Carbonyl Derivatives of Proteins—as Markers of Free Radical Processes in Dairy Products

Valeriy Vysokogorskiy
Department of Food and Food Biotechnology
Omsk State Agrarian University
Omsk, Russia
vve-bio@mail.ru

Galina Kryshkina
Department of Food and Food Biotechnology
Omsk State Agrarian University
Omsk, Russia
gg.kryshkina@omgau.org

Oksana Lazareva
Department of Food and Food Biotechnology
Omsk State Agrarian University
Omsk, Russia
on.lazareva@omgau.org

Natalya Strelchik
Department of Food and Food Biotechnology
Omsk State Agrarian University
Omsk, Russia
nv.strelchik@omgau.org

Abstract—Improving methods for assessing the biological properties of milk is a currently important task. The level of carbonyl derivatives of proteins during iron-induced oxidative degradation of pasteurized milk proteins was used as a marker of the intensity of free radical processes. During incubation of milk under aerobic conditions for 48 hours, a 1.6 - 2.0 times increase of the content of carbonyl derivatives of proteins was found at all studied wavelengths (from 356 to 535 nm). Maximal increase in the level of carbonyl derivatives was observed at absorption wavelengths corresponding to neutral ketone dinitrophenylhydrazones and, especially, to basic ketone dinitrophenylhydrazones (by 80-100%). The addition of dihydroquercetin to the incubation medium significantly inhibited the formation of carbonyl derivatives of proteins; there was only a slight increase in dinitrophenylhydrazones by 15–25% at 428, 430 and 434 nm. Significant inhibition of the formation of carbonyl derivatives of milk proteins in the presence of a standard antioxidant confirms the free-radical nature of the formation of carbonyl derivatives of pasteurized milk proteins and indicates the possibility of using the evaluation of the level of carbonyl derivatives of milk proteins during aerobic incubation to detect the level of antioxidant activity of dairy products.

Keywords—antioxidant activity, oxidative modification of proteins, carbonyl derivatives, pasteurized milk, dihydroquercetin, iron-induced oxidative destruction.

I. INTRODUCTION

Preservation of the nutritional properties of food products during storage and using of various technological processes is the most important task of food biotechnology. The influence of different production factors causes, first of all, the decrease in the level of micronutrients: vitamins, microelements and, especially, antioxidants [1-3]. Antioxidant activity of products and the content of phenolic compounds are determined by cooking methods [4]. Blanching vegetables improves the taste of green leafy vegetables but their antioxidant properties significantly decrease [5]. Ultrafine milling of wheat bran leads to a decrease in antioxidant activity and the total content of phenolic compounds [6]. Heat treatment of products results in the most significant effect on antioxidant activity. Steaming and suspension treatment significantly reduced the content of vitamin C and the total content of phenolic compounds in plants of Brassicaceae family including broccoli, cauliflower, and mustard [7]. However, heat treatment can increase the antioxidant activity of vegetable extracts by increasing the bioavailability of active components, for example, lycopene yield during heat treatment of tomatoes [8-9] or increasing phenolic compounds in citrus peel extracts at higher temperatures [10]. If the bioavailability of components in the products is high in normal conditions without additional processing, then increased temperature causes a decrease in antioxidant activity [11-15]. High temperature destroys polyphenolic compounds and inactivates some oxidases [16-17]. Heat treatment has a significantly greater effect on the antioxidant activity of milk than any other factors of the technological process [18].

Under the stress situations, a deficiency of endogenous antioxidants occurs in the human body. An important pathogenetic factor of many diseases is the development of oxidative stress which is characterized by the imbalance of pro- and antioxidants, overproduction of reactive oxygen species, and a decrease in antioxidant activity [13], [19-22]. These data indicate the need for using antioxidants as therapeutic agents, as well as the importance of exogenous intake of antioxidants with food and increase the antioxidant ability of foods. If previously antioxidants were used in food technologies mainly for increasing the shelf life of products [23-24], recently they are used not only to better preserve food but also to improve the bioactive properties of final product [25-27]. Instead of synthetic agents, alcoholic and aqueous extracts of plants are more often used as sources of antioxidants. A methodology has been developed for the manufacturing of some bakery, dairy and meat products with the powders of natural extracts from aromatic plants, spices and fruits in order to increase their antioxidant activity [28-30].

