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Original scientific paper

THE USE OF GLYCOHYDROLASE IN THE PROCESSING OF HULL-LESS SEED VARIETY OF PUMPKIN IN THE RELATION TO ENHANCED PROTEIN EXTRACTION

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Enzyme hydrolysis optimisation of cell-wall polysaccharides of pumpkin seed with cellulases and pectinases and their mixture, in the relation to enhanced protein extraction was the objective of this study. The individual and combined effects of cell-wall-degrading enzyme activities on protein extraction combined with other process parameters: enzyme concentration and time of hydrolysis were evaluated by the Response Surface Methodology (RSM).

The optimal value protein isolate yield (9 g of soluble protein from 100 g pumpkin seed)is achieved under the following conditions: 2% enzymes (cellulases), reaction time 300 min, pH = 5.0, temperature 45°C.

KEYWORDS: *Cucurbita pepo*; protein isolates; enyzmatic extraction; pectinase; cellulase

INTRODUCTION

The seeds of plants affiliated to the family of *Cucurbitacea* produce a number of proteins and peptides. The proteins exhibit abortificient, antitumur, antifugal ribosome inactiving, immunomodulatory and anti-AID activities (1, 2, 3). In the recent years, more attention has been focused on the utilisation of pumpkin seed proteins in the production of various new foods, drugs, cosmetic ingredients and biodegradable packing films (4, 5).

The proteins are found inside of oil pumpkin (*Cucurbita pepo* L.) seeds cells linked with oil and a wide range of carbohydrates (cellulose, hemi-cellulose and pectin) (6). The cell content is surrounded by a rather thick wall, which has to be opened so that the

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protein and oil can be released. Thus, when opened by enzymatic degradation, downstream processing makes fractionation of the components possible to a degree which cannot be reached when using the conventional techniques (7). In the general conventional techniques of extraction of the proteins from oil seed, after the separation step, the bulk proteins may be recovered as concentrate in the solid phase, or as isolate in the aqueous phase, depending on the pH of the extraction medium (8).

In contrast to conventional processing, the enzyme process is based on the use of water as solvent and cell wall degrading enzymes, glycohydrolase, to facilitate an easy and mild fractionation of oil and protein. Pumpkin seeds (*Cucurbits pepo,* variety Olinka), could be utilized successfully as a good source of edible protein (350 g/kg) and oil (444 g/kg) for human consumption and new dietary supplement (9). In pumpkin seed, the main storage proteins are salt-soluble globulins, accompanied by gluteliens and lesser amoumts of albumines and prolamins (10). Pumpkin storage proteins as such as 2S albumines and 11S globulines are localized in the protein bodies. Protein bodies consist of a matrix and a crystalloid composed of 11S globulin (cucurbitin) (11). Solubility of a protein is one of the critical functional attributes required for its use as a food ingredient; solubility greatly influences other properties, such as emulsification, gelation and foaming (12).

The aim of this study was to investigate optimisation of enzyme hydrolysis of cell-wall polysaccharides of pumpkin seed with cellulases and pectinases, in the relation to enhanced protein extraction. The individual and combined effect of cell-wall-degrading enzyme activities on protein extraction combined with other process parameters: enzyme concentration and time of hydrolysis were evaluated by the Response Surface Methodology (RSM).

EXPERIMENTAL

Pumpkin seeds (*Cucurbita pepo,* cv. Olinka) were provided by the "Pan-Union", Novi Sad, Serbia. The seeds were kept at the temperature of 4°C until used in the experiment. The seeds were ground in a coffee-grinder, providing specks smaller than 2 mm.

Enzymes

Two different types of enzymes-cellulase and pectinase were selected on the basis of the chemical composition of pumpkin seed.

Pectinase

The pectinase used in the study, produced from *Aspergillus niger* by Novo Nordisk and commercially named Pectinex ultra sp, had a declared activity of 26,000 galacturonic acid /ml, measured at pH 5.0 and 45°C, which also coincides with the optimum conditions for maximum enzyme activity.

