Austin Food Sciences

Open Access

Austin Publishing Group

Rapid Communication

Rapid Assay to Evaluate the Total Antioxidant Capacity in Donkey Milk and in more Common Animal Milk for Human Consumption

Beghelli D[1], Lupidi G[2], Damiano S[2], Cavallucci C[1], Bistoni O[3], De Cosmo A[1] and Polidori P[2]*

[1]School of Biosciences and Veterinary Medicine, University of Camerino, Italy

[2]School of Pharmacy and Health Product Sciences, University of Camerino, Italy

[3]Department of Clinical & Experimental Medicine, University of Perugia, Italy

***Corresponding author:** Polidori Paolo, School of Pharmacy and Health Product Sciences, University of Camerino, Camerino (MC), Italy

Received: February 22, 2016; **Accepted:** March 04, 2016; **Published:** March 10, 2016

Abstract

The milk antioxidants, by preventing lipid peroxidation, maintain milk quality, but they also exert a beneficial effect on the consumer's health, in particular that of infants. Donkey Milk (DM), for its nutritional, functional and bioactive components, seems to be one of the best substitutes of breast milk when the latter is not available. However, there are few data about its antioxidant properties. In this study, the Total Antioxidant Capacity (TAC) of donkey milk was determined by means of an in micro-plate assay. DM samples were analyzed at the first, third and fifth month of the lactation period (n 6/period), comparing results to those obtained in milk of different dairy species (goat, ewes, cows) and in breast milk using the same assay. The lactation periods did not affect the TAC of DM, whereas significant different values ($P<0.001$) were observed between species. The breast milk showed the lowest TAC value, followed by its progressive increase in donkey, cow's, goat's and ewe's milk. The rapid test here adopted can be successfully employed for a reliable monitoring of the TAC in DM and, thanks to the constant antioxidant supply, DM can also be sponsored as a valid alternative to infant milk nutrition.

Keywords: Total Antioxidant Capacity (TAC); Donkey milk; Breast milk; Cow milk; Ewe milk; Goat milk

Introduction

Human Milk (HM) besides being the best source of nutrients, also supplies a complex system of defence factors necessary for the health of growing infants [1,2]. The potential of HM to directly affect oxygen-induced tissue injury in the newborn has been demonstrated by experimental studies in animals [2].

The milk antioxidant compounds also exert a beneficial effect on the consumer's health by giving a potentially greater protection from exposure to the oxidative stress that is recognized as a feature of many acute and chronic diseases [3-5].

Therefore, milk antioxidants, including proteins, carotenoids, flavonoids as well as vitamins such as vitamin E and C, not only carry out important roles in preventing lipid peroxidation which in turn is the underlying cause for generation of hydrolytic off-flavors, but they also could help in reducing the loss of important nutrients and bioactive agents that promote health of offspring or of older consumers [6-8].

When breast milk is not accessible, it is very important that infant nutrition fulfils the right antioxidant requirements to resemble natural feeding as much as possible.

Cow milk is widely employed as a substitute, although it is not routinely fed to human infants as it is, but it needs to be modified into formulas that are more comparable to HM. However, some authors [9] stated that in this adaptation process many factors, including antioxidants, are either absent or poorly represented, as in other artificial feedings. Besides the well known infant formula, in the past few years, Donkey Milk (DM) has gained considerable attention in the Scientific Community due to its nutritional, functional and bioactive components [8].

DM seems to be the best substitute for human milk in infant nutrition [10-13] because it is rich in lactose (the taste of ass' milk resembles breast milk), lysozyme, -3 and -6 polyunsaturated fatty acids [14]; the ash residue is similar to that of human milk and the protein profile is adequate for the correct development of infant digestive tract [15,16]. In particular, ass's milk can be consumed by human infants with multiple food allergies or Cow's Milk Protein Allergy [17-20] and elderly people, because of its ability to up-regulate the immune response [21,22].

Nowadays, what is scarcely known is whether the DM, besides the nutritional characteristics, is as so similar to breast milk to also satisfy the antioxidant requirements of infants? In general, it is particularly difficult to compare the different Total milk Antioxidant Capacity (TAC) values reported in literature because they were obtained by means of diverse analytical methodologies [23] and/or not all the possible substitutes to human milk, produced by the most common dairy animals, were compared for this specific feature [5].

