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Oxidative Modification of the Proteins of Breast and Cow Milk

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Abstract

One of the important modern characteristics of the nutritional and biological value of milk and dairy products is the antioxidant properties. The high stability and sensitivity of the determination of carbonyl derivatives of proteins, as well as the informative value of the action of antioxidants, allow using them as the markers of oxidative damage. The purpose of this paper was to compare the level of carbonyl derivatives of proteins in breast and cow milk. The determination of the oxidative modification of proteins was based on the reaction of carbonyl derivatives of amino acid residues with 2, 4-dinitrophenylhydrazine. The content of the products was determined during spontaneous and metal-catalyzed oxidative modification of the proteins. During the determination of the spontaneously formed carbonyl derivatives of the proteins, their significantly higher content in cow milk compared to breast milk was established. This increase ranged from 46% to 83% at different wavelengths. Thus, the determination of carbonyl derivatives of amino acid residues of the proteins made it possible to reveal significant differences in the antioxidant properties of breast and cow milk, manifested in a lower level of carbonyl derivatives in breast milk. The lower level of carbonyl derivatives in the composition of breast milk proteins is likely associated with the increased activity of the antioxidant system of breast milk or the increased rate of removal of damaged proteins upon activation of milk proteases.

Keywords: carbonyl derivatives, oxidative modification, proteins, human milk, breast milk, metal-catalyzed

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1. Introduction

The intensity of free-radical processes in milk and dairy products and their antioxidant properties are one of the important modern characteristics of food and biological value of production. Despite the breakthroughs in medicine and the promotion of breastfeeding, breast milk substitutes are widely used, most often they are based on cow milk [1]. However, for maximum adaptation of substitutes, knowledge of the characteristics of the composition and properties of breast milk is necessary.

The unique biological and nutritional properties of breast milk make it an indispensable food for newborns. Breast milk contains more than 200 different nutrients, all macro- and microelements necessary for the life of a child [2-4].

Among all the essential nutrients, proteins play the greatest nutritional role. At the same time their content in human milk is much lower than in the milk of other mammals [5]. All milk proteins are divided into two main groups: caseins and whey proteins, the ratio of which is different in different periods of lactation: at the beginning of the lactation period their ration is 80:20 and at the end of lactation it is 50:50. This ratio of mature breast milk (60:40) differs from cow milk (20:80). Whereas in breast milk the main share is beta-casein among other caseins, in cow milk it is only half of the caseins[2]. Most of the milk proteins are whey proteins (about 300). In the serum of breast milk, the main protein is alpha-lactalbumin and in cow milk it is beta-lactoglobulin [3].

The fact that breast milk contains a high activity of proteolytic enzymes that promote the formation of specific peptides is of particular interest [6, 7]. In comparison with hydrolytic degradation proteins undergo oxidative damage to an even greater extent and the oxidative modifications of proteins occur both outside the body and in the body. Both peptide bonds and side radicals of amino acids can undergo oxidative modification [8] and, as a result, carbonyl derivatives of proteins [9], nitrotyrosine, bityrosine [10], and various modification products of cysteine residues such as disulfides, glutathione thiols, nitrosothiols, etc. are formed [11].

The metals of variable valence (Cu^{2+}, Fe^{2+}), various free radicals, reactive oxygen and nitrogen species, as well as imbalance in the balance of prooxidants and antioxidants towards the increase in their content can serve as inducers of the formation of oxidatively modified proteins (OMP) [12]. The activation of free radical oxidation, leading to the development of oxidative stress, is not only the important pathogenetic factor in many diseases; it also participates in the development of various physiological and adaptive processes [13].

For many years, lipid peroxidation indicators have been used as a widespread indicator of oxidative stress, since unsaturated fatty acids are readily exposed to oxidative processes. However, the products of lipid peroxidation are unstable and rapidly degraded and the formation of carbonyl derivatives of proteins is irreversible and leads to the aggregation or degradation of proteins. In this regard, the determination of the level of protein carbonyls is considered a reliable indicator of oxidative stress, aging and other pathologies [14–16].