Cellulase

Cellulase purchased from Sigma (commercial code no. C2415) was produced from *Aspergillus niger*. The declared activity at pH 5.0 and 37°C (which is around the optimum

conditions for the enzyme activity) was 5.1 unit/mg of solids. It should be noted that one unit of cellulase activity corresponds to the one which releases 1mol of D-glucose from cellulose per hour.

Enzyme extraction process

Ground pumpkin seeds were resuspended in 0.05 mol/l phosphate buffer, pH 5. The hydrolytic enzymatic treatment to enhance protein extractability was performed during the mixing stage, which was carried out at 150 rpm and 45°C (the optimum to preserve the quality of products and favour the activity and stability of enzymes). The optimum pH for these enzymes is 5.0. The experimental conditions (such as seed/aqueous phase ratio, size of seed specks) were too constant in all experiments of protein extraction. The other experimental conditions (enzyme/seed ratio and extraction time) depended on the experiment. The enzyme preparation was dosed in different concentrations in order to study and optimise the maceration procedure. Separation of the solid phase from the liquid phase was achieved using Centrifuge "Sorvall", 14,000 g, 20 minutes. The solid phase after the first centrifuging was separated, while supernatant was centrifuged again under the same conditions.

Methods

Association of Official Analytical Chemists AOAC (1990) methods were used to determine seed moisture content, oil content, and content of carbohydrates. Concentration of reducing sugars was assayed by DNS method according to Miller (1959). Soluble proteins were determined by Lowry (1951) method.

Response surface methodology (RSM)

Response surface methodology (RSM) is an effective tool for optimizing the process. Independent variable values of the process and their corresponding levels are shown in Table 1.

Table 1. Independent variable values of the process and their corresponding levels

Independent variable		**Levels**		
		-1	0	1
Reaction time (min)	X_1	0	150*	300**
Concentration of enzymes (%)	X_2	0	1	2

* For pectinase: 60 min;

** For pectinase: 120 min

The computer software used for this study was Statsoft statitica 5.0. and the polynomal model:

$$\mathbf{Y = B_0 + B_1X_1 + B_2X_2 - B_3X_1^2 + B_4X_1X_2 + B_5X_2^2}$$

where: B_0, B_1...B_5 – coefficient of the polynomal model, Y- protein content (mg/g) or reducing sugars solulubilized in the liquid phase (mg/g), X_1- reaction time (min), X_2 - concentration of the enzymes.

RESULTS AND DISCUSSION

The optimisation of enzyme hydrolysis of polysaccharides of cell-wall of Olinka pumpkin seed in the relation to enhanced protein extraction was conducted by analysing the following: chemical composition of pumpkin seed and dosage and choice of enzymes.

Chemical composition

Hull–less seed variety of pumpkin c.v. Olinka meal contains 5.49 % moisture and dry residue shows high oil (44.4 %) and proteins (35.29 %) contents 96 % digestibility, which is interesting for the food industry. According to the chemical analysis (Table 2), carbohydrates of hull-less kernel (Olinka) are 0.26 % reducing sugars (simple sugars) and 9.49% unavailable carbohydrates. Cellulose is the major unavailable carbohydrate, present in a concentration of 4.69 %, while the concentration of hemicelluloses and pectin are 4.80 % and 2.7 %, respectively.

Table 2. Chemical composition of carbohydrate of hull-less pumpkin seed

Carbohydrate (%)	9.75 ± 4.2
Row cellulose (%)	4.69
Hemicelluloses (%)	4.80
Reducing sugar (%)	0.26
Pectin (%)	2.7

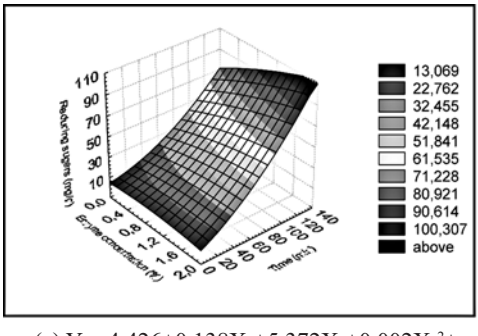
In spite of the low carbohydrate percentage, its composition is interesting for further studies becouse of its high cellulose and pectin contents. Enzyme hydrolysis yielded the degradation of cellulose and pectin in cell wall and released protein from pumpkin seed cells.

