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STUDY OF REDOX PROCESSES - VERY IMPORTANT FACTOR IN THE DESIGN OF FOOD SUPPLEMENTS

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Abstract

Today, under conditions of high population stress, maintaining the immune system's activity is achieved by the addition of very valuable food supplements with high energy density The use of serum proteins from cow's milk - in the design and development of innovative food supplements - gives both added value to the products and an efficient use of resources (respecting viable bio-economy principles)

This work is part of an important group that aimed to study the redox processes occurring in the preservation and processing of cow's milk and/or in the design and development of aqueous extracts - such as "soymilk" - used as the basis for certain food supplements and nutraceuticals

The main objective of this part of the Study was - to determine the changes in oxidation-reduction potential in pre-heat treatments of cow's milk

The conclusions of the Study are necessary to determine the best antioxidant with a protective role in milk oxidation, as well as to design a new preservation technology for cow's milk.

Key words**: **food supplements, cow milk, redox potential

INTRODUCTION

It is well known today the increasing importance given to functional foods, functional food technologies, the diversification of functional food manufacturing recipes - diversification aimed at meeting the increasingly sophisticated demands of consumers.

Now that Romania has been declared an acceding country to the EU, the complex issues related to functional and organic food are of growing concern.

The strategy for functional foods in Europe aims to improve industrial competitiveness in the context of increasing global trade in such products by establishing a multidisciplinary collaborative platform. Again, 6 scientific areas of interest have been identified:

-Gastrointestinal functions;
-physiological and behavioral functions;
-Biotechnology design and development;
- scientific modeling of metabolism in certain protein-rich foods such as soy;
- the impact of food technologies on obtaining and prolonging product viability;
-redox state control.

The redox processes occurring under normal conditions in cow's milk, although very important, have been less studied. Although these chemical oxidation-reduction processes have been less studied in practice, the dairy products industry has in recent years experienced unprecedented development and, above all, diversification, both at home and abroad.

Even though many dairy product recipes have been developed on the basis of traditional methods, today, tradition tends to be increasingly replaced by the application of quality management standards. In the dairy products industry in the European Union, total quality management is applied at all levels "from farm to fork". In September 2005, International Quality Standard 22.000 was introduced, which establishes Food Safety Management, a standard that has been applied in Romania since January 2006.

Today, this important Standard, which is the basis of the Food Safety Management System, has reached its 2018 Edition. In the last period, a number of tools have been developed to help establish the related technical requirements (such as TS 22003 - which sets important rules for the Audit Team).

Due to the fact that milk constitutes a favorable environment for the growth of potentially pathogenic microorganisms, from milking to packaging, a number of physico-chemical factors and hygienic conditions have to be respected. If these hygienic conditions are irreplaceable, temperature and pH protection can also be provided by means of certain chemical substances, which are involved in oxidation-reduction processes.

More than 1/3 of the world's children are known to be 'lactose intolerant'. For this reason, the development of substitute products was initially a necessity, followed by their diversification. Under these conditions, aqueous extracts such as "Soymilk", "Almond milk", "Rice milk" and others were developed.

The study of redox processes, their mechanisms and the activity of oxidoreducing enzymes under certain environmental conditions is essential for developers of innovative products.

This scientific work had as its main objective - to determine the changes in oxido-reduction potential in the case of preliminary heat treatments of cow's milk, and this objective is the first of the four main ones addressed. The other Objectives were quantified in the study of the behavior of the main redox agents under conditions of acidity change; determination of changes in the main electrochemical parameters (pH,Eh) in milk subjected to lactic fermentation with the help of microorganisms; analysis of the activity of the main antioxidants acting in cow's milk, in order to be able to establish the best protection system against milk oxidation.

All this is necessary to determine the best antioxidant with a protective role against milk oxidation and to design a new preservation technology for cow's milk.

Normal, fresh milk has a redox potentialEh between + 0.20 and + 0.30 V. The value of the redox potential of cow's milk can change depending on the degree of freshness, the heat treatments to which it is subjected and the conditions under which the milk is stored.

MATERIALS AND METHODS

In order to determine the changes in the redox potential of cow's milk over time, as a function of freshness, origin, heat treatment and storage conditions, 8 experimental variants were performed.

A fresh cow's milk (maximum 2 hours after mechanical milking), a standardized milk and a reconstituted milk powder were used.

