Int J Pharm Bio Sci 2014 April ; 5 (2) : (B) 400 - 408

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIOXIDANT PROPERTIES OF MILK OLIGOSACCHARIDES FROM VARIOUS RUMINANTS

TULIKA ROY[1*] AND DESH DEEPAK[2]

[1]Department of Biochemistry, Babu Banarsi Das University, Lucknow, India
[2]Department of Chemistry, Lucknow University, Lucknow, India

ABSTRACT

Milk is an unique gift of mammals not only to the neonates but to the entire mankind. Milk is the most advantageous and complete form of nutrition to the neonates, providing energy and a variety of nutrients that are very important for the growth and development. Milk contains several oxidative and antioxidative agents. The antioxidative factors include several vitamins, minerals, enzymes and most importantly oligosaccharides. Antioxidants demonstrate the capacity to prevent the formation of reactive oxygen species (ROS) or scavenging hydrogen peroxide because free radicals attack cellular components leading to the oxidation of lipids, proteins and DNA, thus causing structural and functional changes to these molecules so there is a need to reduce oxidative stress or boost antioxidant defenses in the body. Our main objective was to explore and study the comparative antioxidant properties of milk oligosaccharides from various ruminants. Assays have been performed to study the activity relationship of the milk oligosaccharides as possible antioxidants. The result suggests that milk oligosaccharides derived from certain ruminant species could be used as natural antioxidants and further studies can be done to elucidate the role of milk oligosaccharides as a functional food and potential drug.

Key words: Ruminant milk; free radicals; natural antioxidants; antioxidant enzyme.

TULIKA ROY
Department of Biochemistry, Babu Banarsi Das University, Lucknow, India

INTRODUCTION

Milks were collected from various ruminant species such as buffalo, cow, gaddi sheep, camel, yak, and mare which were processed by kobata&ginsburg method crude mixture of oligosaccharides of milk along with other essential milk components like vitamins, minerals, enzymes, were analysed for various bioactivities mainly antioxidant/oxidant. Antioxidant bioactivity was determined as many reactive oxygen species (ROS), including the superoxide radical, hydroxyl radical, hydrogen peroxide, and the peroxide radical, are known to cause oxidative damage to all living systems. Minute changes in the level of ROS inside cells, affect the rate of metabolism, gene expression, post-translation protein alterations and participate in regulating the cycle of cell division and the programming of apoptosis[1,2]. Due to the presence of reactive oxygen species oxidation of DNA occurs resulting in possible mutations[3]. Recent studies and evidences suggests that reactive oxygen species and their subsequent effect on cellular macromolecules play a significant pathological role in some dreadful human diseases such as cancer, atherosclerosis, hypertension, and arthritis[4,5]. Although the human body naturally has an inherently antioxidative system (i.e., superoxide dismutase, glutathione peroxidase, and uric acid) to protect and prevent itself from the damage caused by peroxidants, but after a certain extent our systems are not sufficiently equipped to totally prevent such damages[6]. Hence, the quench of finding natural antioxidants from food sources is increasing remarkably, because it is believed that antioxidants can protect the living system not only from the attack of free radicals but also retard the progress of many chronic diseases, slow down the process of biological oxidation in the human body as well as retarding the lipid oxidative rancidity in foods[7]. Antioxidants from natural sources are more desirable than those chemically produced, because some synthetic antioxidants have been reported to be carcinogenic[8]. Antioxidants from natural sources would be easier to digest, health promoting and can be taken as supplements for a longer period of time to fight oxidative stress caused by prolonged illness minus the side effects of the synthetically created.

