Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVI, Issue 2 - 2017, pag. 71 - 76

INVESTIGATION ON LACTOSE FERMENTING YEASTS ACTIVITY IN THE WHEY OBTAINED BY COAGULATION OF MILK PROTEINS BY BERRY COAGULANT

Olena GREK[1], *Olena KRASULYA[1], Alla PETRYNA[2]

[1]*Educational and Research Institute of Food Technology, National University of Food Technologies,*
[2]*Branch office of the National University of Food Technologies in Lviv*
01601, Volodymyrska str. 68, Kyiv, Ukraine, olena_krasulya@ukr.net
*Corresponding author
Received 8th May 2017, accepted 27th June 2017

Abstract: *The results of biochemical activity of lactose fermenting yeasts in the wort based on whey, obtained by thermo acid coagulation of milk by berry raw material (sterilized black currant paste) are shown. The extraction of black currant' valuable components occur in the protein foundation and colored whey, which can be used in the production of fermented beverages with high biological and nutrition value. It was found from the analysis of lactose fermenting yeasts' biomass accommodation that the biggest growth of yeasts in the wort based on colored whey was in the samples which are fermented by Zygosaccharomyces lactis 868-K – the general amount of cells was (78.1...79.9)·10^6 CFU/ml for 48 hours. The optimal fermentation temperature (30…32 °C) was established by the parameters of fermentation activity: accumulation of ethyl alcohol and carbon dioxide in the wort, the total amount of yeasts cells.The obtained results were used in the technology development of non-alcocholic beverages based on colored whey.*

Keywords: *colored whey, fermentation activity, lactose fermenting yeasts, wort.*

1. Introduction

Milk whey is the valuable protein-carbohydrate side raw material. The yield of whey is 70...90 %, at the rennet cheeses production, 70...80 % at the cottage cheese and 75 % at the casein [1, 2]. In Ukraine, about 50 % of whey is being processed, the rest of it being recycled, herewith the valuable raw material is lost and the environmental problems are getting worse. The last one is connected with high values of chemical oxygen consumption, which consist of 75 g/l for acid whey and 57 g/l for deprotonated. Besides this aspect, the content of organic nitrogen in acid whey is about 1.03 g/l [3-5]. The additional costs for effluent treatment plants or whey transportation to specialized plants reduce economic indicators of plant generally. There are a lot of industrial methods of whey processing. One of the most effective and the least expensive is the manufacturing of fermented non-alcoholic beverages which already exists in the dairy plants equipment.

The use of milk whey in the capacity of water phase in the beverages manufacturing promotes enrichment with biological components such as γ-casein, β-lactoglobulin, whey albumin, immunoglobulin and proteose-peptone, lactose, glucose, galactose, lactulose, arabinose, microelements and ultramicroelements, vitamins (retinol, tocopherol, thiamine, riboflavin, pirodoxin), milk fat, etc. The energy value of milk whey is somewhat lower than in skimmed milk, and the biological value is approximately the same,

this makes possible to be used in the manufacturing of dietary foods [2, 6 - 8].
There are several methods to get milk whey:

- hard and soft rennet cheeses production;
- casein making process;
- cottage cheese production by acid, acid-rennet and thermoacid methods and etc.

Also the obtaining technology of the protein-berry clots by thermo acid coagulation of milk proteins by berry raw material (black currant paste), with receiving of milk whey was developed [9].
Black currant is one of the most widespread berry crops that grow in Ukraine. Medicinal-and-prophylactic properties are determined by the fact that berries contain vitamins, macro- and microelements, polysaccharides (pectin), polyphenols and others which are necessary for humans. Berries contain a high amount of iron, phosphorus and calcium salts in the form of organic compounds, which are easily digested by the human body.[10, 11].
The extraction of black currant' valuable components occur in the same way as in the protein foundation in whey. The addition of black currant puree with high acidity permits to increase the yield of clot. These berries contain pigments that give light crimson hue to the cheese clot and intensive crimson red to the whey obtained. Colored whey is a valuable raw material for the production of fermented non-alcoholic beverages due to its intensive coloration, which excludes the necessity of adding artificial colourants or flavorants. Also, most of the water-soluble constituents of berry puree – vitamin C, carbohydrates, polyphenols, macro- and microelements, pass into the whey, thus increasing its biological value. Carbohydrates of puree at 95 % are represented by reducing sugars (glucose and fructose), which, probably can be the growing medium for yeasts and consequently they can accelerate the process of colored whey fermentation.
The aim of the work was to investigate the biochemical activity of lactose fermenting yeasts in the wort based on whey, obtained by thermo acid coagulation of milk by berry raw materials.