The variants considered in the experiment are:

-V1- control variant, fresh homogenized milk with 3.5% fat;

-V2- the variant to which a redox color indicator - 2-3 drops of methylene blue - was added to the fresh milk;

-V3-variant containing the fresh milk to which methylene blue has been added and treated, after 30 minutes of stabilization, with 3 ml of 3 % formaldehyde aldehyde solution;

-V4- variant in which the fresh milk has been heavily polluted with substances of an organic nature;

-V5- variant in which the milk was boiled for 10 minutes under sterile conditions;

-V6- standardized cow's milk (3,5 % fat content);

-V7- variant in which the milk was processed UHT ('ULTRA HIGH TEMPERATURE') at very high temperature, characterized by rapid heating of the milk to 145-150oC for 1-2 seconds in special stainless steel containers;

-V8- the variant using milk powder reconstituted from 3.5% milk fat.

The milk was homogenized to avoid separation of fat during storage. For drinking milk it is recommended that homogenization should be carried out at 60-80oC, at a pressure of 11,778-17,613 N/m2. For sample preparation, a 2000mL stock solution was made from which 100mL was taken for each variant.

The use of methylene blue indicator in milk was done in order to determine the degree of contamination of milk with bacteria. Milk generally contains a reducing system consisting of xanthine oxidase or aldehyde reductase. This system does not manifest itself under aerobic conditions and in the absence of a suitable substrate, but it can be revealed by measuring Eh- under inert atmospheric conditions or by reducing methylene blue in the presence of an aldehyde, the dye being reduced to a colorless leucoderivative.

Organic substances such as straw, vegetable waste, fodder in a certain state of decomposition and manure have been used to pollute the milk. These form bonds with cystine radicals (from protein residues) and consume oxygen from the milk. No set protocol was used for milk, 25 g of vegetable waste and manure per 100 mL of milk was used for milk purification.

Pasteurization is a heat treatment of milk, carried out at temperatures below 100oC, used to destroy pathogenic microorganisms and most of the normal flora of microorganisms. Processed UHT milk was subjected to a strong sterilization heat treatment to destroy both vegetative (already existing) and sporulated forms through which micro-organisms multiply in milk.

STAS drinking water, free from impurities, foreign tastes and odors, microbiologically pure, was used to reconstitute the powdered milk. The analysis of the water used was carried out in the specialized Laboratories of the Oltenia Regional Environmental Protection Agency.

For each experimental variant, the redox potential (Eh expressed in volts) was etermined as a function of time and the coefficient of determination (R2) was calculated, which establishes the degree of correlation between the two quantities. A Multitester C535 measuring cell with glass electrode and platinum ring SP60X type SP60X, produced by CONSORT, was used to measure the redox potential.

The concentration of the oxidised and reduced forms directly affects the redox potential value for each experimental variant (according to the Nernst equation).

$$E = E^0 + (0.059/z)\log\frac{[oxidised\ species]}{[reduced\ species]}$$

Figure 1. The Redox Potential Equation (the Nernst Equation)

RESULTS AND DISCUSSIONS

Analyzing the experimental results, it can be observed that during the storage time, especially during the first 3 hours, fresh milk showed a downward curve of Eh as a result of the action of saprophytic microorganisms (flora of microorganisms existing under normal conditions in milk) (Figure 2).

These micro-organisms can participate in oxidative fermentation reactions modifying the Eh of milk. If this type of milk is forcibly deaerated by nitrogen bubbling, the Eh-value drops sharply to +0.05V

Under normal aerobic conditions, the variation of the redox potential in the region of the potential corresponding to the reduced methylene blue system is practically constant and depends on the type of milk analyzed. Depending on the hygienic condition of the milk there is a maximum and a minimum value of redox potential Eh-.

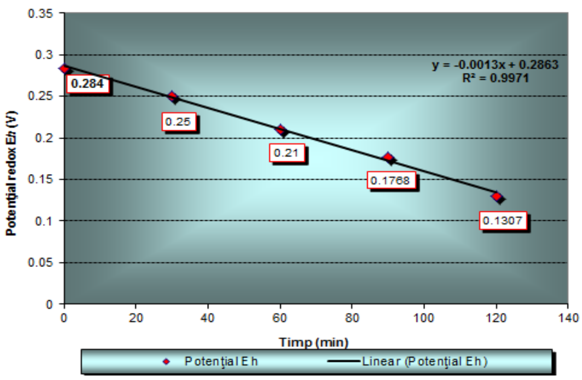

Figure 2. Variation of redox potential over time in fresh milk

In anaerobiosis, when a hydrogen acceptor (an aldehyde) and methylene blue (V3) are introduced into the milk, the activity of xanthine oxidase is stimulated. The oxidized form of xanthine oxidase (XO) transfers two electrons to the aldehyde and converts to the reduced form (XR), thus giving the first decrease in *Eh* due to the increased concentration of the reduced forms of the enzyme *(1).* The redox systems formed are unstable and after about 10 minutes the reduced form of xanthinoxidase accepts two electrons from methylene blue, the redox color indicator changes to the reduced, colorless form and thus decreases *Eh*-ul again*.* Experimental data obtained suggest that formaldehyde/xanthine oxidase and methylene blue/xanthine oxidase couples are active in the measured potential range.

