Biocatalyst Regeneration in Fruit Concentrate Processing: A Study of Enzyme Recovery Techniques

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ABSTRACT

The enzymatic activity of amylase and pectinase in the wastes of apple juice after ultrafiltration.Was studied it was found the in the waste a significant amount of enzymes remains that retain their activity, which made it possible to assume their repeated use after regeneration.

KEYWORDS: fermentation, amylase, pectinase, regeneration.

1. INTRODUCTION

The limited natural resources and environmentally harmful changes in the nature for human raise the question of the development of skeintypically grounded complex and environmentally safe technology for the processing of secondary raw materials and the need for the production of a new generation of food. Products, balanced on the mam ingredients: protein, carbohydrates, fats, enzymes and other biologically active substances [1]. These circumstances require careful consideration of the issues on the study of biotechnology of fruit berry raw material and their wasters in using pectolytic and amilolytic enzymes in food industry, in particular, canning and juice industry [2].

The use of enzyme preparations can be considered one of the most promising areas of intensification the production processes of fruit berry juices. They are used to increase the yield of juice, lighting and stabilization of beverages and prevent oxidative processes in the products made from them [3]. Therefore, the development of regeneration technology of amylase and pectinase from the waste of fruit berry juices to create a waste – free, cost- effective and environmentally safe technologies, improvement of products quality is an actual task.

Of all types of fruit berry preserves the most useful for man are juices. They have a high nutritional and biological value. At the same time in respect of human physiology the most valuable are natural juices lighted.

One of the problems of development of industrial production of drinks based on fruits and berries is related primarily to unsolved problems to ensure their durability. The composition of such drinks include colloidal substances causing turbidity and conditioning their instability (pectin substances, starch, proteins, polyphenol compounds). At present, to increase the yield of juice, lighting and stabilization of beverages and prevent oxidative processes in the products made from them enzymes, are used.

2. MATERIALS AND METHODS

To obtain high-quality and stable apple juice concentrate amylase and pectinase are used. The object of the research was a waste of fruit – burry juice after fermentation, in the production of apple juice concentrates, in which amylase and pectinase with brands Amylase AG 300 LK.F. and Pectinex Ultra AFP contains K.F. were used as enzymes.

3. RESULTS AND DISCUSSION

It is known [4], that a proteinaceous nature provides enzymes, characteristic for proteins, the ability to change protein activity, depending on various factors. The level of changes in activity on various factors can be determined by the enzymatic reaction rate. We have studied the effect of temperature and pH on the rate of enzymatic reactions of the enzymes used. In studying the effect of a factor on the rate of enzymatic reaction, all other factors were held constant and had the optimum values.

To determine the effect of the temperature the selection of apple juice sample after the compression step was carried out. Then the juice was heated to a temperature 92^0C. At the same time the process of pasteurization and destruction of oxiredutase juice occurs. The starch contained in the juice at the temperature falls onto dextrin. Then the juice was cooled to room temperature. To determine the effect the temperature on amylase activity the known method was used [5]. The obtained are shown in Table 1.

To determine the effect of the temperature on the activity of pectinase the ethanol test on pectin was used [6].

Table 1

The effect of the temperature on the activity of amylase KF

Temperature of incubation	Coloring with iodine	Names of the products detected on the basis of color
0	blue	Not found cleavage

22-30	orange	Incomplete cleavage
45-50	yellow	Full splitting
85	blue	Inactivation of the enzyme

Table 2 shows the experimental data for the study of temperature effect on pectinase activity.

Table 2

The effect of the temperature on pectinase KF activity

Temperature of incubation	Detection index	Names of the products detected on the basis of color
0	Gel formation	Not found cleavage
22-30	Big residue	Incomplete cleavage
45-50	Homogeny system	Full splitting
85	Gel formation	Inactivation of the enzyme

On the basis of finding s it is established that the optimal value of the temperature, in which the reaction proceeds at maximum rate, is higher than 37^0C. An increase of extremely optimal temperature leads to the decrease, then to the cessation of the enzyme effect that is related with denaturation. In the transition from optimal to low temperature the rate of enzymatic reaction decreases and at 0^0C stops. The cause is a decrease of the movement rate of the molecules of substrates and enzymes, that reduces formation of enzyme-substrate complex and carrying the reaction out. In an increase of the temperature from negative values an action of the enzymes is reduced, the rate of the reaction catalyzed increases.

The pH effect on enzyme activity was studied. The finding are presented in tables 3 and 4.

Table 3

The effect of pH on amylase KF activity

Indices	Number of test-tubes				
	1	2	3	4	5
pH	2.87	4.10	5.33	6.59	7.96
Coloring with iodine	brown	orange	yellow	orange	brown

Table 4

The effect of pH on pektinase KF activity

Indices	Number of test-tubes				
	1	2	3	4	5
pH	2.8	4.10	5.33	6.59	7.96
Detection	Gel formation	Big residue	Homogeny system	Flaky residue	Gel test-tube

The prices of fermentation of apple juice was carried out by adding enzymes solution, obtained after regeneration and put into thermostat at temperature of 45^0C. After two hours the tests for enzymes stability were conducted.