Mother,s milk is the ideal and a complete planned food during infancy[9]. Breast feeding is the best mode of nutrition for infants whether premature or term. In certain cases when the neonate is devoid of the mother,s milk then formula milk,s can be the only life saving mode of nutrition. Both mothers' milk and formulas contain macronutrients, vitamins and minerals that support healthy growth and development of the infants[10] however, strong evidences suggests mother,s milk specially the first milk known as the colustrum is best for the infants which provides better protection for premature infants and normal infants against various oxidative stress[11]. But in special cases when the neonate is devoid then mother,s milk id replaced by formula milk,s which are formulated from certain ruminant species which matches the nutritional value of human milk to remarkable extents. Human milk contains antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase[12] and non-enzymatic antioxidants lactoferrin, ascorbic acid, and vitamin E that helps the premature infant to cope with ROS-mediated diseases. Cu/Zn-SOD is found in human milk[12, 13]. Cu/Zn-SOD is very resistant to various types of denaturing stress including heating and commercial pasteurized milk retains its SOD activity at a similar level to unpasteurized milk[14]. CAT is one the most heat-labile enzymes of human milk with most of its activity being destroyed by treatment at 72^0 C for 15 sec[15]. Glutathione peroxidase (GSHPx) removes H_2O_2 and other peroxides. GSHPx activity is lost by heating at 80^0C for 10 min[16]. Lactoferrin (Lf) is a member of iron-binding transferrin protein family that inhibits the formation of ROS by binding iron and thus attenuating the conversion of hydrogen peroxide into hydroxyl radical via the Fenton type reaction. Lf is abundant in human milk[17]. Vitamin C is a water-soluble anti-oxidant and a free radical scavenger, able to moderate the oxidative stress effects of various diseases[18]. Vitamin E functions primarily as

an antioxidant. It consists of eight vitamins (related chemical substances that fulfill the same specific vitamin function) four tocopherols and four tocotrienols. a-Tocopherol, the form of vitamin E in human milk is an important fat-soluble antioxidant that acts as a radical scavenger[13]. Both human milk and formulas (F) contain vitamin C and E.

In the human body, lipid peroxides are toxic and capable of damaging most of the body cells. The process of lipid peroxidation is initiated by an attack upon a fatty acid or fatty acyl side chain by any chemical species that features sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain. The resulting lipid radicals then undergo molecular rearrangement, followed by reacting with oxygen to produce peroxyl radicals, which are capable of abstracting hydrogen from adjacent fatty acid side chains and thus propagating a chain reaction of lipid peroxidation[4]. The in vitro trials results demonstrated the highest total antioxidant capacity (TAC) in goats' milk known for its great therapeutic value and easy digestion, especially from Prisca breed. Ex vivo trials showed that Prisca goats' milk inhibits platelet aggregation at lower amounts than milk from other species[19]. Milk fat contains a number of individual components that can be described as having anticarcinogenic properties. In particular, conjugated linoleic acid (CLA) and sphingomyelin have been suggested to have important anticancer properties[20, 21, 22, 23]. McIntosh et al.[24] demonstrated a protective role for dietary dairy proteins against tumour development, showing that dietary whey protein and casein were more protective against the development of intestinal cancers in rats than was red meat or soy bean protein. They concluded that dietary proteins differ in their ability to protect against cancer development and that the proteins in dairy foods, particularly the whey proteins, appear to play a significant role in cancer prevention[24]. Daily ingestion of foods containing peptides with potent ACE-inhibitory activities may be effective at keeping the human blood pressure low. Praveesh et al.[25] have shown that an angiotensin I-converting enzyme-inhibitory cow milk hydrolysate and its *in vitro* antioxidant and anticancer activity. The aim of this study was to investigate the antioxidant properties of different ruminant's milk. The LPO, SOD and catalase assay; antioxidative enzyme activities were used to determine the antioxidant properties of various milks of different ruminants as each ruminant has its own unique property and the antioxidative agents present can give rise to a new horizon of natural antioxidants in the field of neutriceuticals

MATERIALS AND METHODS

Reagents and Consumables

All the specified chemicals and reagents were purchased from Sigma (Sigma St Louis, MO, USA) unless otherwise stated. Culture wares and other plastic wares used in the study were procured commercially from Nunc, Denmark. Milli Q water (double distilled deionized water) was used in all the experiments.

Isolation of milk oligosaccharides

Milk oligosaccharides have been isolated by using the protocol of Kobata and Ginsberg[26]. In brief, 1000 ml of milk was collected and stored at -20^0 C and centrifuged for 15 min at 5000 rpm at 4^0 C. The solidified lipid layer was removed by filtration through glass wool column in cold. Ethanol was added to the clear filtrate to a final concentration of 68% and the resulting solution was left overnight at 0^0 C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0^0 C. The supernatant and washings were combined and filtered through a micro filter to remove remaining lactose and lyophilized affording crude oligosaccharide mixture. The lyophilized material (mixture of oligosaccharides) was further purified by fractioning it on sephadex G-25 column using glass double distilled water as eluant at a flow rate of 3 ml/min. Each fraction was analyzed for the presence of sugars by phenol sulphuric acid reagent.