Phytochemicals play an important role in human health; they protect against oxidative stress, inflammation, and reduce the risk of chronic diseases such as cardiac diseases, cancer, diabetes, and neurodegenerative disorders [31-32].

Prophylactic effectiveness of phenolic compounds in food products is determined by their bioavailability and delivery into cells of different tissues and organs. Absorption potential can be determined by the amount that passes through cell membrane into intestine, thereby becoming available for actions within the cell. Bioavailability means the amount of components that is released from the matrix of solid food into intestine [33]. Phenolic compounds of products are delivered to a different extent and gradually during digestion in stomach and intestine [34-35]. The amount of absorbed phenolic compounds from food can play a key role in protecting gastrointestinal tract from oxidative...
stress and cause a decreased risk of diseases of stomach or large intestine and of colon cancer [36].

Taking into account significant biological and nutritional value of food products as sources of antioxidants, the significance is increasing of developing markers of antioxidant activity that are sufficiently specific and sensitive. Most of the methods for assessing antioxidant activity are based on the study of the concentration of certain substrates: total amount of phenolic compounds, flavonoids and other substrates. However, in order to identify the bioactivity of food products it is necessary to use methods based on different mechanisms of action: electron transport, transport of hydrogen atoms, chelating ability of metals of variable valence, etc. [37], for different products of free radical processes [38].

Polyunsaturated fatty acids easily undergo oxidative processes, and therefore lipids were previously studied as substrates for free radical oxidation. However, in recent years, many works appeared that proved the greater informative value of the products of oxidative degradation of proteins [39] since they persist for a longer period. Proteins perform various biological functions and, accordingly, their oxidative damage lead to changes in these functions [40]. The sensitivity of proteins to the action of free radicals allows us considering their oxidative modification as a marker of activation of free radical processes.

Deficiency of antioxidants during oxidative stress is often reduced by injection of synthetic drugs, but intake of a complex of natural antioxidants with food is the most physiological way [28], [30], [41]. Milk and dairy products can serve as a constant food source of antioxidants. However, according to K. Smet et al (2008), the antioxidant activity of pasteurized milk decreases during the first days of storage [12]. At the same time, an increase level of products of lipid peroxidation which is often used to characterize the activation of free radical processes is recorded only in a week.

In this regard, there is an assumption about the use of other substrates as more sensitive markers of oxidative degradation of milk components, namely the determination of carbonyl derivatives of proteins.

II. RESEARCH OBJECTIVE

Determination of the level of carbonyl derivatives of pasteurized milk proteins during incubation under aerobic conditions.

TABLE I.

| Content of carbonyl derivatives of proteins at the wavelength, nm |
|-------------------|---|---|---|---|---|---|---|---|
|                   | 356 | 363 | 370 | 428 | 430 | 434 | 520 | 535 |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Milk before incubation |
| n=10  | 2.70±0.09 | 2.59±0.08 | 2.49±0.08 | 1.56±0.06 | 1.56±0.06 | 1.52±0.06 | 0.88±0.04 | 0.82±0.04 |
| Milk after 48 h incubation |
| n=6   | 4.33±0.30 | 4.24±0.30 | 4.12±0.30 | 2.82±0.20 | 2.80±0.30 | 2.72±0.30 | 1.75±0.20 | 1.66±0.20 |
|       | 160.3% | 163.7% | 165.5% | 180.8% | 179.5% | 178.9% | 198.9% | 202.4% |
| t     | 4.9 | 4.9 | 5.0 | 4.7 | 4.6 | 4.5 | 4.1 |
| P     | 0.003 | 0.003 | 0.003 | 0.003 | 0.004 | 0.004 | 0.006 | 0.006 |

1.6 - 2.0 times increased content of carbonyl derivatives of proteins was observed at all studied wavelengths but, to a greater extent, an increase in carbonyl derivatives at absorption wavelengths corresponding to neutral ketone

III. RESEARCH OBJECT AND DESIGN

Research object includes samples of pasteurized milk with the fat mass fraction of 2.5% obtained at Manros-M milk processing enterprise (Omsk Branch of Wimm-Bill-Dann JSC). Fresh samples and samples after incubation under aerobic conditions for 48 hours at a temperature of 4 ± 2°C were studied.

