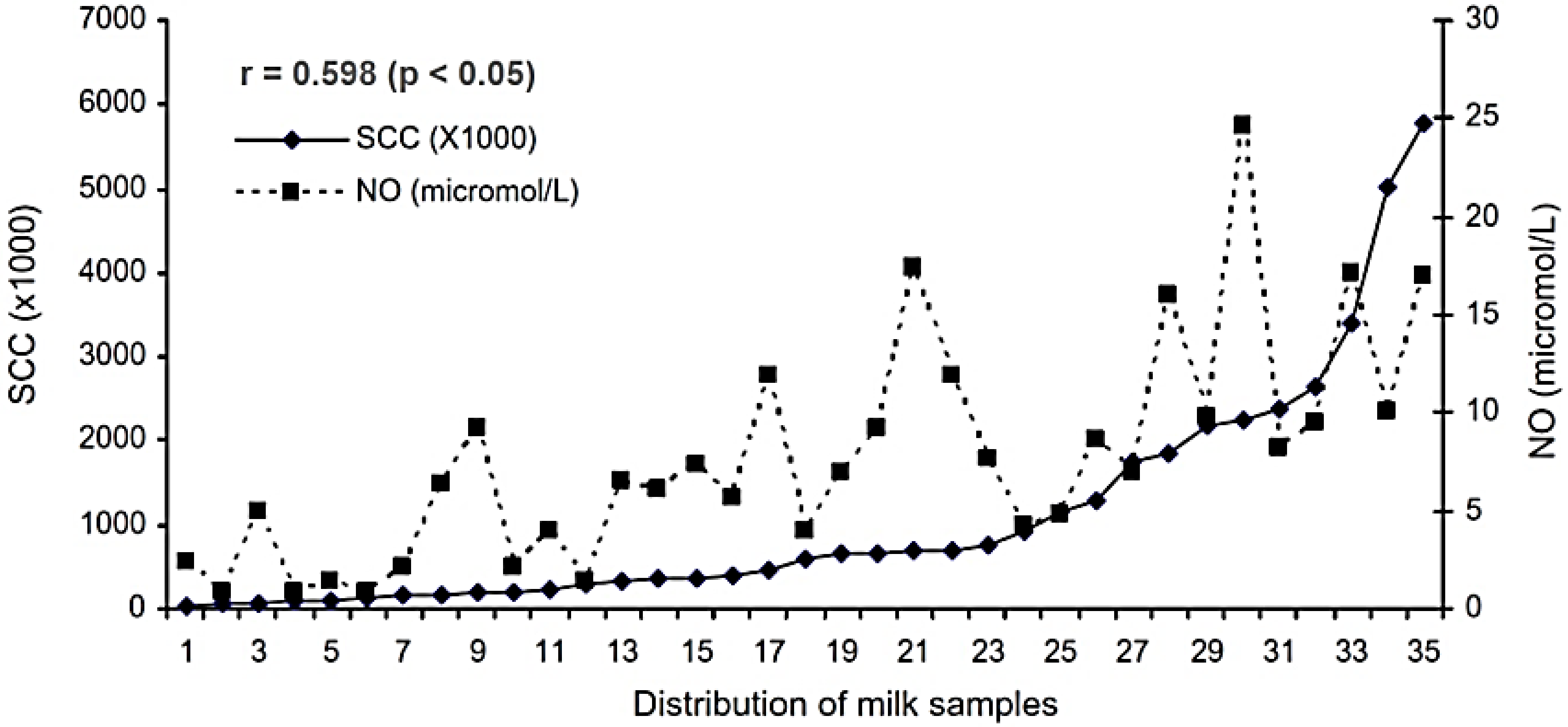
Pearson correlations of milk total antioxidant activity with milk yield and quality traits

(Niero et al., 2019)

Trait	Total antioxidant activity
Milk yield	–0.22***
Fat content	0.13***
Protein content	0.18***
Casein content	0.15***
Lactose content	–0.11***
Somatic cell score	0.13***

Animal health

Coefficient of correlation (r) between somatic cell count (SCC) and nitric oxide (NO) in milk samples with subclinical mastitis (Atakisi et al., 2010)



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Animal health

The supplementation of mastitic dairy cows with antioxidant vitamins (vitamin A, C, E) and β-carotene, is very important to help the animal recover early