2. Materials and methods

Microorganisms
To carry out the experimental research, *Zygosaccharomyces lactis 868-K*, *Kluyveromyces lactis 2452*, *Saccharomyces lactis 9*5 yeasts from the «Collection of microorganism strains and plant lines for food and agricultural biotechnology» of the Research Institution «Institute of Food Biotechnology and Genomics' of the National Academy of Science of Ukraine» were used .

The production of colored whey
Having in view a fermented drink base, the whey was obtained from the milk-protein clots production by the method of thermo acid coagulation and the classic technology from the whole milk was used. The sterilized blackcurrant paste (TM «LiQberry» manufacturer, Ukraine Technical Conditions 15.3-24110704-003:2011), was used as a coagulant. The composition of paste is shown in the table 1 [12].

The paste was prepared in industrial conditions, by improved technology with the use of hydrodynamical (cavitational) processing of raw material at the TEK-CM installation.
The berries were processed in accordance with the developed regime in order to get the necessary degree of homogenization and industrial sterility [12]. The (10±0.5) % of berry coagulant from the general mass was added to the prepared normalized mixture. The whey obtained had the following indicators: the amount of dry matters – (7.47±0.37) %; reducing sugars – (5.57±0.58) %; protein – (1.3±0.07) %; the value of active acidity – (4.8±0.27); color – dark pink, bright, saturated; taste and smell – sour with a taste of berry coagulant.

Table 1.

The composition of homogenized black currant paste

Indicator	Amount
Fats, g/100 g	0.2
Proteins, g/100 g	0.8…1.0
Soluble dry matters, g/100 g	18.9
Cellulose, g/100 g	1.5
Value of pH	3.5
Polyphenols, mg/100 g	350…400
Organic acids, g/100 g	2.0…2.5
Vitamin C, mg/100 g	20…40
Pectin, g/100 g	0.9…1.1
Carbohydrates, g/100 g	
Total amount	14.92
Reducing sugars	14.32
fructose	1.96
glucose	12.36
Minerals, mg/100 g	
K	300…350
Na	25…32
Ca	30…36
Mg	26…31
P	28…33
F_c	1.0…3.0

Preparation of experimental samples

For the wort preparation, the colored milk whey was separated from the residual proteins and other factored particles, pasteurized at the temperature of (78±1) °C without exposure and cooled down to the temperature of (30±2) °C. The lactose fermenting yeasts in amount of at least 40 mln per 1 cm^3 of wort were introduced into the obtained mixture.

The fermentation was carried out at the temperature of 30 °C during 48 hours in accordance with the theoretical data [2]. The control sample – milk whey, obtained from the manufacturing of milk-protein clots by thermo acid coagulation of classical technology without using berry raw materials. The physiological condition of yeast was estimated by the total number of yeast cells (10^6 CFU/ml) and it was directly counted in the Goryaev chamber. The amount of carbon dioxide exhaled during the fermentation process was controlled by gravimetric method [13]. The fermented wort was distilled in order to determine the mass fraction of alcohol in the distillate by conventional methods [14]. The content of reducing matters was investigated by iodometric method [15].

The statistical analysis

All the analytical determinations were performed at least three times and the value reported for determined characteristics was the average value ± of the standard deviation (S.D.). The statistical analysis was performed by the Microsoft Excel statistical software version 2010.

3. Results and discussion

The determination of the lactose-fermenting yeast productivity

The results of lactose fermenting yeasts' biomass accumulation in the colored whey in 48 hours are shown in the fig. 1.

