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Review article

Antioxidants and Antioxidant Capacity of Human Milk

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SUMMARY

Milk contains plenty of enzymatic and non-enzymatic antioxidant components that probably account for the vital antioxidant protection of the infants at early stages of life against the development of complications induced by oxygen free radicals. Indigenous milk enzymes play a key role in regulating lactogenesis, including active involution of mammary gland. Moreover, they are essential constituents of antioxidation and the innate immune system of milk. Among antioxidant enzymes, superoxide dismutase, catalase and selenium-containing glutathione peroxidase have been demonstrated in human milk. Mainly, the enzyme content of colostrum is higher than that in corresponding mature milk. Beside lipophilic antioxidant in human milk, tocopherols, carotenoids and vitamin A are of great interest. Those components demonstrate the highest levels in colostrum and decline during early lactation, despite the fact that total lipids increase. The complete list of active antioxidant components in human milk is not yet known.

This review reports the main findings of enzymatic and non-enzymatic antioxidants, as well as antioxidant capacity of human milk. Synergism of action of several antioxidants helps to eliminate free radicals in newborns. Bearing in mind that milk contains a number of antioxidants, many reactions are possible and it is difficult to define the exact contribution and function of each antioxidant. Besides qualitative and quantitative analysis of human milk antioxidants, the measurement of total antioxidant capacity could be a useful tool for examination of this dynamic, complex fluid.

Key words: **human milk, enzymatic antioxidants, non-enzymatic antioxidants, oxidative stress, antioxidant capacity**

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INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. They are either radicals that contain at least one unpaired electron or reactive non-radical compounds derived from radicals, capable of oxidizing biomolecules and able to damage all biological macromolecules (1, 2). These ROS are normally produced in living organisms at low but measurable concentrations, and may be beneficial or even crucial in processes such as intracellular signalling and defence against microorganisms. Also, ROS are involved in the cell growth, differentiation, progression, and death (3).

Cells which use oxygen and consequently ROS have to evolve complex antioxidant defence systems to neutralize ROS and protect themselves against free radical damaging (4). When the production of ROS exceeds the capacity of the body's antioxidant defence to detoxify them, a condition known as oxidative stress occurs. High concentrations of ROS can damage all major cellular constituents such as lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids, which leads to functional impairment (5, 6).

At birth, the newborn is exposed to a relatively hyperoxic extrauterine environment caused by an increased oxygen bioavailability with greatly enhanced generation of ROS. As a result, human infant is under oxidative stress due to the difficulty of adapting to ambient oxygen, especially because of antioxidative defence mechanisms which are poorly developed in the neonatal period. It is believed that oxidative stress is involved in the pathogenesis of numerous neonatal diseases (7).

Prematurely born infants, who already have a reduced antioxidant capacity, are exposed to oxidative stress which can often be caused by infections, oxygen, mechanical ventilation, intravenous nutrition or blood transfusions. This is the reason why many of the disorders of prematurely born infants are thought to be the result of this imbalance between oxidative stress and antioxidant capacity (8). Furthermore, breastfeeding has been associated with low rates of a variety of illnesses in premature infants, including necrotising enterocolitis, respiratory disease, and retinopathy of prematurity (9).

The aim of this review was to discuss important antioxidant components of human milk which affect the protection of infants against the development of complications induced by oxidative stress. The measurement of total antioxidant capacity could be a useful tool for examination of this media.

NUTRITIONAL AND BIOCHEMICAL PROPERTIES OF HUMAN MILK

The stages in the continuum of human milk are colostrum (1–5 day postpartum), transitional milk (6–15 days postpartum), and mature milk (after 15 days). The content of human milk gradually changes and its relative content is significant for newborns and their physiologic adaptation to extrauterine life. Thus, there can be a significant difference in the concentrations of major constituents of milk, such as fat and protein. For example, the total lipid content notes a significant increase with time of lactation from 1.99 ± 0.25% in colostrum up to 3.89 ±0.28% in mature milk. As for the protein content in colostrum and mature human milk, it decreases from 1.88 ± 0.4% to 1.35 ± 0.3%, respectively. Furthermore, colostrum is rich in protein, fat-soluble vitamins, minerals, and immunoglobulins. During the first few days of life, the role of colostrum for a newborn is not only to provide nutrition, but also to protect it against infections while the immune system is still developing. The ash content is high, and the concentrations of sodium, potassium, and chloride are higher in colostrum than those in mature milk. The content of transitional milk includes high levels of fat, lactose, water-soluble vitamins, and transitional milk contains more calories than colostrum (10).