The redox potential decreases sharply during the first 35 min, decreasing further with time (Figure 3).

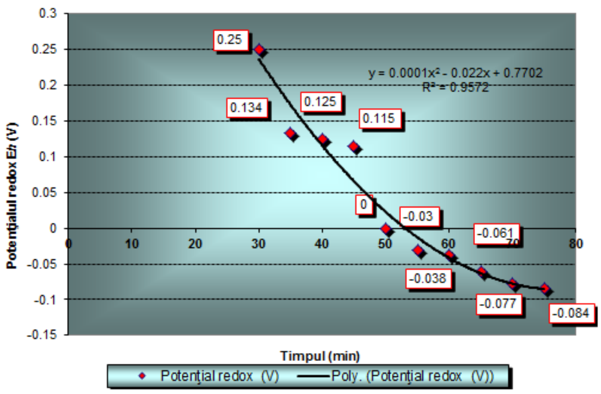

Figure 3 - Decrease in the redox potential of cow's milk as a result of formaldehyde attack

An analysis of the change in redox potential in highly contaminated cow's milk (Figure 4) shows a decrease in Eh over time due to the introduced pollutants consuming dissolved oxygen. As a result, the concentration of oxidised forms in the milk decreases, changing Eh over time according to the Nernst equation.

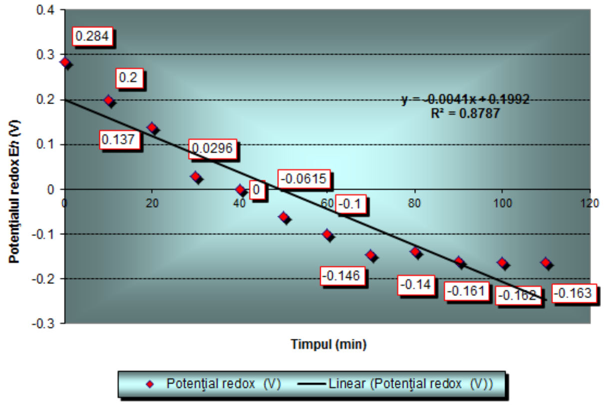

Figure 4. Specific kinetics of redox variation in cows' milk from a polluted area

Boiling the milk for 10 minutes under sterile conditions keeps the Eh- constant over time (0.289V) by destroying pathogenic microorganisms and most of the normal flora. These micro-organisms are no longer able to secrete enzymes that change the Eh-value of the milk. This may also be the reason why the enzymatic fermentation of lactose to lactic acid is prevented after the milk is boiled, with the pH of the milk remaining constant.

The variant in which reconstituted milk powder was analysed has an Eh variation similar to that of the control, its value decreasing over time. At the same time, due to the mechanical stirring used in its production, the Eh values of the variant in which the powdered milk was analysed are higher than those of the fresh milk. Different redox systems occur in UHT milk than in fresh milk. Milk serum proteins (lactoserine) undergo thermal denaturation, releasing thiol groups to the environment as they break disulfide bonds. These groups are then oxidised, leading to an increase in redox potential compared to fresh milk. For this reason, the redox potential of milk processed in this way is higher than that of the other variants. In this case, the Eh- decreases much less over time.

According to the different degrees of milk processing expressed in the 8 experimental variants analysed and the experimental data obtained, the variant in which the milk was processed by UHT stands out as the best. This variant also has the lowest coefficient of determination of the Eh function/time, which indicates the best stability over time.

CONCLUSIONS

During storage, especially during the first three hours, fresh milk shows a decreasing Eh curve as a result of the action of saprophytic microorganisms (normally present in milk); the changes may be greater for fresh milk in the case of forced deoxygenation, and when fresh milk is forcibly deoxygenated by nitrogen bubbling, the Eh value drops sharply to +0.05V;

If the milk is contaminated with bacteria, the bacteria use reducing molecules to produce energy and excrete waste products that increase the redox potential Eh;

Under normal conditions of temperature and pressure (298.15 K and 1 atm), the variation of the redox potential in the reducing zone of methylene blue (V2) is practically constant.

