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# ULTRAFILTRATION OF WHEY FOR PROTEIN CONCENTRATES AND YEAST/PROPIONIC ACID BACTERIAL BIOMASSES PRODUCTION

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The study determined optimum conditions for ultrafiltration of an acid-rennet whey. Experiments were made with the deproteinized ultrafiltrate toward its utilization in the production of the yeast-bacterial biomass. To this end, growth dynamics of *Kluyveromyces fragilis* yeast and *Propionibacterium shermanii* were determined by using different propagation times. It was shown utilization of such combined cultures renders biomasses rich in quality protein and vitamins (mainly vit. $B_{12}$). Initial yeasting of a culture prior to introduction of propionic bacteria makes it possible to obtain higher yields of biomass and vit. $B_{12}$ within a period shorter than the traditional cultures of propionic bacteria require. The project was also concerned with analyzing the composition of the protein concentrates obtained by ultrafiltration. It was indicated that these concentrates should be mixed with the yeast-bacterial biomasses obtained from the same whey.

It has been demonstrated in the earlier papers that whey may be used as a medium for bacteria cultures and for the joined yeast-bacterial cultures, aimed at production of biomass rich in protein and vitamins [11, 12].

A number of papers have recently been published on the application of ultrafiltration (UF), and the reverse osmosis (R O) techniques to the deproteinization of whey, used in the production of whey protein concentrates (WPC) of high nutritional value [17].

Numerous papers on the process mechanism [2], and on the composition, nutritional value and application of protein concentrates [3, 7, 8], as well as on the utilization of deproteinized whey permeate [9, 5, 4], have been published so far. In the investigations carried out within this pro-

ject, conditions of whey ultrafiltration process, and the composition of protein concentrates were estimated, as well as utilization method of deproteinized whey permeate (ultrafiltrate) was elaborated, basing on the yeast-bacterial fermentation technique which had been elaborated earlier.

## MATERIAL AND METHODS

The characteristics of the acid-rennet whey used in the experiments, supplied by Dairy Cooperative in Poznań, was as follows:

| | |
|---|---|
| — dry matter | 5.4 to 5.5 |
| — lactose | 3.9 to 4.3 |
| — total N×6.38 | 12.8 to 12.9 |
| in per cent of dry matter | |
| — pH value | 4.5 to 4.6 |

Ultrafiltration of whey used in preliminary experiments was carried out in the apparatus SARTORIUS type 16896, using cellulose membranes type SM 121-36.

A Danish apparatus of DE Danske SUKKER FABRIKER A/S type II No. DT 7403118 was used, having acetate cellulose membranes type 600, being a barrier to the compounds of molecular weight higher than 6.000. Protein concentrates obtained after ultrafiltration were freeze-dried, and the whey permeate was used as a medium for joined yeast-bacterial cultures. The fundamental chemical analysis involving dry matter, total nitrogen, protein nitrogen, lactose, vitamin $B_{12}$, was carried out in protein concentrates, biomass and whey permeate according to the standard methods. *Kluyveromyces fragilis* CBS-397 yeast of the Centralbureau voor Schimmelcultures Collection of Delft, Holland were used in these investigations. Museum strains of yeasts were kept on slant agar with lactose. *Propionibacterium shermanii*-1, *P. petersonii* J, and *P. freudenreichii* J (1 : 1 : 1) originated from our local collection. Yeast medium was prepared from whey permeate by adding 5 per cent $(NH_4)_2SO_4$ and 0.5 per cent of $K_2HPO_4$, adjusting pH value to 5.7, followed by twofold sterilization in steam flow, with a 24 hours interval.

Propionic bacteria medium was made from whey permeate by adding 16 mg $CoSO_4$ per 1 liter, and adjusting pH value to 7.0 ($\pm$0.2), followed by tyndallization in a Koch apparatus. Joined yeast-bacterial cultures were grown according to the technique elaborated for full whey medium [1], in flasks of 200 ml volume, and afterwards in a fermentor of New Brunswick Scientific N. J. Model MF 114 (7 l) manufacture, with an automatic control of: pH, temperature, aeration and stirring intensity programmes.

In both cases, the yeast cultures were propagated at the 40 l/ 1 l/1 h aeration rate, at pH = 5.7 and at the temperature of 30°C. Subsequently in the partially fermented medium the pH value was adjusted to 7.0, fol-

lowed by heating at 100°C for 30 min. and propionic bacteria inoculum was added. The estimated in this project optimal duration of these two stages was: 4 hours for the first stage, and 74 hours for the second, respectively.

