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Determination of Antioxidant Activity in Milk Extracts

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

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Antioxidants are mainly non-nutrient compounds in both human and animal feed, but have the antioxidant capacity in vitro to provide an artificial power index in preventing the destruction of cells and tissue potential by inhibiting nutrient oxidation. Milk contains lipophilic and hydrophilic antioxidants, which play a key role in maintaining. The efficiency of extraction for determining the antioxidant activity of milk corresponds to the method used for plant extraction and it is in a strong linear positive correlation. For this purpose, the phosphor molybdenum method based on the reduction of Mo (VI) in Mo (B) in samples of milk. Extracts obtained with methanol + ethanol Soxlet method. Green complexes formed at acidic pH value and spectrophotometric in the UV range at wavelength λ = 695 nm measurement. The values of milk primers are compared with respect to the calibration curve of IUPAC (3,4,5-Trihydroxybenzoic acid) or gallic acid, measuring range (0.00 to 14.00 µg / ml, y = 0.0344 + 0.0519, R^2 = 0.9709). Milk samples tested for antioxidant activity. 10 measurements of absorptions were made on each of the 4 samples of milk extracts and statistics in Excel. The results concentration, c = 3, 80; 2.35; 3.78; 4.85 / (µg / ml). The highest value of antioxidant activity in packs milk, which has 3.2% fat, also affects the fat and the presence of vitamin E, which found in fat droplets and has a synergistic effect with vitamin C. It concluded that the highest value of the total antioxidant activity in milk obtained from the first sample due to the use of several types of feed - alfalfa, two types of concentrated and straw, which proves the dependence of antioxidant activity on the impact of nutrients, that is, of their type and quantities.

INTRODUCTION:

Antioxidant activity is the result from the content of antioxidants - substances that inhibit oxidation, prevent or reduce oxidative damage in the body. There are three main types in nature: enzymes, vitamins and phytochemicals. Antioxidants are molecules that easily and safely deliver one or more electrons (Irshad, 2002). According to Pokorny J., and Korczak J., (2001), antioxidants defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting initiation or the propagation of oxidative chain reactions. Studies of total antioxidant activity by Carlsen H.M., et al., (2010), have be performed on more than 3,100 foods, beverages, spices, plants, and supplements used in the human and non-plant world of non-plant foods. In practice, several in vitro tests and procedures are important for

the development of antioxidant activity in specimens of interest today. Some broader comparisons of different in vitro methods have be made by Badarinath A. V., et al., (2010) and discuss that methods and procedures can be grouped. According to Mavromichalis I., (2012), a natural guarantee for animals to get a good amount of food is the daily addition of antioxidants to their food such as vitamin C, vitamin E, selenium and beta-carotene.

Material and Methods:

Samples of milk extracts from four farms used for this purpose. The reaction of the extracts is in the formation of molybdenum. Based on the reduction of the molybdate Mo (VI) to Mo (V) in the samples, green complexes formed at acidic pH and the absorption and concentration of the samples are determined, spectrophotometrically at a wavelength of 695 nm. First the calibration solutions from the standard gallic acid read, a standard gallic acid curve is constructed. The absorbed readings from the samples applied to the standard curve and the concentrations of the samples read. Samples of cow's raw milk from three farms and one commercial milk for sale examined in the following order:

1. Raw cow's milk from farm A,
2. Raw cow's milk from farm B,
3. Raw cow's milk from farm C,
4. Cow's milk pasteurized with 3.2% fat (commercially in tetrapack).

Milk Extraction:

Samples of cow's raw milk from three farms (randomly) from three different sites were extracted with 6% trichloroacetic acid (CCl3COOH 99%, Sigma Aldrich). Preparation of the filtrate (whey fraction) Put 15 ml of 6% trichloroacetic acid (TCA) in a 50 ml vial, add 5 ml of milk and stir with a glass rod until fine suspension. Leave at room temperature for 5 minutes. The supernatant is then separated by centrifugation at 7550 rpm-1 (RCF = 5410g) in a Hettich Universal 320R (Andreas Hettich GmbH - Germany) centrifuge for 10 minutes. The resulting supernatant filtered through Whatman No. filter paper. 1.

Total antioxidant capacity

From the review of NurAlam et al. - NurAlam et al., (2013), who have listed 19 methods for determination of antioxidant activity in vitro and 10 methods in vivo, we used the method for determination of antioxidant activity in samples milk in vitro, phosphomolybdate method.