The interest in determining the TAC in milk is increasing since it is able to give an overall picture of the antioxidant potential of this type of food, and also because TAC measurement requires much less work and methodological infrastructure than analyzing the often complex composition of individual antioxidants. This purpose has highlighted the need to develop reliable, easy and fast methods to quantify this property in a basic food such as milk for human development.

Citation: Beghelli D, Lupidi G, Damiano S, Cavallucci C, Bistoni O and De Cosmo A. Rapid Assay to Evaluate the Total Antioxidant Capacity in Donkey Milk and in more Common Animal Milk for Human Consumption. Austin Food Sci. 2016; 1(1): 1003.

The luminescence switch-on detection assay, based on an iridium complex, could represent an interesting alternative method, having shown its potential in monitoring proteins and being time and cost effective [24]. However, the method here adopted evaluates the ability of milk samples to contrast with the massive oxidative action of a powerful and physiological oxidant (hypochlorous acid) and has the advantage to be faster than all the other methods cited in literature, though keeping the reliability of the results [25].

Aims of the present work were 1) to evaluate the total antioxidant capacity of donkey milk in different lactation periods and 2) compare the results with data from different dairy animals (cows, ewes and goats) and breast milk, using the same assay.

Materials and Methods

Sampling and preparation of milk samples

Donkey milk: Eighteen individual milk samples were manually collected from mammary gland in a semi-extensive herd (pasture and integration with concentrate) of the Southern Italy (Ponte Cagnano, Salerno) from 18 pluriparous jennies of different breeds (Ragusana, Amiantina and mixed breeds), age (5-18 years) and lactation period (at one, three or five months; six ass/lactation period), so that the early, middle and late lactation periods were investigated in the same feeding and seasonal conditions. Samples were collected in spring (April 2014) and transferred into a 'mobile' refrigerator to laboratory at 4°C where they were frozen as individual samples at -20°C until analysis (within the first month of storage). Further three different 50 ml bulk milk samples of eight pluriparous donkeys (Martina Franca breed, Az. Agricola Cambiotti, Gualdo Tadino, Perugia, Italy), were collected in two different days and treated as afore mentioned. The TAC values of the latter samples were compared to those obtained in the milk samples of the dairy animals considered in the present study.

Cow milk: Three bulk milk samples were collected in an intensive herd of 130 Frisona cows (primiparous and multiparous together) situated in the Central Italy (S. Eraclio, Foligno), by three different tanks where daily production is stored (pasteurized) at 4°C until it is withdrawn by local dairy transformers. Refrigerated milk samples (milked 3 hours before) were collected in spring (April 2014) and transferred into a mobile refrigerator to laboratory at 4°C where the same were stored at -20°C individually or by combining two samples in turn to form three pools until analysis (within the first month by time of collection), for a total of six samples.

Ewe and Goat milk: Three individual milk samples were manually collected from mammary gland in a semi-extensive herd (pasture and integration with concentrate) of central Italy (S. Maria Rossa, Perugia), by three different ewes (Sardinian, pluriparous animals) or goats (Umbrian local breed, pluriparous animals) at the middle of their lactation periods (April 2014). Samples were treated as referred for cow's samples.

Breast milk: Three individual milk samples were collected at the S. Maria della Misericordia Hospital (Perugia, Italy) in spring (April 2014) from three voluntary women within their first week of lactation. All the milk donors provided written informed consent in accordance with the declaration of Helsinki. The study was approved by the local Ethics Committee (CEAS Umbria).

Milk samples were withdraw (with a mechanical breast pump) from the mothers into sterile flasks for their hospitalized babies and an aliquot (10 ml)/each was kindly given for this purpose. Samples were transferred to laboratory at 4°C into a 'mobile' refrigerator and stored at -20°C until analysis (within the first month by collection).

TAC assay and milk procedure

The TAC assay was performed using the Oxy Adsorbent Test (Diacron International, Grosseto, Italy) and a spectrophotometric plate reader (FLUO star Omega, Multi-mode microplate reader BMG Labtech, Ortenberg, Germany) at 546 nm wavelength.

