METHODS OF DETERMINATION OF PARAMETERS OF FERMENTED WHEY-MALTY MIXTURES

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Abstract

The article presents main methods of studying restored whey-malty mixtures after fermentation by lactose-fermenting yeast and saccharomyces for getting a beverage of the kvass type.

The methods of accumulation of yeast cells Kluyveromyces lactis 469 and their percent with glycogen from the general concentration at fermentation of wort of the restored mixture at different ratios of dry malt and whey are offered and results are obtained. According to the obtained data, it was established, that yeast cells actively developed in the period from 4 to 16 fermentation hour at the ratio of malt and whey in the restored mixture as 1:1,5 and 1:2. The increment of cells with glycogen in mixtures with the ratio – malt:whey as 1,5:1 and 2:1 was intensive. Thus, at 36 hour of fermentation the amount of yeast was 67,2 and 68,9 % respectively from the total number of cells.

The generative capacity of yeast allowed to specify the fermentation temperature of wort of restored mixtures, cultivated by different races. It was established, that for Kluyveromyces lactis 469 the maximal accumulation of yeast (67…69 mln cell/cm^3 of wort) is observed at fermentation temperature – 30…32 °C, for Saccharomyces cerevisiae P-87 and Saccharomyces cerevisiae M-5 – 30…34 °C.

The gasochromatographic method allowed to identify side products of fermentation of fermented whey-malty wort by both lactose fermenting yeast and saccharomyces. The presented information is enough for the objective assessment of the qualitative composition of fermentation products as a result of the effect of different yeast races. The use of Saccharomyces cerevisiae P-87 for fermentation of whey-malty wort positively influences metabolism of a producer, stimulating biosynthesis or transformation of aromatic substances of the nutritive medium. The obtained research results indicate objective possibilities for the effective functioning of the aforesaid yeast race for fermentation of restored whey-malty wort in production systems.

Keywords: dry whey and malt, side fermentation products, gasochromatographic method, fermentation, whey-malty wort.

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1. Introduction

It is expedient to introduce technologies of products, based on whey, that don't need essential technical re-equipping of an enterprise and establishing of new lines, in production [1, 2]. One of simplest technological ways of processing whey is beverage production.

Whey is a product with a natural set of nutritive and biologically full-value components. The qualitative composition and number of macro- and microelements of whey products essentially exceed traditional refreshing beverages. Whey contains all irreplaceable amino acids. 90 % of carbohydrates are presented by disaccharide – lactose. The content of glucose in whey of sour-milk cheese – 0,7…1,8 %. It is conditioned by hydrolysis of lactose at processing. Among other carbohydrates, arabinose, lactulose and amyloid are present [3]. Fat- and water-soluble milk vitamins are transformed in whey; the latter ones almost completely. At the same time cheese whey contains much more of them than one of sour-milk cheese.

Whey drying is the most popular way of processing for prolonging the storage life. It provides conservation of all nutrients of the raw material. According to the literary data, dry milk whey has the following chemical composition, the mass share in %: moisture – 3…5, carbohydrates – 66,7…73,6, proteins – 12,5…14,0, fats – 0,7…1,5, mineral substances – 4,45…7,90, milk acids – 1,2…2,2 [4, 5].

Dry whey contains almost all milk components and has the low energetic value, can be considerably used for manufacturing products with the combined composition, for example, ice-cream, yogurt, confectionary products and so on. The high biological value of whey is conditioned by protein substances, and also vitamins, hormones, organic acids, immune bodies, microelements. Dry whey on the organoleptic level is combined with different vegetable ingredients, for example, wheat sifting, cellulose, extrudates and grain malts and so on. It is possible to use dry rye malt with mass share (8,0±0,25) % in mixtures with dry whey [6]. Solubility is an important parameter for using dry whey in the composition of such substances for restoring. This parameter for dry whey doesn't exceed 0,8 cm^3 of a damp residue [7]. The existent method of determining this parameter is general and suitable for the objective assessment of dry malt [8, 9].

Fermented beverages, based on whey, of the kvass type combines value components of the main raw material and products of metabolism of microorganisms, created at fermentation (ethyl alcohol, volatile acids, enzymes, different aromatic compounds and so on) [10].

All types of whey beverages have limited storage terms. Prolongation of suitableness and convenience of using this assortment both in production conditions and for the wide circle of con-

sumers needs development of correspondent technologies. Malt with rusks is contained by recipes of dry concentrates of kvass for restoring in water [11]. The use of dry concentrates, based on whey and rye malt, as a base of kvass is possible.

