The International Journal of Engineering and Science (IJES)
|| Volume || 7 || Issue || 5 Ver. II || Pages || PP 77-82 || 2018 ||
ISSN (e): 2319 – 1813 ISSN (p): 23-19 – 1805

Determination of Vitamin C in Different Types of Milk

Julijana Tomovska[1], Mirjana Menkovska[2], M. Ayaz Ahmad[3]
[1]*University "St. Kliment Ohridski'', Bitola, Faculty of Biotechnical Sciences, Macedonia*
[2]*UniversitySs.Cyril and Methodius", Skopje, Institute of Animal Science, Macedonia*
[3]*Department of Physics, Faculty of Science, P.O. Box741, University of Tabuk, 71491, Saudi Arabia*
Corresponding Author' Julijana Tomovska

--*ABSTRACT*--
Mainly consumed food that is rich with antioxidant have a protective role of the human cells against the oxidative damage. Vitamin C represent one of the most known antioxidants, and an examined on it was performed in different types of milk. The antioxidant characteristics of vitamin C stems from the fact that it is exclusively good electron donor, and it represent a reducing agent neutralizing the free radicals. In the investigation as a medium were used a few types of milk: raw cows and sheep milk, sterilized whole milk and skim milk, milk with added of vitamins and chocolate milk. Sample extraction was performed according to the method described by Roe and Kuether with certain modifications. Determination of vitamin C was done by spectrophotometer (Spectro Quant Pharo 300 – Merck) at 520nm using calibration curve according to the method of Al-Ani. The highest content of vitamin C was obtained from vitaminized milk with the maximal value 1.20 mg/dl, while the minimal value was obtained from skim milk 0.1 mg/dl. With added of vitamin C in milk also the antioxidant capacity of milk is improved. In addition of this, daily essential vitamin C intake in the nutrition at children (vitaminized milk) and sports (chocolate milk) had also improved. Taking into regard the above stated, it was concluded that it is reasonable to add vitamin C in milk and to obtain milk with supplements having status of functional food, providing so a number of benefits for the consumer health.
KEY WORDS: *antioxidant activity, free radicals, vitamin C, chocolate milk, raw milk*.

Date of Submission: 30-03-2018 Date of acceptance: 28-05-2018

I INTRODUCTION

Antioxidants

Antioxidants are important for living organisms because they can delay or completely inhibit the formation of free radicals by donating hydrogen atoms, which is a process of neutralizing free radicals. Oxidative stress is involved in the pathology of cancer, atherosclerosis, malaria, and rheumatoid arthritis. Antioxidants can be defined in the broadest sense of the word as molecules that are able to delay or completely prevent oxidation (loss of one or more electrons) to other molecules, usually biological substrates such as fats, proteins, and nucleic acids. The oxidation of these substrates can be caused by two types of reactive species: free radicals and species that are not free radicals but are reactive enough to cause oxidation of the substrates previously mentioned. There are three main types of antioxidants:

1. Primary: prevent the formation of new free radicals by two mechanisms - turning existing ones into less dangerous molecules before they can react or by preventing the formation of free radicals from other molecules. Examples of such antioxidants include: The enzyme superoxide dismutase (SOD) that converts the superoxide radical (O2) into hydrogen peroxide (H_2O_2). The enzyme glutathione peroxidase (GPx) that converts hydrogen peroxide and lipid peroxides into less dangerous molecules before forming free radicals, Catalases, Glutathione reductase, Glutathione S – transferase, Proteins that bind to metals (ferritin, transferrin, and ceruloplasmine) that limit the availability of iron to form a hydroxyl radical through a Fenton reaction.
2. Secondary: neutralize free radicals and thus prevent the occurrence of chain reactions, such as Vitamin E or α-tocopherol, vitamin C or L-ascorbic acid, beta carotene (precursor of vitamin A), uric acid, bilirubin, albumin, ubiquinol-10, methionine.
3. Tertiary: repaired damaged biomolecules by free radicals such as: enzymes for the repair of DNA and methionine sulfoxide reductase (Rivera et al., 2009).

