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IDENTIFICATION OF BY-PRODUCTS OF FERMENTATION OF WHEY AND MALT WORTS

Summary. One of the least unprofitable and non-waste technologies of lactose-fermentation processing is manufacture of fermentation drinks with the addition of rye fermented malt. For conduct of fermentation process it is reasonable to use the special microorganisms able to utilize lactose with formation of alcohol and carbon dioxide.

Influence of different lactose fermentative yeast races (*Zygosaccharomyces lactis* 868-K, *Saccharomyces lactis* 95, *Kluyveromyces lactis* 469) on physical and chemical parameters of fermented wort in comparison with the standard ones was investigated. The results prove the highest fermentative activity of *Zygosaccharomyces lactis* 868-K yeast, in particular: ethyl alcohol content - 1.0 v. %, sodium hydroxide solution acidity with concentration of 3 mol/dm³ per 100 cm³ of drink - 3.5 cm³, drink stability at 20 °C - 5 days. Physical and chemical parameters of wort fermented with *Saccharomyces lactis* 95 and *Kluyveromyces lactis* 469 yeast are considerable lower than the standard ones which points at rather low activity of fennents catalyzing lactose hydrolysis.

For investigation of influence lactose fermentative yeast on organoleptic parameters, there were identified waste products of fermentation in distillate of fermented serum and malt drink by gas and chromatographic method by actual techniques of definition of Q-C5 spirits developed by scientists of Basic Scientific Research Laboratory of National University of Food Technologies. After the contents of waste fermentation products, wort fermented with *Zygosaccharomyces lactis* 868-K yeast, which is characterized by low concentrations of n-propane (1.84 mg/dm³), isobutane (29.30 mg/dm³), acetaldehyde (27 mg/dm³) and high concentrations of 2-methyl-1-butanol (58.59 mg/dm³) and 3-methyl-1-butanol (211.11 mg/dm³) has higher parameters. In wort fermented by *Saccharomyces lactis* 95 and *Kluyveromyces lactis* 469 yeast, high concentrations both of n-propane (157.53 mg/dm³ and 33.29 mg/dm³, respectively), isobutane (261.80 mg/dm³ and 32.27 mg/dm³, respectively), and acetaldehyde (229.04 mg/dm³ and 172.48 mg/dm³, respectively) are accumulated. Moreover, such trial samples have low concentrations of 2-methyl-1-butanol (20.78 mg/dm³ and 173.52 mg/dm³, respectively) and 3-methyl-1-butanol (6.24 mg/dm³ and 17.56 mg/dm³), which influence on formation of a general fermented drink aroma.

As it can be seen from experimental research, biosynthesis of fermentation waste products can be defined as the result of regulatory functions of a yeast cell. Presence of *Zygosaccharomyces lactis* 868-K yeast strain in nutrient medium posi-

tively influences on producer's metabolism by stimulating biosynthesis or transformation of nutrient medium aromatic substances.

Key **words**: by-products of fermentation, whey and malt wort, lactose fermenting yeast, sensory indicators.

Introduction

The wide use of lactoserum as the basis for beverage fermentation is prevented with sensory properties (specific taste and flavor), short storage terms without the additional thermal processing. Such sensor indicators are caused by the complex of substances different in chemical origin which are mainly formed as the result of effect of ferments on milk components in the process of production of milk and protein products. When passing through fermentation processes, casein and fat are subject to the most considerable changes in milk. These components provide the main mass of volatile matters - peptides free amino acids, aldehydes, ketones, fatty acids (butyric, propionic, acetic, formic) etc. defining the specific taste and aroma. Whey proteins also influence on sensory properties, although at receiving of milk and protein products the action of ferments is basically pointed at casein, serum proteins which remain in solution and are subject to conformational and the other changes (reaction between protein and sugars, proteolysis, protein change under influence of light, oxygen, etc.) [1-3].

The rational way of lactoserum sensory indicators improvement is selection of substances which will form composition with better indicators. These can be artificial aromatic substances, other food supplements and herbal raw material. Another perspective way of lactoserum sensory indicators improvement increase of whey beverages biological value is considered to be fermentation. As the results of sugar fermentation, besides the main products (ethyl alcohol and carbonic acid) by-products which are situated in substrate are formed. Higher alcohols (propyl, isoamyl, isopropyl carbinol, etc.) belong to them.

One of influences on composition of higher alcohols of acids and ethers is yeast race. Accumulation of higher alcohols at fermentation is defined by metabolism intensity in the period of yeast reproduction and is connected with animation, formation of keto acids from carbohydrate transmutation products and during the main fermentation and final fermentation with transamination. Higher alcohols can also be formed without participation of amino acids, for example, through acetic acid after the following scheme: acetic acid acetoacetate acetone isopropanol. Higher alcohols are volatile substances with the certain aroma which is shown after formation of ethers from them. Esters are formed as the result of etherification as yeast waste products from volatile or non-volatile organic acids and higher alcohols. Esters are aromatic substances characterizing beverage flavor.

Problem definition

Development of fermentation beverages is in selection of yeast races which would favor alcohol fermentation with the simultaneous fermentation of the main lactoserum carbohydrate, lactose. Physical and chemical indicators on final product characterising the level of fermentation process completeness and content of by products of fermentation of whey and malt wort forming general flavor of a future beverage have an effect upon selection of yeast.

By-products of fermentation include higher alcohols (propyl, isoamyl, isopropyl, etc.) with typical aroma and ability to form esters which have a considerable effect on flavor of a fermented beverage [4-5].

Esters add fruit shades to fermented beverages. Composition of medial ethers of fatty acids includes ethylacetate with threshold concentration 180-200 mg/dm³, which has a sharp aroma of fruit essence. It was established that high concentrations of ethylacetate that it have a negative effect on flavor. Methyl acetate has a mild fruit and flower flavor which can be perceptible at threshold concentrations of 30 mg/dm³ [4-7].