The main redox systems consisting of the oxidoreductase enzymes in milk can be destroyed by heating above 80oC, with the redox potential value for boiled milk remaining constant over time;

According to the different degrees of milk processing, as it appears in the 8 experimental variants analysed and from the experimental data obtained, the variant in which the milk was processed UHT stands out as the best. This variant also shows the least variation in the Eh/time function over the whole range analysed, which indicates the best stability over time. The UHT process inactivates both the enzymes capable of fermenting lactose to lactic acid and those responsible for the oxidation reduction in milk.

REFERENCES

Alfa Xenia Lupea, Dorina Ardelean, Mirabela Padure, (2000) –*Chimia si controlul alimentelor de origine animala*, Editura Politehnica, Timisoara

Belitz, H.D., Grosch, W. (1999). *Food Chemistry*. Translation from the Fourth German Edition by M.M. Burghagen, D. Hadziyev, P. Hessel, S. Jordan and C. Sprinz, Springer Verlag, Berlin, 67-71, 100-102, 104-105;

Berglund, L., Rasmussen, J.T., Andersen, M.D., Rasmussen, M. S., Petersen, T.E. (1996). *Purification of the Bovine Xanthine Oxidoreductase from Milk Fat Globule Membranes and Cloning of Complementary Deoxyribonucleic Acid*. *J. Dairy Sci.* 79, 198–204.

Campbell, P.N., Smith,A.S. (2004) **-** *Biochimie ilustrată*, Editura Academiei Române, Bucureşti;

Ciobanu, G., Marinescu, G. (2002). *Biochimie Practica*, Tipografia Universitatii din Craiova, 98-101

Costin G.M., Segal Rodica (1999)- *Alimente Funcţionale. Alimentele şi sănătatea.*Editura Academica, Galaţi, p 90-99;

Drauz, K., H.Waldmann,2002- *Enzyme Catalysis in Organic Synthesis*, Wiley-VCH, Weinheim;

Enroth, C., Bryan, T., Eger, B.T, Okamoto, K., Nishino, T.I, Takeshi, N., Pai, E.F. (2000). Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: Structure-based mechanism of conversion. *PNAS*, 97(20), 10723–10728

Florea, T. (2001)- *Chimia alimentelor*, vol II, Editura Academiei, Galati;

Klaas D. Kussendrager, (2000)- *Lactoperoxidase: physico-chemical properties, occurrence,mechanism of action and applications,* British Journal of Nutrition nr 84, Suplimentul. 1, p19-S25

Leonte, M., Traian Florea,T, (1998) – *Chimia alimentelor*, vol I, Editura Pax Aura Mundi, Galati;

Petre Săvescu, (2005) - "*Studies concerning the reaction mechanisms for the main redox agents from cow's milk*", *Scientifical Researches Agroalimentary Processes and Technologies*, Vol.XI Nr2,Editura AgroPrint, 2005 Timisoara, pag. 345-350, ISSN 1453-1399

Săvescu, P. Liviu Giurgiulescu L, (2006) – „*Researches regarding the antioxidant effects for the milk xanthine oxidase concentration*", Journal of Agroalimentary processes and Technologies, vol XII, No 1, Editura AGROPRINT, Timişoara, 2006, pag. 21-26

Savescu P. 2006. *Ph.D. Thesys in Chemistry*, Faculty of Chemistry, University of Craiova

Savescu, P., Badescu, G., Milut, M., Ciobanu, A., Apostol, L., Vladut, V. (2019). *Healthy Food – Through Innovative Technologies*. Vol. ISB-INMA-TEH Bucharest. 516-521.

Savescu, P., Iacobescu, F., Poenaru, M.M. (2020). *Study on the Use of Biomaterials as Protective Membranes for Certain Functional Foods*. In Book Advances in Bionanomaterials II, Book Series: Lecture Notes in Bioengineering. Publisher: Springer International Publishing, Print ISBN: 978-3-030-47704-2, Springer Nature Switzerland AG 2020, S. Piotto et al. (Eds.), BIONAM 2019, LNBE, https://doi.org/10.1007/978-3-030-47705-9_16, 1-13.

Savescu, P., Iacobescu, F., Poenaru, M.M. (2020). Patent Application -OSIM A00745, "*Innovative food supplement based on plant products obtained from organic farming*", OSIM, Romania.

Savescu, P. (2021). Chapter *Natural Compounds with Antioxidant Activity-Used in the Design of Functional Foods.* In Book Title -Functional Foods - Phytochemicals and Health Promoting Potential, IntechOpen Eds., London, U.K., DOI: http://dx.doi.org/10.5772/intechopen.97364, 1-25.