Before supplementing rations with vitamins, dairy farmers should have their animal feeds tested and evaluated by nutritionist and a trustworthy laboratory to be sure about supplementation levels

Unjustified supplementation may result in a weakened immune system, animal health problems and can be expensive (Yang and Li, 2015)

Feeding effect

Concentrations and daily output of retinol in milk of Holstein cows receiving control fat or fat containing CLA at weeks 1, 3, 5, 8 and 11 of lactation (Gessner et al., 2015)

	Milk (μg/l)		Milk (μg/g fat)		Daily output via milk (mg/d)	
Week	Control	CLA	Control	CLA	Control	CLA
1	111	260*	1.78	4.33*	2.71	6.80*
3	236	339*	4.83	7.99*	8.12	11.37*
5	281	325*	6.42	8.77*	10.10	11.93*
8	272	326*	6.77	9.87*	9.81	12.55*
11	294	339*	7.55	10.55*	10.08	12.74*
Average	239	318*	5.47	8.30*	8.16	11.08*
SE†	15		0.44		0.60	
Week	<0.0001		<0.0001		<0.0001	
Group	<0.0001		<0.0001		<0.0001	
Week × Group	0.004		0.84		0.39	

*Significantly different from the control group ($p < 0.05$).

3. ANIMALS & FARMING

Feeding effect

Concentrations and daily output of α-tocopherol in milk of Holstein cows receiving control fat or fat containing CLA at weeks 1, 3, 5, 8 and 11 of lactation (Gessner et al., 2015)

	Milk (μg/l)		Milk (μg/g fat)		Daily output via milk (mg/d)	
Week	Control	CLA	Control	CLA	Control	CLA
1	438	848*	6.69	13.75*	11.8	25.9*
3	381	505*	7.80	11.74*	13.7	17.9*
5	366	447*	8.83	12.20*	13.5	16.7*
8	451	594*	11.24	18.00*	16.5	23.8*
11	450	602*	11.43	18.60*	15.8	23.1*
Average	417	599*	9.20	14.86*	14.3	21.5*
SE†	47		1.04		1.87	
Week	<0.0001		<0.0001		0.014	
Group	<0.0001		<0.0001		<0.0001	
Week × Group	0.004		0.17		0.033	

*Significantly different from the control group ($p < 0.05$).

3. ANIMALS & FARMING

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4. DAIRY INDUSTRY

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Milk skimming

Tocopherols content (mg/L) in raw milk (RM), whole UHT milk (WUM), partially skimmed UHT milk (SUM), whole pasteurized milk (WM) and partially skimmed pasteurized milk (SM) (Niero et al., 2018b)

Milk[2]	Mean	Minimum	Maximum
RM			
α-tocopherol	1.35	1.25	1.42
γ-tocopherol	0.21	0.20	0.22
WUM			
α-tocopherol	1.18	1.10	1.26
γ-tocopherol	0.15	0.14	0.16
SUM			
α-tocopherol	0.47	0.44	0.52
γ-tocopherol	0.14	0.10	0.12
WM			
α-tocopherol	1.41	1.33	1.54
γ-tocopherol	0.16	0.15	0.17
SM			
α-tocopherol	0.65	0.59	0.69
γ-tocopherol	0.12	0.11	0.13