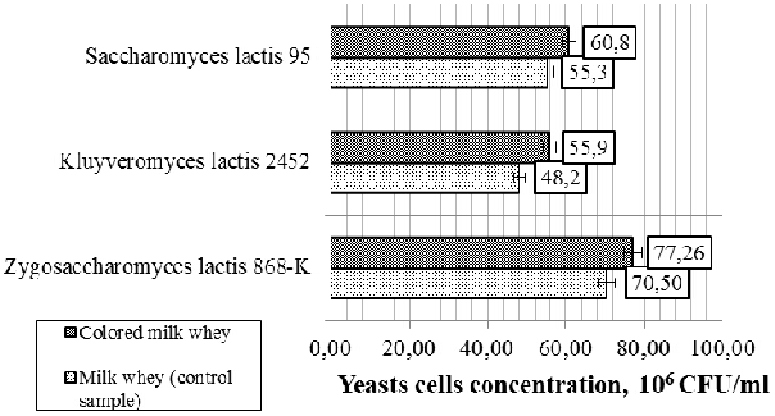

Fig. 1. Accumulation of yeasts cells in the colored milk whey

The research results confirm that yeast cells develop more actively in the medium with colored whey. Obviously, this effect was associated with a high amount of reducing sugars, due to the transfer of glucose and fructose from the berry coagulant. Herewith the amount of growth of the yeast cells varied from $55.9 \cdot 10^6$ to $77.26 \cdot 10^6$ CFU/ml of wort depending on the type of yeasts. The biggest amount of lactose fermenting yeast growth in the medium with colored whey was registered in the samples fermented by *Zygosaccharomyces lactis 868-K* microorganisms – the general amount of yeasts cells was of $77.26 \cdot 10^6$ CFU/ml of wort. Thus, the fermentation of whey wort with colored whey by lactose fermenting yeasts accelerates the process of fermentation. The obtained data confirm the possibility of using such whey in the process of fermented colored whey beverages manufacturing.

The determination of the fermentation rational temperature range

The temperature range of whey wort fermentation was specified according to the *Zygosaccharomyces lactis 868-K* yeasts biomass accumulation, amount of exhaled carbon dioxide and ethyl alcohol. The dependence of the change of these indicators from the temperature of fermentation in wort is shown in the table 2.

Table 2.

The dependence of *Zygosaccharomyces lactis 868-K* yeasts biomass accumulation, the amount of exhaled carbon dioxide and formation of ethyl alcohol in the wort from the fermentation temperature

Fermentation temperature, °C	Yeast cells concentration, (10^6 CFU/ml)		CO_2 content, g/100 ml		Ethanol content, vol. %	
	Whey (control)	Colored whey	Whey (control)	Colored whey	Whey (control)	Colored whey
24	61.2±1.84	65.3±1.96	0.69±0.02	0.73±0.02	0.20±0.01	0.26±0.01
26	65.9±1.98	69.8±2.09	0.73±0.02	0.79±0.02	0.22±0.01	0.28±0.01
28	69.2±2.08	75.7±2.27	0.83±0.02	0.85±0.03	0.25±0.01	0.29±0.01
30	69.3±2.08	78.1±2.34	0.93±0.02	0.96±0.03	0.26±0.01	0.35±0.01
32	71.2±2.14	79.9±2.40	0.95±0.03	0.99±0.03	0.30±0.01	0.38±0.01
34	70.5±2.12	76.4±2.92	0.94±0.03	0.96±0.03	0.25±0.01	0.33±0.01
36	70.1±2.10	78.5±2.36	0.86±0.03	0.88±0.03	0.20±0.01	0.31±0.01

According to the research results, shown in table 2, the optimal temperature of medium growth for the interested yeast species (control and model samples) is 30…32 °C, at which the

maximal accumulation of microorganisms – $(61,2…79,9)\cdot10^6$ CFU/ml can be observed. With the further temperature increase or decrease, the amount of yeast cells reduce, that can be explained by the lowering of their ferments' activity.

Wort substrate from the colored whey with the amount of yeasts biomass $79,9\cdot10^6$ CFU/ml of wort with the temperature 32 C showed high quality of culture medium for lactose fermenting yeasts. The worst result was registered by the wort that was fermented at the temperature of 24 °C – $65,3\cdot10^6$ CFU/ml. In all the samples, a similar dynamics of the biomass accumulation of yeast species *Zygosaccharomyces lactis 868-K* was observed.

The amount of accumulated carbon dioxide varied from 0,69 to 0,99 g per 100 ml of wort. The highest rate was observed at the temperature of 30...32 °C and was 0.73...0.99 g per 100 ml of medium. With the temperature increase or decrease the yeasts fundamentally reduced their activity and consequently the amount of exhaled carbon dioxide decreased. The content of ethanol in all the samples was in the range from 0.2 to 0.38 vol. %. The maximal amount of ethyl alcohol 0.38 vol. % was accumulated at the temperature of 32 °C in the wort with colored whey. In the control sample, this indicator is lower by 16…30 %, that testifies the positive impact of carbohydrate composition of colored milk whey on lactose fermenting yeasts' biological activity. According to the requirements of regulatory documents on non-alcoholic beverages [16], the permissible amount of ethanol in the product is of most 1.2 vol. %, thus the obtained worts can be the foundation for fermented beverages.