Dosage and choice of enzymes

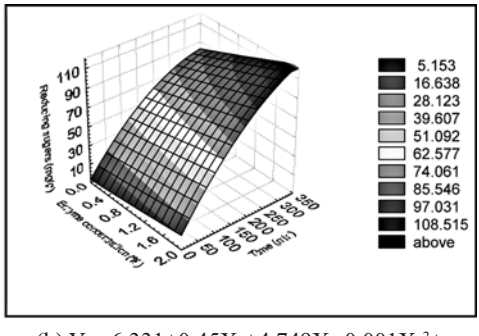
The effect on protein extraction yield of enzymatic treatment was evaluated using two commercial formulations. When many factors and interactions affect desired response, response surface methodology is an effective tool for optimizing the process (16).

Enzymatic degradation of polysaccharides was accompanied by formation of degradation products such as reducing sugars. Simultaneous effect of two independent variable values: concentration of enzymes and reaction time were investigated. Figure 1 shows 3D graphs of carbohydrate degradation by individual and combined activities of enzymes

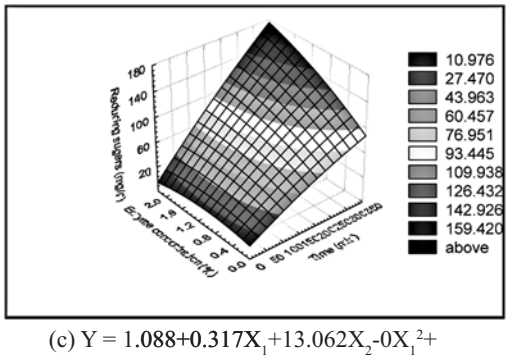
(pectinase and cellulase), measured as reducing sugars in the liquid phase. Similar values of concentration of reducing sugars (100 and 108 mg/1 g dry seed) were achieved by using separately both pectinase and cellulase with concentrations of enzymes 2%, but with different reaction times. However, the reaction time of cell-wall degradation by applying of pectinase was twice shorter than with cellulase. Combined activities of the studied enzymes (1% pectinase and 1% cellulase) produced 142 mg reducing sugars per 1 g dry pumpkin seed under the same reaction conditions (pH=5.0, temperature: 45ºC, reaction time: 300 min). Initial concentration of reducing sugars in 1 g of dry pumpkin seed was 2.6 mg (Table 1) and after enzymatic treatment, this concentration increased by 50-60 times. Generally, the best results were obtained with the enzyme mixture containing pectinase and cellulase activities, showing a synergetic effect as it has been reported for other enzyme- assisted oil extractions (17). This concept has already been commercialized for the production of olive oil and has also been investigated for other oil-bearing materials (17).



(a) $Y = 4.426+0.138X_1+5.372X_2+0.002X_1^2+ + 0.153X_1X_2-2.948X_2^2$



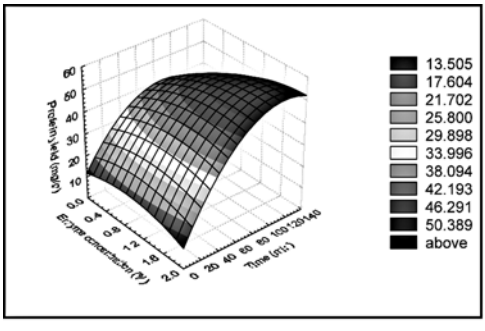
(b) $Y = 6.331+0.45X_1+4.749X_2-0.001X_1^2+ + 0.041X_1X_2+0.571X_2$



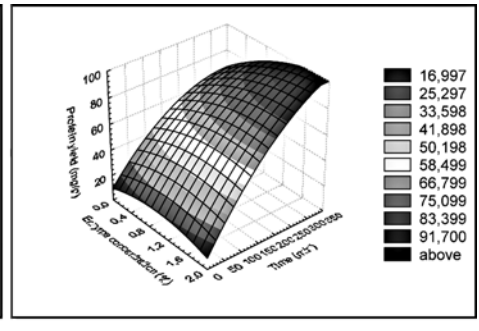
(c) $Y = 1.088+0.317X_1+13.062X_2-0X_1^2+ + 0.124X_1X_2- 7.302X_2^2$

