INFLUENCE OF ACTIVE ENZYMES ON COTTON OIL REFINING PROCESS

R.X.Medatov,
Kh.T.Khasanov
Ferghana Polytechnic Institute, Uzbekistan,
Tashkent chemical-technological institute, Uzbekistan.,
E-mail.: xas.tyr@rambler.ru

Abstract. Effect of cellulases and proteinases on carbohydrates and proteins of black cotton oil is studied. Processing of crude cotton oil neutral proteinase in combination with cellulolytic enzymes positively influences enzymatic processes and increases degree of hydrolysis of proteins and carbohydrates.

Key words: cellulase, proteinase, cottonseed oil, carbohydrates, proteins, hydrolysis, refining.

Introduction

The enzyme production and using them in various sectors of food industry has been significantly expanded in recent years [1,2]. The industrial development of new enzyme technologies in combination with existing technology has a number of advantages: reduce production costs, environmental safety of production, obtain products for various purposes, use raw materials of any quality and type.

Refining process is one of the most important technological processes in production of vegetable oils [3].

Refining process combines many processes, which main purpose is to remove by-products and some impurities from oil. By-products are substances which 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.

There are many methods to refine vegetable oils, which can be divided into physical, physical-chemical, and chemical methods.

Vegetable oils, both used directly for food and which be sent for industrial processing, must be subjected to a full refining cycle in order to remove harmful substances for human body, improve marketable state, increase organoleptic characteristics, and also provide to prevent the oxidation.

The traditional cotton oil refining technology consists of the following stages: hydration, neutralization (often combined with hydration), washing, drying, bleaching and deodorization [4].

The main purpose of hydration is to extract phosphatides and some hydrophilic substances from crude oil. The hydration process is a rather complex process and the results will be determined depending on the further quality changes of oil during storage and processing.

Oil contains various biopolymers, including hydrophobic proteins, carbohydrates. These biopolymers create a diffusion area at oil-water interface, the more significant the more impurities. As a result, after hydration the separation of phases occurs poorly, the yield of the separated oil layer decreases, and the refining process itself becomes difficult.

Recently, many researchers have shown the possibility of using hydrolytic enzymes in oil hydration [5]. For instance, in [6], by using the microbial phospholipase A_2 to hydrolyze non-hydratable phosphatides, the technology of hydration of vegetable oils was improved. Enzymatic hydration not only completely removes phosphatides (both hydratable and non-hydratable), but also transform them into high effective emulsifiers.

It could be assumed that in our case, using cellulases and proteinases for hydrolyze of biopolymers (proteins and carbohydrates) of cottonseed oil increases the hydratability of by-products, which is reflected in the yield of refined oil and refining parameters.

The aim of this research work is to study the effect of cellulolitic and proteolytic enzymes on crude cottonseed oil biopolymers during refining process.

2. Materials and methods

"ViscoStar 150L" as cellulases and neutral proteinase (Neutrase) were manufactured by Novozymes, Denmark. The crude cottonseed oil was obtained from “Toshkent Yog-moy combinati” JSC, Uzbekistan.

2.1. Enzymatic treatment of cottonseed oil. 200 g of testing oil was heated to 45-50^{0}C with stirring, then the stirrer speed was increased and a predetermined amount of a solution of "ViscoStar 150L" and neutrase diluted in water was added slowly in a ratio of 1:1, and kept stirring for 60 min. The total water content was 6% and the enzyme was 0.1% of oil weight. Then a temperature of the mixture was increased to 85^{0}C and kept at this temperature for 20 minutes. At this temperature, the mixture was stirred for 60 minutes.

2.2. Determination of carbohydrate hydrolysis products. An equal volume of distilled water was added to the fermented oil and after thorough mixing, it was centrifuged at 5000 rpm for 10 min. Then, the aqueous part was separated and the content of reducing sugars was determined by Nelson and Shomody method [7].

2.3. Determination of protein hydrolysis products. 2 ml were taken from the aqueous portion of centrifuge centrate, 0.3 M TCA was added and filtered. Then, 0.2 ml aliquots were taken from the filtrate, 0.8 ml distilled water, 5 ml of 0.5 M Na_2CO_3 solution were added. The mixture was shaken and 1 ml of Folin solution was added. The content of amino acids was determined by optical density of the samples [8].

2.4. Neutralization of cottonseed oil. 200 g hydrated oil was heated to 50^{0}C. Upon reaching the setting temperature, without stopping mixing, the calculated amount of alkali was added. Then the oil temperature was raised by 10-15^{0}C, the rotation frequency was reduced, and stirring was carried out until well-formed flakes formed. The neutralized oil was decanted, washed with hot distilled water (60-65^{0}C), filtered and dried at 100-110^{0}C.

Chemical analysis of refined oil was carried out according to generally accepted method [9].

Results and discussion

It has always to be faced to formation and destruction of highly dispersed systems, which properties can be often determined by chosen technology, during the extraction and processing of oil. The high dispersity occurs due to by-compounds in oil.

The by-compounds include: free fatty acids, phospholipids, waxes (esters of higher monohydric alcohols and higher fatty acids), hydrocarbons, vitamins, proteins and compounds which formed during heat moisture-heat treatment of cotton meal.

It is known that the amount of carbohydrates and proteins in cotton meal are 12-14% and 25-38%, respectively. Some part of proteins and carbohydrates, together with other substances, pass to the oil, during the forepression of cotton meal.

By-compounds demonstrate surface-active properties and due to the oil processing is complicate. These properties of by-compounds can be weakened by enzymatic influence. To increase the degree of hydratability of the by-compounds, we have studied the hydrolysis of carbohydrates and proteins of unrefined oil with cellulolytic and proteolytic enzymes.

Table 1. shows the content of products of hydrolysis of proteins and carbohydrates after fermentation of crude cottonseed oil with enzyme preparations such as: ViscoStar 150L , neutral proteinase (Neutrase) and their mixtures.

Table 1

The effect of neutral proteinases with amylases on hydrolyzable of proteins and carbohydrates of crude cottonseed oil at pH 7.0

No.	Enzymes used	Reducing sugar, mg/g in oil	Protein hydrolysis products, µmol/g in oil
1	Without enzymes	1,44±0,06	0,55±0,02
2	Neutrase	1,46±0,06	1,2±0,04
3	Neutrase +"ViscoStar 150L"	4,65±0,24	1,28±0,04
4	ViscoStar 150L	4,82±0,24	0,62±0,02

It can be seen from the table that the treatment of cottonseed oil with enzymes leads to accumulation of hydrolysis products of macromolecular compounds. For instance, under the treatment with Neutrase and ViscoStar 150L, the formation of reducing sugars was 4.65 ± 0.24 mg/g in oil, while the products of hydrolysis of proteins was 1.28±0.04 µmol/g in oil.

4. Conclusion

Enzymatic solutions consisting of Neutrase and cellulases (ViscoStar 150L) significantly effect on the refining process of cottonseed oil. Therefore, the physical - chemical parameters of refined oil were better than, when these enzymes were used separately[10].

This is achieved due to the enzymatic breakdown of carbohydrates and hydrophobic proteins contained in oils and preventing refining. Using enzymes in cottonseed oil refining, makes it possible to improve the vegetable oils technology.

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