-54 %
in total tocopherols

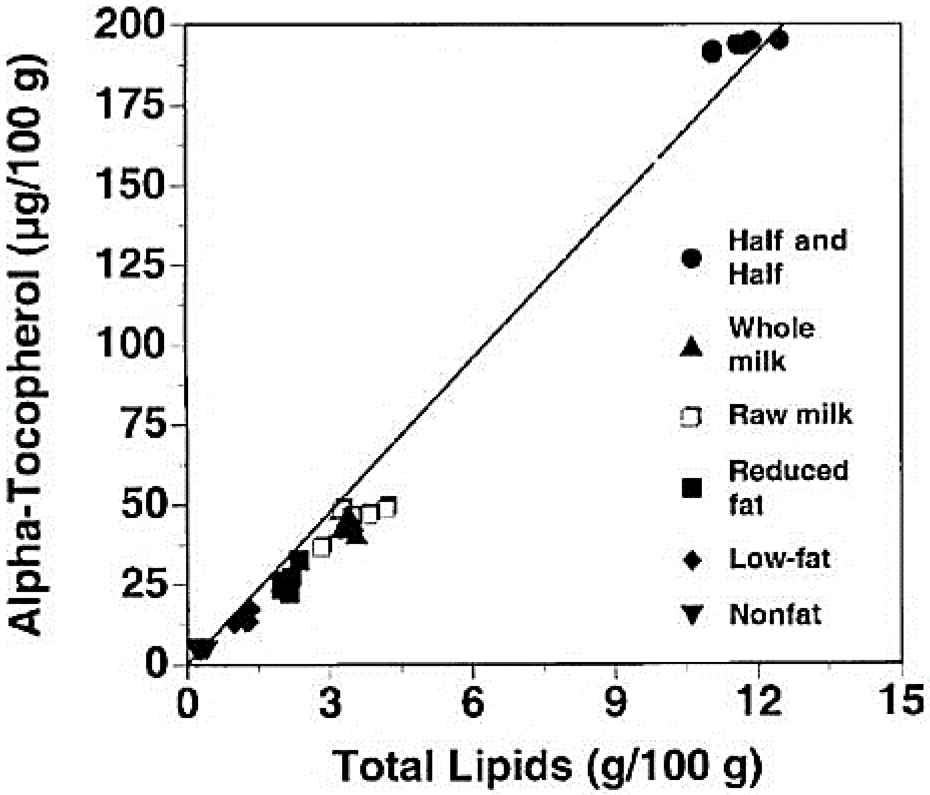
-51 %
in total tocopherols

4. DAIRY INDUSTRY

Milk skimming

Correlation between α-tocopherol and total lipids in milk samples of different lipid content ($R^2 = 0.985$)

(Kaushik et al., 2001)



4. DAIRY INDUSTRY

Thermal treatments

Tocopherols content (mg/L) in raw milk (RM), whole UHT milk (WUM), partially skimmed UHT milk (SUM), whole pasteurized milk (WM) and partially skimmed pasteurized milk (SM) (Niero et al., 2018b)

Milk[2]	Mean	Minimum	Maximum
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SUM			
α-tocopherol	0.47	0.44	0.52
γ-tocopherol	0.14	0.10	0.12
WM			
α-tocopherol	1.41	1.33	1.54
γ-tocopherol	0.16	0.15	0.17
SM			
α-tocopherol	0.65	0.59	0.69
γ-tocopherol	0.12	0.11	0.13