PHOSPHOMOLYBDATE METHOD:

The molybdate test used for this purpose based on the reduction of Mo (VI) to Mo (V) from the sample and the subsequent formation of a green phosphate / Mo (V) complex at an acid pH (NurAlam et al. NurAlam et al., 2013; Prieto et al., 1999; George et al., 2016; Houten, Raman - Houghton, Raman, 1998). Place 1 ml of test extract and 1 ml of reagent (0.6 M sulfuric acid - 95%, AnalaRNormapur, VWR Chemicals), 28 mM sodium phosphate, Merck and 4 mM ammonium molybdate (Merck) in a test tube. The tubes are incubated in a water bath at t = 950C for 90 minutes. The mixture then cooled to room temperature and the wavelength absorption of 695 nm per spectrophotometer (SpectroquantPharo 300 Merck) measured at each test. The spectrophotometer subjected to self-testing, zeroed with a blank test - water, and then for each test, which contains a test sample, a blank test is made containing 1 ml of reagent solution and an approximate volume of the same solvent. The procedure takes place under the same conditions as the analyzed samples only without a sample. The standard curve is prepared with known concentrations (0.2-14 μg / ml) of gallic acid (Galic acid, Cayman Chemical Company). The antioxidant capacity of the extracts expressed as the ratio of the gallic acid equivalent per gram of dry extract (m GAE / g). According to Prieto et al. - Prieto et al. (1999), ascorbic acid (2 mM) used as a positive control, corresponding to the value of 30.80 mM GAE.

Standard gallic acid curve:

Preparation of basic solution of gallic acid $C_6H_2(OH)_3COOH$ or (C7H6O5) with Mt = 170.02 g / mol or 1 M solution: weigh 0.170 g of gallic acid in a 100 ml flask (90 ml H2O and 10 ml of absolute methanol , CH3OH). Appropriate solutions with concentrations are prepared from the standard solution: 0.2 mM - 1.4 mM. Place 1 ml of standard extract and 1 ml of reagent (0.6 M sulfuric acid - 95%,

AnalaRNormapur, VWR Chemicals), 28 mM sodium phosphate, Merck and 4 mM ammonium molybdate (Merck) in a test tube. The tubes are incubated in a water bath at t = 950 C for 90 minutes. The mixture then cooled to room temperature and the wavelength absorption of 695 nm per spectrophotometer (SpectroquantPharo 300 Merck) measured at each test.

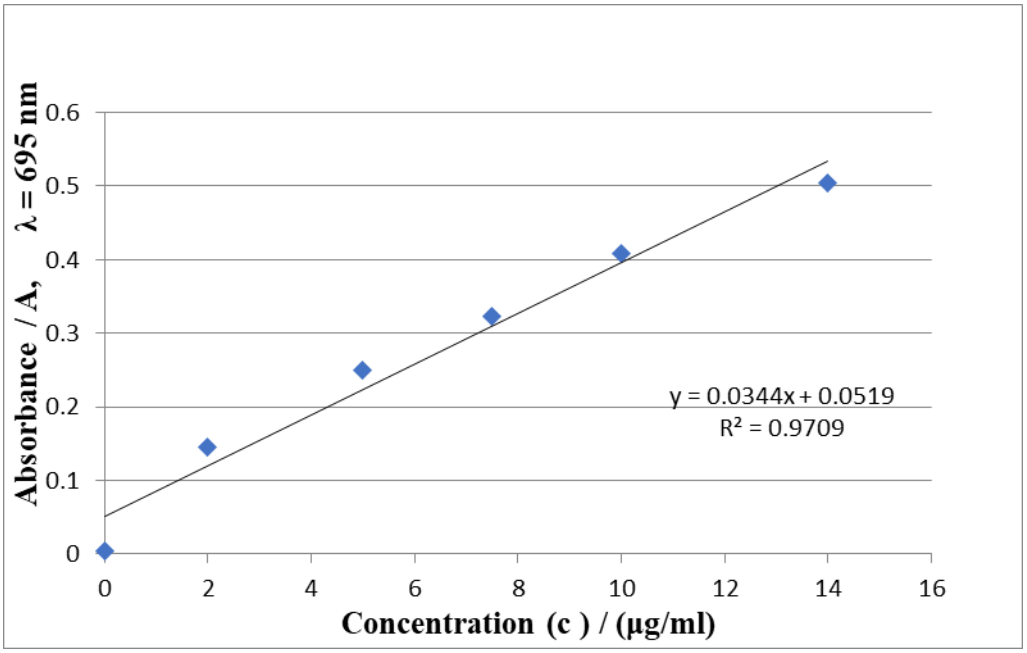
Statistical analysis:

The results expressed as mean ± standard deviation and the statistical significance of the differences was determined using one-way analysis of variance, student t-test. Differences are considered significant if p <0.05. The values are displayed as mean ± SD (n = 3).