This test, normally used on a serum or plasma matrix, was adapted to a microplate assay by Brambilla et al. [26]; whereas, Bianchi et al. [25] reported its use in the evaluation of milk antioxidant activity. Each milk sample was tested in triplicate. Briefly, the TAC assay evaluates the ability of samples to contrast with the massive oxidative action of a Hypochlorous acid (HClO) solution and TAC values are expressed in μ mol neutralized HClO/ml of sample. Bilirubin, uric acid, vitamins C and E, albumin and in general, the macromolecular complexes (e.g. as glycoproteins) that act as shock absorber against free radicals, help to buffer the oxidizing action of hypochlorous acid. HClO was selected among other oxidant agents because it is not only a powerful but also a physiological oxidant. As soon as the 'free' HClO reacts with a correctly buffered chromogenic substrate (N,N-diethylparaphenylendiamine), a colored complex develops. The optical density generated by the colored complex is directly proportional to the concentration of HClO and indirectly related to the antioxidant capacity.

In the present study the micro-plate assay has been slightly modified. In particular, the optical densities at the beginning of the assay (absorbance blank: A blank 0) were subtracted to the values obtained after the incubation period (10 minutes) and the suggested sample dilutions (1:100in distilled water) for blood samples were reduced to 1:75, for whole milk samples.

Statistical analysis

The data were analyses using the GLM procedure of SPSS® 13 (Chicago, IL, SPSS Inc 2004). An ANOVA model, with the dairy animal milk/breast milk as fixed variable, was used. For the DM samples collected in three different lactation periods, the sampling time was also included as a fixed effect. Data were reported as least squares means and Standard Error (SEM). Differences were considered to be significant when $P \leq 0.05$.

Results and Discussion

The TAC of DM did not significantly vary during the different lactation periods evaluated (Table 1) and these results could be an important index of good nutritional quality of donkey's milk. Milk TAC values obtained from different dairy animals and breast milk (Table 2). Goat's and ewe's milk showed significantly higher TAC values compared to that from donkey, cow's and human milk, being the latter significantly lower.

Although to measure all the antioxidant activity present in biological fluids, various methods have been devised, the essential features of any test are a suitable substrate, an oxidation initiator, and

Table 1: Total Antioxidant Capacity (TAC), expressed as μ mol neutralized HClO/ml of sample, evaluated in donkey milk at different lactation periods (at the first, third or fifth month).

Lactation period				
Month	1st (n =6)	3rd (n =6)	5th (n =6)	P<
TAC (μ mol neutralized HClO/ml sample)	221.7	219.1	210.9	n.s.
SEM	9.7	21.2	16.8	

Table 2: Milk Total Antioxidant Capacity (TAC) evaluated in different dairy animals and in breast milk.

TAC (μ mol neutralized HClO/ml sample)						
	Donkey milk	Ewes milk	Cow milk	Goat milk	Breast milk	P<
	(n =6)	(n =6)	(n =6)	(n =6)	(n =3)	
WM	213.6[a]	294.6[b]	226.2[a]	281.2[b]	140.1[c]	0.001
SEM	4.9	6.5	9.2	6.5	10	

Note: [a,b,c]Post Hoc Test: Multiple comparisons. The mean difference is significant at the 0.05 level.

an appropriate measure of endpoint. Therefore, these aspects should be taken into consideration when selecting a test for measuring antioxidant activity related also to the model food system used for the test.

As far as our knowledge, there are no available data in literature comparable to our results: that is because both the number of 'dairy' species enrolled in the study and the type of assay utilized were different. Bianchi et al. [25], with the same assay we adopted, obtained TAC values slightly lower than our in ewe's milk; but they tested only this specie.

However, the TAC values obtained in this study partially agree with results reported by others, which used, as a measure of TAC, the 'Ferric Reducing Antioxidant Power' (FRAP) method, adapted to milk [27,28].

Indeed, although they observed that the milk TAC values were within the same range in cow and sheep, the glutathione peroxidase values were remarkably higher in sheep colostrum/milk compared to those found in cow colostrum/milk. According to Stangsted [29], the glutathione peroxidase activity appears to have any functional relevance for the oxidative stability of bovine milk and it could at least in part explain the lower TAC of cow's milk *vs.* ewe's ones.

