Effect of hydrolytic enzymes pretreatment on the oil extraction from pumpkin seeds

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Abstract

Introduction. The aim of the present study was to evaluate the effect of enzyme mixture on the cell integrity, oil yield of cold-pressing and dynamics of solvent extraction of pumpkin seeds oil.

Materials and Methods. Protolad, Alkaline, acid proteases and Cellulad (ENZIME, Ukraine) were used for pumpkin seeds pretreatment. The cells integrity was evaluated by the method of immediate "shaking". The cold pressing was carried out on the laboratory screw press. The solvent oil extraction rate was estimated using a Soxhlet extractor as oil quantity extracted after one extraction cycle.

Results and discussion. It was detected that main increase of pumpkin seed cells integrity destruction comparing with a control sample had been happened after 2-hour of enzyme pretreatment. Further incubation of ground seeds with enzymes did not lead to significant increase of "open" cells content in the mixture. It was shown that using of different kind of proteolitic enzymes for pumpkin seeds pretreatment resulted in increase of destroyed cells quantity from 3 to 10.4%. Using of proteases and cellulase mixture for pumpkin seeds pretreatment had resulted in further increase of level of pumpkin seed cells "revealing" by 10%. The oil yield of cold pressed pumpkin oil after enzyme pretreatment with protease (70%.) and cellulase (30%) mixture was increased from 62.3 (control sample) to 70.0%. from total oil content of seeds. The rate of solvent extraction of oil from pumpkin seeds had increased after enzyme pretreatment, that means 25.4 and 17.7% of oil were extracted after 80 min extraction from mass of enzyme pretreated and control seeds, respectively. There was no difference of peroxide content between enzyme pretreated sample and control.

Conclusions. Using of proteases and cellulases mixture for pumpkin seeds pretreatment had resulted in increase of destroyed cells quantity, following by increase of cold pressed pumpkin oil as well as rate of solvent extraction of oil from pumpkin seeds.

Introduction

Pressing and solvent extraction are the two methods for oil obtaining from oil seeds. The solvent extraction is more effective for oil yield, that gives possibility to recover almost total oil content. The disadvantages of this method are process safety and environmental issues associated with solvent extraction process. The pressing is more environment friendly but does not provide the whole oil extraction. Thus it is important to develop the effective and environment friendly technology of oil recovering from oil materials.

In this context, different enzymatic technologies were proposed for oil extraction from raw materials. Firstly, the enzyme assisted aqua extraction was used to obtain oil and protein from oil seeds. In order to increase the efficiency of water extraction of oils and proteins from oilseeds, it was proposed to use hydrolytic enzymes to destroy cell walls, as well as protein frames surrounding oleosomes [1-10]. The oil yield had varied from about 60 to 90%. depending from proteases and cellulases used and kind of oil material. The weakness of enzyme assisted aqua extraction is the necessarity to separate of oil-in-water emulsion and low oil yield. Enzyme assisted technology was proposed to obtain the partly hydrolyzated protein from sunflower meal [11,12].

In parallel, enzyme assisted technologies were used for oil seeds pretreatment before their processing. The cellulase, hemicellulase, pectinase and proteases are usually used for this purpose. It was shown that press oil yield had increased from 72 to 90-93%. after pretreatment of rape seeds by carbohydrases and proteases [13–16]. The increase of press soy bean oil yield after enzyme pretreatment was shown in [17–19].