The determination of the reducing matters amount

The metabolism of lactose-fermenting yeasts is due to the course of chemical reactions, catalyzed by enzymes and connected with hydrolysis of lactose and monosaccarides of berry puree as an additional source of carbohydrates. Therefore, the amount of reducing matters, left over after the completion of the fermentation of wort from the colored whey was determined.

According to the research results, the initial content of reducing matters in colored whey differs essentially from the control sample and was of 5.57 % and 4.6 % correspondingly. After the wort fermentation during 48 hours at the temperature 30...32 °C the amount of reducing sugars decreased significantly to 0.07 % – for the wort based on the colored whey and 1.1 % for the control sample, respectively. This accounts for an almost complete process of carbohydrates fermentation and utilization, as well as for high activity of enzymes that catalyze the hydrolysis of lactose. The obtained data prove that the *Zygosaccharomyces lactis 868-K* yeasts have a high fermentation activity not only in relation to carbohydrates of whey, but probably to monosaccarides of black currant paste as well.

4. Conclusions

In the process of research, the possibility of lactose–fermenting yeasts usage for the fermentation of wort based on whey, obtained by thermo acid coagulation of milk by berry raw materials – sterilized black currant paste was proved. The basic regularities of multiplication of the diferrent yeast species on the base of colored whey were studied. It was determined that *Zygosaccharomyces lactis 868-K* are the most effective ones for the fermentation at the temperature of 30…32 °C and maximately accumulate the yeast biomass (the amount of cells – $(78,1...79,9)\cdot10^6$ CFU/ml) for 48 hours.The obtained results can be used in the processing technology of non-alcoholic beverages based on colored whey.

5. References

[1]. RAMOS O.L., PEREIRA R.N., RODRIGUES R.M., TEIXEIRA J.A., VICENTE A.A., MALCATA F.X., Whey and Whey Powders: Production and Uses / Reference Module in Food Science, from *Encyclopedia of Food and Health*: 498–505, (2016).

[2]. HRAMTHSOV A.G., The phenomenon of milk whey, monograph, *SPb: Profession*, 802 p., (2011).

[3]. KLASNJA M.T., SCIBAN M.B., Osnovi procesa anaerobnog prečišćavanja otpadnih voda

prehrambene industrije i industrije pića, *Acta Periodica Technologica*, 31: 740–748, (2000).
[4]. JELEN P., Whey processing. Utilization and Products, *Reference Module in Food Science, from Encyclopedia of Dairy Sciences (Second Edition)*, 731–737, (2011).
[5]. SHERSHENKOV B., SUCHKOVA E., The Direct Microbial Synthesis of Complex Bioactive Compounds as Perspective Way of Milk Whey Utilization, *Energy Procedia*, 72: 317–321, (2015).
[6]. CUI S.W. NIE S., ROBERTS K.T., Functional Properties of Dietary Fiber, *Comprehensive Biotechnology (Second Edition)*, 4: 517–525, (2011).
[7]. JELIČIĆ I., BOŽANIĆ R., TRATNIK L.J., Napitci na bazi sirutke − nova generacija mliječnih proizvoda, *Mljekarstvo*, 58: 257–274, (2008).
[8]. SMITHERS G.W., Whey and whey proteins – from «gutter-to-gold», *International Dairy Journal*, 18: 695–704, (2008).
[9]. GREK O., PSHENYCHNA T., KRASULYA O., PAKHOMOVA Y., IVASHCHENKO K., The influence of berry puree on microbiological indicators of Cheese product during storage, *Food and Environment Safet*, XV, Is. 4: 334–340, (2016).
[10]. MAKARKINA M.A., YANCHUK T.V., The characteristics of the black currant grades by sugars and organic acids content, *Modern gardening*, 2: 9–12, (2010).
[11]. SHEVCHUK L.M., The quality of black currant in the Carpathian region, Ukrainian Academy of agricultural Sciences, 10: 24–26, (2008).
[12]. IVASHCHENKO K.Y., Improving of homogenized fruit products technology using hydrodynamic processing: synopsis of a thesis..phd,speciality 05.18.13. «Technology of canned and chilled products» NUFT: 20 p., (2015).
[13]. GOST (All Union State standard) 6687.3-87 Carbonated soft drinks and drinks from bread raw materials. The methods of carbon dioxide determination, (1987).
[14]. GOST (All Union State standard) 6687.7-88 Non-alcoholic beverages and kvasses. A method of alcohol determination, (1988).
[15]. GOST (All Union State standard) 3628-78 Milk products. The methods of sugar determination, (1978).
[16]. DSTU 4069:2002 «Non-alcoholil drinks. General technical conditions», (2002).