Estimation of lipid peroxidation (LPO) levels

Lipid peroxidation (LPO) was performed using the thiobarbituric acid reactive

substances (TBARS). In brief, 0.2 ml of saliva was taken and added with 2 ml of thiobarbituric acid reagent (15% TCA, 0.7% TBA, and 0.25 N HCl) and heated at 100^0 C for 15 min in a water bath. The sample were then placed in cold and centrifuged at 1000xg for 10 min. Absorbance of the supernatant was measured at 535 nm using a microplate reader (Synergy HT, BioTek, USA). The data are expressed as nmol MDA/ml.

Superoxide dismutase (SOD) activity

The activity was measured following the protocol described earlier by Mc Cord and Fridovich[27]. I'm brief, the experiment was carried out in two setups. In one setup, 1.1 ml pyrophosphate buffer, 0.2 ml NBT, 0.2 ml PMS and 20 µl enzyme source were taken. Second setup received all the above reagents minus the enzyme source. The reaction was started simultaneously in two sets by the addition of 0.2 ml NADH. After a interval of 90 seconds, 0.5 ml glacial acetic acid was taken to each tube for checking the reaction, after this same amount of enzyme source was added in reference tubes. The absorbance was read at 560 nm using a microplate reader (Synergy HT, BioTek, USA) against reagent blank. Difference between reference and experimental optical density (OD) gives the inhibition of NBT reduction by an enzyme source. Protein was also estimated in enzyme source. The unit of SOD enzyme activity was defined as the amount of enzyme required to inhibit the OD at 560 nm of NBT reduction by 50% in one minute under the assay conditions.

Estimation of catalase levels

The experimental setup for catalase activity was quite similar to the LPO. The activity was measured using commercially available kit for catalase activity (Catalog no. 707002; Cayman Chemicals, USA) following the protocol provide by the manufacturer. In brief, 100 µl of assay buffer (supplied in the kit), 30 µl of methanol, and 20 µl of sample were mixed in the 96-well plate. Reaction was initiated by adding 20 µl of hydrogen peroxide (0.882 M) and incubated in shaker for 20 min at room temperature. Reaction was stopped by adding 30 µl of potassium hydroxide. Then chromogen (30 µl) was added and incubated for 10 min followed by addition of potassium periodate (10 µl). The plates were kept at room temperature for 5 min and read at 540 nm using multiwell micro plate reader (Synergy HT, BioTek, USA).

Statistical analysis

The results are expressed as mean and standard error of means (Mean±SE) for at least three experiments. One way ANOVA followed by post hoc Tukey's test was employed to detect differences between the groups of treated and control. $P < 0.05$ was taken to indicate significant differences.

RESULTS

Estimation of lipid peroxidation (LPO) levels

A significant decrease in the levels of LPO was found in sheep and yak i.e. 5.60±0.11 and 5.082±0.16; 1 mg/500 µl saliva group respectively as compared to controls. No significant changes were observed in the levels of LPO in camel and mare. No significant changes were found in the levels of LPO in 2 mg/500 µl saliva groups.

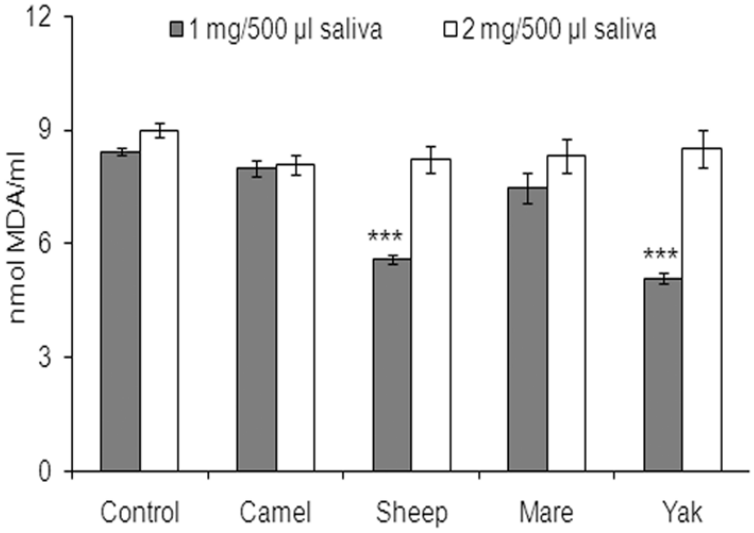

Figure 1
Levels of lipid peroxidation in different ruminants.
Values are given as mean ± SE of the data obtained from three independent experiments.
****= p < 0.05, **= p < 0.01 and ***= p < 0.001.***