The effect of different enzyme preparations (Viscozyme L, Kemzyme, and Feedzyme) on the oil yield, physicochemical and antioxidant properties of cold pressed flaxseed oil were assessed in [20]. The oil yield from enzyme-treated cold pressed flaxseeds, although lower than Soxhlet extracted oil yield, was considerably higher when compared with the control Most of the physicochemical parameters such as refractive index, density, iodine number, free fatty acid contents, saponification value, color and fatty acid profile did not differ significantly between the control and enzyme pretreated oil. At the same time the peroxide value, *para*-anisidine value, conjugated dienes, triens and induction period (Rancimat method) of oil from enzyme-treated flaxseeds were superior compared with the control. The effects of enzyme-assisted cold-pressing (EACP) on the oil yield and physicochemical properties of hemp seed oil were also investigated using five enzyme preparations [21]. The oil yield from enzyme-treated hempseeds were found to be significantly higher than that determined for the control. There were no significant variations observed for the values of iodine number, refractive index, density, unsaponifiable matter and fatty acid composition between the enzyme-extracted and control hempseed oils. The values of saponification value, free fatty acids, iodine value and peroxide value were slightly varied between the oils tested. A relatively higher content of tocopherols (4.8–14.1%) as well as improved Rancimat profiles were observed in the enzyme extracted oils compared to the control.

To the best of our knowledge, there are no reports about pumpkin seeds oil recovering using EACP. The aim of the present study was to evaluate the effect of proteases and cellulases on the cell integrity, oil yield of the EACP and dynamics of solvent extraction of pumpkin seeds oil.

Materials and methods

Materials

Pumpkin (*Cucurbita pepo*) seeds were purchased from a local market (Kyiv, Ukraine). Neutral protease from *Bacillus subtilis* (Protolad, 70 FIP-U/g, optimum pH 6.5-7.0, ENZIME, Ukraine), alkaline protease from *Bacillus licheniformis* (Alkalase, 2.4 AU/g, optimum pH 8.5-9.0, ENZIM, Ukraine) and acid protease (ENZIM, Ukraine) were used for hydrolysis of cell proteins. Cellulad (ENZIM, Ukraine) – a complex enzyme preparation of cellulases for the hydrolysis of non-starch polysaccharides, obtained by directed fermentation of the breeding strain *Tr. reesei*. In addition to the main activity of cellulase, the preparation contains significant amounts of hemicellulase and xylanase. All chemicals used for experiments were at least analytical grade.

Enzyme treatment and pressing

Clean seeds were ground using a coffee grinder and then subjected to incubation with 0.6%. (by seed weight) of the enzyme preparations over a period of 2 h at (51±3) °C and 50%. moisture level. The hydrolyzed sample was dried at 100 °C in a drying oven to inactivate the enzymes and to readjust the moisture to the desired level (6–7%) prior to pressing. Pressing of the hydrolyzed and dried seed sample for oil extraction was done in a laboratory screw press at (60±5) °C. A control sample of pumpkin seeds was also processed under the same set of conditions, except for the enzyme adding during treatment.

Evaluation of the cells integrity by the method of immediate "shaking"

10 g of ground pumpkin seeds were placed in a flask, 200 cm^3 of petroleum ether was added and the contents of the flask were shaken for 3 seconds (exactly). Then the flask was left for 10 seconds (exactly), after which the ether solution of oil was quickly and carefully transferred to another flask. The obtained solution was filtered to a weighed flask. The filter was washed by several portions of the ether. The ether from the weighed flask was evaporated and the resulting oil was dried to a constant weight. Simultaneously another 10 g of ground pumpkin seeds were taken to determine the oil content by the extraction in the Soxhlet apparatus. The mass fraction of oil extracted from the instantaneous culling and expressed as a percentage of its total content corresponds to the number of "exposed" cells.

The number of destroyed cells was determined as a percentage of oil extracted by the immediate "shaking" from the total oil content in the ground pumpkin seeds:

$$X = \frac{a_1 \cdot 100}{a_2},$$

where a_1 - oil extracted by the immediate "shaking",%; a_2 - the total oil content of ground pumpkin seeds, determined by the extraction in the Soxhlet apparatus,%.

Measurement of dynamics of oil solvent extraction

The enzyme pretreatment of pumpkin seeds was carried out as described in 2.2. The pretreated and control seeds were taken to filtering paper shells, weighted and placed in a Soxhlet extractor. The extractor was fitted with a condenser and a 0.5 L round bottomed flask. The extraction of oil was done in a water bath for 80 min, using about 350 mL *n*-

hexane. The duration of extraction cycle was about 10 min. After every extraction cycle the three filtering paper shells were withdrawn from extractor, hexane was evaporated, dried and weighted. The level of oil extraction was calculated using the following equation:

$$O = \frac{(m_0 - m_n)}{m_0} \cdot 100,$$

where m_0 - the initial mass of filtering paper shell, m_1 - the mass of filtering paper shell after every extraction cycle, n - the number of extraction cycle.

