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PH AND TEMPERATURE EFFECT ON WHEAT GERM CAKE CATALASE ACTIVITY AND RIGOR[1]

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The paper presents the results of the study considering the role of catalase as the governing factor at wheat germ storage. The pH and temperature effect on wheat germ cake catalase activity and rigor has been considered and analyzed.

Keywords: *catalase; wheat germ cake.*

ВЛИЯНИЕ РН И ТЕМПЕРАТУРЫ НА АКТИВНОСТЬ И УСТОЙЧИВОСТЬ КАТАЛАЗЫ ЖМЫХА ЗАРОДЫШЕЙ ПШЕНИЦЫ

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Представлены результаты исследований касательно роли каталазы как определяющего фактора при хранении жмыха зародышей пшеницы. Рассмотрено и проанализировано влияние pH среды и температуры на активность каталазы жмыха зародышей пшеницы.

Ключевые слова: *каталаза; жмых зародышей пшеницы.*

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Introduction

In the Russian Federation there is a number of state-run programs aimed at developing production of foods enriched with essential components, functional use products, distribution of advanced processing of agricultural raw materials on the principles of non-waste production, sustainable use of secondary products, as well as food industry waste reduction. The above-mentioned programs are as follows: the comprehensive program of biotechnologies development in the Russian Federation, health nutrition state policy in the period through to 2020, state program of agriculture development and agricultural commodities, raw materials and foods market regulation.

From this point of view, wheat germ cake (WGC), being the product of advanced wheat processing, seems to be of great interest. WGC is a native plant component that has high nutritional and biological value. It contains vitamins A and E, vitamin B complex as well as 20 macro- and microelements. Thanks to its rich biochemical composition and high functional and technological properties WGC can be widely used in food, confectionery, perfumery and medical industry. However, WGC has low storage stability as it is characterized by a high level of lipids (8-10%), which contain up to 80% of polyunsaturated fatty acids (including ω-3, ω-6 fatty acids) [1, 2].

Catalase (EC 1.11.1.6) is an enzyme of the oxidoreductase group that catalyses a reduction-oxidation reaction; it is within this type of reaction that two molecules of hydrogen peroxide are transformed into water and oxygen. When storing and processing WGC it is catalase that has a negative effect. Lipase starts a process of WGC fats getting rancid (EC 3.1.1.3) as it triggers lipids hydrolysis alongside with free fatty acids formation as well as their further oxidation. Lipoxygenase (EC 1.13.11.12) affects the parallel process as fatty acids hydroperoxides are decomposed to acidic products. As it takes part in the reaction, catalase leads to oxygen evolution; the latter intensifies the oxidation processes in the product. The result of the chain reaction leading to hydroperoxides formation in WGC as well as other advanced types of oxidation products and lipid decomposition is objectionable odour and rancid taste of the product [3, 5].

The aim of the paper is to study the way pH and temperature affect WGC catalase activity and rigor as these are significant factors influencing the storage parameters.

Materials and study methods

For the aims of the study the following type of wheat germ (WG) was used, namely, the one industrially produced (TS 9295-010-00932732-08 ‘ed-

ible wheat germ flakes'). The authors studied batches of WG obtained as a result of processing wheat grain of various types coming from Belgorod, Lipetsk and Voronezh oblasts (Starooskolsky bread and cereal products factory JSC, Buturlinovsky flourmill factory JSC, Tonex JSC, Lipetsk bread and cereal products JSC). WG processing was performed using a cold pressing technique at Tonex JSC (Belgorod oblast). WGC, being the product of WG advanced processing met all the requirements of the relevant technical specifications (TS 9295-014-18062042-06 'VITAZAR edible wheat germ flour').

A WGC specimen was obtained by mechanical grinding in a laboratory mill and its further homogenization with phosphate-citrate buffer (pH 7.4). The first purification step involved the precipitation of the enzyme specimen using 96.5% ethanol at a temperature of 2–4°C. The precipitate was separated using a cold centrifuge at 5000 g and then it was vacuum dried. The ballast proteins were eliminated from the enzyme by fractionation with the use of ammonium sulfate. The precipitate was obtained at the level of ammonium sulfate saturation of 60–80%. The latter was dissolved in a minimum amount of buffer. Low-molecular impurities were removed from the enzyme solution using gel-filtration on Sephadex G-25. The final purification step involved gel-filtration on Sephadex G-100 (Pharmacia, Sweden). Finally, the enzyme specimen was obtained, being characterized by 80-fold purity and specific activity of 545.2 units/1 mg protein.

An activity unit was thought to be the amount of mm hydrogen peroxide being decomposed during the incubation process to 1 ml of the enzyme specimen at 37°C and pH = 7.4 [3, 4].