The advantage of the determination of carbonyl derivatives as indicators of oxidative stress lies in their appearance at early stages of impairment, high stability and sensitivity, as well as informativeness about the action of antioxidants [15].

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Thus, the determination of the content of carbonyl derivatives is a fairly sensitive and reliable indicator of the oxidative modification of milk proteins.

Table 1: Content of carbonyl derivatives of milk proteins with spontaneous oxidative modification (OD/ ml)

Wavelength, nm	Breast milk n=10	Cow milk n= 8	t	P	% variance
356	0.846±0.09	1.235±0.14	2.34	0.03	145.9
363	0.781±0.09	1.169±0.13	2.45	0.03	149.7
370	0.728±0.09	1.105±0.13	2.38	0.03	151.8
428	0.368±0.08	0.673±0.12	2.11	0.05	182.9
430	0.362±0.08	0.656±0.12	2.04	0.06	181.2
434	0.344±0.08	0.643±0.12	2.07	0.06	186.9
520	0.189±0.06	0.417±0.10	1.96	0.07	220.6
535	0.220±0.06	0.385±0.10	1.41	0.18	175.0

Table 2: Content of carbonyl derivatives of milk proteins with metal-catalyzed oxidative modification (OD/ ml)

Wavelength, nm	Breast milk n=10	Cow milk n= 8	t	P	% variance
356	2.438±0.57	6.236±0.61	4.55	0.001	255.8
363	2.439±0.59	5.715±0.45	4.41	0.001	234.3
370	2.402±0.60	5.755±0.45	4.47	0.001	239.6
428	1.283±0.28	3.958±0.27	6.88	0.001	308.5
430	1.250±0.28	3.907±0.27	6.83	0.001	312.6
434	1.206±0.21	3.796±0.27	7.57	0.001	314.6
520	0.428±0.08	1.436±0.17	5.37	0.001	335.5
535	0.351±0.07	1.166±0.17	4.43	0.001	332.2

2. Problem Statement

The nutrition of infants determines the health of a person throughout entire life. The ideal way to provide newborns with all the necessary nutritional components is undoubtedly breastfeeding, since breast milk is the main source of all essential nutritional components for the full growth and development of children. However, in the absence of

the possibility of breastfeeding, the quality of breast milk substitutes is of particular importance. Most often they are made on the basis of cow milk with the addition of various components, bringing the composition of the nutrients of the mixtures closer to the composition of breast milk.

For maximum approach to human milk, breast milk substitutes include vitamins, minerals and other biologically active substances. Apart from the most important nutrients, the components of antioxidant protection should also be included in the composition of infant formula. The existing differences in the antioxidant activity of infant formula for baby food and breast milk indicate the need for further optimization of the antioxidant component of infant formula in order to approximate their biological and nutritional adaptation to breast milk.

3. Research Questions

Milk proteins, like other substrates, are subject to oxidative damage. The determination of carbonyl derivatives of proteins is a reliable indicator of oxidative damage, characterized by stability, sensitivity and informational content on the action of antioxidants [17].

Proteins undergo oxidative damage, with oxidative modifications of proteins occurring both outside the body and in the body. Both peptide bonds and side radicals of amino acids can undergo oxidative modification [8] and, as a result, carbonyl derivatives of proteins [9], nitrotyrosine, bityrosine [10], and various modification products of cysteine residues such as disulfides, glutathione thiols, nitrosothiols, etc. are formed [11].

Metals of variable valence (Cu^{2+}, Fe^{2+}), various free radicals, reactive oxygen and nitrogen species, as well as imbalance in the balance of prooxidants and antioxidants towards the increase in their content can serve as inducers of the formation of oxidatively modified proteins (OMP) [12]. The activation of free radical oxidation, leading to the development of oxidative stress, is not only an important pathogenetic factor in many diseases, it also participates in the development of various physiological and adaptive processes [13].