Problems that need a scientific substantiation include identification of such side fermentation products as higher alcohols (propyl, isoamyl, isobutyl and so on) with a typical smell and ability to create esters that essentially influence a fragrance of a fermented beverage [12, 13].

The aim of the work is determination of effective methods of studying restored whey-malty mixtures after fermentation for developing dry concentrates and selection of yeast that allows to improve the technology of fermented beverages.

2. Materials and Methods

Whey-malty mixtures were prepared on the base of dry whey and rye malt. Physical-chemical parameters of dry whey, according to SSU 4552:2006 (European analogue – «Sweet whey powder» ISO 9001; ISO 14001; FSSC 22000) are presented in **Table 1**.

Table 1
Physical-chemical parameters of dry whey

Parameter	Value
Mass share, %	
moisture	5,0±0,33
lactose	50,0±2,5
fat	2,0±0,05
Titrated acidity of whey, restored to mass share of dry substances 6,5 %, cm^3 of damp residue	0,8±0,04

Physical-chemical parameters of dry rye malt according to SS 29272-92 “Malt for bread and kvass” (European analogue – «Barley Malt Powder» ISO 9001, ISO 9000, ISO 14001, ISO 14000, ISO 20000) are presented in **Table 2**.

Table 2
Physical-chemical parameters of dry rye malt

Parameter	Value
Mass share, %	
moisture	8,0±0,25
Extract in the dry substance of malt	42±2,1
Sodium hydroxide with concentration 0,1 mol/dm^3 for 100 g of the dry substance of malt, cm^3	35±1,75
Iodine solution with concentration 1 mol/dm^3 for 100 g of the dry substance of malt, cm^3	17±0,85

At the first stage of the experimental studies there were prepared the dry mixtures with different ratios of malt and whey 1:1,5; 1:2; 1,5:1; 2:1 by mixing. The choice of proportions was conditioned by organoleptic parameters of the restored mixtures. At the second stage there was produced whey-malty wort with introducing dry substance 10 %, restored at temperature 35…45 °C, intensively mixing, the temperature was increased to 75...80 °C for transforming extract substances into the solution (reducing sugars, soluble pentosans, nitrogen-containing compounds and so on). Then the mixture, cooled to 25…30 °C, was set for decanting for eliminating residues of denatured proteins of whey to the malt residue. For malt fermentation there were used lacto-fermenting yeast *Kluyveromyces lactis 469* with amount 40 mln cell/cm^3 of wort. The effectiveness of aforesaid yeast in whey was proved by the previous studies [14, 15]. Fermentation was realized to temperature 30 °C during 36 hours.

Yeast cultures, used in the work, were received from the "Collection of strains of microorganisms and lines of plants for food and agricultural biotechnology" SI "Institute of food biotechnology and genomics" NAS of Ukraine.

Correspondent races of lactose-fermenting yeast and saccharomyces were selected by methods that objectively estimate the effectiveness of fermentation.

The physiological condition of yeast was estimated by the general number of cells with glycogen with coloration by Lugol solution. The amount of yeast in 1 cm^3 was determined by the direct calculation in Goryaev chamber [16–18].

According to the literary data [19], normal yeast contains no more 10 % of dead cells. The high content of such cells causes decelerated fermentation, favors the development of side microflora and autolysis of yeast. For functioning normally, yeast must contain 70…75 % of cells with glycogen. Less number characterizes insufficient nutrition of yeast, starvation. Such yeast multiply slower, lag-phase is longer, fermentation process is decelerated.

The following stage was the study of fermentation activity of different yeast races in whey-malty wort and suitableness for getting fermentative beverages with the standardized content of ethyl alcohol. The raw material – is a restored mixture of dry whey and rye fermented malt.

The control parameters of yeast biochemical activity were the amount of accumulated ethyl alcohol, depth of sugars utilization in the process of cultivation by the content of reducing substances for the final term of fermentation. The research objects were selected as yeast *Saccharomyces casei*, *Saccharomyces cerevisiae M-5*, *Kluyveromyces lactis 2452*, *Kluyveromyces lactis 469*, *Saccharomyces lactis 95*. The control – wort, based on water and malt, produced according to the classic technology using yeast *Saccharomyces cerevisiae-P-87.*

Then there was estimated the ability to development in different races of yeast in fermented whey-malt wort. For comparing the ability to accumulation at different variations, there was selected the monoculture *Saccharomyces cerevisiae P-87* [16]. Such yeast is not able to metabolize lactose independently or to stimulate the development of lactose-fermenting yeast. There was also studied the consistent cultivation of *Saccharomyces cerevisiae P-87* with lactose-fermenting yeast *Saccharomyces lactis 95* on the nutritive medium – restored mixture of whey and malt. During the experiment there was determined the number of yeast cells at fermenting wort of the restored mixture (ratio of malt to whey– 1:2). The process was fixed during 36 hours.