The level of carbonyl derivatives was determined according to the method of Reznick A.Z. and Parker L. in the modification for milk proteins [39]. Formation of carbonyl derivatives under normal conditions occurs spontaneously but the level of autooxidation is low, and the introduction of Fe2+ ions into the system is accompanied by a significant acceleration of free radical oxidation [42]. The intensity of metal-catalyzed oxidation of milk proteins was evaluated by inducing free radical oxidation by Fe2+/H2O2 system. Products of metal-catalyzed oxidative degradation of proteins were determined using UNICO 2800 spectrophotometer at the following wavelengths: 356, 363, 370, 428, 430, 434, 520, 535 nm. From the literature data, ranges are known at which dinitrophenylhydrazones are registered. Neutral aldehyde-dinitrophenylhydrazones are registered at the range of 230–558 nm, basic aldehyde-dinitrophenylhydrazones at the range of 258–264 nm, the absorption spectrum for neutral ketone-dinitrophenylhydrazones is 363–367 nm, for basic ketone-dinitrophenylhydrazones – 430–434 and 524–535 nm [42]. To determine the antioxidant effect on the formation of carbonyl derivatives of proteins, dihydroquercetin was added to the incubation medium at a dose of 25 mg per 100 mL of test sample, preheated to t=35°C for better solubility.

Statistical processing of the data obtained was carried out using STATISTICA 6 program. Differences between the values of parameters in the compared groups were assessed using parametric Student’s t-test. Critical level of significance of differences during testing statistical hypotheses was adopted as p=0.05.

IV. RESULTS AND DISCUSSION

Induction of free radical processes with iron with hydrogen peroxide revealed an increase in the content of carbonyl derivatives of pasteurized milk proteins after 48 hours of milk incubation under aerobic conditions at the temperature of 4±2°C (Table I).
Incubation of pasteurized milk for 48 hours at the temperature of 4±2°C under aerobic conditions with the addition of dihydroquercetin caused no significant increase in the level of carbonyl derivatives at most part of studied wavelengths, only a slight increase of 15–25% was observed at 428, 430 and 434 nm.

Unchanged level of carbonyl derivatives of pasteurized milk proteins in the presence of dihydroquercetin indicates that incubation of milk under aerobic conditions without dihydroquercetin leads to the formation of carbonyl derivatives due to the activation of free radical processes.

Metal-catalyzed oxidative modification of proteins allows one to determine not only damage to polypeptide chain amino acids but also disorder of protein molecule conformation, in contrast to spontaneous modification which characterizes only the number of modified amino acids [43].

V. CONCLUSION

1) Aerobic incubation of pasteurized milk for 48 hours at the temperature of 4±2°C caused a significant increase in the content of carbonyl derivatives of proteins.

2) Inhibition of the formation of carbonyl derivatives of milk proteins in the presence of dihydroquercetin confirms their free-radical nature.

REFERENCES

[1] V.B. Spirichev, “Scientific rationale for the use of vitamins in the prophylactic and therapeutic purposes. Report 1. Lack of vitamins in the diet of modern human: reasons, consequences and correction,” Voprosy pitania (Problems of Nutrition), Vol. 79, No. 5, pp. 4-14, 2010. (in russ.)

[2] V.M. Kodentsova, O.A. Vrzhesinskaya, D.V. Risnik, D.B. Nikiityuk and V.A. Tutelyan, “Micronutrient status of population of the Russian Federation and possibility of its correction. State of the problem,” Voprosy pitania (Problems of Nutrition), Vol. 86, No. 4, pp. 113-124, 2017. (in russ.)

