FOOD TECHNOLOGY

DOI - 10.32743/UniTech.2021.86.5.11807

REDUCTION OF FREE FATTY ACIDS IN COTTON OIL WITH IMMOBILIZED LIPASE**Rustamjon Medatov**

Assistant,
Ferghana Polytechnic Institute of the Republic of Uzbekistan,
Uzbekistan, Ferghana
E-mail: rustam_m9090@mail.ru

Bobir Kayumov

Assistant,
Tashkent Chemical-Technological Institute of the Republic of Uzbekistan,
Uzbekistan, Tashkent
E-mail: b.qayumov@bk.ru

Hasan Hasanov

Assistant professor,
Tashkent Chemical-Technological Institute of the Republic of Uzbekistan,
Uzbekistan, Tashkent
E-mail: xas.tyr@rambler.ru

**СНИЖЕНИЕ СВОБОДНЫХ ЖИРНЫХ КИСЛОТ ХЛОПКОВОГО МАСЛА
С ПОМОЩЬЮ ИММОБИЛИЗОВАННОЙ ЛИПАЗОЙ****Медатов Рустамжон Хошимович**

ассистент,
Ферганский политехнический институт Республики Узбекистан
Республика Узбекистан, г. Фергана

Каюмов Бобир Собирович

ассистент,
Ташкентский химико-технологический институт Республики Узбекистан,
Республика Узбекистан, г. Ташкент

Хасанов Хасан Турсунович

доцент,
Ташкент химико-технологический институт Республики Узбекистан,
Республика Узбекистан, г. Ташкент

ABSTRACT

It has been shown that the use of sorbents based on silica gel, polyamide and fofatidylethanolamine in lipase immobilization promotes an increase in the stability of the enzyme 2-2.5 times and the retention of activity in media with ethanol and acetone. It was found that the content of free fatty acids in cottonseed oil with immobilized lipase can be reduced by 60-70%.

АННОТАЦИЯ

Показано, что использования сорбентов на основе силикагеля, полиамида и фосфатидилэтаноламина при иммобилизации липазы способствует повышению стабильности фермента 2-2,5 раза и сохранению активности в средах с этанолом и ацетоном. Установлено, что содержание свободных жирных кислот хлопкового масла с иммобилизованной липазой можно снизить на 60-70%.

Keywords: lipase, fungus, *Rhizopus microspores*, immobilization, stability, use, neutralization, cottonseed oil.

Ключевые слова: липаза, грибная, *Rhizopus microspores*, иммобилизация, стабильность, применение, нейтрализация, хлопковое масло.

Recently, in many countries, the production and use of enzyme preparations in the oil and fat industry has been expanding. Enzymes are used both at the stage of processing oil seeds [1] and at the stage of refining and modification of oils [2].

In the production of vegetable oils, one of the most critical technological processes is oil refining.

Refining combines processes, the main purpose of which is to remove substances from oils, accompanying it, and some foreign impurities. Related substances include substances contained in seeds and passing into oil in a minimally altered state and substances that have changed during extraction under the influence of external factors [3].

Recently, many authors have shown that the use of enzymes is promising for the hydration and neutralization of vegetable oils using various enzymes [4].

Enzymatic hydration of vegetable oils using proteolytic and lipolytic enzymes has been successfully carried out by many researchers [1]. Research is also being conducted on the use of lipolytic enzymes to neutralize free fatty acids in the refining of vegetable oils [5]. The essence of the method lies in the fact that lipase is used to attach free fatty acids to mono- and diglyceride molecules. It is known that lipases in systems with a low water content catalyze the synthesis of glycerol esters with fatty acids and a number of other reactions.

Especially, much attention is paid to the use of immobilized enzymes, since the free lipases used for this process turned out to be less stable and lost activity due to the heterogeneity of the system [6]. Therefore, immobilized enzymes with high catalytic activity and stability are widely used.

The aim of this work is to study the decrease in the content of free fatty acids in cottonseed oil with immobilized lipolytic enzymes on hydrophobic sorbents obtained on the basis of silica gel, polyamide and phosphatidylethanolamine.

Methodology

Reagents. We used a lipase preparation from the fungus *Rhizopus microsporus* (Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan), purified according to a previously published method [7].

To prepare buffer solutions, we used one- and two-substituted potassium phosphate (chemically pure "Reakhim"), rhodamine 6Zh (chemically pure "Reakhim"), acetic acid (chemically pure "Reakhim"), rectified alcohol (JSC "Biokimyo") and hexane (reagent grade "Reakhim"), silica gel KSK (reagent grade "Reakhim").

