ANTIOXIDANT ACTIVITY OF COMPLEXES OF B-CYCLODEXTRIN WITH WHEY PROTEIN HYDROLYSATE

A. Nosik[1], E. Tarun[1], T. Halavach[2], A. Butina[2]

[1]Belarusian State University, ISEI BSU,
Minsk, Republic of Belarus
[2]Belarusian State University,
Minsk, Republic of Belarus
nosik_anastasiya@mail.ru

The comparative characteristic of antioxidant activity of 4 complexes of β-cyclodextrin with hydrolysate of whey proteins Peptigen IF 3080 WPH is presented. According to fluorimetric studies the increase in inhibition of free radical oxidation of fluorescein was shown for all complexes as compared with peptides of whey proteins. The antioxidant activity of complexes that were obtained at 25 and 50 ºC increased by 1.3 and 1.6 times, respectively.

Keywords: antioxidant activity, β-cyclodextrin, hydrolysate of whey proteins, fluorescein.

Milk is a unique product, providing the body with a variety of essential nutrients and possessing antioxidant properties. Enzymatic hydrolysis of the proteinaceous component of milk is aimed at obtaining products with low allergenic potential and high nutritional value [1, 2]. The purpose of creating hydrolyzate complexes of whey proteins with β-cyclodextrin was to eliminate the bitter taste of the hydrolyzate. At the same time, it is relevant to study the properties of complexation on the functional properties of peptides, in particular, the antioxidant activity of hydrolyzed milk proteins.

Antioxidant activity of 4 complexes of β-cyclodextrin with hydrolyzate of whey proteins of milk Peptigen IF 3080 WPH from Arla Foods Ingredients Group (Denmark) was studied. Complexes were obtained with a 5% hydrolyzate content, β-cyclodextrin (β-CD) application in an amount of 3 and 5%, and also under different temperature regimes:

– Complex (I) – hydrolyzate 5 % + β-CD 3 % – 25 ºC;
– Complex (II) – hydrolyzate 5 % + β-CD 5 % – 25 ºC;
– Complex (III) – hydrolyzate 5 % + β-CD 3 % – 50 ºC;
– Complex (IV) – hydrolyzate 5 % + β-CD 5 % – 50 ºC.

In the first series of experiments, access complexes at a temperature of 25 ° C, in the second series – at 50 °C. At the same time, the ratio of "hydrolyzate: β-CD" in each series of experiments was 5: 3 and 1: 1.

The method for determining the antioxidant activity with respect to activated forms of oxygen is based on measuring the fluorescence intensity of the oxidizable compound and its decrease under the influence of ROS. In the present work, fluorescein was used to detect free radicals. Generation of free radicals was carried out using the Fenton system, in which hydroxyl radicals are formed during the interaction of iron (Fe2 +) complex with ethylenediaminetetraacetic acid (EDTA) and hydrogen peroxide [3, 4].

The antioxidant activity of Peptigen IF 3080 WPH hydrolyzate, β-cyclodextrin and the resulting inclusion complexes of the cyclic oligosaccharide and peptides was determined.

In the study of inhibition of the reactions of free radicals generated in the Fenton system, the dependences of fluorescence intensity on the logarithm of the concentration of complex samples, peptides of whey proteins and β-CD were obtained. The positive effect when all the samples were added appeared at their concentration in the sample of 0,01 mg/ml. With a subsequent increase in the concentration of the samples, an increase in the suppression of the action of free radicals and an increase in the fluorescence were observed. Studies were carried out in a wide range of concentrations of 0,01–10 mg/ml. The test samples restored fluorescence to 86–92% (Table). Graphically the defined are indicators of IC50 – the concentration of the samples, at which the achievement of 50% inhibition of activated forms of oxygen.

It is known that the AOA peptides are caused by the reducing properties of the amino acid radicals tryptophan, tyrosine, methionine and histidine [1], whereas antiradical properties of β-CD are associated with additional hydroxyl groups included in the cyclic oligosaccharide [5]. In this connection, the calculation of IC50 for the complexes was carried out both on the dry matter content and the amount of the protein fraction (Table).

Table

Indicators of antioxidant activity of β-CD complexes with hydrolyzed whey proteins of milk Peptigen IF 3080 WPH

Sample Name	A_{max}, %	C_{max}, mkg (dry matter)/ml, $\times 10^{-3}$	IC_{50}, mkg (dry matter)/ml	IC_{50}, mkg(protein)/ml
Complex (I)	89	10	68,0±2,4	42,5±1,5
Complex (II)	87	10	80,1±5,7	40,1±2,9
Complex (III)	90	10	50,3±1,2	31,5±0,7
Complex (IV)	86	2	66,2±4,8	33,1±2,4
hydrolyzate Peptigen	92	10	51,9±3,5	51,9±3,5
β-CD	87	10	66,0±3,0	–

Note – The results of independent experiments are presented as the arithmetic mean ± confidence interval

The IC50 value of the sample of peptides of whey proteins was 51,9 ± 3,5 mkg/ml, while β-CD was 66,0 ± 3,0 mkg/ml. In the case of the calculation for dry matter, a comparison of the complexes obtained with a different ratio of components made it possible to establish a decrease in the antioxidant activity with a decrease in the amount of the peptide fraction. In addition, an increase in the antiradical properties is shown with an increase in the complexation temperature. When calculating the IC50 values on the content of the protein component in the complexes, there is no significant effect of the amount of β-CD introduced on the ability to inhibit the free radical oxidation of fluorescein. At the same time, an increase in the antioxidant activity complexes, obtained at 25 and 50 °C, in 1,3 and 1,6 times, respectively, was revealed. According to experimental data, it is expedient to calculate IC50 values for the amount of peptide fraction in complexes to evaluate the effect of interaction of peptides with β-CD on the level of their antiradical action.

In general, an increase in the antiradical potential of the inclusion complexes of β-CD with hydrolyzate of whey proteins of milk has been established. The maximum increase in the level of antioxidant activity (1,6 times as compared with peptides) is shown for complexes obtained at a temperature of 50 °C.

BIBLIOGRAPHY

1. *Zulueta, A.* Antioxidant capacity of cow milk, whey and deproteinized milk / A. Zulueta, et al. // Int. Dairy J. – 2009. – Vol. 19, № 6–7. – P. 380–185.

2. *Halavach, T. M.* Enzymatic hydrolysis of milk proteins as a basis of specialized food products biotechnology / T. M. Halavach, V. P. Kurchenko, A. I. Albulov // Nauka i studia. – 2016. – Vol. 3. – P. 1196–1207.

3. *Cao, G. H.* Oxygen-radical absorbance capacity assay for antioxidants / G. H. Cao, H. M. Alessio, R. G. Cutler // Free Radic. Biol. Med. – 1993. – Vol. 3, № 14. – P. 303–311.

4. *Тарун, Е. И.* Ингибирование свободных радикалов, генерируемых в системе Фентона, под действием флавоноидов / Е. И. Тарун, Е. В. Чудновская // Труды БГУ. – 2014. – Т. 9, ч. 1. – С. 114–121.

5. *Иванова, Л. А.* Ферментные системы и технологии получения циклодекстринов / Л. А. Иванова // В мире науки. – 2006 – №1 1. – С. 80–82.