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Full Length Research Paper

Stability of water soluble antioxidants in the blood of dairy cattle under different temperatures

Firas Mahmoud Faleh Hayajneh

Al Taif University, College of Pharmacy, Department of Pharmacology, Kingdom of Saudi Arabia.
Free University of Berlin- college of veterinary medicine, Berlin- Germany.

Email: firashope@yahoo.com

Abstract

In the current study attempts were made to test the effect of different temperatures on the stability of water soluble antioxidants (ACW) in the blood of dairy cattle, because sometimes it takes long time for the blood samples taken in the field to be transported to the laboratory where different biochemical tests are made, in this time during transport the samples underlies many factors that have an effect on the content of the samples under study like light, temperature, and time itself.

Keywords: Antioxidant, Free radical, Serum, Refrigerator, Temperature

INTRODUCTION

There is no animal life without oxygen consumption and its conversion to water with the production by leakagefrom mitochondrial electron transport of free radicles (Mark, 2013) in the course of oxidative phosphorylation and the production of ATP as the ultimate and immediate source of energy (Antioxidant From Wikipedia, the free encyclopedia, 2013).

Living organisms are continuously exposed to reactive species since the generation of ATP from molecular oxygen demands electrons. This paradoxical need for a toxic source of energy molecule is central to the Life Sciences since organisms must continuously battle to keep an appropriate balance of prooxidants and antioxidants, which results in a status of good health and high milk production of cows (Popov and Lewin, 2000; Haiying et al., 2003).

Free radicals, which are reactive chemical species with an odd number of electrons inducing damage to lipids, proteins, carbohydrates and DNA, are involved in the aetiopathogenesis of civilization diseases such as atherosclerosis, cancer and diabetes. In physiological processes they are normally in a steady state with antioxidants. Reactive species can also play roles as cellular secondary messengers and regulators or ignaling molecules as with nitric oxide (Halliwell, 2006; (Kimura et al., 2005) or gaseous sulphur dioxide (Liu et al., 2010; Kimura et al., 1979).

This antioxidative homeostasis is maintained by the antioxidative system of the organism, regulating absorption, synthesis, activation, release and excretion of exogenous and endogenous antioxidants (Popov and Lewin, 2000).

During oxidation electrons or hydrogen are transferred from one molecule to another, the latter serving as an antioxidant. Antioxidants, therefore, can stop the formation of free radicals and the chain reactions, which would otherwise result in cell damage or even death (Mark, 2013).

The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health. In fact, the discovery of the role of free radicals in chronic degenerative diseases is as important as the discovery of the role of micro-organisms in infectious diseases (Maples and Mason, 1988).During the last 3 decades, much attention has been focused on determining the mechanisms by which antioxidants protect cells from oxygen radicals and other activated oxygen species

Table 1. Stability of the ACW value after 24 h under the five different temperatures /original values

Temperature	n	Mean value	s	s_x
+25C˚	10	5.2	3.02	0.95
+4C˚	10	7.1	3.36	1.06
-20C˚	10	8.4	3.02	0.95
-24C˚	10	7.0	3.64	1.15
Liquid nitrogen	10	8.4	2.33	0.74

*Dependant variable ACW (µg/ml)

Table 2. Stability of the ACW value after 24 h under the five different temperatures

Temperature (I)	Temperature (J)	n	Mean difference (I-J)	s_x	p	95% Confidence interval	
						Lower limit	Upper limit
+25C˚	+4C˚	10	-1.8	0.51	0.006	-2.98	-0.68
	-20C˚	10	-3.1	0.79	0.003	-4.89	-1.31
	-24C˚	10	-1.8	0.59	0.015	-3.12	-0.44
	Liquid nitrogen		-3.2	0.43	0.000	-4.13	-2.20
+4C˚	+25C˚						
	-20C˚	10	-1.3	0.85	0.17	-3.20	0.66
	-24C˚	10	0.1	0.48	0.919	-1.03	1.13
	Liquid nitrogen	10	-1.3	0.58	0.046	-2.64	-0.03
-20C˚	+25C˚						
	+4C˚						
	-24C˚	10	1.3	0.79	0.130	-0.47	3.11
	Liquid nitrogen	10	-0.1	0.70	0.932	-1.64	1.52
-24C˚	+25C˚						
	+4C˚						
	-20C˚						
	Liquid nitrogen	10	-1.4	0.72	0.088	-3.02	0.25
Liquid nitrogen	+25C˚						
	+4C˚						
	-20C˚						
	-24C˚						

*Means difference is significant p≤ 0.05
*Dependant variable ACW (µg/ml)

(Popov and Lewin, 1994).

MATERIALS AND METHODS

The method used for the determination of the water soluble antioxidative capacity in this study is the method described by (Haiying et al., 2003) which is a method for testing and quantification of non-enzymatic antioxidants, such as ascorbic acid and uric acid and of poly component systems like plasma. This system is based on a photochemical generation of free radicals combined with their chemiluminescent detection.

For the determination of the water soluble antioxidative capacity the following reagents were used

- Reagent 1: ACW-Diluent (sample solvent).
- Reagent 2: Reaction buffer.
- Reagent 3: Stock solution (Photo sensitizer and detection reagent), 250µl/vial.
- Reagent 4: Calibration standard for the quantification of water soluble antioxidants in equivalents of ascorbic acid.