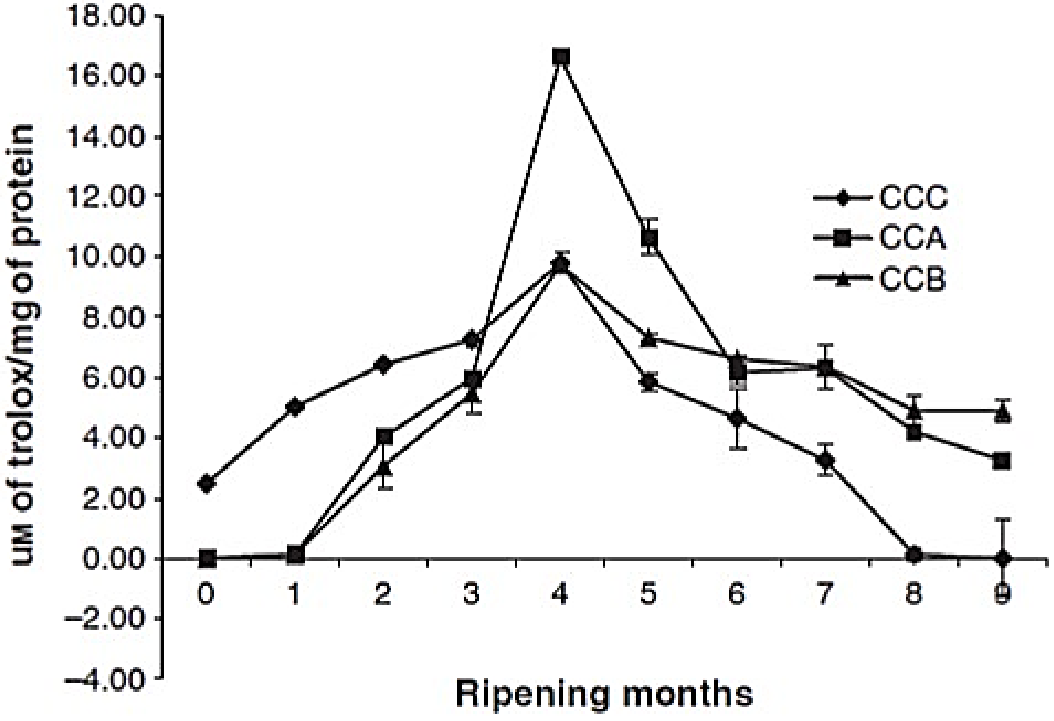
-18 %
in total tocopherols

-21 %
in total tocopherols

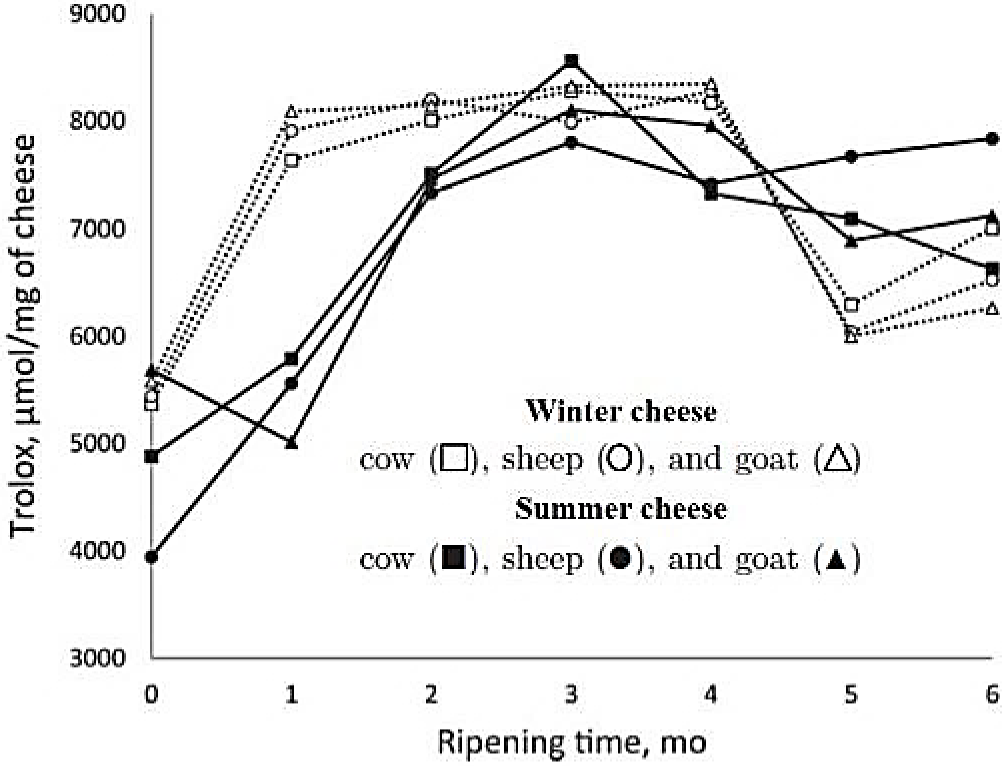
4. DAIRY INDUSTRY

Cheese ripening

Radical scavenging activity of Cheddar cheeses A (CCA), B (CCB) and C (CCC) during ripening (Gupta et al., 2009)



Antioxidant activity of cow, sheep and goat milk cheeses during ripening (Revilla et al., 2016)