Simos et al. [5] observed that a particular Greek goat race (Prisca) had a higher milk TAC than cows and donkey and they measured the total antioxidant milk capacity by the Blue CrO_5 assay.

However, in the same study, Sannen and Ionica goats showed an antioxidant capacity of milk comparable to that of donkey milk.

Other factors than breed/genotype may affect milk TAC properties. For instance, as natural pasture, feeding and season influence nutritional composition of milk of several species [23], the same factors could also affect its antioxidant properties. Indeed, by varying the food intake of vitamins, polyunsaturated fatty acids and trace minerals, acting these nutrients as antioxidants or important covalent factors of antioxidant enzymes (such as gluthatione peroxidase and superoxide dismutase), the antioxidant properties of milk could vary [30-34].

Zulueta et al. [34] have provided evidence that the major contributors of TAC in whole milk are the casein fractions and the hydrophilic antioxidant compounds, such as vitamin C and uric acid, in the deproteinised milk. Total casein content is similar in cow milk and goat milk but their fraction composition differs to a great extent since the major casein fraction of cow milk is a αS1 - casein and of goat milk is b1- and b2-caseins [5,36]. In literature there are some reports on the antioxidant properties of caseins [37-40] but, as far as we know, it has been not investigated yet whether the different types of casein correspond to a different total milk antioxidant capacity. According to Oner et al. [41] goat milk contains the highest concentrations of scavengers of free radicals than other and this aspect could contribute to explain the different milk antioxidant properties in different dairy species. According to Bucevic Popovic et al. [39] that studied the antioxidant activity of different milk components (fat, casein, whey) from cow, goat, sheep and donkey milk, the fat isolated from the milk of cow and donkey exhibit the highest oxidative stability. However, the asinine whey exhibit also a radical scavenging activity comparable with the strong synthetic antioxidant butylated hydroxyanisole and butylated hydroxytoluene. In our study, the donkey milk showed a TAC lower than that of goats and ewes but similar to that one of bovine milk and, most important, higher than the one of breast milk. Furthermore, the lower TAC observed in breast milk agrees with the report of Oner et al. [41]. The nutrient and antioxidant compositions of breast milk are affected by many factors such as the dietary intake of nutrients by the mother, the different geographic areas of the lactating women, different ethnic groups they belong to and whether or not they smoke. The attainment of appropriate plasma levels of some antioxidants in early infancy is dependent upon the feeding of human milk [9]. In this study, DM has shown a TAC at an adequate level for infants, which does not undergo inflection during the entire production cycle. Furthermore, DM does not need to be modified before consumption, and it can be stored at -20°C up to four months without losing its antioxidant properties (unpublished data). Since donkey milk is considered a good substitute for HM, these data support the increasing interest and sponsorship for the use of donkey milk as alternative in babies' milk nutrition, having the adequate antioxidant levels to satisfy the infant requirements.

Conclusion

In conclusion, the use of this easy, fast and inexpensive commercial TAC assay has proven satisfactory in describing the total antioxidant properties of human and animal milk. This analytical method can be used to improve the quality of production, working technologies and storage of milk, ameliorating the quality of this product that is fundamental in the human food chain. Donkey milk might be considered one of the best breast milk substitutes, when the latter is not available and that not only because of its nutritional characteristics, but also for its antioxidant properties that might help reducing the oxidative-stress-mediated disease in early human life.

References

1. Goldman AS, Goldblum RM. Defence agents in human milk. In Handbook of Milk Composition. 1st edn. New York: Academic Press. Jensens RG. 1995.

2. Friel JK, Martin SM, Langdon M, Herzberg GR, Buettner GR. Milk from Mothers of Both Premature and Full-Term Infants Provides Better Antioxidant Protection than Does Infant Formula. Pediatric Res. 2002; 51: 612-618.

3. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006; 52: 601-623.

4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Inter J of Biochem and Cell Biol. 2007; 39: 44-84.

