



# Antioxidant Properties of Milk and Dairy Products







attributed to the difference in amino acid profile [17]. Antioxidant activities of superoxide dismutase, catalase and glutathione peroxidase, casein and certain peptides are well established [18].

### Antioxidant properties of whey proteins

In recent years, utilization of whey in food and nonfood applications is mounting across the globe. Whey protein has higher biological value and despite the fact that about 30–35% of the whey is still discarded [19]. In food industries, whey proteins are used as emulsifying, gelling and bulking agent. Antioxidant activity of whey protein is scientifically established and antioxidants of whey can efficiently inhibit the lipid oxidation [20]. Antioxidant activity of whey protein is due to the chelation of transition metals by lactoferrin and scavenging of free radicals by sulphur containing amino acids [21]. Whey proteins boost the level of glutathione peroxidase which is regarded as one of the most significant water soluble antioxidant system [22]. Whey proteins have antioxidant activity and addition of whey protein in soybean oil emulsion increased the oxidative stability [12]. Antioxidant characteristics of salmon oil emulsion increased as a function of addition of whey protein [20]. Food containing whey proteins have better antioxidant activity. Lactoferrin and casein can inhibit lipid peroxidation, generation of peroxide radicals, thiobarbituric acid reacting substances, uptake of oxygen and iron oxide free radicals [23]. Casein fraction, composition of whey proteins and amino acids profile of cow, buffalo, sheep and goat milk have been presented in Tables 1, 2 and 3, respectively.

### Antioxidant characteristics of carotenoids

Carotenoids are lipophilic molecules with a tendency to accrue in membrane or lipoproteins [24]. Milk fat globule membrane is considered as the most volatile site for auto-oxidation [24].  $\beta$ -carotene is regarded as preventive antioxidant, it can quench singlet oxygen

**Table 1** Casein fraction of cow, buffalo, goat and sheep milk

Parameters	Cow	Buffalo	Sheep	Goat
TPC (g/L)	27.8	49.2	59.4	33.4
$\alpha$ S <sub>1</sub> -casein (%)	37	90	33	99
$\alpha$ S <sub>2</sub> -casein (%)	7	13	14	8.52
$\beta$ -casein (%)	42	28	30	63
$\gamma$ -casein (%)	6	22	9	18
$\kappa$ -casein (%)	9	7	14	8

TPC Total Protein Content

Source of Data

Cow Milk: [104]

Buffalo Milk: [105]

Sheep Milk: [104]

Goat Milk: [104]

**Table 2** Composition of whey proteins in cow, buffalo, goat and sheep milk

Parameters	Cow Milk	Buffalo Milk	Sheep Milk	Goat Milk
Whey proteins (g/L)	6.46	6.46	10.76	6.14
$\beta$ -Lactoglobulin (%)	59.3	59.3	61.1	54.2
$\alpha$ -lactalbumin (%)	16.2	16.2	10.8	21.4
Immunoglobulin's (%)	15.0	15.0	20.0	11.5
Serum albumin/lactoferrin (%)	9.5	9.5	8.1	12.8

Source of Data

Cow Milk: [104]

Buffalo Milk: [105]

Sheep Milk: [104]

Goat Milk: [105]

and one molecule of  $\beta$ -carotene can quench 250 to 1000 molecules of singlet oxygen [26]. Carotenoids act as scavengers of singlet oxygen and other reactive oxygen species [25]. Among the various antioxidant systems in milk, carotenoids act a scavenger of singlet oxygen and peroxy radicals [27]. Dairy lipids may suffer from oxidation, which leads to the negative impact on quality and sensory characteristics of finished products. Auto-oxidation and light induced oxidation is affected by a complex interaction of pro and

**Table 3** Amino acids profile of cow, buffalo, sheep and goat milk

Amino Acid (g/100 g)	Cow Milk	Goat Milk	Buffalo Milk	Sheep Milk
Aspartic acid	7.8	7.4	7.13	6.5
Threonine	4.5	5.7	5.714	4.4
Serine	4.8	5.2	4.65	3.4
<sup>a</sup> Glutamic acid	23.2	19.3	21.4	14.5
Proline	9.6	14.6	12.0	16.2
<sup>a</sup> Cystine	0.6	0.6	0.586	0.9
Glycine	1.8	2.1	1.93	3.5
Alanine	3.0	3.6	3.03	2.4
<sup>a</sup> Valine	4.8	5.7	6.760	6.4
<sup>a</sup> Methionine	1.8	3.5	0.928	2.7
<sup>a</sup> Isoleucine	4.2	7.1	5.714	4.6
<sup>a</sup> Leucine	8.7	8.2	9.792	9.9
<sup>a</sup> Tyrosine	4.5	4.8	3.858	3.8
Phenylalanine	4.8	6.0	4.713	4.3
<sup>a</sup> Histidine	3.0	5.0	2.73	6.7
Lysine	8.1	8.2	7.497	7.8

<sup>a</sup>Amino acid has antioxidant activity in milk and dairy products

Source of Data

Cow Milk: [106]

Goat Milk: [107]

Buffalo Milk: [108]

Sheep Milk: [107]

antioxidants. Photo-oxidation is predominantly inhibited by  $\beta$ -carotene, it absorbs light that would otherwise be absorbed by riboflavin, which may give rise to quality related issues.  $\beta$ -carotene absorbs light in a concentration dependent manner [28]. Results of an earlier investigation regarding the migration of carotenoids from milk to cheese and butter have shown that concentration of carotenoids was intensified in cheese and butter [29].