4. DAIRY INDUSTRY

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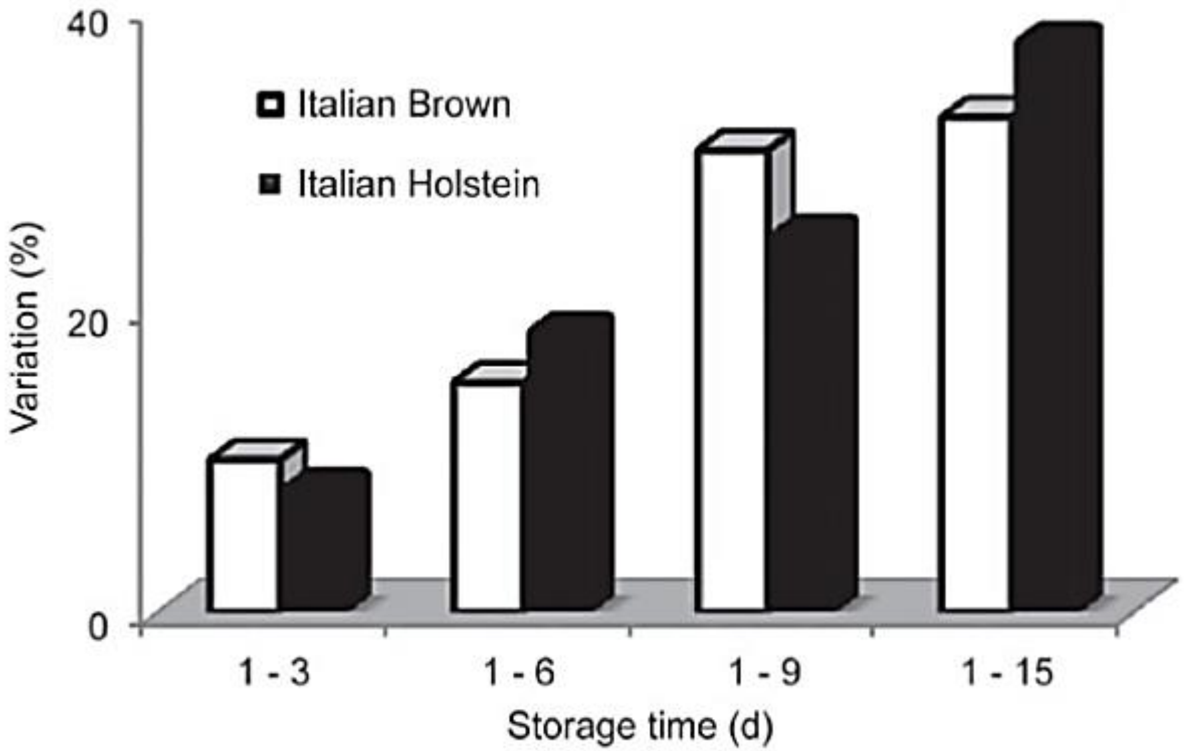
Fermented dairy products: yogurt

Percentage variation of antioxidant activity in Italian Brown and Italian Holstein yogurt during storage for 15 days (Perna et al., 2013)



4. DAIRY INDUSTRY

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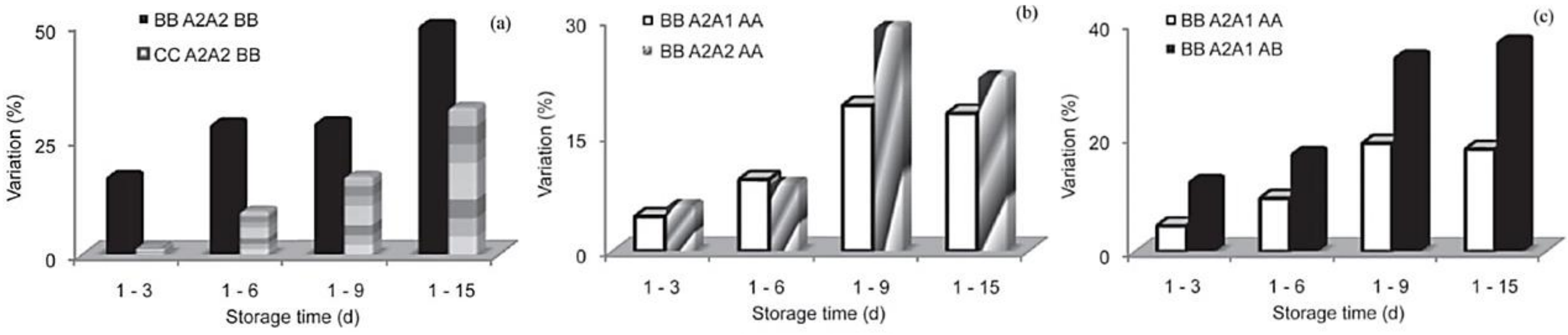
Fermented dairy products: yogurt

Percentage variation of antioxidant activity for different haplotypes of
α_{S1}-CN / β-CN / κ-CN (Perna et al., 2013)



4. DAIRY INDUSTRY

Fermented dairy products: kefir

Inhibition ability of linoleic acid peroxidation and reducing power of cow and goat milk and kefir

(Liu et al., 2005)