Antioxidants are classified according to where they perform their function, their background and their biochemical characteristics. Thus antioxidants are divided into two more general groups, in terms of whether they are soluble in water (hydrophilic) or are liposoluble (hydrophobic).

In the body, there is a contradiction of oxygen, which states that although almost all living organisms need oxygen for life, it is highly reactive and damages living organisms by creating ROS. That's why the organisms possess a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to biomolecules such as DNA, proteins and fats. Typically, antioxidant systems neutralize reactive species at the very site where they are created before they have the ability to damage the vital components of the cell (Devasagaya et al., 2004).

Exogenous antioxidants

Antioxidants are synthetic or natural substances that are present at low concentrations compared to biomolecules that they need to protect. Antioxidants protect by inhibiting harmful effects caused by free radicals. Intake of exogenous antioxidants through the diet can increase the protection of the body and help endogenous antioxidants in the fight against various diseases (Camacho-Luis et al., 2009). Fortunately, much of the foods we eat is rich in antioxidants that protect the cells from oxidative damage. For example, vitamin C is abundant in citrus fruits and various vegetables and is one of the most famous antioxidants. Vitamin E, which is liposoluble, can be found in nuts, unrefined vegetable oils, wheat germ and integral cereals. Beta carotene, which turns into vitamin A in the body, is found in leafy vegetables, carrots and other dark colored fruits (A. Chehue et all.2013).

Vitamin C

Vitamin C, also known as ascorbic acid (enantiomer, L-ascorbic acid), is an antioxidant water-soluble vitamin. The antioxidant feature of this vitamin stems from the fact that it is an extremely good electron donor, which explains that it is a reducing agent that directly neutralizes or reduces the damage caused by electronically unbalanced, unstable reactive species, i.e. free radicals. The presence of vitamin C is required for a number of metabolic reactions in all organisms, and is created in all organisms except humans (Tedesco et al., 2000). Vitamin C is essential for the biosynthesis of collagen proteins, carnitine (which is a pro-catabolic fatty acid transporter in mitochondria) neurotransmitters (mediators of cellular communication) neuroendocrine peptides as well as in angiotensin control, helps in the development and construction of teeth, cartilage, iron absorption, growth and repair of normal connective tissue. Ascorbate is considered a powerful antioxidant due to its ability to donate electrons in various enzymatic and non-enzymatic reactions (Fernandez-Pachon et al., 2004).

Mechanism of antioxidant action of Vitamin C

Vitamin C is an electron donor and therefore a reducing agent. All the known physiological and biochemical features of this vitamin are due to this fact. Ascorbic acid donates two electrons from the double bond between the second and third carbon atom of a molecule composed of 6 carbon atoms. Vitamin C is called an antioxidant because by donating its own electron prevents other compounds being oxidized. However, due to the nature of this reaction, vitamin itself is oxidized in the process. It is important to note that when vitamin C donates electrons they are donated sequentially. The form that is formed therein is a free radical of semi dehydroascorbic acid (SDA) or an ascorbyl radical (Fig.1). Compared with other free radicals, the ascorbyl radical is relatively stable with a half-life of 10-5 seconds and is very poorly reactive. This property ascorbate makes it a highly needed antioxidant and is preferred by the body (Buettner and Moseley, 1993).
Ascorbyl radical with its unpaired electron is a component with a short life span. When losing an electron, dehydroascorbic acid is formed. The stability of dehydroscorbic acid (DHA) depends on factors such as pH and temperature, but most often the survival time is several minutes (Washko et al., 1993).