Biomasses obtained were separated from the post-fermentation fluids using centrifugation at 1900×g for 50 min.

## RESULTS AND DISCUSSION

Whey ultrafiltration is a complex process mainly due to the high concentration of lactose, as it has been reported in the literature. At a certain concentration level of proteins, the concentration of lactose in the product is also increasing, resulting in an elevated viscosity and in a considerable reduction in concentration rate [10]. Taking this into acount, experiments were conducted on the duration of concentration process as influenced by the predetermined concentration of protein concentrate and its composition. Results are shown in Table 1.

Table 1. Duration of ultrafiltration process and composition of whey protein concentrate as influenced by degree of concentration

| Degree of concentration per vol. | Duration of process UF per hour | Dry matter of the concentrate % | Total N×6.38 | | Total protein N×6.38 | | Lactose % |
|---|---|---|---|---|---|---|---|
| | | | % | % of dry matter | % | % of dry matter | |
| 2 | 2 | 5.75 | 0.92 | 16.4 | 0.81 | 8.21 | 4.1 |
| 4 | 5 | 6.82 | 1.25 | 31.3 | 1.66 | 18.01 | 4.35 |
| 6 | 10 | 8.02 | 3.15 | 29.21 | 2.31 | 25.00 | 4.71 |
| 7 | 12 | 12.95 | 4.15 | 32.01 | 3.55 | 27.43 | 5.27 |
| 8 | 16 | 13.51 | 4.71 | 32.50 | 3.57 | 28.01 | 5.41 |
| 10 | 24 | 14.52 | 5.12 | 33.10 | 3.69 | 29.60 | 5.82 |

The results in Table 1 indicate that the increase in ultrafiltration time was proportional to the increase in protein content in the concentrate, only up to six to eight fold concentration of the product. For that reason, a sevenfold (per vol.) degree of concentration procedure was applied, resulting in production of concentrates and ultrafiltrates (permeates), which composition in shown in Table 2.

In the further part of this article preliminary trials are presented, aimed at utilization of the ultrafiltrate as a medium (substrate) for propagation of *Kluyveromyces fragilis* CBS-397 yeast-cultures, and propionic bacteria cultures. The results demonstrated that growth of these microorganisms on whey ultrafiltrate was not significantly different from

that on the full whey, or on whey deproteinized by traditional methods.

Average yields obtained were as follows:

— yeasts 5 g per 1 liter of culture medium
— propionic bacteria 7 g per 1 liter of culture medium
— yeasts+bacteria 9 to 11 g per 1 liter of culture medium.

Table 2. Basic characteristics of whey ultrafiltration products obtained by sevenfold concentration (per vol.) of whey proteins

| | Dry matter % | Total N × 6.38 | | Protein N × 6.38 | | Lactose | | Ash in dry matter |
|---|---|---|---|---|---|---|---|---|
| | | % | % of dry matter | % | % of dry matter | % | % of dry matter | |
| Concentrate | 11-13 | 2.8-4.2 | 31.0-34.0 | 2.5-3.8 | 26.5-28.8 | 3.9-5.3 | 29.0-33.0 | 7-9 |
| Ultrafiltrate | 4.2-4.8 | 0.2-0.4 | 7.2-8.9 | 0.05-0.99 | 0.5-1.3 | 3.7-3.8 | 78.0-81.0 | 12-15 |

To elaborate optimal technique of joined yeast-bacterial biomass production, experiments were carried out on the dynamics of yeast-bacterial cultures, propagated under conditions described in appropriate section of "Material and Methods". Various growing times were applied for yeast cultures, ranging from 2 to 16 hours, after which propionic bacteria were introduced each time. Propagation time of bacteria on the partially yeast fermented medium attained in all cases 72 hours. Corresponding separate cultures of yeasts and of bacteria, being reference populations in the experiments, paralleled the joined yeast-bacteria cultures. Results obtained are shown in Fig.