Determinations of peroxide values

Determinations of peroxide values of the enzyme extracted and control pumpkin seed oils were made following AOCS official methods [24].

Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data was performed using the statistical software Statistica [25]. A probability value at $p< 0.05$ was considered statistically significant. Data are presented as mean values±standard deviation calculated from triplicate measurings.

Results and discussion

Influence of enzyme pretreatment of pumpkin seeds on the cell integrity

To establish the appropriate conditions for seeds pretreatment firstly we have study the influence of treatment duration by proteases on the cell integrity. It was detected, that after first hour of treatment about 5.5%. increase of destroyed cells were observed comparing with a control sample (Fig. 1) and next hour of treatment have resulted in next 9.6%. of destroyed cells content increase. Further incubation of ground seeds with enzymes did not lead to significant increase of "open" cells content in the mixture. It was decided to use two-hour enzyme treatment for the next study. At the same time enzyme pretreatment duration of hemp seeds was up to 6 hours in study of Latif S. and Anwar F. [21].

The data obtained have demonstrated that using of different kind of proteolitic enzymes for pumpkin seeds pretreatment resulted in increase of destroyed cells quantity from 3 to 10.4%. (Fig. 2). The acid proteases were the most effective enzymes in "revealing" of pumpkin seeds cells, showing the highest increase of destroyed cells content in oil material.

Obviously, that proteins are important substances for the integrity of pumpkin seeds cells, which play a significant role in the building of the intracellular membranes, including that surrounding the oleosomes and in the adhesion of cell membrane to cell walls. Enzyme pretreatment facilitates the breakdown of the protein network surrounding the lipid bodies and also supports the conversion of the complex seed lipoprotein molecules into simple lipid and protein molecules, thereafter enhancing both the oil availability and extractability [22, 23].

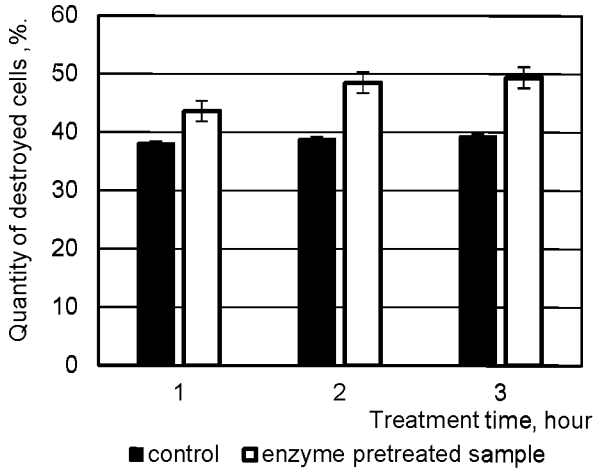

Figure 1. The influence of proteases treatment duration on the quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds

The accessibility of cell oil depends from the integrity of cell walls, that are building from cellulose. It was supposing, that adding of cell-wall-degrading enzymes to the enzyme cocktail for seeds pretreatment has to enhance the enzymes effect on the cell structure and improve the accessibility of oil.

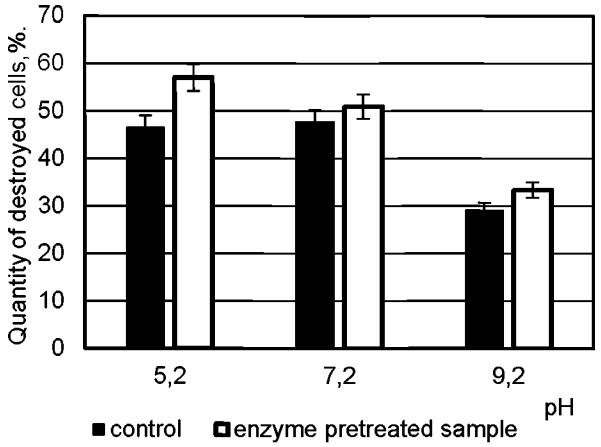

Figure 2. The influence of different proteases on the quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds

The data obtained have shown that using of protease and cellulase for pumpkin seeds pretreatment had resulted in some increase of destroyed cells in ground pumpkin seeds mixture (Fig 3). The maximum level of "revealing" of pumpkin seeds cells had increased to about 61%. under influence of mixture of 30% neutral protease and 70% cellulase that is 10%. higher than after treatment by this protease itself. The growing of content of destroyed cells was about 3%. under using of acid protease and cellulase mixture (Fig 3, b).

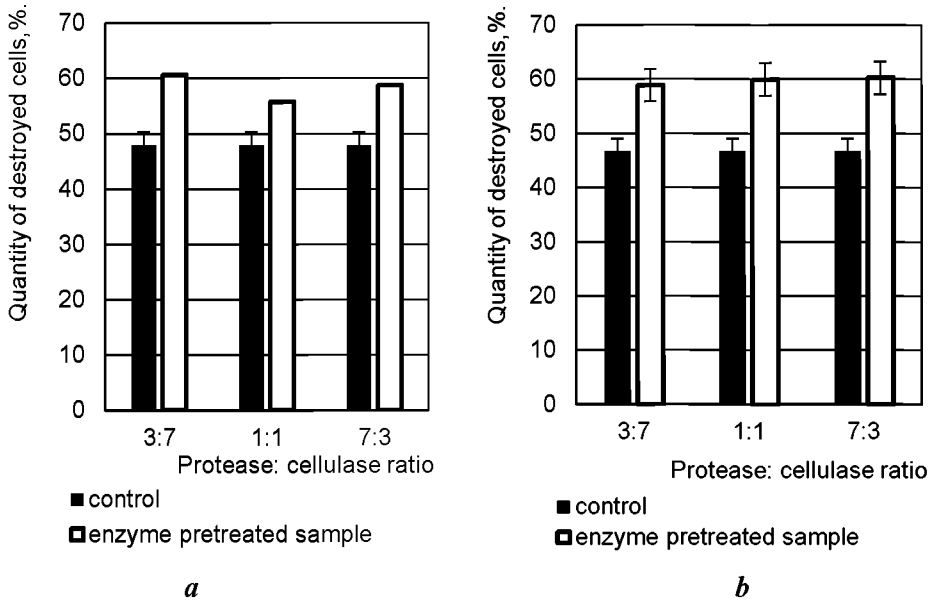

Figure 3. The quantity of destroyed cells in control and enzyme pretreated ground pumpkin seeds on dependence the proteases kind and protease: cellulase ratio (a - neutral protease and cellulase mixture, b - acid protease and cellulase mixture)

Influence of enzyme pretreatment of pumpkin seeds on oil recovery

The cold pressing of dried pumpkin seeds (seeds moisture was 7.0±0.1) were carried at (60±5) °C. The oil yield of cold pressed pumpkin oil after enzyme pretreatment of seeds with neutral protease (70%.) and cellulase (30%.) mixture was increased from 62.3 (control sample) to 70.0%. from total oil content, which was 42.4%. Our data are in accordance with the results obtained for hemp seeds with the enzyme of complex activity (mainly a-amylase, b-glucanase, cellulase complex, hemicellulase complex, protease and xylanase activities) [21] as well as with results obtained for flax seeds [20] and rape seeds [13] cold pressing after enzyme pretreatment.

Commonly, the enzyme pretreatment of oil seeds are using as a technique to increase the press oil yield, mainly cold pressed oil, as heat pressing and solvent extraction of oil seeds give a high recovering of oil on their own. But in our study we have researched the dynamics of oil solvent extraction from oil material under effect of enzyme pretreatment.