Milk is the only source of nutrients for the neonatal mammal during the early stage until weaning. Thus, in addition to providing macronutrients (protein, carbohydrate and lipid) and water, the role of milk is also to supply sufficient vitamins and minerals to support the growth of the neonate. Additionally, human milk contains various bioactive compounds including numerous immunologic factors, enzymes, growth factors, and hormones (11). Moreover, human milk contains higher concentrations of scavengers of free radicals than cow's milk (12). The antioxidants are divided into enzymatic antioxidants and non-enzymatic antioxidants (small molecules). Both groups of

antioxidants in human milk exert synergistic actions in scavenging free radical and help to eliminate free radicals in infants (3, 13, 14).

Unlike infant formula, which is standardized within a very narrow range of compositions, human milk composition is dynamic, and varies between feeding, diurnally, over lactation, and between mothers and nations. Further, the composition of this dynamic, multifaceted fluid varies between term and preterm infants.

It has been found that breast milk has many advantages over formula. Breast milk contains hydrophilic as well as lipophilic antioxidants, including certain vitamins and a whole range of antioxidant enzymes. On the contrary, formulas contain only few supplemented antioxidants, for instance, vitamin E (8). The precise balance of several antioxidants in breast milk, rather than any single factor, determines its oxidative stability (15).

ENZYMATIC ANTIOXIDANTS IN HUMAN MILK

Among antioxidant enzymes, superoxide dismutase for dismutation of superoxide anion, catalase for degradation of hydrogen peroxide (H_2O_2) and selenium-containing glutathione peroxidase for destruction of H_2O_2 have been demonstrated in milk (16). Also, breast milk has some supporting enzymes such as glucose-6 phosphate dehydrogenase and glutathione reductase (2). In addition, there are numerous specialized antioxidant enzymes reacting with and, in general, detoxifying oxidant compounds.

Some human milk enzymes have dual functions when it comes to breaking down nutrients into products which can be used: bile salt associated lipase activity releases free fatty acids which have antimicrobial activity; catalase is known for its anti-inflammatory effects because of the degradation of H_2O_2, and glutathione peroxidase is responsible for decreasing inflammation by preventing lipid peroxidation (17).

What distinguishes antioxidant enzymes in human milk from other antioxidant enzymes of different origin is their tertiary structure. It is said that they are more hydrophobic and less sensitive to proteolysis and denaturation, which has to do with their physiological role in the gastrointestinal system of a newborn (18). Generally, the enzyme content of human or ruminant colostrum is higher than that in corresponding mature milk (19, 20) or ruminant milk (21).

Superoxide dismutase (SOD)

Superoxide dismutase catalyses the removal of the superoxide radicals $O_2^{\cdot-}$ and protects cells from potentially harmful oxidizing effects according to the reaction:

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

A coproduct of SOD is H_2O_2, which is converted to H_2O by catalase, glutathione peroxidase, or suitable reducing agents. Hydrogen peroxide produced from $O_2^{\cdot-}$, and directly by specific oxidases, must be eliminated. This is accomplished by catalases and peroxidases (11).

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) (3). SOD inhibits lipid oxidation in model systems. Cow milk contains a low level of SOD (150 times less than in blood), which is present exclusively in the skim milk fraction. The activity of SOD detected in milk corresponds to between 0.15 mg (22) and 2.4 mg of enzyme per litre milk when compared to the activity of purified bovine erythrocyte SOD (23). SOD content in human milk is 2.0 to 2.3 times higher than in bovine milk (24). Savić *et al.* (25) found that SOD activity was higher in human colostrum than in milk.

Catalase (CAT)

Catalase is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa (3). Milk catalase is a hem protein with a molecular weight of 200 kDa, and an isoelectric pH of 5.5; it is stable between pH 5 and 10 but rapidly loses activity outside this range (11).

