UTILIZATION OF ENZYME PEREPARATIONS FROM YARROWIA LIPOLYTICA FOR COLLAGEN ISOLATION FROM PIG SKIN

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Background

Pig skin containing "younger", less crosslinked collagen than beef hide is mainly utilized in meat industry as a common ingredient in sausage formulations in the form of collagen-fat emulsions (Calhoun et al.,1996). However pig skin collagen crosslinked by acid labile aldol bands can be also isolated in the form of fibres, being raw material for protein isolates manufacturing, or production of higher class (A) of parent gelatins obtained after acid hydrolysis. Enzymatic treatment with proteases, e.g. using pepsin, can increase the effectiveness of collagen or gelatin isolation and/or purity of obtained preparations due to hydrolysis of non-collagenous proteins (albumins, globulins) and for cleavage of globular - non-helical regions (telopeptides) of collagen molecules (Bailey & Light,1989) . In the process of collagen fibres isolation, contrary to gelatin production with acid-thermal procedure, to high collagenase-like activity of enzymes is undesirable. Many sources of proteolytic enzymes are tested as a substitute of pepsin in previous studies (Krasnowska at al.,1997) i.e.,proteases from fungi, trypsin-like enzymes from pumpkin or pancreas were applied. Mentioned enzymes show high proteolytic activity but do not reveal lipolytic properties, which are important for lowering high fat content in pig skin . Enzymes produced by Yarrowia lipolytica yeast meet the requirements.

Objectives

Research on the possibility of application of enzymes produced by Yarrowia lipolytica yeast strain instead of pepsin, for collagen isolation from pig skin, has been conducted. It was assumed that enzymatic preparations from Yarrowia lipolytica choosen for studies should be characterised by high proteolytic but low collagenase activity. The aim of the study was also the recognition of the effect of comminution degree on the action of proteases on pig skin proteins in the citric acid solutions.
Thermal characteristics of the collagen fibres isolated from pig skin applying acid-enzymatic procedure to optimize conditions of gelatin extraction was subject of the experiments, in which also thermorheological characteristics of obtained gelatins has been determined.

Methods

Yarrowia lipolytica yeasts (strain P II 6a) were cultivated on the medium containing: 4% glucose, 0.4% Bactopeptone, 0.2% yeast extract (YG) or 4% soy oil (YO) instead glucose, in batch shake-flask system. Fluid obtained after cultivation was centrifuged at 7000 x g and used for pig skin corium hydrolysis. Pig skin was defatted, tempered to –2.0°C, cut into 1x1 cm pieces or ground using grinder plate of 3 mm hole diameter. Cut or ground skin was washed 3 times with 5% NaCl and 0.02 M $NaHCO_3$ solution, pH=7.5. In first experiment comminuted skin was soaked in ratio 1:10 with solutions containing enzymes: pepsin (P) dissolved in citric acid (pH=2) or crude enzyme preparation from Yarrowia lipolityca in citrate-phosphate buffer (pH=3). The incubation with proteases (2.5 mg/g skin) was conducted for 8 h or 24 h at 20°C. The samples were centrifuged (7000 x g) and free amine groups according to Kucharoo & Fox (1982) and hydroxyproline content (A.O.A.C.,1990) in supernatant were determined. In second experiment collagen was extracted after enzymatic-acid treatment but procedure included also precipitation of fibres in 10% NaCl solution. Thermal properties of isolated fibres using differential scanning calorimeter DSC 22 (Seiko) at a heating rate of 3°C/min was determined. On the basis of DSC results, thermohydrolysis of fibres with water in ratio 1:5 at 60°C or 90°C to obtain gelatin, was conducted. Thermorheological properties (viscoelastic behaviour) of gelatins was studied using thermomechanical analysis (TMA/SS Seiko) at heating rate of 3°C/min, oscillating force of 45 N with 2N amplitude and 0.1 Hz frequency.