### Antioxidant characteristics of ascorbic acid, vitamin E and minerals

Nutraceuticals and functional food ingredients that are beneficial to vascular health may represent useful compounds that are able to reduce the overall cardiovascular risks [30]. Ascorbic acid is one of the most strong and least toxic natural antioxidant. It is the main water soluble antioxidant present in milk and free radical scavenging activity of ascorbic acid is due to low oxidation-reduction potential (330 mV). Ascorbic acid is the major water-soluble antioxidant in milk and can act as strong free radical scavenger [31]. Ascorbic acid can scavenge superoxide anion radicals, alkoxyl radicals and singlet oxygen [31]. Ascorbic acid can scavenge superoxide, iron oxide, nitric oxide and alkoxyl radicals [32]. Ascorbic acid significantly inhibited the degradation of riboflavin in cream in presence of 1000 Lux light for four days [33]. Ascorbic acid and tocopherol were added in milk to enhance the flavor and photo-oxidative stability. Ascorbic acid and tocopherol supplemented samples revealed better flavor and photo-oxidative stability as compared to non-supplemented samples [34]. Ascorbic acid significantly inhibited the degradation of riboflavin in light exposed milk, antioxidant activities were mainly attributed to the scavenging effect on singlet oxygen [35]. A study was conducted to determine the effect of tocopherol and vitamin C against the development of atopy in infants. Increased concentration of vitamin C in breast milk reduced the risk of atopy in infants [36]. Ascorbic acid is extremely helpful for the infants as it plays a pivotal role in the formation of neuro transmitters, synthesis of carnitine and improves the absorption of iron. Human and cow milk contains about 40 and 20 mg/Liter [37, 38]. Oxidation of ascorbic acid depends upon temperature, light, oxygen and amount of catalysts. Vitamin A and E are regarded as primary lipid soluble antioxidants and main job of these vitamins is to protect the polyunsaturated fatty acids and associated bio-chemical compounds from peroxidation (Table 4).  $\alpha$ -tocopherol can be considered one of the most important lipid-soluble antioxidants in milk, due to its presence in milk fat globule membrane [39]. It can act as a preventative, chain breaking antioxidant and quencher of singlet oxygen in

**Table 4** Vitamin content of cow, buffalo, goat and sheep milk

Vitamins	Cow Milk (mg/100 g)	Buffalo Milk (mg/100 g)	Goat Milk (mg/100 g)	Sheep Milk (mg/100 g)
Vitamin A <sup>a</sup>	46	69	185	146
Vitamin E <sup>a</sup>	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 <sub>6</sub>	0.04	0.33	0.046	0.08
Vitamin B <sub>12</sub>	0.45	0.40	0.665	0.712
Biotin	2.0	13	1.5	0.93
Vitamin C <sup>a</sup>	0.09	2.5	1.29	4.16
Vitamin D	2.0	2.0	1.33	1.18

<sup>a</sup>Vitamin possesses antioxidant activity

Source of Data

Cow Milk: [37]

Buffalo Milk: [107]

Goat Milk: [108]

Sheep Milk: [107]

milk [40]. Milk can develop off flavor as a result of photo-oxidation and contamination with copper. The existence of antioxidants in milk can inhibit the free radical mechanism by donating the proton and thus inhibit the onset of auto-oxidation. Vitamin E can inhibit the activity of plasmin; a proteolytic enzyme and secondly it can directly scavenge the free radicals [41]. Among the tocopherols,  $\alpha$ -tocopherol is regarded as more powerful scavenger of free radicals and antioxidant activity of  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol is about 80–90% less than  $\alpha$ -tocopherol [42].  $\gamma$ -tocopherol is of high functional value as it can trap the nitrogen oxide species. It helps the body to prevent cardiovascular diseases and cancers. The concentration of vitamin E in cow milk has been reported about 0.9 mg/mL while summer milk possessed higher concentration than winter milk. Concentration of vitamin in human milk ranges from 3 to 13 mg/mL [43]. Addition of 100 mg  $\alpha$ -tocopherol/kg milk fat and 100 mg ascorbyl palmitate/kg milk fat to UHT milk decreased the concentration of hexanal during the storage period of 4 weeks [34]. Khan et al. [44] studied the effect of vitamin E supplementation on oxidative stability of sheep butter and supplementation of sheep butter with 60 mg/kg efficiently inhibited the lipid peroxidation and raised the shelf stability. Antioxidant activity of zinc and selenium for the inhibition of superoxide dismutase is scientifically proven [45]. Glutathione and selenium enhanced the functional value and antioxidant capacity of milk [46]. Mineral content of cow, buffalo, goat and sheep milk have been presented in Table 5.