Most catalases are enzymes which contain heme. They are considered to be responsible for a dismutation reaction. It is a reaction in which one H_2O_2 oxidizes another, so that one is converted to O_2 and the other to two molecules of H_2O (26). The average catalase activity in raw cow milk, determined by a polarographic method, was 1.95 U/ml, (U-

enzymeunit) (27). The level of catalase in human milk is 10 times higher than in bovine milk (28).

Glutathione peroxidase (GSHPx)

GSHPx is a selenium containing enzyme that contributes to protect cells against lipid peroxidation induced damage (29). GSHPx catalyses the decomposition of hydrogen peroxide (H_2O_2) and organic hydroperoxides (R-OOH) by glutathione (γ-Glu.Cys.Gly) according to the following reaction:

$$ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$$

Milk contains a low level of GSHPx, more that 90% of which is the extra-cellular type. The function of this enzyme in milk is not yet fully known, it is the only known enzyme that fixes 30% of total selenium, an important element of diet. It is also known that milk GSHPx content varies among different species (human >caprine> bovine) and diet (30, 31).

GSHPx activity has been detected in raw cow's milk at levels between 12 and 32 U/ml, and its activity correlates significantly with selenium concentration. This suggests that selenium glutathione peroxidase is one of the biologically active forms of selenium in cow's milk (30, 32). Both GSHPx activity and selenium contents of human milk have been shown to decrease with time of lactation and reach a plateau at one month postpartum (33).

NON–ENZYMATIC ANTIOXIDANT IN HUMAN MILK

Non-enzymatic antioxidants can be formed in the animal body or need to be supplied through diet as essential nutrients. Breast milk non-enzymatic antioxidant are glutathione (GSH), arginine, citrulline, taurine, creatine, selenium, zinc, ascorbic acidβ-carotene, coenzyme Q10**,** vitamins E and A. Lacofferin, the iron-binding protein, can function as an antioxidant. Also, there are a few carotenoids which have provitamin A action, but they can also function as antioxidants. Several non-enzymatic antioxidants, such as vitamin E, carotenoids and coenzyme Q10, can take the role of radical scavengers in the lipid phase, while vitamin C acts in the water phase. Furthermore, there are non-enzymatic antioxidants which can react in both lipid and water phase, for instance, flavonoids, which function as radical scavengers as well as metal ion binders (14).

Lipophilic antioxidants such as carotenoids, vitamin A and α-tocopherol demonstrate the highest levels in colostrum and decline during early lactation, despite the fact that total lipids increase. A lipoprotein-associated transfer mechanism is carried out from the blood plasma into the breast milk (34). The yellow colour of colostrum results from β-carotene. Further, colostrum is rich in fat-soluble vitamin A, carotenoids, and vitamin E. The average vitamin A level on the third day can be three times that of mature milk. Similarly, carotenoids in colostrum may be ten times the level in mature milk, and vitamin E may be two to three times greater than in mature milk (35).

Lactoferrin

Lactoferrin is an 80-kDa glycoprotein composed of about 690 amino acid residues involved in innate immune host defences and exhibits a wide range of biological activities. Human breast milk, in contrast to cow's milk, contains a high concentration of this iron-binding whey protein, which may facilitate absorption of iron from human milk. Actually, a major proportion of iron in human milk is bound to lactoferrin, which is capable of binding 2 ferric ions. Lactoferrin is fairly stable against intestinal proteolytic digestion, and binds to specific receptors in human intestinal mucosa (36). Also, human milk lactoferrin has anti-infective properties (37).

In the research performed by Nagasawa *et al.* (38), lactoferrin was measured immunologically in human colostrum and in milk from 2 to 197 days after parturition. Results showed that lactoferrin decreased during the first two weeks, paralleling a decrease in total and in whey protein nitrogen. Then the curve sloped more gradually. Colostrum, transitional milk, and mature milk contained (mean ± standard deviation), (4.9 ± 0.6 mg/ml), (4.5 ± 0.8 mg/ml), (1.6 ± 0.3 mg/ml) of lactoferrin (38).

L-ascorbic acid (Vitamin C)

Ascorbic acid (vitamin C) is considered to be one of the most powerful, the least toxic natural antioxidants. Moreover, ascorbic acid is an effective reducing agent. This nutrient is very important and it is considered to be vital to the health and

development of infants. It is also crucial for the synthesis of collagen, which develop rapidly during the period of infancy (39). It has diverse biochemical functions, being involved in the neurotransmitter formation, carnitine synthesis, and enhancing iron absorption.