Specification of the temperature of wort fermentation was realized in samples, cultivated by different yeast races at temperatures 24…36 °C with interval 2 °C.

At the fermentation process the amount of emitted carbon dioxide was controlled by the weight method. Mature mash was distilled for determining the mass share of alcohol in the distillate. The image of the laboratory device for extracting ethyl alcohol is presented on **Fig. 1**.

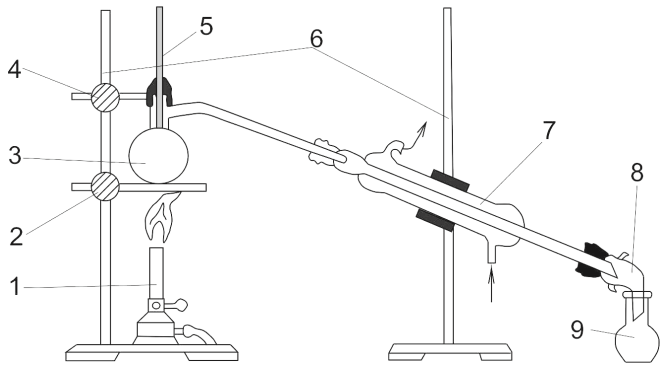

Fig. 1. Device for simple distillation of liquid substances: 1 – Bunsen burner; 2 – ring with clutch and asbestos net; 3 – distillatory flask (Wurtz flask); 4 – claw with clutch; 5 – thermometer; 6 – supports; 7 – Libich refrigerator; 8 – allonge; 9 – receiving flask

The content of reducing substances in whey-malty wort was determined by the iodometric method (this index before fermentation was 5,24 %). The main parameter of the intensity of this

process is the amount of emitted carbonic acid in the time unit, so fermentation continued up to the stop of emission of carbon dioxide [16].

Identification of side fermentation products of fermented whey-malty wort.

The characteristic of the smell of higher alcohols and esters depending on construction is presented in **Table 3** [12].

Table 3

Characteristic of smells of alcohols depending on molecular construction

Fermentation product	Structural formula	Smell
Methanol	$H–CH_2OH$	Wine
Ethanol	$CH_3–CH_2OH$	–
n-propanol	$CH_3–CH_3–CH_2OH$	Unpleasant, sharp, more pleasant at dilution
Isoamyl alcohol	$(CH_3)_2 – CH – CH_2 – CH_2CH$ $\vert$ CH_3 $\vert$ $CH_3 – CH_2 – CH – CH_2OH$	Fusel tone
Acetaldehyde	$CH_3–COH$	Sharp, specific, unpleasant bouquet
Propion aldehyde	$CH_3–CH_2–COH$	Sharp, specific, unpleasant bouquet
Methyl acetate	$CH_3–COO–CH_3$	Sweet, ether aroma
Ethyl acetate	$CH_3–COO–CH_2–CH_3$	Acetic-sour tone, fruit smell
Methyl propionate	$CH_3–CH_2–COO–CH_3$	Weak aromatic smell with sour tint
Isobutanol	$CH_3 – CH – CH_2CH$ $\vert$ CH_3	Fusel tone

Identification of side fermentation products in the distillate of fermented whey-malty wort was realized by the gasochromatographic method by determining higher alcohols C_1–C_5 [20]. Conditions of the chromatographic analysis of determination of volatile substances are presented in **Table 4**.

Table 4

Conditions of the chromatographic analysis of determination of volatile substances

Conditions of analysis	Value
Chromatography column (d/L)	D-mannit with solid carrier of chromosorbs W 80…100 mesh (1,5 mm/2 m)
Gas-carrier	nitrogen
Temperature of column, °C	90
Temperature of injector, °C	150
Temperature of detector, °C	200
Consumption of gas-carrier, cm^3/min	33
Type of detector	Flame-ionization, sprayer with internal diameter 0,25 mm
Consumption of hydrogen, cm^3/min	33
Consumption of air, cm^3/min	330
Volume of sample	0,2

The image of the laboratory equipment Chrom 5 (plant "Laboratory devices" Czechia) for the gasochromatographic analysis is presented on **Fig. 2**.