[3] Yu.P. Fomichev, L.A. Nikanova, V.I. Dorozhkin, A.A. Torshkov, A.A. Romanenko, E.K. Es’kov, A.A. Semenova, V.A. Gonotskiy, A.V. Dunaev, G.S. Yaroshchev, S.A. Lashin and N.I. Stolnaya, "Dihydroquercetin and arabinogalactan – natural bioregulators in human and animal life, in crop production and food industry. Moscow: Scientific Library, 2017.

[4] D. Kumari, A. Chandrasekara and F. Shahidi, “Bioaccessibility and antioxidant activities of finger millet food phenolics,” Journal of Food Bioactives, Vol. 6, pp. 100-109, 2019. https://doi.org/10.31666/JFB.2019.6187

[5] G. Oboh, “Effect of blanching on the antioxidant properties of some tropical green leafy vegetables,” Food Science and Technology, Vol. 38, No. 5, pp. 513-517, 2005. https://doi.org/10.1016/j.lwt.2004.07.007

[6] K.X. Zhu, S. Huang, W. Peng, H.F. Qian and H.M. Zhou, “Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber,” Food Research International, Vol. 43, No. 4, pp. 943-948, 2010. https://doi.org/10.1016/j.foodres.2010.01.005

[7] T. Lafarga, I. Viñas, G. Bobo, J. Simó and I. Aguilo-Aguayo, “Effect of steaming and sous vide processing on the total phenolic content, vitamin C and antioxidant potential of the genuss Brassica,” Innovative Food Science & Emerging Technologies, Vol. 47, pp. 412-420, 2018. https://doi.org/10.1016/j.ifset.2018.04.008

[8] V. Dewanto, X. Wu, K. Adom and R. Liu, “Thermal processing enhances the nutritional value of tomatoes by increasing the total antioxidant activity,” Journal of Agricultural and Food Chemistry, Vol. 50, No. 10, pp. 3010-3014, 2002.

[9] A. Patras, N. Brunton, S.D. Prieve, F. Butler and G. Downey, “Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées,” Innovative Food Science and Emerging Technologies, Vol. 10, No. 1, pp. 16-22, 2009. https://doi.org/10.1016/j.ifset.2008.09.008

[10] S.M. Jeong, S.Y. Kim, D. Kim, J.C. Jo, C. Ahn and S.C. Lee, “Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels,” Journal of Agricultural and Food Chemistry, Vol. 52, No. 11, pp. 3389-3393, 2004. https://doi.org/10.1021/jf040166d

[11] S. Calligaris, L. Mantzocco, M. Anese and M.C. Nicolì, “Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk,” International Dairy Journal, Vol. 14, No. 5, pp. 421-427, 2004. https://doi.org/10.1016/j.idairyj.2003.10.001

[12] K. Smet, K. Raes, J.De Block, L. Herman, K. Dewettinck and K. Coudijzer, “A change in antioxidative capacity as a measure of onset to oxidation in pasteurized milk,” International Dairy Journal, Vol. 18, No. 5, pp. 520-530, 2008. https://doi.org/10.1016/j.idairyj.2007.11.012

[13] V.E. Vysokogorskiy and G.V. Ignatieva, “Chemiluminescence analysis of pasteurized milk,” Pishchevaya promyshlennost (Food Industry), No. 10, pp. 34-35, 2012. (in russ.)

[14] V.E. Vysokogorskiy and P.V. Veselov, “Estimation of antioxidizing properties of goat and cow milk,” Voprosy pitania (Problems of Nutrition), Vol. 79, No. 1, pp. 3389-3393, 2004. https://doi.org/10.1021/pf040166d

[15] Yu.V. Balakireva, F.Yu. Akhmadullina, A.A. Lapin and F.G. Karinova, “Changes of the antioxidant activity of milk at pasteurization,” Molochnaya promyshlennost (Dairy Industry), No. 9, pp. 74, 2010. (in russ.)