Superoxide dismutase (SOD) activity

A significant increase in the activity of superoxide dismutase in Mare (15.82±0.51; 1 mg/500 µl saliva group) was observed as compared to controls. However, a significant decrease in the activity of SOD was observed in camel (7.54±0.14; 1 mg/500 µl saliva group) and yalk (6.10±0.21; 1 mg/500 µl saliva group) as compared to control but no significant changes were observed in the activity of SOD in sheep. No significant changes in the activity of SOD were found in 2 mg/500 µl saliva group.

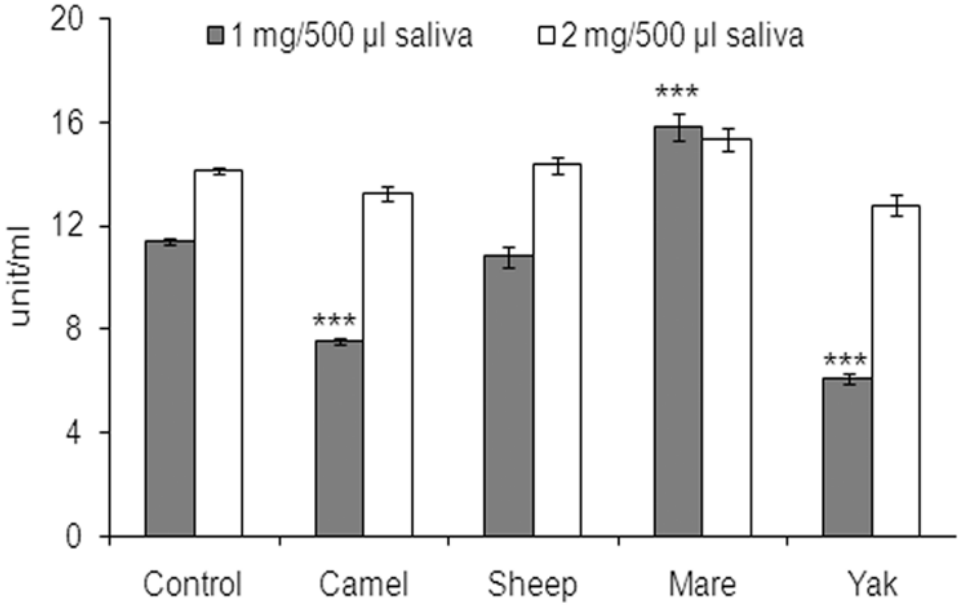

Figure 2
Levels of superoxide dismutase in different ruminants.
Values are given as mean ± SE of the data obtained from three independent experiments.
****= p < 0.05, **= p < 0.01 and ***= p < 0.001.***

Estimation of catalase levels

A significant increase in the levels of catalase was also found in Mare (0.87±0.08; 2 mg/500 µl saliva group) as compared to controls. However, a significant decrease in the levels of catalase was observed in camel (0.15±0.01; 2 mg/500 µl saliva group) and yalk (0.33±0.03; 2 mg/500 µl saliva group) as compared to control but no significant changes were observed in the levels of catalase in sheep. No significant changes were found in the levels of catalase in 1 mg/500 µl saliva groups.