It was visually found that a homogeneous system was formed after addiction of enzyme solution, which shows pectin decomposition.

The results of iodine test have shown the complete decomposition of starch. In the result of conducted regeneration process of enzymes a mixture of enzymes containing amylase and pectinase was obtained.

Thus, on the basis of findings the opportunity of regeneration of amylase and pectinase has been shown and their repeated use in fermentation of apple juice.

It is found that optimal pH value is 5.33, in which the reaction proceeds with maximum rate. In the production of apple juice after ultrafiltration a waste of enzymes, that is a mixture of carbon adsorbent and residue of apple juice is formed. The adsorbent we have formed in the result of ultrafiltration was extracted from the process line and studied the enzymatic activity of amylase and pectinase sorbet on it.

Pectolytic activity in the waste of apple juice was determined using an interferometry method. For the unit of pectolytic activity was accepted an amount of enzyme, which catalyzes the hydrolysis of 1 gram of pectin to the products, not precipitated with zinc sulfate in conducting the hydrolysis under strictly defined conditions: temperature 50^0C, the hydrolysis time 1 hour, the pH of the reaction medium-4. Pectolytic activity has been expressed in the number of units 1 gram of the test preparation.

Table 5

Activity of pectinase KF in waste after ultrafiltration

Number of tubes	Activity of pectinase

	PkC	%
1	77.5	98.7
2	77.3	98.5
3	77.6	98.9

To determent the amylase activity in the filtrate was used Wohlgemuth method [3], based on finding the maximum dilution of liquid containing amylase KF, at which under standard conditions complete decomposition of a certain amount of starch occurs. The analyses were carried out at 37^0C for 60 minutes. As a control, a starch solution in distilled water in the range of concentrations studied was used. Based on these results established that the control solutions in the absence of amylase had blue color, and in test solutions at concentrations up to 0.75%, this reaction does not occur due to the extremely low levels of starch. On the hand, in the experiment, which uses waste of apple juice after ultrafiltration starting with starch content of 12.5% amylase activity of this waster was clearly revealed. The lack of a positive reaction to the amylase activity demonstrates that the enzyme contained in the waste is not able to decompose all entered starch at a large number into the reaction mixture.

Thus, our studies have shown that after ultrafiltration the wastes of apple juice have significant amount of enzyme, which retains its activity and is able to decompose the starch at a concentration of 12.5%. In practice the starch content in apple juice is from 1% to 1.5%.

Table 6

Determination of amylase activity in the waste of apple juice after ultrafiltration

Number of samples	Dilution of 1% starch in%	Color solution	
		Experiment (waste)	Control (waster)
1	50	blue	blue
2	25	blue	blue
3	12.5	brown	blue
4	6.2	brown	blue
5	3.1	yellow	blue
6	1.5	yellow	blue
7	0.75	yellow	yellow

Thus, it is found that after the ultrafiltration process, the fermentative activity of enzymes in the waste of production of apple juice remains, which allows to suggest their reuse after regeneration.

To study regeneration prowess of enzymes the waste was separated by centrifugation into solid and liquid phase. Then after that the fermentative activity of the enzymes was determined in solid and liquid phases. It is found that the liquid phase does not contain enzymes. On the basis of the obtained results it could be suggested that the enzymes are adsorbed on activated carbon. To determine the fermentative activity of the enzymes in solid phase, the solid phase was washed witch acetate buffer solution witch pH=5,5. Then it was filtered witch a water pump. After filtration, the fermentative activity was determined in the newly formed liquid and solid phases. The obtained results are shown in Tables 7 and 8.

Table 7

Determination of the activity of pectinase in solid phase of the waste

Number of tubes	Activity of pectinase	
	PKS, U\g	%
1	25.59	30.0
2	27.12	31.8
3	26.10	30.6

Table 8

Determination of amylase activity in the waste of apple juice in the solid phase of the waste after centrifugation

Number of samples	Dilution of 1% starch in %	Solution color	
		Experiment (waste)	Control (water)
1	50	blue	blue
2	25	blue	blue
3	12.5	brown	blue
4	6.2	sv. brown	blue
5	3.1	yellow	blue
6	1.5	yellow	blue
7	0.75	yellow	yellow

On the basis of the obtained results it is established that the enzymes are adsorbed mainly on activated carbon and in washing with acetate buffer it may be separated to the liquid phase. To study the opportunity of reuse of enzymes for the regeneration of pectinase and amylase, we have conducted their deposition in with the solution of NaCl. The enzymes formed in deposition were filtered and dissolved in water. Then, sampling of apple juice from the tank was carried out after cooling up to 50^0 C. Application of the developed technology allows to increase juice production efficiency by precipitation and regeneration of enzymes sorbents.

4. CONCLUSION

1. The possibility of isolating adsorbed enzymes on activated carbon in the liquid phase by washing with acetate buffer solution was investigated.
2. It is established that the activity of enzymes after ultrafiltration in waste apple juice is preserved and contribute to the breakdown of starch at the concentration of 12.5%
3. The possibility of amylase and pectinase regeneration after enzymeation of apple juice was shown.

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