Measuring principle

Free radicals are being produced by irradiation of a photosensitizer (dye) substance. These radicals are parti-

Table 3. Stability of the ACW value after 168 h under the five different temperatures /original values

Temperature	n	Mean value	s	s_x
+25C˚	10	3.5	2.52	0.80
+4C˚	10	5.4	2.65	0.84
-20C˚	10	7.3	2.58	0.82
-24C˚	10	7.2	2.75	0.87
Liquid nitrogen	10	8.4	2.60	0.82

*Dependant variable ACW (µg/ml)

Table 4. Stability of the ACW value after 168 h under the five different temperatures

Temperature (I)	Temperature (J)	n	Mean difference (I-J)	s_x	p	95% Confidence interval	
						Lower Limit	Upper limit
+25C˚	+4C˚	10	-1.9	0.91	0.064	-3.99	0.14
	-20C˚	10	-3.8	1.27	0.015	-6.70	-0.95
	-24C˚	10	-3.7	1.30	0.018	-6.69	-0.80
	Liquid nitrogen	10	-4.9	1.36	0.006	-7.97	-1.81
+4C˚	+25C˚						
	-20C˚	10	-1.9	0.68	0.021	-3.44	-0.35
	-24C˚	10	-1.8	0.67	0.024	-3.34	-0.30
	Liquid nitrogen	10	-3.0	0.83	0.006	-4.83	-1.09
-20C˚	+25C˚						
	+4C˚						
	-24C˚	10	0.1	0.29	0.799	-0.59	0.74
	Liquid nitrogen	10	-1.1	0.37	0.018	-1.89	-0.23
-24C˚	+25C˚						
	+4C˚						
	-20C˚						
	Liquid nitrogen	10	-1.1	0.37	0.014	-1.99	-0.30
Liquid nitrogen	+25C˚						
	+4C˚						
	-20C˚						
	-24C˚						

*Means difference is significant p≤ 0.05
*Dependant variable ACW (µg/ml)

ally eliminated from the sample after they react with the antioxidants normally present in the sample, the remaining radicals in the measuring cell cause luminescence to the detector substance. In this way the antioxidant capacity of the sample is being determined. Quantification of the antioxidative capacity of the sample is determined by comparison with the standard (calibration curves are constructed using ascorbic acid in case of ACW measurement, and trolox in case of ACL measurement).

RESULTS

Stability under five different temperatures within 168 hours

In this experiment blood samples were taken using Serum tubes, kept for 30 minutes in the refrigerator and then centrifuged for 10 minutes, 4000R/min. The outcoming Serum was divided into five groups, which were kept at different temperatures (room temperature (+25C˚), +4C˚,

-20C˚, -24C˚ and under liquid nitrogen, further each of these groups was also divided to be tested after 24 hours and 168 hours. The first measurement was done after one hour because of technical reasons.

In the samples kept at under room temperature (+25C˚) there was a statistically significant decrease noticed after 24 hours whereas the samples kept under other temperatures (+4C˚, -20C˚, -24C˚ and under liquid nitrogen) remained without a statistically noticed decrease. In the samples kept under +4C˚, compared with the samples kept under liquid nitrogen conditions, there was a significant decrease Tables 1, 2.

After 168 hours the decrease in the ACW values in the samples kept at the room temperatures (+25C˚) continued and was significant under both room (+25C˚) and refrigerator (+4C˚) temperatures. The highest values were measured under liquid nitrogen conditions Tables 3, 4.

DISCUSSION

Regarding the effect of temperature on the stability of ACW, it was found that the water-soluble antioxidative capacity is most stable under liquid nitrogen with no decrease within 168 hours, and the highest decrease was under room temperature conditions, and in the refrigerator +4C˚. The ACW values were also stable under -24C˚ and -20C˚ conditions within 168 hours.

Similar results were found by (Haiying et al., 2003) who reported that the concentration of vitamin C did not change during the 6-day storage at -20 C˚ in the plasma of fattening cattle treated with dithioerythritol (DTE), but the vitamin C level in untreated samples decreased significantly (P<0.05) during storage at -20C˚. Also (George et al., 1995) reported that under -70C˚ ascorbic acid is stable over 4 years in human plasma.

(Halliwell, 2006) demonstrated that total ascorbic acid (TAA, the sum of ascorbic acid and dehydroascorbic acid) in properly prepared human plasma is stable at -70 C˚ at least 6 years when preserved with dithiothreitol.

TAA in human plasma or Serum preserved with metaphosphoric acid degrades slowly, at a rate of no more than 1% per year. It is believed that the biochemical

changes, that samples undergo, which in turn have an effect on the antioxidant contents of the samples is stopped under low temperatures (-20C˚, -24C˚ and liquid nitrogen), which might have been the reason behind the stability of water soluble antioxidative capacity in the samples kept under these temperatures in this study. These effects and changes could not be stopped under room temperature conditions and under +4C˚ conditions.

CONCLUSION

The water soluble antioxidative capacity (ACW), measured in Serum tubes is most stable without statistical detectable changes, under liquid nitrogen, at -24C˚, -20C˚ within 168 hours, and is not stable when measured under room temperature conditions (+25C˚), or at +4C˚. In these cases there is a decrease, that is first noticed after one hour from the first measurement.

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