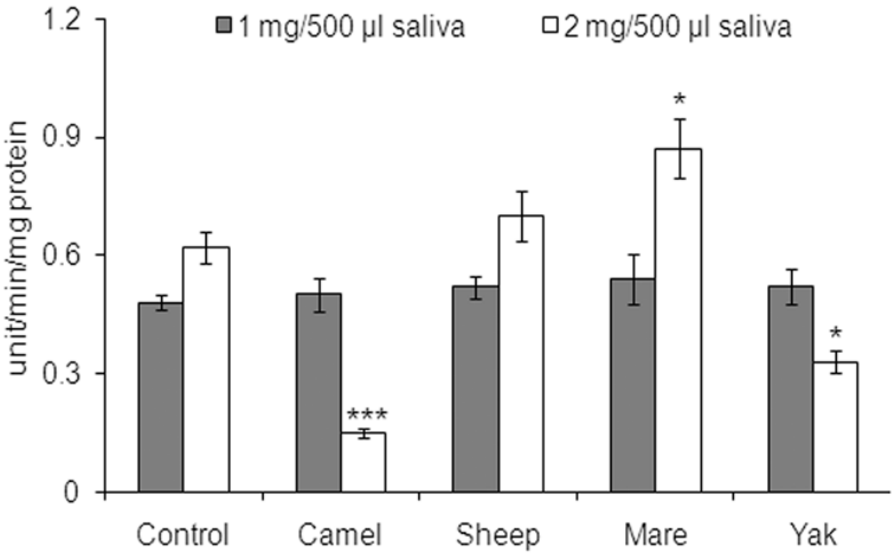

Figure 3
Levels of catalase in different ruminants.
Values are given as mean ± SE of the data obtained from three independent experiments.
****= p < 0.05, **= p < 0.01 and ***= p < 0.001.***

DISCUSSION

Intake of milk oligosaccharides was found positively associated with health promoting factors and lower chances of many diseases analyzed by epidemiological studies and clinical trails. Antioxidant capacity was believed to be the antidote of various dreadful and incurable diseases like Cancer, AIDS and tumors. Antioxidant capacity of various milk oligosaccharides are demonstrated be collection of milk samples from various ruminants like cow, yak, buffalo, camel, mare, gaddi sheep and was processed by Kobata and Ginsburg method and then the crude oligosaccharides was assessed for their bioactivities. When an excess of free radicals is formed they can analyze protective enzymes like superoxide dismutase, catalase and peroxidase cause destructive and lethal cellular effects for examples apoptosis. It has been known that oxidation also plays a significant role at the cellular level, in addition oxidative stress is known to cause age related diseases. The factors involved in these diseases are the lipid peroxides and it has been observed increased oxidative damage leads to incurable diseases like Alzheimer's in human. Cancer is probably a consequence of DNA damage. Various antioxidative agents are artificially induced to lower the lipid oxidation rate but natural antioxidants like milk oligosaccharides could also be used and to inhibit the oxidation rate. The effect of various ruminant milks such as cow, camel, buffalo, mare sheep show oxidant/antioxidant properties against markers such as MDA, SOD and catalase in Assimilated saliva sample which was assessed spectrophotometrically. The results indicated a significant decrease in the concentration of saliva in both the groups (1

mg/500 µl saliva and 2 mg/500 µl saliva) as compared to the control group of the samples. More significant decrease in MDA level was found in sheep and chauri cow crude oligosaccharide mixture as compared to the control in 1 mg/500 µl saliva. No significant change was observed in camel and mare MDA activity. In group 2 mg/500 µl saliva, there was difference found. All compounds (camel, sheep, mare, chauri cow) showed a variation, it could be assessed that the protective effect of milk against oxidative stress in all groups was due to its antioxidant properties. Camel milk in group 2 mg/500 µl saliva and sheep & chauri cow milk in group 1 mg/500 µl saliva was found to contain high concentration of antioxidant property. Vitamins and enzymes found in milk act as antioxidants and have been found to be useful in decrease of oxidative stress. It has been previously reported that proteins deriving from dairy products reveal some antioxidant potential[28]. Pena-Ramos and Xiong[29] found that peptides deriving from milk protein hydrolysates inhibited lipid oxidation, suggesting that the specific amino acid residue side-chain groups or the specific peptide structure of the antioxidative peptides may be attributable to chelation of prooxidative metal ions and termination of the radical chain reactions. The antioxidant assays like SOD and Catalase in groups 1 mg/500 µl and 2 mg/500 µl saliva was found antioxidant specially the mare milk showed more antioxidant property in both groups 1 mg/500 µl and 2 mg/500 µl saliva in different concentrations. The catalase assay camel, sheep, mare, chauri cow showed less significant changes when compared to the control in group 1 mg/500 µl and 2 mg/500 µl saliva. SOD scavenging capacity of saliva is considered very important in reducing the oxidative stress caused by day to day oxidation .The elevation of antioxidant assay like SOD and Catalase in milk of ruminants may be due to the association with a loss of balance between pro oxidation. It has been found that oligosaccharides present in milk have positive health promoting factors which not only help against oxidative stress but also have remarkable immune stimulant properties. It has been reported previously that some milk-derived proteins and peptides demonstrate some level of antioxidative activity[30]. Wong and Kitts[28] found that the reducing activity of certain buttermilk solids was mainly attributed to the sulfhydryl content of the group and that free hydroxyl groups could have also contributed, in part, to the observed reducing activity. Further, it has also been previously reported that some lactic acid bacteria may exhibit excellent reducing power[31]. Highly reactive free radicals formed by exogenous chemicals or endogenous metabolic processes in the human body or in food systems are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Almost all organisms are well-protected against free-radical damage by antioxidative enzymes such as SOD, catalase, peroxidase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, GSHPx, and GR. Among a number of different antioxidative enzymes, catalase, GSHPx, and SOD have been demonstrated to be present in milk[13]. Catalase is one of the most heat-labile enzymes known, with most of the activity of the enzyme being destroyed by even modest heat treatment. In vitro antioxidant activities of cow milk were studied using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging and total reducing power assay. The observed result showed that cow milk fermented with the combination of Lactobacillus plantarum and Lactobacillus casei have good antioxidant property. Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and biological systems[25]. The reduction of DPPH absorption is indicating the capacity of the milk hydrolysate to scavenge free radicals, independently of any enzymatic activity. The antioxidant activity of several species and strains of milk bacteria contained in fermented milk can significantly affect human health. This has been confirmed also by clinical studies of milk fermented with a starter culture Lactobacillus fermentum ME-3[32, 33]. Our pilot study of various ruminant milks suggested that various milks can be used as natural antioxidants, therapeutics, nutricueticals and most importantly potential drugs to existing dreadful diseases such as tuberculosis,