[16] V.P. Shidlovskaya and E.A. Yurova, “Antioxidants in milk and their role in assessing milk quality,” Pishchevaya promyshlennost (Food Industry), No. 2, pp. 24-26, 2010. (in russ.)

[17] V.P. Shidlovskaya and E.A. Yurova, “Antioxidant activity of enzymes,” Molochnaya promyshlennost (Dairy Industry), No. 12, pp. 48-49, 2011. (in russ.)

[18] Z. Caplan and D.M. Barbano, “Shefl life of pasteurized microfiltered milk containing 2% fat,” Journal of Dairy Science, Vol. 96, No. 12, pp. 8035-8046, 2013. https://doi.org/10.3168/jds.2013-6657
[19] E.B. Menshikova, N.K. Zenkov, V.Z. Lankin, I.A. Bondar and V.A. Trufakin. Oxidative stress: pathological conditions and diseases. Novosibirsk: ARTA, 2008.

[20] W.Kh. Khavinson, V.A. Barinov, A.V. Arutyunyan and V.V. Malinin, Free radical oxidation and aging. Saint Petersburg: Nauka, 2003.

[21] N.L. Samuseva, N. Kurch, V.E. Vysokogorskiy, O.Z. Mkrtchan, S.Zh. Walter and A.V. Sverdlova, “Intensity of free-radical processes and organogenesis disorders in prenatally alcoholized rats,” Siberiskij vestnik psihiatrj i narkologij (Siberian Bulletin of Psychiatry and Narcology), No. 1, pp. 69, 2003. (in russ.)

[22] B. Hallwell and J.M.C. Gutteridge, Free Radicals in Biology and Medicine. NewYork: Clarendon Press, 2008.

[23] M. Carocho, M.F. Barreiro, P. Morales and I.C.F.R. Ferreira, “Adding molecules to food, pros and cons: A review of synthetic and natural food additives,” Comprehensive Reviews in Food Science and Food Safety, Vol. 13, No. 4, pp. 377-399, 2014. https://doi.org/10.1111/1541-4337.12065

[24] K.H.G. Freitas and O. Fatibello-Filho, “Simultaneous determination of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food samples using a carbon composite electrode modified with Cu(II)2-, immobilized in polyester resin,” Talanta, Vol. 81, No. 3, pp. 1102-1108, 2010. https://doi.org/10.1016/j.talanta.2010.02.004

[25] I. Rasooli, “Food preservation – A biopreservative approach,” Food, No. 1, pp. 111-136, 2007.

[26] H. Ye, S. Shen, J. Xu, S. Lin, Y. Yuan and G.S. Jones, “Synergistic interaction of cinnamaldehyde in combination with carvacrol against food-borne bacteria,” Food Control, Vol. 34, No. 2, pp. 619-623, 2013. https://doi.org/10.1016/j.foodcont.2013.05.032

[27] A. Pasqualone, A.M. Bianco, V.M. Paradiso, C. Summo, G. Gambacorta, F. Caponio and A. Blanco, “Production and characterization of functional biscuits obtained from purple wheat,” Food Chemistry, Vol. 180, pp. 64-70, 2015. https://doi.org/10.1016/j.foodchem.2015.02.025

[28] C. Caleja, L. Barros, A.L. Antonio, M.B.P.P. Oliveira and I.C.F.R. Ferreira, “A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits,” Food Chemistry, Vol. 216, pp. 342-346, 2017. https://doi.org/10.1016/j.foodchem.2016.08.075

[29] S. Bajaj, A.Urooj and P. Prabhansanker, “Effect of incorporation of mint on texture, colour and sensory parameters of biscuits,” International Journal of Food Properties, Vol. 9, No. 4, pp. 691-700, 2006. https://doi.org/10.1080/10992910600547632

[30] C. Caleja, L. Barros, A.L. Antonio, M. Carocho, M.B.P.P. Oliveira and I.C.F.R. Ferreira, “Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives,” Food Chemistry, Vol. 210, pp. 262-268, 2016. https://doi.org/10.1016/j.foodchem.2016.04.114