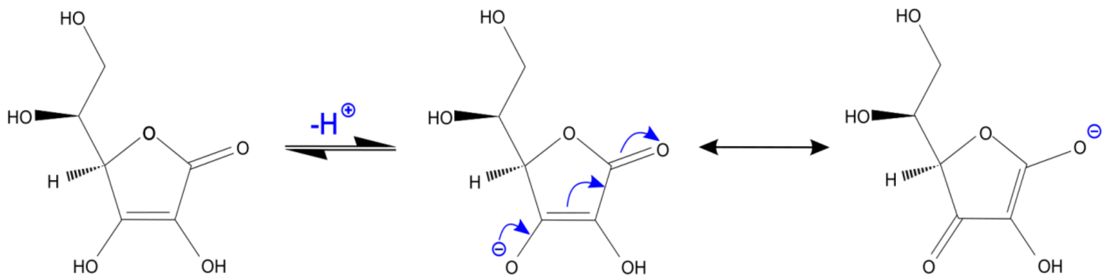
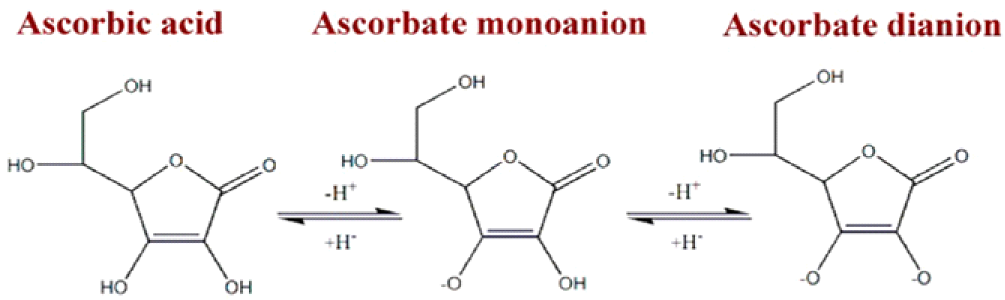


Fig.1:- Ascorbic acid can donate a hydrogen atom and form a relatively stable (SDA) Ascorbyl free radical



	Ascorbic acid	Ascorbate monoanion	Ascorbate dianion
pH 7	**Lower Concentration**	**Higher Concentration**	**Lower Concentration**
acid pH	**Higher Concentration**	**Lower Concentration**	**Lower Concentration**

Fig. 2:- The stability of SDA and DHA depends of pH

Antioxidant mechanisms in milk

According to the question of which are the largest antioxidants in milk, a study by Chen et al. (2002; 2003) shows that urate is an important low molecular weight antioxidant in milk that coincides with data provided by Østdahl et al. (2000). Quite interesting is the research of Clausen et al. (2009), which includes the antioxidant role of various milk components. Namely, beta lactoglobulin and alpha lactalabumine contributed much less to total antioxidant capacity, and in the low molecular weight range, urate and ascorbate were present as antioxidant factors. Using another approach, in the research of human milk Tijerina-Sáenz et al. (2009) found that the antioxidant capacity is strongly correlated with alpha tocopherol but not with vitamin A as well as the content of polyunsaturated fatty acids. In another study, Kristensen et al. (2004), when examining two types of buttermilk with a different content of unsaturated fatty acids, found that the antioxidative capacity in the serum was similar to the two samples, although the buttermilk with more unsaturated fatty acids was less oxidative stable.

Lipid autooxidation in Milk is a complex network of pro-oxidants and antioxidants. Antioxidant vitamins in milk make a major contribution to the daily intake of these components with the diet. For example, vitamin E and carotenoids represent liposoluble antioxidants found in the fat globules membranes, where auto-oxidation of milk fat actually occurs. Vitamin C is an important water-soluble antioxidant and has a complex interaction with iron and liposoluble antioxidants. Vitamin C as part of the antioxidant system of milk is of great importance as it participates in the conversion of the tocopheryloxy radical into tocopherol.

The aim of this research is to examine the effects of milk supplements on the total antioxidant capacity of milk as a complete biological system. Vitamins, especially vitamin C, are one of the most important antioxidant systems in the human body as additives to which special attention is paid.

II MATERIALS AND METHODS

Materials

As a medium for this research, several types and types of milk are used: raw cow and sheep's milk, sterilized whole and skimmed milk, milk with vitamin supplement (vitaminized milk) and chocolate milk. All samples except raw milk were purchased from the free commercial network. The average chemical composition of the milk is shown in Tables 1 - 4.