tumors. Studies further suggests that mare milk may be contain more antioxidative agents than cow, buffalo, chauri-cow, gaddi sheep which are equally biologically active and health promoting.

CONFLICT OF INTEREST

We have no conflict of interest with anybody working in the area and among the authors in the manuscript.

Funding

Financial support from Babu Banarasi Das College Of Dental Sceinces, Department of Biochemistry, Babu Banarsi Das University, Lucknow, India.

ACKNOWLEDGEMENT

The authors are grateful to the Vice chancellor, Babu Banarsi Das University, Lucknow, for his support. Thankful to the Dean Dental college Dr Shobha tandon & to Dean PhD Dr Ahmed Ali Babu Banarasi Das University, Lucknow, India for his constant encouragement. Dr Desh Deepak Assistant Professor, Lucknow University, Lucknow, India for his constructive critisism , help and advice without whom this paper would not have been possible. Dr.AB Pant, Sr. Scientist, CSIR-Indian Institute of Toxicology Research , Lucknow, India for his able guidance and support in my study. Last but not the least I would like to thank Babita Singh and Vinay Kumar Tripathi for their unending support and valuable help at every stage of this study of milk oligosaccharides.

REFERENCES

1. Sliwa-jóźwik A., Jóźwik A., Kołataj A., Influence of exogenous glutathione [GSH], as stress factor, on the activity of lysosome enzymes in some organs of mice. Archiv fur Tierzucht-Archives of Animal Breeding, 45 (3): 307-314, (2002)
2. Crujeiras AB., Parra D., Milagro FI., Goyenechea E., Larrarte E., Margareto J., Martínez JA., Differential expression of oxidative stress and inflammation related genes in peripheral blood mononuclear cells in response to a low-calorie diet: a nutrigenomics study. Omics: A Journal of Integrative Biology, 12: 251-61, (2008)
3. Poulsen HE., Oxidative DNA modifications. Experimental and Toxicologic Pathology, 57: 161-169, (2005)
4. Halliwell B., Gutteridge JMC., Oxygen toxicity, oxygen radicals, transition metals, and disease. Biochem J, 219: 1-4, (1984)
5. Frenkel K., Carcinogen-mediated oxidant formation and DNA damage: Pharmacol Ther, 53: 127-166, (1992)
6. Simic MG., Mechanisms of inhibition of free-radical processed in mutagenesis and carcinogensis. Mutat Res, 202: 377-386, (1988)
7. Pryor WA., The antioxidant nutrients and disease preventions what do we know and what do we need to find out? Am. J. Clin. Nutr, 53: S391-S393, (1991)
8. Imaida K., Fukushima T., Shivai T., Ohtani M., Nakanishi K., Ito N., Promoting activities of butylated hydroxyanisole and bultylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of ç-glutamyl transpeptidase-positive foci development in the liver of rats. Carcinogenesis, 4: 895-899, (1983)
9. Anderson GH., Human milk feeding. Pediatr Clin North Am, 32: 335-353, (1985)
10. Turoli D., Testolin G., Zanini R., Bellù R., Determination of oxidative status in breast and formula milk. Acta Paediatr, 93: 1569-74, (2004)
11. Friel JK., Martin SM., Langdon M., Herzberg G., Buettner GR., Human milk provides better antioxidant protection than does infant formula. Pediatr Res, 51: 612-8, (2002)
12. L'Abbe M., Friel JK., Glutathione peroxidase and superoxide dismutase