[31] J. Fieder and K. Burda, “Potential role of carotenoids as antioxidants in human health and disease,” Nutrients, No 6, pp. 466-488, 2014. https://doi.org/10.3390/nu6020466

[32] K. Griffiths, B. Aggarwal, R. Singh, H. Buttar, D. Wilson and F.De Meester, “Food antioxidants and their anti-inflammatory properties: A potential role in cardiovascular diseases and cancer prevention,” Diseases, Vol. 4, No. 3, pp. 28, 2016. https://doi.org/10.3390/diseases4030028

[33] F. Shahidi and H. Peng, “Bioaccessibility and bioavailability of phenolic compounds,” Journal of Food Bioactive, Vol. 4, pp. 11-68, 2018. https://doi.org/10.3166/jfba.2018.4162

[34] A. Chandrasekara and F. Shahidi, “Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated in vitro digestion and microbial fermentation,” Journal of Functional Foods, Vol. 4, No. 1, pp. 226-237, 2012. https://doi.org/10.1016/j.jff.2011.11.001

[35] M.R. Prajapati, V. Patel, T. Parekh and R. Subhash, “Effect of in bioprocessing on antioxidant activity of selected cereals,” Asian Journal of Plant Science and Research, Vol. 3, No. 2, pp. 66-72, 2013. https://doi.org/10.13140/2.1.3077.2161

[36] A.M. Jenner, J. Rafter and B. Hallwell, “Human fecal water content of phenolics: The extent of colonic exposure to aromatic compounds,” Free Radical Biology and Medicine, Vol. 38, No. 6, pp. 763-772, 2005. https://doi.org/10.1016/j.freeradbiomed.2004.11.020

[37] D. Granato, F. Shahidi, R. Wrolstad, P. Kilminar, L.D. Melton, F.J. Hidalgo, K. Miyashita, J.Van Camp, C. Alasalvar, A.B. Ismail, S. Elmore, G.G. Birch, D. Charalampopoulos, S.B. Astley, R. Pegg, P. Zhou and P. Finglas, “Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods?”, Food Chemistry, Vol. 264, pp. 471-475, 2018. https://doi.org/10.1016/j.foodchem.2018.04.012

[38] V.V. Khasanov, G.L. Ryzhova and E.V. Mal'tseva, “Antioxidant research methods,” Himiya rastitelogo syr'ya (Chemistry of Plant Raw Material), No. 3, pp. 63-75, 2004. (in russ.)

[39] Yu.A. Podolnikova, V.E. Vysokogorskiy, T.D. Voronova and O.N. Lazareva, “Characteristics of the parameters of milk proteins lipid peroxidation and carboxylation of the cattle of the urbanized region,” Fundamentalnyje issledovaniya (Fundamental Research), No. 9, pp. 2687-2691, 2014. (in russ.)

[40] A. Garcia-Garcia, H. Rodriguez-Rocha, N. Madayspuiithya, A. Pappa, M.I. Panayiotidis and R. Franco, “Biomarkers of protein oxidation in human disease,” Current Molecular Medicine, Vol. 12, No. 6, pp. 681-697, 2012. https://doi.org/10.2174/156652412800392543

[41] C. Caleja, L. Barros, A.L. Antonio, M. Carocho, M.B.P.P. Oliveira and I.C.F.R. Ferreira, “Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives,” Food Chemistry, Vol. 210, pp. 262-268, 2016. https://doi.org/10.1016/j.foodchem.2016.04.114

[42] E.E. Dubinina, Products of oxygen metabolism in the functional activity of cells (life and death, creation and destruction). Saint Petersburg: Medical Press, 2006.

[43] G.K. Rubtsov, “Complete application of model biological systems for assessment of oxidative modification of proteins in the pool of average weight molecules,” Vestnik Tomskogo GPU (TSPU Bulletin), No. 8(136), pp. 81-90, 2013. (in russ.)