**Table 5** Mineral content of cow, buffalo, goat and sheep milk

Minerals	Cow Milk	Buffalo Milk	Goat Milk	Sheep Milk
Calcium	122 (mg/100 g)	112 (mg/100 g)	134 (mg/100 g)	195–200 (mg/100 g)
Phosphorus <sup>a</sup>	119 (mg/100 g)	99 (mg/100 g)	121 (mg/100 g)	124–158 (mg/100 g)
Potassium	152 (mg/100 g)	92 (mg/100 g)	181 (mg/100 g)	136–140 (mg/100 g)
Magnesium	12 (mg/100 g)	8 (mg/100 g)	16 (mg/100 g)	18–21 (mg/100 g)
Sodium	58 (mg/100 g)	35 (mg/100 g)	41 (mg/100 g)	44–58 (mg/100 g)
Zinc <sup>a</sup>	530 (µg/100 g)	410 (µg/100 g)	56 (µg/100 g)	520–747 (µg/100 g)
Iron <sup>b</sup>	80 (µg/100 g)	161 (µg/100 g)	7.22 (µg/100 g)	72–122 (µg/100 g)
Copper <sup>b</sup>	60.58 (µg/100 g)	35 (µg/100 g)	5.13 (µg/100 g)	40–68 (µg/100 g)
Manganese	20 (µg/100 g)	27 (µg/100 g)	3.2 (µg/100 g)	5.39 (µg/100 g)
Iodine	2.1 (µg/100 g)	4 (µg/100 g)	2.2 (µg/100 g)	10.41 (µg/100 g)
Selenium <sup>a</sup>	0.96 (µg/100 g)	6 (µg/100 g)	1.33 (µg/100 g)	3.14 (µg/100 g)

<sup>a</sup>Chemical constituents has antioxidant activity in milk

<sup>b</sup>Chemical constituent has pro-oxidant activity

Source of Data

Cow Milk: [46]

Buffalo Milk: [107]

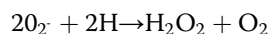
Goat Milk: [109]

Sheep Milk: [107]

## Enzymatic antioxidants

### Superoxide dismutase

Superoxide dismutase (SOD) catalyzes the removal of superoxide free radicals ( $O_2^-$ ) and safeguards the cells from harmful effects by the following reaction.



Catalase, glutathione peroxidase or other reducing agents converts  $H_2O_2$  to  $H_2O$ , hydrogen peroxide formed from  $O_2^-$  and oxidases is eliminated by catalases and peroxidases [47]. Cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD and extracellular EC-SOD are the major forms of SOD [48]. SOD can inhibit lipid peroxidation. In cow milk SOD is exclusively present in skim milk fraction, with a concentration of 0.15 mg to 2.4 mg/L [49]. Human milk has 2.0 to 2.3 time higher concentration of SOD than cow milk.

### Glutathione peroxidase (GSHPx)

GSHPx is a selenium encompassing enzyme that provides protection against lipid peroxidation. It catalysis the breakdown of  $H_2O_2$  and organic hydroperoxides (R-OOH) by glutathione ( $\gamma$ Glu.Cys.Gly) as per following chemical reaction [50].



More than 90% of GSHPx exists in milk as extra cellular enzyme and it is only enzyme which fixes selenium (about 30% of the total). Its concentration varies among the mammals and concentration is in the order of human > caprine > bovine [51]. Concentration of GSHPx in cow milk ranges from 12 to 30 U/mL and its activity

is mainly dependent upon the concentration of selenium. Antioxidant activity and selenium content decreases with the progression of lactation [52].

### Catalase

Milk catalase is a heme protein and molecular weight of catalase is 200 kDa with isoelectric pH of 5.5. This enzyme is stable in a wide range of pH 5–10 and however, it rapidly loses activity out this pH range [53]. Most of the catalases contain heme and catalase causes the dismutation of  $H_2O_2$  (a chemical reaction in which  $H_2O_2$  causes oxidation of the other  $H_2O_2$  molecules, consequently, one is converted to  $O_2$  and the other two are converted to two molecules of  $H_2O$ ) [54]. A polarographic method showed that average catalase activity in cow milk was 1.95 U/mL [55]. Concentration of catalase in human milk is approximately ten times greater than cow milk [56].

### Oxidative stability of milk and milk products

The oxidative stability of milk and dairy products is of concern to the dairy industry. Oxidation in milk can result in strong off-flavors and in deterioration of the nutritional quality of milk. The oxidative stability of milk and dairy products is the result of a delicate balance between the anti- and pro-oxidative processes in milk. Oxidative stability of milk and dairy products depends upon fatty acid composition (Tables 6 and 7), contamination with metal ions, concentration of tocopherols and carotenoids [57]. Processing, packaging, storage conditions and period have a pronounced effect on the extent of natural antioxidants, which is directly connected with oxidative stability of pasteurized milk and dairy products [58].













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"This course was developed and edited from the open access article: Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge - Khan et al. *Lipids in Health and Disease* (2019) 18:41, (<https://doi.org/10.1186/s12944-019-0969-8>), used under the Creative Commons Attribution License."