Determination of lipolytic activity. Lipolytic activity was measured by photocolometric method using rhodamine 6G as a chromophore agent [8]. Specific activity was expressed in μmol of fatty acids released in 1 hour per 1 mg of protein.

Refined cottonseed oil emulsified with an equal volume of 1% polyvinyl alcohol was used as a substrate. The reaction mixture with a volume of 2 ml, containing 0.2 ml of the substrate in 0.1 M phosphate buffer, pH 7.5, and 20 mM CaCl_2 , was incubated at 37 $^\circ\text{C}$ in the enzyme solution for 1 h. 0.25 ml of the reaction mixture

was taken and added 1 ml 0.1 M HCl in ethanol to stop the reactions. The rest of the operations were performed according to the method [8].

Synthesis of sorbents for immobilization of lipolytic enzymes. To 100 g of SiO_2 with a particle size of 160-250 μm was poured 10 g of polyamide dissolved in concentrated HCl (200 ml), the mixture was kept under stirring for 1.5-2.0 hours, then 200 ml of 40% aqueous acetone was added ... The supernatant was removed by decantation, and the sorbent was washed with distilled water until neutral pH of the medium. Then the resulting sorbent was activated with 2 N HCl at a temperature of 45 $^\circ\text{C}$ for 1.5-2.0 hours, after which it was washed with distilled water and modified with a 1% solution of glutaric dialdehyde in 0.1 M phosphate buffer pH 8.0 for 2 hours ...

The unbound portion of glutaric dialdehyde was removed by washing with distilled water and 0.1 M phosphate buffer, pH 8.0. Then, a solution of cephalin (phosphatidylethanolamine) with a concentration of 40 mg / ml in a 0.1 M buffer, pH 8.0, containing 50% ethyl alcohol was added to the sorbent. The mixture was kept under constant stirring for 24 h, then washed with ethanol and dried in air.

The immobilization of lipases on such sorbents was carried out by incubating the enzyme solution in 0.1 M phosphate buffer pH 7.4-8.0 with the carrier for 3-5 hours at a temperature of 4-10 $^\circ\text{C}$. The ratio of carrier and enzyme preparation was 10: 0.5. The unbound part of the enzyme was removed by washing with 0.1 M buffer solution pH 8.0 [9].

Hydration of cottonseed oil. 200 g of the test oil was heated to 45-50 $^\circ\text{C}$ with stirring, then the stirrer speed was increased and the specified amount of H_2O was slowly introduced into water and kept stirring for 60 minutes. The total water content was 6% by weight of the oil. Then the temperature of the mixture was raised to 85 $^\circ\text{C}$ and kept at this temperature for 20 minutes. At this temperature, the mixture was stirred for 60 minutes. The hydrated oil was decanted, filtered and dried at a temperature of 100-110 $^\circ\text{C}$.

Treatment of hydrated cottonseed oil with immobilized lipases. 150 g of the test oil, heated to 45-50 $^\circ\text{C}$ with stirring, then the stirrer speed was increased and a predetermined amount of immobilized enzyme, 5% by weight of the oil, was slowly introduced. Then it was kept at this temperature for 12 hours.

Chemical analysis of refined oil was carried out according to the generally accepted method [3].

Results and discussion

The oil extracted from oilseeds is a mixture of free fatty acids (FFA), mono-, di- and triglycerides, phosphatides, pigments, sterols and tocopherols. Traces of metals, flavonoids, tannins and glycolipids may also be present [3,10].

FFA in raw cottonseed oil varies depending on harvesting methods, storage conditions, up to processing in oil and fat factories.

It should be noted that FFAs are more susceptible to oxidation than the glycerol esters of these fatty acids; and this lipid oxidation leads to oxidative rancidity in

edible oils. Therefore, any increase in the acidity of the oil must be absolutely ruled out.

The resulting immobilized lipase from the fungus *Rhizopus microsporus* on a hydrophobic carrier to reduce the acid number of cottonseed oil had a number of advantages over free lipase.

Figure 1 shows the thermal stability of free and immobilized lipase. From the data presented, Fig. 1 shows that after immobilization, the thermal stability of the enzyme increases significantly. For example, when the enzyme was incubated at 50 °C for 40 minutes, the decrease in the activity of the free enzyme was 50%, for the immobilized lipase it was 20%.

1 - free lipase, 2 - immobilized lipase.

Figure 1. Thermal stability of free and mobilized lipase from the fungus *Rh.microsporus* at 50 °C

When obtaining immobilized enzymes, the main goal is not only to increase resistance to high temperatures, but also to reduce the inactivating ability of various denaturing agents, which occurs when the enzyme is used in various technological processes.