Fig. 2. Gasochromatographic equipment Chrom 5

Determination of quantitative ratios of higher alcohols, aldehydes and esters in the distillates was realized by the method of internal normalization using "Chromprocessor" software [21].

3. Experimental procedures

The results of the experimental studies of accumulation of yeast cells *Kluyveromyces lactis 469* and their percent with glycogen from the general concentration at fermentation of wort of the restored mixture at different ratios of dry malt and whey are presented in **Table 5**.

Table 5

Dynamics of accumulation of yeast cells and their percent with glycogen from the general concentration

Ratio of malt and whey	Fermentation duration, hour									
	0	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**36**
Concentration of yeast cells, mln/cm³										
2:1	30	37	45	58	65	66,8	67,2	68,4	69	69,3
1,5:1	30	39	49,5	60	66,3	67,9	68,2	69	70	70,9
1:1,5	30	43	50	63	72	72,3	73,1	73,5	74	74,5
1:2	30	40	52	65	72,5	73	73,6	74	74,5	74,9
% of cells with glycogen from the general concentration										
2:1	71	70,8	70,1	70	69,8	69,8	69,5	69,4	69,1	68,9
1,5:1	69,7	69,5	69,5	69,2	68,9	68,2	68,1	67,8	67,5	67,2
1:1,5	68	66,8	66,7	66,4	63,4	63,1	62,7	60,8	59,1	59
1:2	65,1	63,8	60,1	58,4	57,9	57,2	56,8	55,4	54,8	54,1

The most increment of cells *Kluyveromyces lactis 469* is observed in the restored mixture of malt to whey as 1:1,5 and 1:2 from 4 to 16 hour of fermentation. Indices are an evidence of the effectiveness of utilization of lactose by yeast.

The increment of cells with glycogen (table 5) was more intensive in samples with the other ratio of malt to whey – 1,5:1 and 2:1. Thus, at 36 hour of fermentation the general amount of yeast *Kluyveromyces lactis 469* was 67,2 and 68,9 % respectively.

Taking into account the fact that the concentration of yeast cells at 36 hour of fermentation was maximal at level 74,9 mln/cm^3, wort of the dry mixture with malt: whey ratio as 1:2 was selected for studying fermentation activity.

The method of distillation of wort, fermented by yeast races allowed to get distillates with different contents of ethyl alcohol. The quantity of ethanol, accumulated in wort, fermented by yeast of race *Saccharomyces casei* – 1,8 %, *Saccharomyces cerevisiae M-5* – 1,5 %, *Saccharomyces cerevisiae P-87* – 1,3 %, *Saccharomyces lactis 95* – 1,2 % *Kluyveromyces lactis 469* – 0,5 %, *Kluyveromyces lactis 2452* – 0,3 %. The content of reducing substances was determined by the iodometric method. The lowest index was fixed in wort, fermented by *Saccharomyces lactis 95* – 0,8 %, that characterizes almost finished process of fermentation and utilization of carbohydrates. The higher indices were fixed in worst, fermented by the other types of yeast: *Saccharomyces casei* – 3,77 %, *Saccharomyces cerevisiae M-5* – 3,75 %, *Saccharomyces cerevisiae P-87* – 3,43 %, *Kluyveromyces lactis 469* – 3,56 %, *Kluyveromyces lactis 2452* – 4,19 %.

Specification of the temperature of wort fermentation was realized in samples, cultivated by different yeast races at temperatures 24…36 °C with interval 2 °C, fermentation duration 36 hours. The initial concentration of yeast cells in all samples was 40 mln/cm^3 of wort. The dependence of accumulation of yeast cells on the cultivation temperature is presented on **Fig. 3**.