5. Simos Y, Metsios A, Verginadis I, D'Alessandro AG, Loiudice P, Jirillo E, et al. Antioxidant and anti-platelet properties of milk from goat, donkey and cow: An *in vitro*, *ex vivo* and *in vivo* study. Inter Dairy J. 2011; 21: 901-906.

6. Van Zoeren-Grobben D, Moison RM, Ester WM, Berger HM. Lipid peroxidation in human milk and infant formula: effect of storage, tube feeding and exposure to phototherapy. Acta Paediatr. 1993; 82: 645-649.

7. Saenz AT, Elisia I, Innis SM, Friel JK, Kitts DD. Use of ORAC to assess antioxidant capacity of human milk. J of Food Comp and Anal. 2009; 22: 694-698.

8. Salimei E, Fantuz F. Equid milk for human consumption. Intern Dairy J. 2012; 24: 130-142.

9. Goldman AS, Goldblum RM, Hanson LA. Anti-inflammatory systems in human milk. Adv Exp Med Biol. 1990; 262: 69-76.

10. Davis TA, Nguyen HV, Garcia-Bravo R, Fiorotto ML, Jackson EM, Lewis DS, et al. Amino acid composition of human milk is not unique. J Nutr. 1994; 124: 1126-1132.

11. Salimei E, Fantuz F, Coppola R, Chiofalo B, Polidori P, Varisco G. Composition and characteristics of ass's milk. Anim Res. 2004; 53: 67-78.

12. Monti G, Bertino E, Muratore MC, Coscia A, Cresi F, Silvestro L, et al. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an *in vivo* and *in vitro* study. Ped All Immun. 2007; 18: 258-264.

13. Vincenzetti S, Polidori P, Vita A. Nutritional characteristics of donkey's milk protein fraction. In Dietary protein research trends. New York: Nova Science Publisher, Inc. Ling JR edn. 2007.

14. Chiofalo B, Azzara V, Venticinque L, Piccolo D, Chiofalo L. Variations of fatty acids in Ragusana Ass's milk. In Proceedings of 55th Annual Meeting of the EAAP, 2004 September 5-9; the Netherlands: Wageningen Academic Publisher. 2004.

15. Cunsolo V, Saletti R, Muccilli V, Foti S. Characterization of the protein profile of donkey's milk whey fraction. J Mass Spectrom. 2007; 42: 1162-1174.

16. Criscione A, Cunsolo V, Bordonaro S, Guastella AM, Saletti R, Zuccaro A, et al. Donkey milk protein fraction investigated by electrophoretic methods and mass spectrometry analysis. Intern Dairy J. 2009; 19: 190-197.

17. Iacono G, Carroccio A, Cavataio F, Montalto G, Soresi M, Balsamo V. Use of ass's milk in multiple food allergy. J Pediatr Gastroenterol Nutr. 1992; 14: 177-181.

18. Tidona F, Sekse C, Crescione A, Jacobsen M, Bordonaro S, Marletta D, et al. Antimicrobial effect of donkeys' milk digested *in vitro* with human gastrointestinal enzymes. Intern Dairy J. 2011; 21:158-165.

19. Vincenzetti S, Polidori P, Mariani P, Cammertoni N, Fantuz F, Vita A. Donkey's milk protein fractions characterization. Food Chem. 2008; 106: 640-649.

20. Vincenzetti S, Foghini L, Pucciarelli S, Polzonetti V, Cammertoni N, Beghelli D, et al. Hypoallergenic properties of donkey's milk: a preliminary study. Vet Ital. 2014; 50: 99-107.

21. Tafaro A, Magrone T, Jirillo F, Martemucci G, D'Alessandro AG, Amati L, et al. Immunological properties of donkey's milk: its potential use in the prevention of atherosclerosis. Curr Pharm Des. 2007; 13: 3711-3717.

22. Amati L, Marzulli G, Martulli M, Tafaro A, Jirillo F, Pugliese V, et al. Donkey and goat milk intake and modulation of the human aged immune response. Curr Pharm Des. 2010; 16: 864-869.

23. Cloetens L, Panee J, Akesson B. The antioxidant capacity of milk-the application of different methods *in vitro* and *in vivo*. Cell Mol Biol (Noisy-le-grand). 2013; 59: 43-57.