The rate of solvent extraction of oil from pumpkin seeds was influenced by enzyme mixture pretreatment also (Fig. 4). Enhancement of oil extraction as result of enzyme mixture pretreatment was observed from the beginning of the extraction process. After first 10 min of extraction about 6.6%. of oil were extracted from control seed samples, whereas 10.3%. from enzyme pretreated seeds, respectively. The difference between two samples was 7.7%. after 80 min extraction, that is 25.4 and 17.7%. of oil were extracted from mass of enzyme pretreated and control seeds, respectively. These were about 60 and 40%. of total oil content for enzyme pretreated and control seeds, respectively.

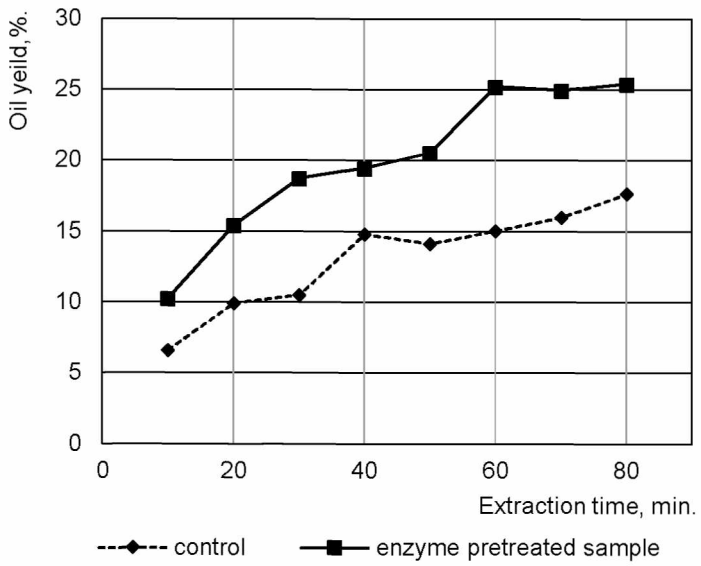

Figure 4. Dynamics of solvent extraction of oil from control and enzyme pretreated pumpkin seeds

It was possible to suggest that enzyme treatment at 40 °C and 50%. moisture level of oil seeds for 2 hours could result in increase of oil quality and probably its biological value. To elucidate this phenomena the main chemical parameters of oil samples had been evaluated. There were not any differences in acidity of oil, obtained from the pretreated seeds and from control seeds, the acid value of pumpkin oil was in the range from 2 to 2.4 mg KOH/g. The similar results were obtained concerning the measuring of oil oxidation level. It had not been demonstrated significant ($p<0.05$) difference of oil peroxide values, they were 2.47 and 2.44 mmol 1/2 O/kg for control and enzyme pretreated pumpkin seed oils, respectively. These results are corresponded to the data obtained for cold presss oils from enzyme pretreated hemp seeds [21], flax seeds [20] and rape seeds [13].

There were no reliable diversity in the composition of fatty acids between the studied samples of oils [26]. The ratio between the individual representatives of sterols in the studied samples of pumpkin oil almost did not differ. The analysis of the tocopherol homologies and their total content in the oil samples shows that tocopherol content in the oil obtained from the seeds after processing with hydrolytic enzymes was 68% higher than in the control [26]. Thus, these results mean that enzyme pretreatment of pumpkin seeds did not decrease the oil quality and improve the biological value of oil.

Conclusions

The results of the present study have showed that the enzymes added during the pretreatment of pumpkin seeds resulted in considerably higher content of destroyed cells in the mixture of ground pumpkin seeds. The mixture of 30%. neutral protease and 70%. cellulase was the most effective, increasing the content of destroyed cells in enzyme pretreated seeds by 10%. comparing with a control. The increase of cold pressed pumpkin

seed oils from enzyme pretreated seeds was observed as result of enzyme influence on the cells integrity. The enzyme pretreatment of pumpkin seeds had accelerated solvent extraction of oil comparing with untreated seeds. The enzyme pretreatment of pumpkin seeds had not adversely affect the quality of the oil. This suggests the use of proteases and cellulases mixture in preparing for the extraction of oil from pumpkin seeds.

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