It is known that adsorbed lipases on hydrophobized surfaces retain a higher catalytic activity [6]. Lipase activation during adsorption on hydrophobic surfaces is

associated with the provision of optimal orientation of the active site of enzymes in relation to the substrate. It can be assumed that in such systems, when the enzyme is located between the lipid layers (the substrate phase and the hydrophobic cavity of the carrier), due to the protective effect of the substrate, the high catalytic activity and stability of the enzymes are retained.

Figure 2. Effect of ethanol (a) and acetone (b) on the activity of free (1) and immobilized lipase from the fungus *Rh.microsporus* (2)

In fig. 2 shows the effect of ethanol and acetone on the activity of free lipase and immobilized on silica gel modified with polyamide, cephalin or palmitic acid. It can be seen that the activity of free lipase decreases with an increase in the concentration of ethanol and acetone in the reaction medium. Free lipase completely lost its catalytic activity in the presence of 30% ethanol, while the decrease in the activity of immobilized lipase was insignificant. The rate of lipid hydrolysis by immobilized lipase increased with an increase in the acetone

content in the reaction medium to 25–40 vol. %. In this case, 1.5–1.9 times more free fatty acids were formed than in the absence of acetone (Fig. 2b).

Immobilized lipase was effective in the treatment of cottonseed oil to reduce the content of free fatty acids.

In fig. 3 shows the effect of enzymatic treatment of cottonseed oil on the content of free fatty acids with free and immobilized lipase. The presented data show that the treatment of cottonseed oil with free lipase does not lead to a decrease in FFA (Fig. 3, curve 1).

1- free lipase, 2 = immobilized lipase

Figure 3. Decrease in the acid number of cottonseed oil with free and immobilized lipase

Immobilized lipase is able to carry out the reaction of addition of FFA to mono- and diglyceride molecules with the formation of triglycerides, which leads to a decrease in the acid number of cottonseed oil. In this case, a decrease in the acid number of the oil begins after 90–100 minutes of incubation with immobilized lipases. the content of free fatty acids of cottonseed oil with immobilized lipase decreases the acid number of the oil by 60–70%.

Thus, the data obtained allow us to conclude that by processing cottonseed oil with immobilized lipases, it is possible to carry out the process of neutralizing cottonseed oil. This will lead to a decrease in the acid number of cottonseed oil. In this case, the state of the enzyme plays a significant role. Lipases immobilized on hydrophobic surfaces have higher activity and stability due to better orientation of the active center of the enzyme and substrate.

Literature:

1. Latif S., Anwar F., Ashraf M. Characterization of enzyme-assisted cottonseed oil by cold pressing // J Food Lipid. - 2007. -V.14. -№4.-P.424-436.
2. Bezborodov A.M., Zagustina N.A. Lipases in catalysis reactions in organic synthesis // Prikl. and microbiol. -2014. -T.50. -No 4. -C.347-373.
3. Guidelines for the technology of obtaining and processing vegetable oils and fats / Under. edited by A.G. Sergeeva, -L.: VNIIZh. -1974. -Volume1, -580 p.
4. Davydova E.M., Petrovichev V.A. Practical experience of using enzyme technology // Oil and fat industry -2002. - Number 3. -S. 24-25.
5. Abd El-Salam A.S.M., Doheim M.A., Sitohy MZ, Ramadan M.F. Deacidification of High-acid Olive Oil // J Food Process Technol 2011, S5-001, p. 1-7.
6. Khasanov Kh.T., Davranov K., Rakhimov M.M. The state of lipases of fungi *Rhizopus microsporus*, *Penicillium* sp. and *Oospora lactis* in the boundary layers of water – solid phase, factors affecting the catalytic properties of enzymes. Applied Biochemistry and Microbiology, 2015, Volume 51, No. 5, P.511-519.
7. Gulomova K., Ziomek E., Schrag J.D., Davranov K., Cygler M. // Lipids. 1996. V. 31. No. 4. P. 379-384.

8. Anderson M.M., McCarty R.E. Rapid and sensitive assay for free fatty acids using rhodamine 6G // Anal. Biochem., 1972, v. 45, p. 260-270.
9. A.C. 1510859. A method of obtaining a sorbent for immobilization of lipolytic enzymes // Khasanov Kh.T., Rakhimov M.M., Yakubov I.T., Kas'yanov S.P., Latyshev N.A., Epshtein L.M., Akulin V.N. , 1989, BI 15.
10. Neklyudov A.D., Ivankin A.N. Biochemical processing of fats and oils into new lipid products with improved biological and physicochemical properties // Applied Biochemistry. and microbiol. -2002. -T. 38.- S.469-481.