24. Lin S, Gao W, Tian Z, Yang C, Lu L, Mergny JL, et al. Luminescence switch-on detection of protein tyrosine kinase-7 using a G-quadruplex-selective probe. Chem Sci. 2015; 6: 4284.

25. Bianchi L, Nunzi C, Pauselli M, Moscati M, Cestola E, Duranti E, et al. Evaluating the antioxidant capacity in different fractions of sheep milk. Milchwiss. 2011; 66: 410-413.

26. Brambilla G, Fiori M, Archetti LI. Evaluation of the oxidative stress in growing pigs by microplate assays. J Vet Med A Physiol Pathol Clin Med. 2001; 48: 33-38.

27. Lipko-Przybylska J, Albera E, Kankofer M. Comparison of antioxidant defence parameters in colostrum and milk between Berrichon du Cher ewes and Uhrusk ewes. J Dairy Res. 2010; 77: 117-122.

28. Albera E, Kankofer M. The comparison of antioxidative/oxidative profile in blood, colostrum and milk of early post-partum cows and their newborns. Reprod Domest Anim. 2011; 46: 763-769.

29. Stangsted J. Absence of both glutathione peroxidase activity and glutathione in bovine milk. Intern Dairy J. 2006; 16: 662-668.

30. Butler G, Nielsen JH, Slots T, Seal C, Eyre MD, Sanderson R, et al. Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. J of the Sci of Food and Agric. 2008; 88: 1431-1441.

31. Chilliard Y, Bauchart D, Lessire M, Schmidely P, Mourot J. Product quality: Modulation of milk and meat fatty acid composition by animal feeding. Prod Anim. 2008; 21: 95-106.

32. Clausen MR, Connolly C, Skibsted LH, Stagsted J. Oxidative stability of bovine milk determined by individual variability in herd irrespective of selenium status. Intern Dairy J. 2010; 20: 507 -513.

33. Weiss WP. Antioxidant nutrients and milk quality. 2010.

34. Castillo C, Pereira V1, Abuelo A, Hernandez J. Effect of supplementation with antioxidants on the quality of bovine milk and meat production. ScientificWorldJournal. 2013; 2013: 616098.

35. Zulueta A, Maurizi A, Frigola A, Esteve MJ, Coli R, Burini G. Antioxidant capacity of cow milk, whey and deproteinized milk. Intern Dairy J. 2009; 19: 380-385.

36. Slacanac V, Bozanic R, Hardi J, Szabo JR, Lucan M, Krstanovic V. Nutritional and therapeutic value of fermented caprine milk. Intern J of Dairy Techn. 2010; 63: 171-189.

37. Tsopmo A, Romanowski A, Banda L, Lavoie JC, Jenssen H, Friel JK. Novel anti-oxidative peptides from enzymatic digestion of human milk. Food Chem. 2011; 126: 1138-1143.

38. Power O, Jakema P, FitzGerald RJ. Antioxidative peptides: enzymatic production, *in vitro* and *in vivo* antioxidant activity and potential applications of milk-derived antioxidative peptides. Amin Ac. 2013; 44: 797-820.

39. Bucevic Popovic V, Delas I, Medugorac S, Pavela-Vrancic M, Kulisic-Bilusic T. Oxidative stability and antioxidant activity of bovine, caprine, ovine and asinine milk. Intern J of Dairy Techn. 2014; 67: 394-401.

40. Piovesana S, Capriotti AL, Cavaliere C, La Barbera G, Samperi R, Zenezini Chiozzi R, et al. Peptidome characterization and bioactivity analysis of donkey milk. J Proteomics. 2015; 119: 21-29.

41. Oner Z, Sandlidere-Aloglu H, Dedebas T. Determination of antioxidant in milk from various animals and humans. Milchwiss. 2011; 66: 133-135.

Austin Food Sci - Volume 1 Issue 1 - 2016
Submit your Manuscript | www.austinpublishinggroup.com

Citation: Beghelli D, Lupidi G, Damiano S, Cavallucci C, Bistoni O and De Cosmo A. Rapid Assay to Evaluate the Total Antioxidant Capacity in Donkey Milk and in more Common Animal Milk for Human Consumption. Austin Food Sci. 2016; 1(1): 1003.