Table 1. Average chemical composition of full fat sterelized milk

Samples	Fat / %	Proteins /%	Carbohydrates/%
1	3.5	3.2	4.6
2	3.2	3.1	4.55
3	3.2	3.4	4.6
4	3.2	2.9	4.6

Table 2. Average chemical composition of skim milk

Samples	Fat / %	Proteins /%	Carbohydrates /%
1	1.6	3.2	4.6
2	0.5	3.4	4.6
3	1.5	3.1	4.55
4	0.9	3.3	4.4

Table 3. Average chemical composition of milk with vitamins

Type of milk	Fat / %	Proteins /%	Carbohydrates /%	Na-sodium/ mg	Ca-calcium/ mg	Zn-zink/ mg	Se- selen/ μg
1	1.6	3.2	4.6	0.04	120	1.5	8.25
2	2.8	3.3	4.6	0.04	121	1.5	7.95
3	2.8	3.3	4.4	0.04	119	1.4	8.15
4	2.8	3.2	4.5	0.04	119	1.5	7.50

Vitamins composition								
Vit. A	Vit. D	Vit. E	Vit. B1	Vit. B9	Vit. B3	Vit. B5	Vit. B6	Vit. Б7
120μg	0.75μg	1.8 mg	0.17 mg	30μg	2.50 mg	0.9 mg	0.28 mg	7.2 μg
120μg	0.75 μg	1.8 mg	0.21 mg	30μg	2.70 mg	0.9 mg	0.30 mg	7.5 μg
120 μg	0.75μg	1.8 mg	0.16 mg	30μg	2.40 mg	0.9 mg	0.21 mg	7.5μg
120 μg	0.75 μg	1.8 mg	0.20 mg	30 μg	2.50 mg	0.9 mg	0.25 mg	7.5 μg

Table 4. Average chemical composition of chocolate milk

Type of milk	Fat / %	Proteins /%	Carbohydrates /%	Chocolate powder /%	Additive sugar /%	Additive cocoa/%
1	1.1	2.4	10.2	2.1	5	1
2	1.0	2.9	10	0.25	5	1
3	2.0	3.3	11.5	1.8	5	1
4	1.0	3.2	1.5	1.5	5	1

The average chemical composition of the milk is taken from the manufacturer's declaration of quality. Each sample is examined or repeated 5 times and the value taken is the mean value.

Method of determination vitamin C

The Extraction of the sample was done using the method described by Roe and Kuether (1942) with some modifications:

- Preparation of the filtrate (whey fraction): 15 ml of 6% trichloroacetic acid (TCA) is placed in a 50 ml vial, added 5 ml of milk slowly and stir until a fine suspension made. After 5 minutes kept at room temperature was centrifuged at 6000 rpm (RCF = 5126 g). The obtained supernatant was filtered.
- Preparation of Calibration curve of standard: in a 25 ml vial, were prepared with the following concentrations of 0.10, 0.40, 0.80, 1.20, 2.0, 3.0 and 4.0 mg / dl. than was added 6% TCA and other needed solutions.

Procedure: three samples of 1.2ml of the clear supernatant and standard solutions in a special test tube with a cap were pipetted 1.2 of 6% TCA , 0.4 ml of 2,4-Dinitrophenylhydrazine was added to all test tubes. The tubes were closed and incubated in a water bath at 37ºC for 3 hours. Then the tubes were cooled in an ice bath for 10 minutes. By constant stirring, gradually 2 ml of cold Sulphuric acid (12mol / L) were added and mix to the vortex. Measurement was done by Spectrophotometer (SpectroQuant Pharo 300 - Merck) at 520nm. (Al-Ani et al., 2007, and N. Gjorgievski et all., 2014).

III RESULTS

In this study, the results for Vitamin C present in different types of milk are presented in tabular and graphical form. Table 5 shows the amount of vitamin C in all types of milk tested.