Analysis of experimental findings presented in Table 3 can lead to the following remarks. Yeasts utilized dynamically lactose present in the

Table 3. Basic characteristics of yeast-bacterial biomasses and whey proteins concentrates produced by ultrafiltration technique

| Preparation | Yield in gms of dry matter from 1 l whey | Dry matter | Total N × 6.38 | | Protein N × 6.38 | | Vit. $B_{12}$ µg/gram of dry matter | Ash % of dry matter |
|---|---|---|---|---|---|---|---|---|
| | | | % | % of dry matter | % of dry matter | % of total N | | |
| Yeast-bacterial biomass | 7.1 | 95.02 | 40.5 | 42.6 | 28.6 | 70.2 | 0.35 | 14.6 |
| Protein concentrate | 15 | 96.6 | 30.9 | 32.0 | 25.4 | 82.3 | — | 9.6 |

substrate, and nearly complete fermentation (0.08%) was observed after 12 hours period. Maximal yield of yeast-biomass (0.73 g dry matter per 100 ml) was achieved after 16 hours growth. However, a maximal yield

of yeast-bacterial biomass (1.04 g dry matter per 100 ml) was obtained when propionic acid bacteria, were introduced after 4 hours of yeast growth, at lactose content of 2.7%. Maximal accumulation of vitamin $B_{12}$ was observed in the same experiments when propionic bacteria were introduced after 6 hours of yeast growth to the substrate. In this case the quantity of 3.29 μg/ml of culture for vitamin $B_{12}$ was obtained.

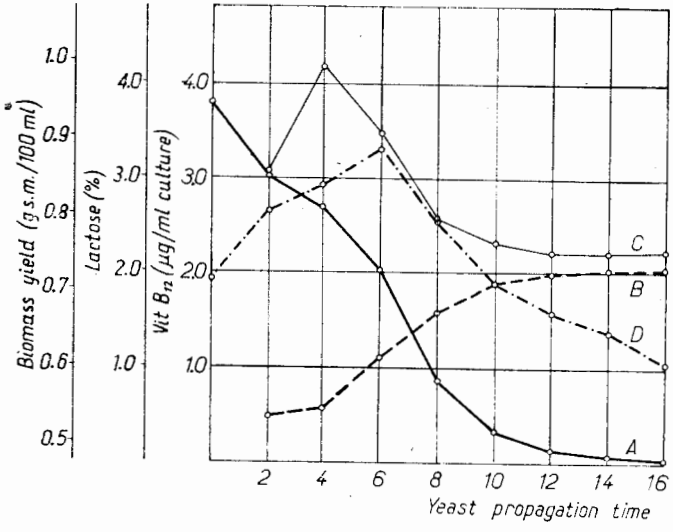


Fig. The influence of the yeasts propagation time on the yield of biomass derived from mixed cultures; A. Concentration of nonfermented lactose after the first step of fermentation. B. Yield of yeast biomass. C. Yield of mixed yeast — bacterial biomass. D. Yield of vitamin $B_{12}$

Experimental findings concerning the yield of both biomasses and vitamin $B_{12}$ lead to the conclussion, that similarly as for the full whey [10], initial whey treatment by yeasts contributes greatly to production of substrate constitutents necessary for a dynamic metabolism of propionic bacteria. These constituents present in the yeast cells are conveyed into the extract during thermal processing, thus providing an abundant growth of propionic bacteria, being in this case a predominating constituant of the biomass. This is an efficient technique in which two valuable products are obtained (biomass and vitamin $B_{12}$).

Moreover the experimental results indicate, that the initial treatment of the whey ultrafiltrate substrate by yeasts, prior to introducing the propionic bacteria contributed to higher yields of biomass and higher quantities of vitamin $B_{12}$ were achieved, in a 50 per cent shorter time, as compared with the traditional system of sole propionic bacteria cultivation (the time of fermentation being 6-7 days). The results outlined above encouraged to commencing yeast-bacterial processes in larger volumes and in optimal conditions using a Brunswick fermentor.

Observations on growth dynamics in propionic bacteria indicate that under a continuous adjustment of pH value to the level of 7.0, an intensive utilization of lactose resulting in considerably high production of biomass and vitamin $B_{12}$ is taking place, in a shorter time (at a higher rate) than in flasks cultures. It seems, that under automatic control of the principal parameters of this process, the propagation time of propionic bacteria could be reduced to 48 hours.

In Table 3 basic composition of the freeze-dried yeast-bacterial biomass is shown, produced by the technique outlined above using whey ultrafiltrate. The composition of whey protein concentrate, obtained after ultrafiltration and freeze-drying was also studied and compared with that of the yeast-bacterial biomass related to the whey permeate. For comparative purpose in Table 4 the content of amino acid in both products is demonstrated.