concentrations in milk from mothers of premature and full-term infants. J Pediatr Gastr Nutr, 31: 270-4, (2000)
13. Lindmark-Mansson H., Akesson B., Antioxidative factors in milk. Br: J. Nutr, 84: S103-S110, (2000)
14. Asada K., Occurrence of superoxide dismutase in bovine milk. Agric Biol Chem, 40: 1659-60, (1976)
15. Hirvi Y., Griffiths MW., Milk catalase activity as an indicator of thermization treatments used in the manufacture of cheddar cheese. J Dairy Sci, 81: 338-45, (1998)
16. Hojo Y., Selenium concentration and glutathione peroxidase activity in cows milk. Biol Trace Elem Res, 4: 233-9, (1982)
17. Raghuveer TS., Mcguire EM., Martin SM., Lactoferrin in the preterm infants' diet attenuates iron-induced oxidation products. Pediatr Res, 52: 964-72, (2002)
18. Halliwell B., Gutteridge JM., Cross CE., Free radicals, antioxidants, and human disease: Where are we now? J Lab Clin Med, 119: 598-620, (1992)
19. Yannis S., Apostolos M., Ioannis V., Angela-Gabriella D., Pasquale L., Emilio J., Pavlos C., Valantis K., Athina B., Giovanni M., Spyridon K., Antioxidant and anti-platelet properties of milk from goat, donkey and cow: An in vitro, ex vivo and in vivo study. International Dairy Journal, 21(11): 901–906, (2011)
20. Ip C., Chin SF., Scimeca JA., Pariza MW., Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res, 51: 6118-6124, (1991)
21. Ip C., Singh M., Thompson HJ., Scimeca JA., Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res, 124: 615-620, (1994)
22. Ip C., Briggs SP., Haegele AD., Thompson HJ., Storkson J., Scimeca JA., The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcin, 17: 1045-1050, (1996)
23. Dillehay DL., Webb SK., Schmelz E., Merrill AHJr., Dietary sphingomyelin inhibits 1, 2-dimethylhydrazine-induced colon cancer in CF1 mice. J Nutr, 51: 6118-6124, (1991)
24. McIntosh GH., Regester GO., Le RK., Royle PJ., Smithers GW., Dairy proteins protect against dimethylhydrazine induced intestinal cancers in rats. J Nutr, 125: 809-816, (1995)
25. Praveesh BV., Angayarkanni J., Palaniswamy M., Antihypertensive and anticancer effect of cow milk fermented by lactobacillus plantarum and lactobacillus casei. International Journal of Pharmacy and Pharmaceutical Sciences, 3(Suppl 5): 452-456, (2011)
26. Kobata A., Ginsburg V., Oligosaccharides of human milk. II. Isolation and characterization of a new pentasaccharide, lacto-N-fucopentaose 3. J Biol Chem, 244 (20): 5496-502, (1969)
27. McCord JM., Fridovich I., Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem, 244: 6049-55, (1969).
28. Wong PYY., Kitts DD., Chemistry of buttermilk solid antioxidant activity: J. Dairy Sci, 86: 1541-1547, (2003)
29. Pena-Ramos EA., Xiong YL., Antioxidative activity of whey protein hydrolysates in a liposomal system: J. Dairy Sci, 84: 2577-2583, (2001)
30. Ye XY., Ng TB., Purification and characterization of glycolactin, a novel glycoprotein from bovine milk: Life Sci, 66: 1177-1186, (2003)
31. Lin MY., Yen CL., Antioxidative ability of lactic acid bacteria: J. Agric. Food Chem, 47: 1460-1466, (1999)
32. Kullisaar T., Songisepp E., Mikelsaar M., Zilmer K., Vihalemm T., Zilmer M., Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Brit J Nutr, 90: 449–456, (2003)
33. Songisepp E., Kals J., Kullisaar T., Mandar R., Hutt P., Zilmer M., Mikelsaar M., Evolution of the functional efficacy of an antioxidative probiotic in healthy volunteers. Nutr J, 4: 22–31, (2005).