Table 5. Average amount of vitamin C in all types of milk

Samples	Full Fat milk / mg/dL	Samples(skim milk) /%	Skim milk/ mg/dL	Vitaminized milk/ mg/dL	Chocolate milk/ mg/dL
1	0.25	1	0.15	1.20	0.22
2	0.35	0.5	0.10	0.75	0.17
3	0.23	0.5	0.11	0.90	0.18
4	0.36	1.5	0.25	0.68	0.21

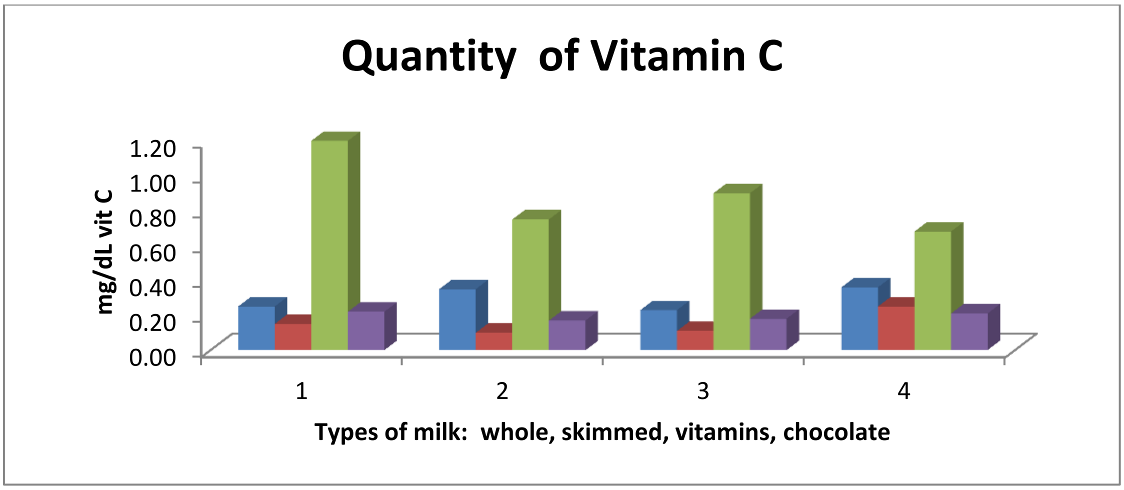


Fig. 3:- The quantity of milk in all type of milk

IV DISCUSION

From the analysis of the milk of vitamin C content, it is understandable that its content is greatest in vitaminized milk because its deliberate addition is made and the largest quantity is observed in milk 1vit, i.e., 1.20 mg / dL. But even chocolate milk contains a substantial amount of vitamin C maximal 0.22 mg / dL, which is probably derived from the cocoa itself used in the process. The full fat milk has a vitamin C content of maximum 0.35 mg / dL, which is higher than that of chocolate milk, which is probably due to a higher amount of vitamin C in whole milk. Further, in skimmed milk, with 1% milk fat, which milk is most often used in the production of chocolate milk. In skimmed milk, we generally have the lowest vitamin C content of 0.25 mg / dL and a minimum of 0.1 mg / dL, both in samples 4 and 2 respectively. The reduced vitamin C content in skim milk is probably due to degreasing processes where, together with fat, it is likely to remove the larger amount of this vitamin that is closely related to vitamin E and which is most concentrated in the fat membranes. Vitamin C together with Vitamin E plays a synergistic role in protecting cells in oxidative activity; ascorbic acid reversible oxidizes to dehydroascorbic acid, thus binds many free radicals to itself and regenerates the reduced form of α-tocopherol.

V CONCLUSION

From this research the main general conclusion is that the additives used in the dairy industry to produce supplemented products such as vitaminized milk and chocolate milk are quite justified because they are proven to increase the biological value of milk as food, and contribute to these products being included in the category of rice i.e functional food.

Highest content of vitamin C is found in vitaminized milk, lowest value is found in skim milk.

The Development of new products of milk with supplements in modern production is aimed at producing foods that have a greater nutritional value from the aspect of useful nutrients, such as vitamins and enzymes.

