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RESEARCHES ON OBTAINING PRODUCTS WITH ADDED VALUE THROUGH SUPERIOR CAPITALIZING OF WHEY

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Abstract

The theme of the paper was chosen because we considered it important that people know not only the negative part about whey - it is a waste, but also the positive side - that it can be successfully used in the production of value-added products. The purpose of the paper was to obtain a probiotic product by superior capitalizing of whey. The objectives of the paper were: (1) Processing of whey in a slightly fermentable form for the cultivation of Saccharomyces cerevisiae and Lactobacillus bulgaricus strains; (2) Optimizing the composition of the whey culture medium in order to obtain the highest biomass productivity; (3) Obtaining probiotics based on Saccharomyces cerevisiae and Lactobacillus bulgaricus strains. Several media variants with different glucose concentrations (0%, 0.5%, 1%, 2%) were used for the biosynthesis of probiotics. In the case of yeasts, glucose supplementation of culture media increases the amount of wet biomass by 21% compared to glucose-free media, and 16% for lactobacilli. However, good results were also obtained on whey as such, without additional glucose, yielding 52 g/l yeast and 42 g/l lactobacilli.

Key words: *whey, probiotic, biomass.*

INTRODUCTION

Whey, a bio product obtained from cheese and sweet cheese was once considered a waste. The discovery of whey as a functional food with nutritional value has made it recognized as a co-product in cheese making (Banu et al., 2000).

Components of whey include α-lactalbumin, lactoferrin, β-lactoglobulin, glycoacropeptides, immunoglobulins, bovine serum albumin, lactoperoxidases, lactose and minerals (Geogescu et al., 2000; Pescuma et al., 2008).

Today, whey is considered a product that has antimicrobial activity, has an immunemodulatory role, improves muscle strength, and prevents cardiovascular disease and osteoporosis (Banu et al., 2000).

Advancing technology in ultrafiltration processes, microfilters, reverse osmosis, ion exchange have resulted in the discovery of new whey products (Costin et al., 2005).

Protein whey concentrate (80-95% protein), whey lactose, whey protein isolates, demineralised whey and hydrolysed whey are products currently available in the world market. Each whey product varies in amount of protein, carbohydrates, immunoglobulins, lactose, minerals and fats.

These variables are important factors in the selection of whey fractions for specific applications (Panayotov et al., 2015; Oprea et al., 2001).

Whey, a complex of milk-derived proteins has been recognized as having a large number of health benefits.

Why has the ability to behave like an antioxidant, antihypertensive, antitumor, antiviral, antibacterial agent (Pescuma et al., 2010).

The purpose of this work is to capitalize on whey and aims to:

1) Processing of whey in a easily fermentable form for the cultivation of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus* strains; 2) Optimizing the composition of the culture medium based on whey in order to obtain as high productivity in biomass (Ştefan et al., 2016); 3) Obtaining probiotics based on *S. cerevisiae* and *L. bulgaricus* strains (Champagne et al., 2002; Bîjnea et al., 2015).

MATERIALS AND METHODS

Raw materials

The main raw material is bovine whey.

The whey used in the experiments was purchased from a private manufacturer and was stored at 4^0C until processing.

Microorganisms

For the biosynthesis of probiotics, strains of *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus* from the Microorganism Collection of the Faculty of Biotechnologies were used.

Culture media

For maintenance of yeast culture we used YPG medium and for lactobacilli we used MRS medium.

Yeast control medium = Medium S (g/v of whey)

Peptone	1%
Yeast extract	1%
Magnesium sulphate	0.2%

pH=4,8

Lactobacilli control medium = Medium L (g/v of whey)

Peptone	0.5%
Meat extract	0.3%
Sodium chloride	0.5
Magnesium sulphate	0.02%

pH=5,5

For the cultivation of microorganisms, several variants of culture media with the same components as control media were tested, to which glucose was added at different concentrations: 0.5%, 1%, 2%.

Processing of whey

1. Centrifugation. The purpose of centrifugation was to semipurification of whey by removing solid residues and grease (8000 rpm, 15 min, 3^0C).

2. Filtration was aimed at removing residual impurities after centrifugation. Filtration was performed under vacuum using a Buchner funnel and high quality filters.

3. Deproteinization aimed at removing residual protein left in whey after obtaining the cheese. Deproteinization was performed by treating the whey filtrate with a 20% trichloroacetic acid solution added at a concentration of 10%, based on the volume of processed whey. The precipitate was removed by vacuum filtration.

Obtaining the vegetative preparation

The solid media was inoculated with a cell suspension from a lyophilized preparation. The culture was statically grown, 24-48 hours at 30-32^0C, then stored at 4^0C.

Preparation of laboratory inoculum

From the maintenance culture, a microbial suspension is prepared in sterile distilled water. 2 ml of cell suspension is inoculated into 25 ml of liquid medium. The inoculum culture was grown in 100 ml Erlenmeyer flasks for 24 hours at 30-32^0C at 200 rpm for yeast and static for lactobacilli.

Obtaining probiotics

For the biosynthesis of probiotics, control media S and L and variants with different concentrations of glucose (0.5%, 1%, 2%) were used. The main objective of making different variants of culture media was to highlight the role of whey as the only carbon source for the cultivation of microorganisms.

Fermentations with yeast were carried out under the following conditions: 48 h, 30^0C, 200 rpm, pH = 4.8.

Fermentations with lactobacilli were carried out under the following conditions: 48 h, 35^0C, 20 rpm, pH = 5.5

Analytical control of the process of biomass production

1. Determination of wet biomass (WCW= Wet Cell Weight). In a centrifuge tube, an exactly measured volume of the sample (10 ml) was introduced and then centrifuged at 4000 rpm for 20 minutes. After the supernatant was removed, the biomass was weighed.