Ascorbic acid is readily oxidized at the pH milk. The rate of oxidation is influenced by factors including temperature, light, the concentration of oxygen and the presence of catalytic trace element. Human milk and colostrum contain about 40 to 70 mg/l ascorbic acid, respectively (11). Bates and Prentice (40) estimated the mean L-ascorbic acid content of human milk in 11 studies on unsupplemented women from western countries as 55 mg/l. However, there is a great variation of ascorbic acid content, from 30 to 100 mg/l with a progressive decrease during the course of lactation. The L-ascorbic acid concentration in cow's milk is lower, about 8 to 20 mg/l.

Vitamin E

Vitamin E consists of eight naturally occurring substances (α-, β-, γ- and δ-tocopheroland the corresponding tocotrienols) of varying biological activity. All isomers feature a chromanol ring, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain which allows for penetration into biological membranes. Vitamin E is considered to act primarily as a lipid-soluble antioxidant, protecting polyunsaturated fatty acids and related substances from peroxidation and, thus, from rancidity. Especially in newborns, vitamin E prevents oxidative damage in neonates suddenly exposed to higher oxygen levels than those in intrauterine environment. α-Tocopherol is the most active one, whereas the activity of the other tocopherols is some 70-90% less (40, 41). However, some recent studies have shown that there are some other vitamin E isoforms which are crucial for human health. Despite the fact that little is known about the physiological role of some isomers, it has been proposed that γ-tocopherol is a useful agent which functions as a radical-trapping antioxidant of nitrogen oxide species. It has a preventive role in cardiovascular diseases, as well as in several types of cancer (42).

The concentration of vitamin E (mainlyα-tocopherol) in cow's milk is quite low (0.9 mg/ml) and is higher in summer than in winter milks. Human milk and colostrum have somewhat higher concentrations of this compound, (~3 and 13 mg/l, respectively) (11, 41). Macias and Schweigert (34) examined colostrum, transitory and mature milk and found that α-tocopherol levels (11.8 ± 6.3 μg/ml) were the highest in colostrum and declined significantly during the first weeks of lactation (2.7 ± 1.1 μg/ml).

Moltó-Puigmartí *et al.* (42) found (Table 1) that concentrations of tocopherols isomers in colostrum were much higher than in mature milk. This trend is in accordance with previous study, which also reports a significant decrease in tocopherol concentration with the progression of lactation. These findings show that early breastfeeding would improve the vitamin E intake of neonates, in particular because vitamin E does not cross the placenta in appreciable amounts.

Table 1. **Mean δ, β + γ and α-Tocopherol content in colostrum and mature human milk samples obtained from 10 Spanish women (Date from Moltó-Puigmartí et al. with modifications (42))**

Sample	δ-tocopherol[a]	β + γ-tocopherol	α- tocopherol
Colostrum	0.14* ± 0.09	0.90* ± 0.53	37.84* ± 24.52
Mature milk	0.03 ± 0.03	0.23 ± 0.17	3.39 ± 2.12

[a]*Values are expressed as μg tocopherol/ml milk ± SD*

**Significantly different from mature milk (p≤0.005)*

In the study of Quiles *et al.* (43), coenzyme Q_{10}, α-, γ- and δ-tocopherol, fatty acids and the total antioxidant capacity of the milk of preterm and full term infants were analyzed. Concentrations of α- and γ-tocopherols were higher in the full term group and decreased over time. Studies carried out by Ortega *et al.* (44) have pointed to inverse correlation between mothers' age and the level of vitamin E in their milk.

Coenzyme Q10 (CoQ10)

Coenzyme Q10 is considered to be an essential component of the mitochondrial respiratory chain for adenosine triphosphate (ATP) synthesis. CoQ10 is an intracellular antioxidant that protects membrane phospholipids, mitochondrial membrane protein, and low-density lipoprotein from free radical-induced oxidative damage. CoQ10 is present in breast milk with higher concentration in mothers of full-term infants in comparison to preterm infants and decreases through lactation (43).

