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EFFECT OF PHOSPHORYLATION AND SUCCINILATION OF YEAST PROTEIN ON ITS ENZYMATIC DIGESTIBILITY AND FUNCTIONAL PROPERTIES

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Key words: protein of the yeast *Torula utilis*, protein phosphorylation, protein succinilation, functional properties, enzymatic digestibility.

It was found that succinilation and phosphorylation of protein lead to its greater yield from yeast, compared to the traditional alkaline extraction, causing a more effective reduction of nucleic acids content in the final product, and a considerable improvement of the functional properties of isolated substances. At the same time phosphorylation caused a smaller reduction of enzymatic digestibility of the protein accompanying all the advantageous effects.

INTRODUCTION

Among the important factors determining the usefulness of microbiological protein as food is the adaptability of its functional properties to technological requirements, and its susceptibility to enzymatic digestion. The fundamental literature describes several methods of modifying the functional properties chemically [2, 10, 11], but it were only the works of Siu and Thompson [11] followed by those of Schwenke et al. [12, 13] and Giec et al. [4] which drew attention to the effect of these methods on the biological value of the obtained preparations in nutrition.

In this research the effectiveness of the methods involving succinilation and phosphorylation of yeast protein was compared with the effectiveness of traditional extraction following alkaline hydrolysis of nucleic acids.

MATERIAL AND METHODS

The experiments were performed with the yeasts *Torula utilis* obtained from the Yeast Production Plant in Wołczyn.

The isolates of chemically modified proteins and the alkaline isolate (control) were obtained according to the following scheme:

Torula utilis yeasts
after cell disintegration, Dyno-Mill, KDL

chemical modifications		control (K)
phosphorylation (F)	succinilation (B)	alkaline hydrolysis of nucleic acids and extraction of protein
sodium trimetaphosphate (STMP), pH 11.5, 35°C, 3 h [10]	succinic acid anhydride, pH 8.5, 20°C, 1 h [8]	sodium hydroxide, pH 10.5, 80°C, 3 h [7]

centrifugation (20 000 g, 20 min, 0-4 C), precipitation of protein in isoelectric point (pH 4.0)

The content of nucleic acids in the product was determined by the method of Herbert et al. [5], and enzymatic digestibility *in vitro* according to the classical method of Mauron [6] consisting in preliminary digestion of protein with pepsin for 3 h and then, after adjusting pH to the optimal value, further digestion with trypsin or pancreatin for 24 h; the digestibility was expressed in per cent as the ratio of amino nitrogen released by this method to amino nitrogen determined after acid hydrolysis (6 n HCl, 24 h, 110°C):

$$\text{Digestibility } (\%) = \frac{\text{amino nitrogen after enzymatic hydrolysis}}{\text{amino nitrogen after acid hydrolysis}} \times 100$$

The functional properties (water sorption capacity, emulsification activity, foam yield) were determined according to Shetta and Kinsella [10].

RESULTS AND DISCUSSION

The conclusion from three repetitions of experiments is that both phosphorylation and succinilation increase the effectivity of protein extraction from yeasts and facilitate the process.

As can be seen in the Table, the applied chemical modifications reduce the content of nucleic acids in protein isolated from yeast. This corroborates the results of Kinsella et al. [2, 9, 10] who stress the effect of the shift of the isoelectric point of modified protein leading to a drop of nucleic acids content. These authors, as well as Schwenke et al. [10] greatly improved the functional properties of microbiological or vegetable protein by chemical modifications, similary as we did in this research (Table). The decreased susceptibility of succinilated protein to digestive enzymes was already noted by other authors. Matoba and Doi [5] found that succinilation inhibits only the activity of trypsin, given the specific nature of this enzyme's activity in the presence of lysine's free $-NH_2$ group. The slight decrease of enzymatic digestibility of succinilated

Table. Characteristic of modyfied proteins isolated from the yeasts *Torula utilis* (means from three repetitions of experiments)

Isolated protein	Protein (% dry mass)		Nucleic acids (% dry mass)	Functional properties		
	total[a]	specyfic[b]		water absorption (g/g dry mass)	emulsification activity (%)	foam yield (cm^3)
F	81.12	77.22	1.72	4.99	82.5	85
B	78.60	75.32	1.80	4.72	75.5	70
H	76.50	66.60	4.22	4.71	72.0	55

F — after phosphorylation
B — after succinilation
H — after alkaline hydrolysis
a — total protein — Kjeldahl's N × 6.25
b — specific protein — protein insoluble in 20% TCA determined by Kjeldahl's method: N (separated on a filter) × 6.25

preparations, also in the case of digestion with pancreatin, is probably due to the inhibition of trypsin, its constituent part. Unlike this phenomenon, the small decrease of enzymatic digestibility of phosphorylated protein is hard to account for. It may be caused by changes in proteins brought about by the alkaline pH accompanying phosphorylation [1]. The technological advantages of chemically modified protein must be assessed against its biological value. This is best done in experiments on animals which we are planning to undertake.

CONCLUSIONS

The experiments reported here indicate that phosphorylation with STMP is worthy of recommendation as a method of modifying protein, especially protein of microbiological origin. This process leads to dissociation of nucleic complexes from proteins which in turn favours an increased yield of protein free from nucleic acids having an adverse effect in nutrition. According to Ellinger [3], the STMP used in the process is least toxic and physiologically hazardous, and, what is more, it can be used on an industrial scale. Phosphorylated proteins are most similar to those occurring naturally, and phosphoramidation of lysine radicals at high pH values may temporarily prevent lysine's $-NH_2$ groups from entering reactions unwanted in food technology. A decrease of pH below 5 allows to reclaim this $-NH_2$ group which to be reclaimed again makes lysine biologically assimilable. The irreversibility of this reaction in the case of succinilation casts doubts on the applicability of this process in modifications of edible proteins. Its usefulness is further challenged by the fact that all the studied parameters indicate phosphorylation as a much superior method in the technology of edible proteins obtained from microorganisms.

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WPŁYW FOSFORYLACJI I SULECYNYLACJI BIAŁKA DROŻDŻOWEGO NA JEGO STRAWNOŚĆ ENZYMATYCZNĄ I WŁAŚCIWOŚCI FUNKCJONALNE

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S t r e s z c z e n i e

Włączenie białka mikrobiologicznego do żywności wymaga dostosowania jego właściwości fizykochemicznych. W literaturze opisano wiele metod osiągnięcia tego celu dzięki różnym modyfikacją chemicznym. Brak jest analizy porównawczej ich skuteczności oraz wpływu na wartość biologiczną uzyskanych preparatów białkowych. Celem niniejszej pracy było porównanie efektywności zabiegów sulecynylacji i fosforylacji białka drożdżowego z tradycyjną estrakcją alkaliczną po hydrolizie kwasów nukleinowych.

Badania wykazały, że proces sulecynylacji oraz fosforylacji zwiększa ekstraktywność białka wskutek przesunięcia punktu izoelektrycznego, co wpływa istotnie na obniżenie zawartości kwasów nukleinowych w produkcie o ok. 60% i poprawę właściwości funkcjonalnych (tabela). Fosforylacja w znacznie mniejszym stopniu obniżyła strawność enzymatyczną preparatu niż sulecynylacja, a dodatkowo, z uwagi na swą odwracalność, wydaje się być godną polecenia metodą uzyskiwania z homogenatów drożdżowych białka przeznaczonego do żywności.