Influence of Rapeseed Protein Hydrolysis Conditions on Animal Cell Growth in Serum-free Media Supplemented with Hydrolysates

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Abstract: Different protein hydrolysates have been prepared from enzymatic hydrolyses of a rapeseed isolate (> 90% protein content) using commercial enzymes of non-animal origin. The extent of hydrolysis has been controlled to prepare various hydrolysates corresponding to different hydrolysis degree (DH) from 5 to 30. The results showed CHO promoting-growth effects of several hydrolysates especially those obtained at high hydrolysis degree. However, different hydrolysates exhibiting same peptide size patterns and similar amino acids compositions did not improve the cell growth in the same way.

Key words: rapeseed, hydrolysates, culture medium, CHO, supplements, extensive hydrolysis, protease specificity

1. INTRODUCTION

One of the key steps for the production of any recombinant proteins of clinical or diagnostic value is the optimization of culture medium. Total elimination of any animal or human derived substances becomes the main objective for the development of serum-free media. Peptones of soy, rice, wheat gluten are yet commercially available. Rapeseed is a potential source of protein containing well-balanced amino acids and until now, a single study reported the use of rapeseed protein hydrolysates as cell culture medium supplements (Deparis *et al.*, 2003). The aim of this present work is

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to study the influence of various rapeseed hydrolysates obtained by enzymatic hydrolyses of a rapeseed isolate on CHO cell growth.

2. RESULTS

A protein isolate is obtained from rapeseed cakes and is composed of proteins (91.4%), lipids (6.6%), fibers and sugars (1.0%), ash (0.8%), polyphenols (0.2%) and phytic acid (0.02%). Apparently, this protein isolate is almost free of non protein components which could play a role in the cell growth.

Hydrolyses of this isolate were carried out with different enzymes and the hydrolysis extent was evaluated by the degree of hydrolysis (DH). Chineese Hamster Ovary cells (CHO-C5) cultures were performed at 37°C in 96 wells plates. Media were composed of a reference one (RPMI 1640, albumin, transferrin, insulin, glutamine and minerals) added of either rapeseed protein hydrolysate or water (reference). Cell growth was followed by measuring total cell density using the Cellscreen system (Innovatis) by image analyses (Table 1).

Table 1. Molecular size distribution of hydrolysate peptides (SE-HPLC) and percent of increase or decrease of cell density in media supplemented with hydrolysates at 2 g/L compared to cell density in the reference at 70 h of culture, measured by image analyses in microplates.

Protease	DH (%)	> 10kDa (%)	1-10 kDa (%)	<1 kDa (%)	Cell density increase (%)
Alcalase 2.4L	5.4	26	56	18	-15
	8.5	15	59	26	3
	12.5	9	49	42	8
	20.4	4	39	57	17
	31.6	1	22	77	33
Esperase 7.5L	5.9	18	61	21	-15
	9.6	12	61	27	-8
	15.1	6	51	43	-15
	27.9	2	29	69	25
Purified Pronase	11.4	8	40	52	0
	18.1	4	28	68	31
	23.4	1	18	81	56
Neutrase 0.8L	10	13	48	39	-5
	14.7	9	41	50	11
	23.2	4	25	71	33
Orientase 90N	6.4	16	54	30	0
	10.8	13	50	37	12
	15.6	8	42	50	4
	34.2	1	20	79	20

After 70 h of culture, hydrolysates characterized by a high degree of hydrolysis (DH > 20%) significantly increase the cell density. Cell growth kinetics were then performed with hydrolysates composed of 70-80% < 1 kDa peptides (Figure 1).

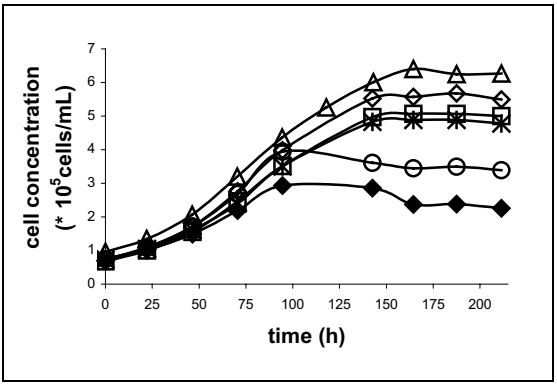

Figure 1. Effect of the supplementation at 4 g/L of a serum-free medium with different rapeseed protein hydrolysates obtained with different proteases: (Δ) Pronase and DH = 23.4%. (◊) Alcalase 2.4L and DH = 31.6%. (□) Esperase 7.5L and DH = 27.9%. (*) Orientase 90N and DH = 34.2%. (○) Neutrase 0.8L and DH = 23.2%. (♦): without hydrolysate.

The supplementation with hydrolysates from extensive hydrolysis process allowed a dramatic increase of the maximal cell density. Some peptides seemed to play the role of growth or survival factors.

3. CONCLUSION

The results show that rapeseed protein hydrolysates, especially those characterized by an extensive degree of hydrolysis, support the growth of CHO cells cultivated in a simple and defined serum-free medium. The increase of the cell density and survival duration depend on the enzyme specificity and consequently on the composition of produced peptides.

REFERENCES

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