Niklowitz *et al.* (45) studied CoQ10 milk concentration in colostrum between 24 to 48 hours postpartum, and again at 7 (transitional milk) and 14 days postpartum (mature milk). At those times, reported CoQ10 levels were 1.1, 1.0 and 0.9 mg/l, respectively. The highest concentrations of fat-soluble CoQ10 are found in human colostrum, despite the lipid content being low.

Carotenoids

Vitamin A represents a micronutrient of great importance to health. In their study, Dimenstein *et al.* (46) drew attention to its involvement in reproduction, the visual cycle and the differentiation of cells, and it also has its effects on physiological processes such as growth, fetal development and the immune system integrity. Since it plays an important part in embryo development and normal differentiation of epithelial tissues, vitamin A is crucial during periods of growth and development, that is, gestation and lactation.

There are two ways in which vitamin A can be transferred from a mother to her child: the first is via the placenta during gestation, and the second via mammary gland during lactation (through breast milk). The latter is quantitatively more significant, and normally, 60 times more vitamin A is transferred from mother to infant during 6 months of lactation than it is accumulated by the fetus during 9 months of gestation (47). Colostrum is particularly rich in vitamin A, containing approximately 2 μg/ml of this vitamin, and therefore, it is an excellent dietary source of the vitamin during the infant's first days of life (46). In addition, vitamin A in human milk is uniquely well absorbed, partly because of the presence of a lipase in milk that helps the infant to digest the vitamin (47).

An important source of vitamin A for infants comes from breast milk carotenoids which can protect nursing infants from respiratory and gastrointestinal infections. Due to its provitamin A activity, a lot of attention has been paid to human milk β-Carotene, but several other dietary carotenoids can also serve as the source of provitamin A, for instance α-carotene, β-cryptoxanthin, and γ-carotene (48). The levels of total carotenoids (236.7 ± 121.9 ng/ml) and vitamin A (1.02 ± 0.56 μg/ml) were the highest in colostrum and declined significantly during the first weeks of lactation, (63.2 ± 23.3 ng/ml) and (0.33 ± 0.14 μg/ml), respectively (34).

DETERMINATION OF ANTIOXIDANT CAPACITY IN BREAST MILK

The anti-oxidative status of biological samples can be analysed by the determination of single components of the system or by measurement of total antioxidant capacity. Due to the fact that anti-oxidative systems act synergistically, a single analysis may not reflect the total potential. There are many methods to evaluate antioxidant capacity of tested compounds. Two of the widely used detection procedures, which facilitate analysis of various antioxidants, were used in the study of Zarban *et al.* (16). Total antioxidant capacity of samples was measured by Ferric Reducing/Antioxidant Power (FRAP) assay and free radical scavenging activity were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Table 2).

Table 2. Total antioxidant capacity, DPPH radical scavenging activity values for colostrum, transitional and mature milks (Date from Zarban et al. with modifications (16))

	Colostrum	Transitional milk	Mature milk
	2 ± 1 days	**3 days**	**30 ± 3 days**
	(n = 115)	**(n = 97)**	**(n = 102)**
Total antioxidant capacity (μmol/l)[b]	1061.6 ± 500.6[a]	915.3 ± 511.4*	816.3 ± 379.4*
DPPH radical scavenging activity (%) (μmol/l)	50.4 ± 19.7	40.8 ± 20.0*	41.9 ± 19.4*

n - number of samples
[a]Values are presented as Mean ± SD
[b]Significantly different from mature milk (p≤0.005)
**Results were expressed as FRAP value (μM Fe (II)) of the samples*

Total antioxidant capacity was obviously higher in colostrum than in transitional and mature milk. In the DPPH test for radical scavenging activity, colostrums were more potent (50.4 ± 19.7%) to reduce stable DPPH radical in comparison to transitional and mature milk. There was a high significant correlation between the results of these two methods.

The antioxidant capacity (AC) of human milk obtained from 60 breastfeeding women at one month postpartum was analysed by the oxygen radical absorbance capacity (ORAC) assay. The average milk AC value was 3.41 ± 0.07 μmolTE⁄ml (Table 3). From the tocopherols, α-Tocopherol was the major isomer found in milk with a mean value of 2.32 ± 0.11 μg⁄ml milk. Relatively small amounts of all-*trans*-retinol were also present, and the average fat content of milk samples from different subjects was 3.12 ± 0.12%. AC of human milk was significantly attributed to the presence of milk α-tocopherol, emphasizing the importance of this particular antioxidant vitamin, a finding complementary to the important role of vitamin E as a radical scavenger in many other biological systems (49). Levels of CoQ10 and α- and γ-tocopherols in human milk directly correlate with the antioxidant capacity of the milk (50).