Results of the study and their discussion

Temperature and pH are considered to be the two most important factors that must be taken into consideration when catalase inactivation takes place for the purpose of WGC long-term storage, as well as when making specimen (Fig. 1 and 2). The enzyme activity was determined within pH 4–9 at the optimum temperature and was expressed as a percentage of the maximum (Fig. 1). The catalase optimum pH is thought to be within the area of 7.3–7.8.

With the optimum pH being found, the temperature effect on the enzyme activity was determined, the optimum temperature being 37 ± 2°C.

The study of thermal and pH stability of the enzyme is of considerable interest as these indications are considered to be important criteria when selecting WGC storage conditions as well as processing conditions of food products that contain WGC. When studying WGC catalase inactivation kinetics,

samples were taken from the incubation medium regularly after certain time intervals, then the residual activity of the enzyme was determined.

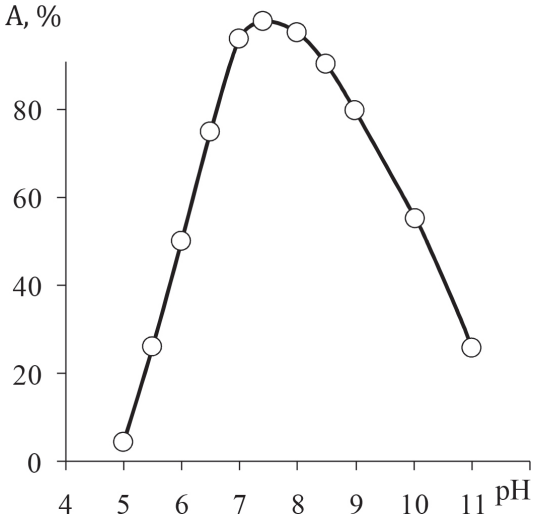

Fig. 1. WGC catalase activity dependence on the medium pH at the temperature of 37°C

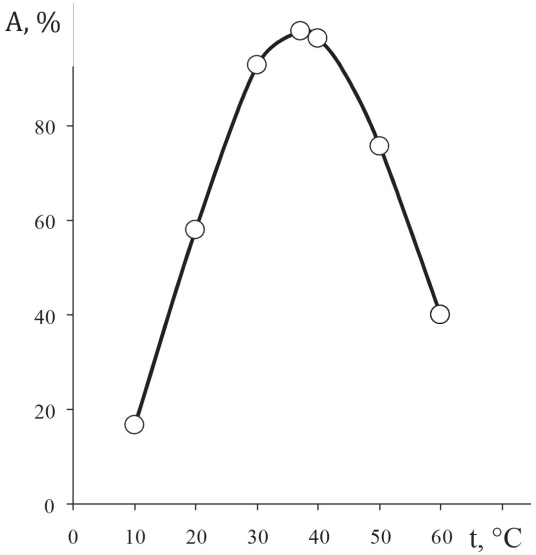

Fig. 2. WGC catalase activity dependence on temperature at the medium pH 7.4

With the use of the inactivation dynamics data we calculated the inactivation rate invariables. The greatest stability of the enzyme was recorded at pH 8.0 and the temperature of 20°C (Table 1).

Increase of temperature resulted in a significant increase of the average rate, which increased more dramatically at hydrogen ions higher concentrations level and the temperature increase up to 40°C and above. We calculated the entropy variation in $\Delta S^{\neq}$ of the enzyme transition state from the active form to the inactivated one.

Table 1.

WGC catalase inactivation rate invariables

Temperature, °C	$K \cdot 10^2$, hr^{-1}				
	pH 4	pH 5	pH 6	pH 7	pH 8
20	4,1	3,3	2,4	1,9	1,4
40	95,9	41,8	27,1	19,6	12,9
60	630,9	524,8	409,3	344,4	289,1

The data obtained show that at low temperatures the high concentration of H^+ ions leads to the enzyme protein globule intensive transition to a chaotic tangle; apparently, it is due to the active destruction of the electrostatic bonds (Table 2).

Table 2.

Temperature effect on pH and entropy value ($\Delta S^{\neq}$) of the WGC catalase transition state

Temperature range, °C	pH	$\Delta S^{\neq}$, J · degree · mol^{-1}
20-40	4,0	170,079
40-60		40,943
20-40	8,0	33,965
40-60		190,717

The opposite situation is observed at pH 8.0. At high temperatures, one can see an intensive destruction of the protein globule. It can be a result of the process in which the hydrophobic interactions of molecule non-polar parts are destroyed.

Conclusion

Based on the data obtained it can be concluded that it is the H^+ ions that greatly affect the WGC catalase inactivation process at low temperatures,

whereas it is heat that affects the same way at high temperatures. The enzyme has a low acid and thermal rigor, which makes it possible to use these characteristics to suppress the enzyme activity at WGC storage as well as find proper technological parameters for processing foods that contain WGC.

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