Results and discussion

Higher degree of skin comminution (grinding versus cutting) enabled more intensive protease action after 8 h enzymatic-acid treatment using both kind of Yarrowia enzymes (YG, YO) and pepsin (Tab.1). Hydrolysis with pepsin caused higher than Yarrowia enzymes degree of skin protein degradation, resulted in significant increase in both free amino groups and hydroxyproline contents in solutions. When pieces of skin were soaked in acid solutions containing enzymes, smaller effect of proteases, than for ground skin was observed. Proteolysis of collagen in pieces of skin was intensified after 24 h treatment (Tab.1). Generally, pepsin was more effective than both of Yarrowia enzymes, especially in degradation process of non-colagenous proteins (free amino groups content). No differences between two tested crude preparations from Yarrowia lipolytica were observed. Effectiveness of gelatin extraction from collagen was the highest for fibres isolated from skin with pepsin and YO enzymes (Tab.1). If fibres were isolated from skin hydrolysed by YO, less gelatin after heat treatment at both of studied temperatures was extracted. The reason were probably the differencies in subunits of collagen (data not presented), which affects gelation process of gelatin (Takahaski at al., 1988).
DSC curves (Fig.1) show a single endotherm characterised by temperature of: the onset, peak i.e. melting point (T_{MAX}) and the end of transition (Tab.2). Enzymatic treatment with pepsin lowered the temperatures of transitions, especially T_{MAX}, of about 3°C in relation to characteristics of fibres isolated in acid solutions, which is probably caused, according to Bailey and Light (1989), by cleavage of telopeptides. Fibres isolated with Yarrowia lipolityca enzymes are more thermal resistant and characterized by higher T_{MAX} (54.3°C) and end temperature of melting i.e., about 60°C, than those derived from skin treated with pepsin. On the basis of DSC evaluation, two temperatures for collagen denaturation to obtain gelatin in the studies were chosen, i.e. 60°C and 90°C. Gelatins obtained from fibres isolated using pepsin (GP) and Yarrowia enzymes (GY) show different thermorheological (viscoelastic) behaviour during their sol/gel transition. In rheothermographs of GP gelatins plateau (weakness of elasticity) before final gel network creation, at temperature over 80°C was observed (Tab.2). GY gelatins were characterised by two sol/gel transitions: first in the region of 30-40°C and second at 60-80°C. So, gelling of GY is possible at both low temperatures, i.e. below 40°C and in the range of 60-80°C.

Conclusions

1.Crude the enzyme preparations of Y. lipolytica can be used as a partial substitute for pepsin in collagen isolation from pig skin.
2. Collagen fibres isolatated from pig skin using enzymes of Y. lipolytica cultivated on oil are the same good source for gelatin extraction as fibres isolated by pepsin.
3. Gelatins derived from fibres isolated using Yarrowia lipolytica enzymes demonstrade good thermal gelling characteristics, e. i. are able to create stable gels in low and high temperature.

Acknowledgements
The authors thank Mrs. Krystyna Drozdowska for her excellent assistance in preparation of enzymes.

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Table 1. Indices of pig skin collagen hydrolysis and effectiveness of gelatin extraction from collagen fibres

Enzymes		Free amino acid groups (µg/g skin)			Hydroxyproline (µg/g skin)			Gelatins (% of extracted collagen)		
		YG	YO	Pepsin	YG	YO	Pepsin	YG	YO	Pepsin
graund ∅ 3	8h 20 °C	268^{cd}	255^{bc}	325^{e}	25.4^{c}	23.7^{c}	29.3^{d}	60°C 56.7^{a}	88.5^{c}	90.6^{c}
Pieces 1x1 cm	8h 20 °C	215^{ab}	187^{a}	247^{bc}	6.8^{a}	7.2^{ab}	5.6^{a}	90°C 62.5^{b}	87.9^{c}	91.2^{c}
	24h 20 °C	329^{e}	311^{de}	422^{f}	7.6^{ab}	7.8^{ab}	9.6^{b}			
LSD		46			2,7			5,2		

a, b, c- different superscripts for , one indice, stand for significant differences at P<0,05; LSD- least significant differences at P<0,05

Table 2. Thermal characteristics of collagen fibre melting (DSC) and thermal rheology (TMA) of gelatins during sol-gel transitions (°C).

Treatment	Collagen fibres -DSC			Gelatines - TMA			
	T_{onset}	T_{MAX}	T_{end}	1st transition		Plateau (1) 2nd transition (2)	T_{end}
Acid pH =2	41.7	50.2	55.2	-		-	-
Acid-enzymatic pepsin pH=2	39.5	47.5	55.0	60°C 90°C	- -	77-83 (1) 51-75 (1)	89 81
Acid-enzymatic Yar.lip. pH=3	45.9	54.3	60.9	60°C 90°C	31-41 36-41	60-82 (2)	-

Fig. 1 Thermogram of collagen fibres isolated with YG enzymes

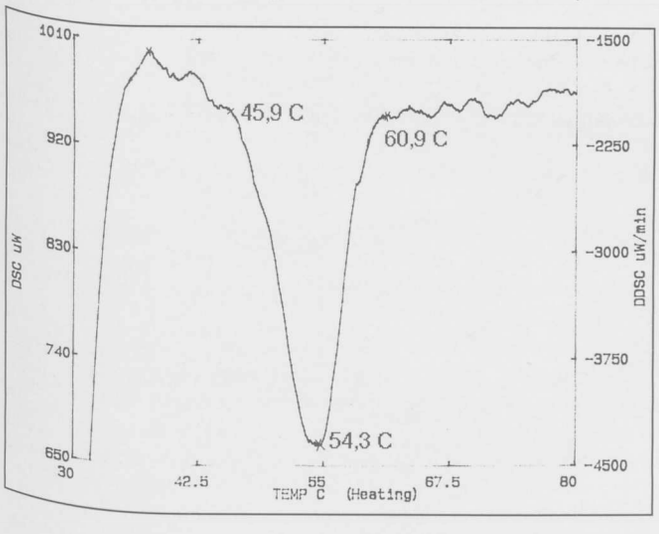

Fig. 2. Rheothermogram of gelatin extracted from YG collagen fibres