*Table 3. Vitamin A and E, fat contents and antioxidant capacity of human milk**
(Date from Tijerina-Sáenz et al. with modifications (49))

Human milk variable	Concentration
all-*trans*-retinol (μg⁄ml)	0.08 ± 0.01
α-Tocopherol (μg⁄ml)	2.32 ± 0.11
δ-Tocopherol (μg⁄ml)	0.11 ± 0.01
γ-Tocopherol (μg⁄ml)	0.46 ± 0.03
AC (μmol TE⁄ml)	3.41 ± 0.07
Fat (%)	3.12 ± 0.12

TE= trolox equivalents
**Values are presented as Mean ± SD, n = 60*

CONCLUSION

The oxidative stress is involved in the pathogenesis of numerous neonatal diseases. Given the growing role of oxidative stress in newborn preterm morbidity, one of the goals is to minimize free radical production and promote the development of adequate antioxidant systems through the adequate nutrition.

The complete list of active antioxidant components in human milk is not known, but lactoferrin, ascorbic acid, (α-, β-, γ- and δ-tocopherol), carotenoids and coenzyme Q10 have been found, as well as antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. The antioxidants in breast milk exert synergistic actions in scavenging free radical and help to eliminate free radicals in a newborn. Lactoferrin, ascorbic acid, and lipophilic antioxidants demonstrate the highest levels in colostrum and decline during lactation. The enzyme content of colostrum is higher than that in the corresponding mature milk. As a result of those findings, total antioxidant capacity was higher in colostrum than transitional and mature milk.

Human milk is essential for protecting a newborn against oxidative stress. The precise mechanisms for this protection are not yet clear, however, this potential appears to be heterogeneous. This review provides ample evidence to strongly support the exclusive breastfeeding, whenever possible.

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Antioksidansi i antioksidativni kapacitet humanog mleka

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SAŽETAK

Mleko sadrži mnoge enzimske i neenzimske antioksidanse koji verovatno utiču na vitalnu antioksidativnu zaštitu dece u ranoj fazi života od razvoja komplikacija izazvanih slobodnim kiseoničnim radikalima. Enzimi koji se prirodno nalaze u mleku imaju ključnu ulogu u regulaciji laktogeneze, uključujući aktivnu involuciju mlečnih žlezdi. Štaviše, oni su esencijalni činioci antioksidativnog procesa i urođenog imunog sistema mleka. Među antioksidativnim enzimima u humanom mleku određeni su superoksid dismutaza, katalaza i glutation peroksidaza koja sadrži selen. Uglavnom je sadržaj enzima u kolostrumu veći od sadržaja u odgovarajućem zrelom mleku. Od lipofilnih antioksidanasa u humanom mleku, od najvećeg značaja su tokoferoli, karotenoidi i vitamin A. Sadržaj ovih komponenti je najveći u kolostrumu, koji zatim opada tokom rane laktacije, bez obzira na činjenicu da sadržaj masti raste. Svi aktivni antioksidansi u humanom mleku još uvek nisu poznati.

U ovom radu su prikazana najznačajnija saznanja o enzimskim i neenzimskim antioksidansima kao i o antioksidativnom kapacitetu humanog mleka. Sinergističko delovanje pojedinih antioksidanasa pomaže u eliminaciji slobodnih radikala kod novorođenčadi. S obzirom da mleko sadrži mnoštvo antioksidanasa, mnoge reakcije su moguće, pa je teško odrediti precizan doprinos i funkciju svakog antioksidansa. Pored kvalitativne i kvanititativne analize antioksidanasa humanog mleka, određivanje ukupnog antioksidativnog kapaciteta bi mogao da bude koristan metod ispitivanja ovog dinamičnog, složenog fluida.

Ključne reči: **humano mleko, enzimski antioksidansi, neenzimski antioksidansi, oksidacioni stres, antioksidativni kapacitet**