More specific products are developed for children is vitaninized milk and for sports is chocolate milk. Vitamin C with 200 ml milk daily consumes satisfied up to 14% of the daily requirement for children and up to 10 % in adults.

REFERENCES

[1]. Rivera Arce E, Morales González J A, Fernández Sánchez A M, Bautista Ávila M, Vargas Mendoza N, Madrigal Santillán E O. (2009). Chemistry of Natural Antioxidants and Studies Performed with Different Plants Collected in Mexico, 227-238 .
[2]. Devasagaya T P, Tilak J C, Boloor K K. (2004). Free Radicals and Antioxidants in Human Health: Currens Status and Future Prospects. *J. Assoc. Physicians India*.; 52:794-804.
[3]. Camacho Luis A, Mendoza Pérez J A, (2009). The Ephemeral Nature of Free Radicals: Chemistry and Biochemistry of Free Radicals.
[4]. Tedesco I, Russo M, Russo P, Iacomino G, Russo G L, Carraturo A. (2000). Antioxidant Effect of Red Wine Polyphenols on Red Blood Cells. *The Journal of Nutritional Biochemistry*.; 11(2):114–119.
[5]. Fernandez-Pachon, M S, Villano D, Garcia-Parrilla M C, Troncoso A M. (2004). Antioxidant Activity of Wines and Relation with their Polyphenolic Composition. *Analytica Chimica Acta*.; 513(1):113–118.
[6]. Buettner GR, Moseley PL (1993): EPR spin trapping of free radicals produced by bleomycin and ascorbate. Free Radic Res Commun 19:S89–S93.
[7]. Washko PW, Wang Y, Levine M (1993): Ascorbic acid recycling in human neutrophils. J *Biol Chem* 268:15531–15535.
[8]. Chen, J, Gorton, L. and Åkesson, B. (2002). Electrochemical studies on antioxidants in bovine milk. *Anal. Chim. Acta*, 474 : 137-146.

[9]. Chen, J., Lindmark-Månsson, H., Gorton, L. and Åkesson, B. (2003). Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. Int. Dairy J., 13 : 927-935.
[10]. Østdal, H., Andersen, H.J. and Nielsen, J.H. (2000). Antioxidative activity of urate in bovine milk. *J. Agric. Food Chem*., 48, 5588-5592
[11]. Clausen, M.R., Skibsted, L.H. and Stagsted, J. (2009). Characterization of major radical scavenger species in bovine milk through size exclusion chromatography and functional assays. *J. Agric. Food Chem*. 57: 2912-2919.
[12]. Tijerina-Sáenz, A., Innis, S.M. and Kitts, D.D. (2009) - Antioxidant capacity of human milk and its association with vitamins A and E and fatty acid composition. *Acta Paediatr*. 98: 1793-1798.
[13]. Kristensen, D, Hedegaard, R.V., Nielsen, J.H. and Skibsted, L.H., (2004). Oxidative stability of buttermilk as influenced by the fatty acid composition of cows' milk manipulated by diet. *J. Dairy Res*., 71: 46-50.
[14]. 114. R. Yamauchi (1997). Vitamin E: Mechanism of Its Antioxidant Activity. *Food Sct Technol. Int. Tokyo*, 3 (4), 301-309.
[15]. M. Al-Ani, L. U. Opara, D. Al-Bahri and N. Al-Rahbi (2007). Spectrophotometric quantification of ascorbic acid contents of fruit and vegetables using the 2,4-dinitrophenylhydrazine method. *Journal of Food, Agriculture & Environment* Vol.5 (3&4) :165-168.
[16]. N. Gjorgievski, J. Tomovska, G. Dimitrovska, B. Makarijoski, M. Ali Shariati **(**2014). Determination of the antioxidant activity in yogurt. *Journal of Hygienic Engineering and Design*, Vol.8 pp 88-92.

Julijana Tomovska et.al., "Determination of Vitamin C in Different Types of Milk." The International Journal of Engineering and Science (IJES) 7.5 (2018): 77-82