Table 4. The amino acid compositions of yeast-bacterial biomass obtained on whey permeate and of whey protein concentrate (freeze-dried)

| Amino acid | Yeast-bacterial biomass | | Whey protein concentrate | |
|---|---|---|---|---|
| | Amino acid contents in mg per 1 g total protein | Amino acid contents in per cent of the total amino acid composition | Amino acid contents in mg per 1 g total protein | Amino acid contents in per cent of the total amino acid composition |
| Lysine | 52.5 | 5.9 | 85.9 | 9.5 |
| Histidine | 11.4 | 1.3 | 11.6 | 1.3 |
| Arginine | 41.5 | 4.7 | 8.9 | 1.0 |
| Aspartic acid | 81.6 | 9.2 | 108.1 | 11.9 |
| Threonine | 39.9 | 4.5 | 61.5 | 6.8 |
| Serine | 35.0 | 4.0 | 47.0 | 5.2 |
| Glutamic acid | 166.5 | 18.8 | 197.4 | 21.8 |
| Proline | 23.7 | 2.7 | 42.0 | 4.6 |
| Glycine | 39.9 | 4.5 | 15.9 | 1.7 |
| Alanine | 104.9 | 11.9 | 48.2 | 5.3 |
| 1/2 Cystine | 5.5 | 0.6 | 11.5 | 1.3 |
| Valine | 43.5 | 4.9 | 51.7 | 5.7 |
| Methionine | 55.5 | 6.3 | 15.8 | 1.7 |
| Isoleucine | 36.1 | 4.1 | 59.5 | 6.6 |
| Leucine | 67.3 | 7.6 | 102.8 | 11.3 |
| Tyrosine | 54.6 | 6.2 | 19.2 | 2.1 |
| Phenyloalanine | 24.4 | 2.8 | 20.0 | 2.2 |
| Total | 883.8 | 100.0 | 907.0 | 100.0 |

Experimental results shown in Tables 3 and 4 demonstrate that the two-stage utilization of whey contributes to the production of two highly valuable nutritional preparations which can be used for various purposes.

The utilization of whey protein concentrates, obtained by ultrafiltration technique to the production of dietetic foods [3, 7] as well as a component in carbohydrate diets [6] has been extensively reported in the literature. Utilization of the yeast-bacterial biomasses rich in vitamin $B_{12}$ and protein has also been reported [11, 12]. Thus conditions proposed in this paper make also the mixing of both whey preparations possible, according to the suggestions reported by some authors [5]. This technique is superrior to the direct yeast fermentation of whey in respect of a possible choice of the nutritionally optimal proportions of both preparations, one of which contains non-dentaturated and highly valuable whey proteins.

## CONCLUSIONS

1. Sevenfold concentration of whey proteins by means of ultrafiltration under the conditions employed was found to be optimal in terms of the nutritional composition of the product and the economy of this process.
2. The whey permeate can be successfully used as growth media for selected strains of yeast and propionic acid bacteria to obtain biomasses rich in proteins and vitamins (predominantly vitamin $B_{12}$).
3. Both the whey protein concentrates and the yeast bacterial biomasses represent high nutritional value and therefore can be recommended as food and feed supplements.

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ULTRAFILTRACJA SERWATKI DO PRODUKCJI KONCENTRATÓW BIAŁKOWYCH I BIOMAS DROŻDŻOWO-BAKTERYJNYCH

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Streszczenie

W pracy ustalono optymalne warunki prowadzenia procesu ultrafiltracji serwatki kwasowo-podpuszczkowej. Następnie przeprowadzono doświadczenia nad możliwością wykorzystania odbiałczonego ultrafiltratu do produkcji biomas drożdżowo-bakteryjnych.

W tym celu określono dynamikę wzrostu drożdży *Kluyveromyces fragilis* i bakterii *Propionibacterium shermanii* — stosując zróżnicowane czasy namnażania. Wykazano, że zastosowanie takich skojarzonych hodowli pozwala na uzyskanie biomas bogatych w wartościowe białko i witaminy (głównie $B_{12}$). Wstępne zdrożdżowanie podłoża przed wprowadzeniem bakterii propionowych pozwala na uzyskanie wyższych wydajności biomasy i witaminy $B_{12}$ w krótszym czasie niż w przypadku prowadzenia samej hodowli bakterii propionowych metodą tradycyjną. W pracy zbadano ponadto skład uzyskanych w wyniku ultrafiltracji koncentratów białkowych i wskazano na celowość mieszania ich z uzyskanymi z tej samej serwatki biomasami drożdżowo-bakteryjnymi.