RESULTS:

Standard gallic acid curve:

Concentrations of reduced Mo (VI) valence in Mo (V) valence in feed extracts are read on a standard gallic acid curve, the concentration values are graphically shown in Graph 1, in the measuring range (from 0.00 to 14, 00 µg / ml, y = 0.0344 + 0.0519, R2 = 0.9709).Graph 1 shows the absorptions and concentrations of gallic acid from which read the corresponding concentrations of reduced molybdenum Mo (V) for all samples, both for animal feed extracts and milk extracts.



Graph 1: Standard gallic acid curve

Table 1 shows the absorption values of 4 samples of milk extracts and statistics are made in Excel. The mean $(x)^{-}$,standard deviation s as well as the relative standard deviation RSD or coefficient of variation (CV) are calculated.

Milk samples				
No. measurements	**1**	**2**	**3**	**4**
1	0,215	0.17	0.167	0.209
2	0.211	0.171	0.167	0.219
3	0.178	0.171	0.173	0.219
4	0,173	0.139	0.172	0.188
5	0.188	0.14	0.150	0.248
6	0.198	0,139	0.155	0.24
7	0.178	0.14	0.153	0.247

8	0.175	0.17	0.160	0.198
9	0.19	0.13	0.170	0.21
10	0.18	0.132	0.177	0.22
n = 10				
$\bar{x}$	0.187	0.151	0.164	0.220
s	0.012	0.018	0.009	0.020
RSD /%	6.576	12.149	5.664	9.154

Table 1: Statistical analysis of reduced Mo (VI) absorbents in Mo (V) in milk extracts

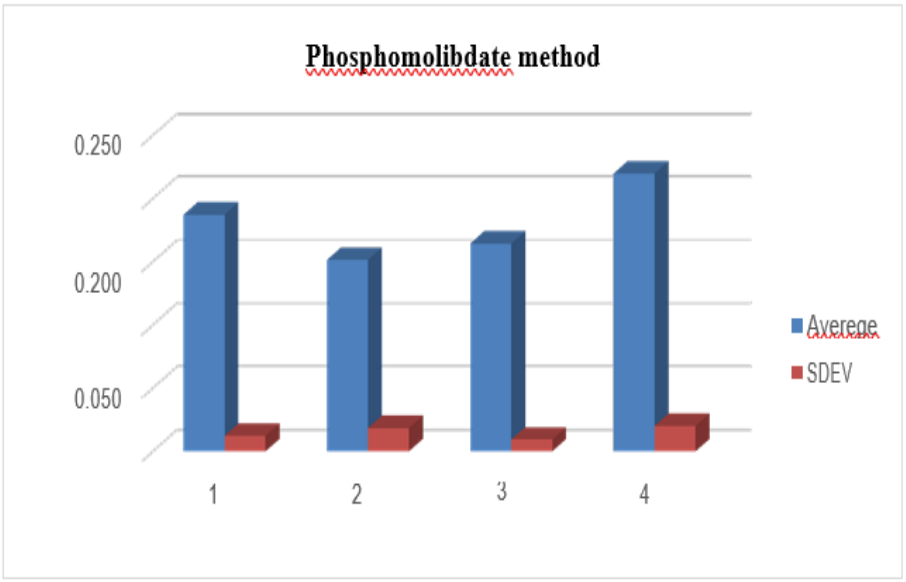
Table 2 shows the concentration and absorbance of reduced molybdenum Mo (VI) to Mo (VI) in milk extract samples.

Concentration (c) / (µg/ml)	**Absorbance (A) λ = 695 nm**
3.80	0.187
2.35	0.151
3.78	0.164
4.85	0.22

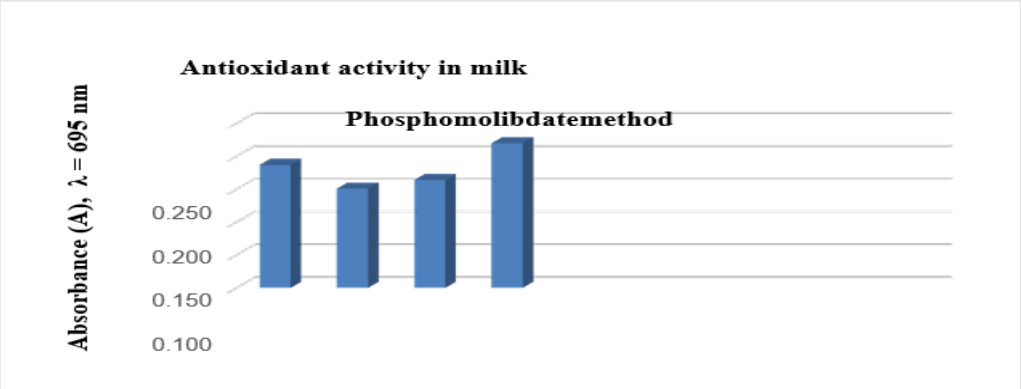
Table 2: Reduced molybdate concentrations in milk extracts

The mean values of the absorbance (A), at a wavelength λ = 695 nm of reduced molybdenum in the samples from the milk extracts, their deviations from the mean value, as well as the standard deviation are shown in Graph 2.