2. Determination of dry biomass (DCW= Dry Cell Weight). In a centrifuge tube, an exactly measured volume of the sample (10 ml) was introduced and then centrifuged at 4000 rpm for 20 minutes. After removing the supernatant, the biomass tube was dried step by step first at 60^0C for 4 hours, then at 105-110^0C, to constant weight.

Post-biosynthesis processing of fermentation media

After completion of the fermentation process, the first post-biosynthesis operation was the separation of biomass from the liquid medium by centrifugation at 4000 rpm for 20 minutes in the cold conditions. After centrifugation, wet biomass was collected and weighed, placed in the trays and dried in the oven at 105^0C. Finally, the total amount of dry biomass was weighed.

RESULTS AND DISCUSSIONS

Purity of cultures

Cultures of *S. cerevisiae* and *L. bulgaricus* used as inoculum in laboratory fermentations were examined microscopically and macroscopically, highlighting the characteristics mentioned in the literature for these species. The microscopic and macroscopic appearance of the cultures is shown in Figures 1-4.

Figure 1. Macroscopic appearance *S. cerevisiae*

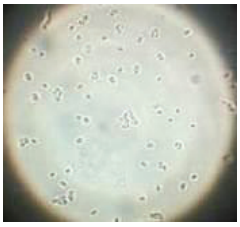

Figure 2. Microscopic exam *S. cerevisiae*

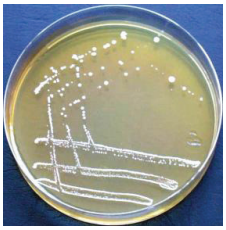

Figure 3. Macroscopic appearance *L. bulgaricus*

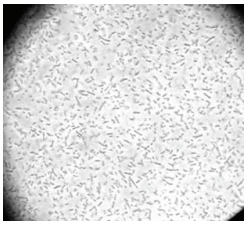

Figure 4. Microscopic exam *L. bulgaricus*

Processing of whey

From the data presented in the graph below (Figure 5), it can be concluded that whey deproteinization had a positive effect on microbial growth, as a higher amount of biomass was obtained in the trichloroacetic acid treated media (Table 1). This effect was more obvious in the case of yeasts.

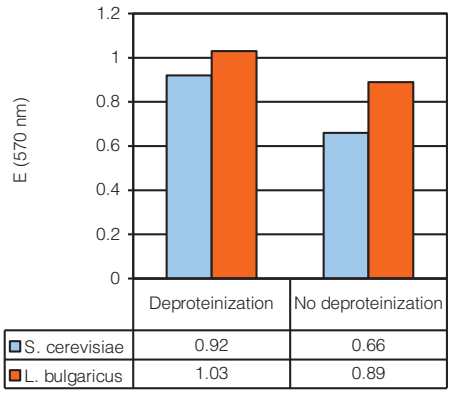

Figure 5. Influence of deproteinization treatment on microbial growth

Table 1. Influence of deproteinization treatment on the amount of biomass

Strain / Culture medium	*S. cerevisiae*		*L. bulgaricus*	
	Deproteinized medium	Undeproteinized medium	Deproteinized medium	Undeproteinized medium
WCW (g/100 ml)	5.23	3.86	4.2	3.51
DCW (g/100 ml)	1.02	0.77	0.85	0.72

From the examination of the data presented in table 1 and figure 5 it can be concluded that the whey deproteinization treatment is cost-effective since it results in much better results in the obtained biomass, by 33.7% in *S. cerevisiae* and 19% higher for *L. bulgaricus*.

Influence of glucose concentration on the accumulation of biomass

For probiotic biosynthesis, *control media S, control media* L and variants with different glucose concentrations were used (0.5% = test 1, 1% = test 2, 2% = test 3).

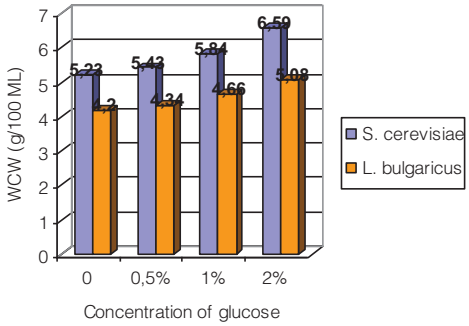

Figure 6. Influence of glucose concentration on the amount of biomass

Comparing the values of the wet biomass quantities obtained in the case of glucose-supplemented media to glucose-free media, the different glucose efficiency is clearly observed with the increase in its concentration (Figure 6). Thus, in the case of yeasts, the increase in the amount of wet biomass is with 26%, compared to the glucose-free media, and in the case of lactobacilli, the increase is with 21%.

However, it can be concluded that good results were obtained also on whey without glucose, 52 g/L of yeast and 42 g/L of lactobacilli, results that cannot be neglected and which, from the economic point of view does not justify additional costs for glucose use.

An idea to make the whole process worthwhile would be to use sources of carbohydrate that also result as residues from various industrial processes.

CONCLUSIONS

The following conclusions can be drawn from present researches:

- ✓ the deproteinization of whey is profitable as it results in much better results in the amount of biomass obtained, with over 33.7%, in case of *S. cerevisiae* and with approx. 19% higher for *L. bulgaricus* compared to unprocessed whey;
- ✓ by comparing the values of the wet biomass quantities obtained by supplementing the media with glucose vs the glucose-free media, the different glucose efficiency is clearly observed with the increase in its concentration. Thus, in the case of yeasts, the increase in the amount of wet biomass is 26%, and in the case of lactobacilli, 21%;
- ✓ good results were obtained also on whey without glucose, 52 g/L of yeast and 42 g/L of lactobacilli, results that cannot be neglected and which, from the economic point of view does not justify additional costs for glucose use.

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