Fig. 1. 3D graphic surface optimisation of reducing sugars production in the liquid phase (mg/g) versus reaction time (min) and enzyme concentration (%) during maceration of pumpkin seed (a) pectinase (b) cellulase (c) enzyme mixture (2 % pectinase, cellulase). (Reaction conditions: 45°C, pH 5.0)

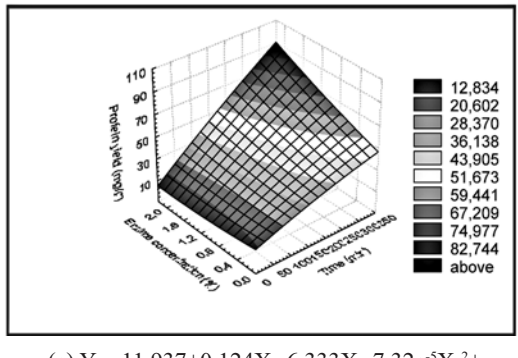
Under the same reaction conditions of cell-wall hydrolysis, the concentration of solubilized proteins was also measured (Figure 2). Results show a slight difference between enzymatic treatment with pectinase and cellulase in protein concentrations. Higher values of solubilized protein concentrations were achieved during enzymatic hydrolyses with cellulase (over 91 mg/ 1 g dry seed) than in the case with pectinase activities (50 mg/ 1 g dry seed). Treatment with enzyme mixture which contains 1% pectinase and 1% cellulase activities has no effect on protein concentration increase in comparison with individual enzyme activities.



(a) $Y = 11.723+0.625X_1+8.677X_2-0.003X_1^2 + + 0.001X_1X_2-7.506X_2^2$



(b) $Y = 9.428+0.327X_1+14.738X_2 – – 0X_1^2+0.052X_1X_2-7.552X_2^2$



(c) $Y = 11.937+0.124X_1-6.333X_2-7.32e^{-5}X_1^2+ + 0.066X_1X_2+1.459X_2^2$

Fig. 2. 3.D graphic surface optimisation of protein yield (mg/g) versus reaction time (min) and enzyme concentration (%) during maceration of pumpkin seed (a) pectinases (b) cellulase (c) enzyme mixture (2 % pectinases, cellulase). (Reaction condition: 45 °C, pH 5.0)

By comparing the predicted and experimental values of protein yield and reducing sugars, solulubilized in the liquid phase during enzymes process maceration of pumpkin seed, it can be seen that they are in good agreement. The values for the coefficient of correlation R^2 are 0.976 and 0.989 for protein yield and reducing sugars, respectively, which indicates the adequacy of the applied model.

CONCLUSIONS

The enzymatic treatment of pumpkin seed during aqueous extraction enhanced considerably protein extraction efficiency. The degradation effect on the cell wall, measured as reducing sugars in the liquid phase, could be correlated with the protein released during this treatment. The final protein product can be considered for human consumption.

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ПРИМЕНА ГЛИКОХИДРОЛАЗА У ПРОЦЕСОВАЊУ СЕМЕНА УЉАНЕ ТИКВЕ ГОЛИЦЕ РАДИ ОПТИМИЗАЦИЈЕ ЕКСТРАКЦИЈЕ ПРОТЕИНА

Драгиња М. Перичин, Љиљана М. Радуловић, Светлана З. Тривић и Етелка Б. Димић

Циљ овог рада је био испитивање услова ензимске хидролизе полисахарида ћелијског зида семена уљане тикве голице, применом ензима пектиназа и целулаза као и њихове смеше, ради оптимизације екстракције протеина. Испитано је деловање појединачних и комбинованих активности ензима пектиназе и целулазе на екстракцију протеина, комбиновано са другим процесним параметрима: концентрација ензима и време хидролизе, применом методологије одзивних површина.

Оптимална вредност приноса протеинског изолата (9 g солубилизованих протеина из 100 g семена уљане тикве) је постигнута применом следећих реакционих услова: 2% ензима (целулазе), време реакције 300 мин., pH = 5,0 и температура 45°C.

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