4. Research Methods

The authors studied the milk received from women breastfeeding infants from 3 to 6 months, the patients of Children municipal polyclinic No. 2 named after V.E. Skvortsov in Omsk. Milk samples were collected in the morning. All women had a preliminary survey,

which took into account the intake of vitamins. Women agreed to process personal data. The average age of new mothers was 27.9 ± 1.17 years. 37.5% of them were primiparous, 62.5% had second and third child births.

Natural raw cow milk was obtained from animals of the black-and-white breed of the forest-steppe zone of Omsk region. All cows were of full age (2–3 lactation), they were in the same conditions of feeding (according to the ration of the farm) and keeping. The milk was cooled to 5 ° C and stored at this temperature until further research within 12 hours.

The assessment of the oxidative modification of the protein was carried out by the method of Reznick A.Z., Parker L. modified by Dubinina E.E. et al. (1995), based on the reaction of interaction of carbonyl derivatives of amino acid residues with 2,4-dinitrophenylhydrazine with the formation of 2,4-dinitrophenylhydrazone derivatives. The content of products was determined during spontaneous and metal-catalyzed oxidative modification of proteins at 356, 363, 370, 428, 430, 434, 520, and 535 nm on a UNICO 2800 spectrometer.

At these wavelengths, aldehyde-dinitrophenylhydrazones (ADNPH) of a neutral character, ketone-dinitrophenylhydrazones (KDNPH) of a neutral character, aldehyde-dinitrophenylhydrazones (ADNPH) of a basic character and ketone-dinitrophenyl-hydrazones (KDNPH) of a basic character are determined.

In order to determine the metal-catalyzed modification of proteins, milk was incubated with 10 mM $FeSO_4$ and 0,3 мМ H_2O_2 for 15 minutes at 37 ° C. The resulting ROS initiate the processes of oxidative degradation of proteins. Statistical processing of the obtained data was carried out using the STATISTICA 6 program. The differences between the values of indicators in the compared groups were assessed using the parametric Student's test. The critical level of significance of differences when testing statistical hypotheses was taken at the level of p = 0.05.

5. Findings

During the determination of the spontaneously formed carbonyl derivatives of proteins, their significantly higher content in cow milk compared to breast milk was established at 356, 363, 370 and 428 nm. These differences range from 46 to 83%. At other wavelengths, the differences are statistically insignificant (Table 1). The induction of free radical processes using iron ions caused the increased formation of carbonyl derivatives of proteins in both breast milk and cow milk, but the degree of increase was significantly different. If at wavelengths 356, 363, 370, 428.430, 434 nm the content of carbonyl

derivatives of breast milk proteins increased 3-3.5 times, then at the same wavelengths their level in cow milk increased 5-5.9 times. Only at wavelengths of 520 and 535 nm, this increase was less pronounced: 1.6 and 2.3 times in breast milk respectively and 3.0 and 3.4 times in cow milk (Table 2). A lower level of carbonyl derivatives in the composition of breast milk proteins is probably associated with the increased activity of the antioxidant system of breast milk, in particular, a higher level of alpha-tocopherol [18]. The antioxidant activity contributes not only to the increase in the biological value, but also to the prolongation of the safety of dairy products [19]. In addition, the decrease in the level of carbonyl derivatives of proteins in breast milk may be reasoned by the increased rate of removal of damaged proteins during the activation of milk proteases. Proteolytic utilization is the important component of the defense system against the accumulation of oxidatively damaged proteins [20], which can be marked with ubiquitin [21].

6. Conclusion

The determination of carbonyl derivatives of amino acid residues with 2,4-dinitrophenylhydrazine allowed revealing significant differences in the antioxidant properties of breast and cow milk, manifested in a lower level of carbonyl derivatives in breast milk. During the induction of free radical processes by iron ions, the antioxidant properties of breast milk are manifested to a greater extent. In order to clarify the mechanisms for the reduction of the intensity of free radical processes in breast milk, it is necessary to conduct research in conjunction with the collective use center “Agricultural and technological research” of Omsk State Agrarian University. The increase in the content of carbonyl derivatives of amino acid residues is more pronounced in cow milk and, to a much lesser extent, in breast milk. The research results indicate the need to increase the content of antioxidant system components in breast milk substitutes.

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