Graph 2 Absorption of reduced molybdenum from (VI) to (V) in milk extracts

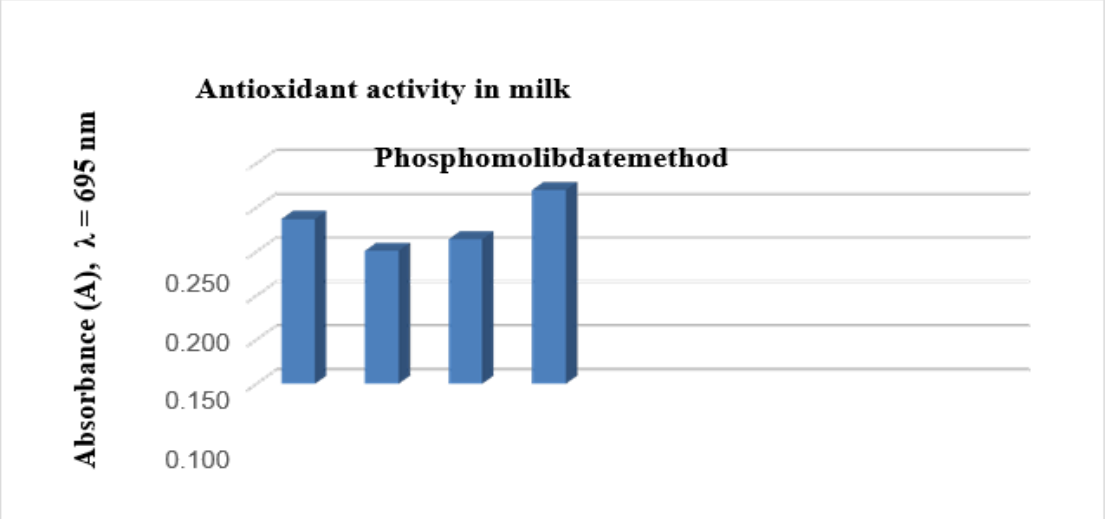


The concentration of reduced molybdenum from (VI) to (V) in the milk extract samples shown in Graph 3. By reducing the molybdenum, the antioxidant activity expressed in µg / mL at λ = 695 nm is read.



Graph 3: Concentration of reduced molybdenum from (VI) to (V) in milk extracts

The concentrations of reduced molybdenum from (VI) to (V) in milk extracts and the standard gallic acid curve shown in Graph 4, which shows the antioxidant activity of milk samples in relation to gallic acid.



Graph 4: Concentration of reduced molybdenum from (VI) to (V) in extracts of milk relative to the standard curve

Analysis of the total antioxidant capacity:

The analysis of total antioxidant capacity is determined as described by Prieto et al., (1998). Concentrations of milk extracts were extracted with 6% trichloroacetic acid and the addition of the same reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In our case, ascorbic acid is used as the standard, and the total antioxidant capacity is expressed as the equivalent of ascorbic acid. By applying the method of molybdate reduction, the values in the milk samples for antioxidant activity are observed significantly low values. According to the phosphomolybdate method, the highest value is in the milk in tetrapack 4.85 µg / mL, and then in the milk from farm A 3.8 µg / mL. We believe that the higher value of antioxidant activity in tetrapack milk, which has 3.2% fat, is due to the fat and the presence of vitamin E, which is found in fat droplets and has a synergistic effect with vitamin C.

CONCLUSION:

From the results obtained for the antioxidant activity with the phosphomolybdate method in milk extracts, it is concluded that the values of reduced Mo (VI) in Mo (V) in milk extracts compared to

pasteurized milk extract - tetrapack taken as standard are low. The highest value with the Phosphomolybdate method was measured in pasteurized milk - tetrapack, and the lowest value in raw milk from farm B. The highest value for the total antioxidant activity in raw milk is obtained from farm A due to the application of several types of food - alfalfa, two types of concentrates and straw, which proves the dependence of antioxidant activity on the impact of nutrients, ie their type and quantities.

Competing Interests Disclaimer:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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