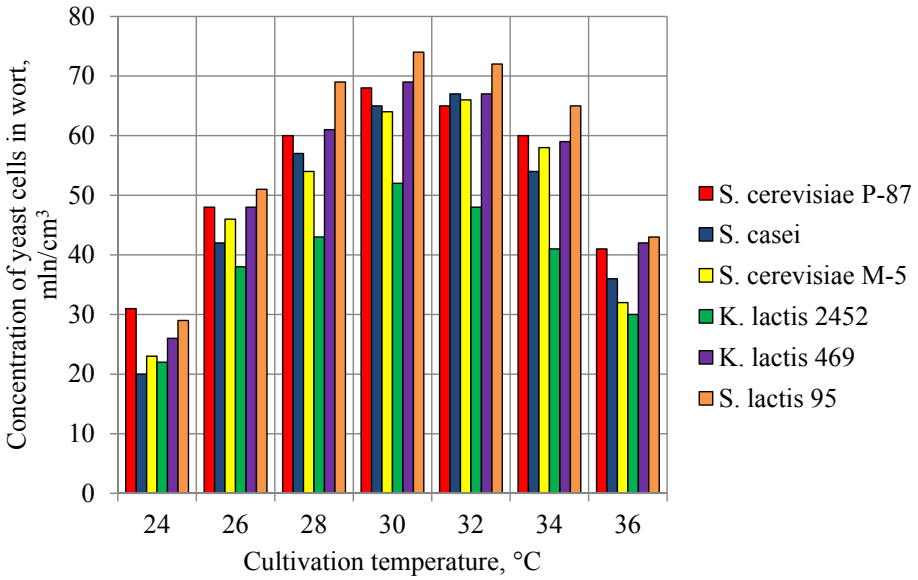

Fig. 3. Dependence of accumulation of yeast cells on the cultivation temperature

The data of fig. 3 testify that the active growth of yeast cells of all races takes place at the temperature from 28 to 32 °C. For *Kluyveromyces lactis 469* the maximal yeast accumulation (67…69 mln cell/cm^3 of wort) is observed at fermentation temperature – 30…32 °C, for *Saccharomyces cerevisiae P-87* and *Saccharomyces cerevisiae M-5* – 30…34 °C.

According to the results, in further studies by the aforesaid methodologies it is possible to use lactose fermenting microorganisms and saccharomyces, although lactose fermenting yeast demonstrated the lower result. Their dynamics of biomass accumulation is enough, so it is expedient to use them for fermentation of restored whey-malt wort. According to generative capacity, the following yeast races were selected for fermentation: *Saccharomyces cerevisiae P-87, Kluyveromyces lactis 469* and *Saccharomyces lactis 95.*

As to the studies by the gaschromatographic method of side fermentation products of whey-malt wort, fermented by *Saccharomyces cerevisiae P-87,* there were fixed values of methyl acetate concentration at level (11,72±0,59) mg/dm^3 and ethyla cetate (92,17±4,61) mg/dm^3. Such indices are optimal for forming a harmonious smell [9]. According to organoleptic parameters, wort is an opaque dense liquid of the dark-brown color, sour-sweet, malty taste without an expressed bitterness. Its smell is harmonious malty with fruit and flower tones.

Wort, fermented by yeast *Kluyveromyces lactis 469*, has the analogous taste, but with the more expressed bitterness. There are observed lower concentrations of methyl acetate (8,03±0,40) mg/dm^3) comparing with the limit one, ethyl acetate ones are essentially higher. At the same type there is observed the malty smell with weakly expressed fruit and sharp sour tones.

Taking into account the aforesaid, wort, fermented by yeast *Saccharomyces cerevisiae P-87,* is suitable by the content of higher alcohols and aldehydes . The content of side fermentation products, in mg/dm^3: n-propanol – 9,89±0,50, isobutanol – 27,39±1,40, acetaldehyde – 32,05±1,60, 2-methyl-1-butanol – 52,29±2,61 and 3-methyl-1-butanol – 207,19±10,36. Wort, fermented by yeast *Kluyveromyces lactis 469*, is characterized with higher concentrations of volatile components that influence formation of the general smell of the fermented beverage.

4. Conclusions

Determination of fermentative activity of yeast by the concentration of yeast cells (mln/cm^3) and their percent with glycogen of the general number is effective at selecting yeast races for fermenting restored whey-malty mixtures.

The results of the gasochromatographic studies of determining side fermentation products of whey-malty wort allows to estimate their qualitative composition objectively as a result of the effect of *Saccharomyces cerevisiae P-87.* Presence of this yeast race in the nutritive medium positively influences metabolism of a producer, stimulating biosynthesis or transformation of aromatic substances of the nutritive medium. The use of such lactose fermenting yeast for fermentation at temperature 30...34 ºC of restored whey-malty wort indicate objective possibilities for the effective use in production systems.

The given information is recommended to be used for substantiating parameters in the technology of fermented whey-malty beverages of the kvass type.

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