Sample	Absorbance at 500 nm	Inhibition ability (%)	Absorbance at 700 nm
Cow-milk	1.075±0.003	51.37±0.11[d]	0.690±0.002[c]
Goat-milk	0.955±0.024	56.80±1.06[c]	0.709±0.002[b]
Cow-milk kefir	0.253±0.010	88.57±0.45[a]	0.760±0.001[a]
Goat-milk kefir	0.531±0.017	75.96±0.78[b]	0.763±0.015[a]

4. DAIRY INDUSTRY

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5. HUMAN HEALTH

In vitro study

Radical scavenging activity of water soluble peptides extracts of Buffalo (BCC) and Cow Cheddar Cheese (CCC) at different ripening stages (Huma et al., 2018)

	Ripening days (D)					
	D30	D60	D90	D120	D150	D180
BCC_5	1.76 ± 0.12^{uv}	3.42 ± 0.10^{o}	6.86 ± 0.07^{j}	13.82 ± 0.33^{ef}	15.44 ± 0.21^{d}	15.73 ± 0.20^{c}
BCC_{10}	2.08 ± 0.04^{t}	3.82 ± 0.02^{n}	7.33 ± 0.02^{i}	14.74 ± 0.14^{e}	15.73 ± 0.13^{c}	15.85 ± 0.09^{bc}
BCC_{15}	2.34 ± 0.07^{rs}	4.42 ± 0.01^{m}	7.92 ± 0.06^{h}	15.22 ± 0.11^{d}	15.95 ± 0.01^{a}	15.94 ± 0.02^{a}
CCC_5	1.71 ± 0.01^{uv}	2.88 ± 0.01^{r}	4.52 ± 0.01^{m}	12.45 ± 0.26^{g}	15.35 ± 0.30^{d}	15.67 ± 0.18^{bc}
CCC_{10}	1.88 ± 0.01^{u}	3.12 ± 0.02^{q}	5.00 ± 0.01^{l}	13.83 ± 0.11^{ef}	15.68 ± 0.18^{bc}	15.72 ± 0.21^{c}
CCC_{15}	2.28 ± 0.03^{s}	3.31 ± 0.01^{p}	5.86 ± 0.06^{k}	14.63 ± 0.22^{e}	15.88 ± 0.11^{b}	15.86 ± 0.10^{b}

In vitro study

Effect of water soluble peptides extracts of Buffalo (BCC) and Cow Cheddar Cheese (CCC) at different ripening stages on reactive oxygen species in human colon adenocarcinoma cells (Huma et al., 2018)

	Ripening days (D)					
	D30	D60	D90	D120	D150	D180
BCC_{100}	57.47 ± 0.23^{a}	56.37 ± 0.30^{bcd}	53.68 ± 0.17^{e}	47.64 ± 0.17^{ij}	40.20 ± 0.12^{uv}	$39.41 \pm 0.23^{u-x}$
BCC_{200}	57.42 ± 0.17^{ab}	56.29 ± 0.12^{cd}	53.55 ± 0.31^{e}	44.24 ± 0.13^{o}	39.77 ± 0.20^{uvw}	$39.39 \pm 0.30^{u-x}$
BCC_{300}	56.23 ± 0.23^{c}	56.09 ± 0.01^{d}	53.30 ± 0.24^{e}	40.42 ± 0.23^{tu}	39.27 ± 0.19^{vw}	$39.03 \pm 0.19^{v-y}$
CCC_{100}	53.32 ± 0.18^{e}	48.36 ± 0.01^{hi}	44.83 ± 0.10^{m}	42.66 ± 0.18^{qr}	$39.22 \pm 0.11^{v-y}$	38.42 ± 0.23^{xyz}
CCC_{200}	51.63 ± 0.30^{f}	46.85 ± 0.02^{jk}	44.42 ± 0.23^{no}	42.26 ± 0.14^{qrs}	$39.16 \pm 0.09^{v-y}$	$38.22 \pm 0.32^{w-z}$
CCC_{300}	49.03 ± 0.01^{g}	45.20 ± 0.19^{l}	43.35 ± 0.21^{pq}	40.42 ± 0.23^{t}	38.28 ± 0.15^{z}	38.01 ± 0.17^{z}

5. HUMAN HEALTH

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THANK YOU!

Antioxidants in milk and cheese: An insight along the dairy chain stakeholders

1. Milk antioxidants overview
2. Analytical methods
3. Animals and farm management
4. Dairy industry
5. Human health

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