E. Peino, U. Gamberto and A. Webb, R. Kepner proved that higher alcohols form 85% of the whole fusel oil complex and form flavor of products of fermentation but their excessively high concentrations add roughness to fermented beverages. Threshold concentrations of higher alcohols are rather high (isopropanol - 2000 mg/dm³, n-propanol - 100-500 mg/dm³, isobutanol - 100-200 mg/dm³, isoamylol - 50-100 mg/dm³), and their effect on flavor formation is negligible. The only exception is isoamylol the presence of which in the form of two isomers, 2-methyl-1-butanol and 3-methyl-1-butanol provides the desirable rose flavor shades.

Smith and Kofman established the considerable effect of aldehydes on flavor of products of fermentation. Scientific studies proved that aldehydes in their pure form have sharp flavor with fruit shades. At dilution, sharpness of aldehydes flavor decreases. Excessively high concentrations of acetaldehyde add oxidation shades to beverages which has an effect on the general flavor (acetaldehyde threshold concentration is 50-100 mg/dm³) [4-7].

Zygosaccharomyces lactis 868-K, *Saccharomyces laclis* 95 and *Kluveromyces lactis* 469 lactose fermenting yeast races from «Collection of microorganism strains and plant lines for food and agricultural biotechnology» of Research Institution in Institute of Food Biotechnology and Genomics' of National Academy of Science of Ukraine» were selected as test subjects.

Research results and discussion

The selected yeast races were used at receiving whey and malt worts in the following way: dry mixture of lactoserum and fermented rye malt in 2:1 ratio was recovered at temperature 35...45 C with initial dry substance content in wort of 10,0 %. The mixture obtained was intensively blended with gradual temperature increase to 75...80 °C for transfer of extractive substances into a solution. Then mixture cooled to

25...30 °C was sent for decantation with purpose to **remove** denaturated laoto serum proteins and malt deposit. For wort fermentation, compressed yeast with mass fraction of moisture 75 %; dosage 1,25 g per 100 cm³ were used. Wort fermentation was conducted at temperature 30 °C till dry substance content decrease by 1,0... 1,5 %, at ethandl content 0,3...0,6 % rpm and pH'3,5...:3,7.

Identification of by-products of fermentation in fermented whey and malt beverage distillate was performed with the use of gas chromatographic method according to efficient method of C1-C5 higher alcohols definition which was developed in Fundamental Scientific Research Laboratory' of National Institute of Food Technologies.

For conduct of gas chromatographic study of whey and malt worts, fermented with different *Zygosaccharomyces lactis* 868-K, *Saccharomyces lactis* 95, *Kluyveromyces lactis* 469 microorganism races, samples were selected in the end of their fermentation process, and their distillates, which characterized contents of all by-products of fermentation, were obtained.' Definition of quantitative ratios of higher alcohols, aldehydes and esters in distillates was conducted by inner normalization method with the use of *Chrome Processor* software.

Characteristic chromatograms of definition of the by- products of fermentation in distillates of whey and malt worts fermented with *Zygosaccharomyces lactis* 868-K, *Saccharomyces lactis* 95, *Kluyveromyces lactis* 469 yeast races are showed on Fig. 1, 2, 3.

Conents of the by- products in distillates of whey and malt worts fermented with *Zygosaccharomyces lactis* 868-K, *Saccharomyces lactis* 95, *Kluyveromyces lactis* 469 lactose fermenting yeast races are summarized in table 1.

Table 1 Conents of the by- products in distillates of whey and malt worts

Substance	Concentrations, mg/dm ³		
	<i>Saccharomyces lactis</i> 95	<i>Zygosaccharomyces lactis</i> 868-k	<i>Kluyveromyces lactis</i> -469
acetaldehyde	229,04	27,00	172,48
methylacetate	6,69	10,61	8,03
ethylacetate	186,12	85,11	498,17
methanol	19,73	14,55	67,48
n-propane	157,53	11,84	33,29
isobutane	261,80	29,30	32,27
2-methyl-1-butanol	20,78	58,39	6,24
3-methyl-1-butanol	173,52	211,11	17,56

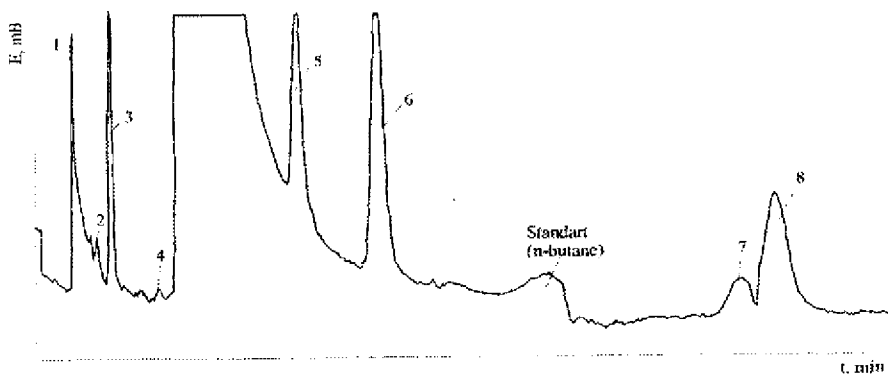


Fig. 1. Characteristic chromatograms of definition of the by- products of fermentation in distillates of whey and malt worts fermented with *Zygosaccharomyces lactis* 868-K: 1- acetaldehyde, 2 - methylacetate, 3- ethylacetate, 4 -methanol, 5 - n-propane, 6 - isobutane., 7 - 2-methyl-1-butanol, 8 - 3-methyl-1-butanol.

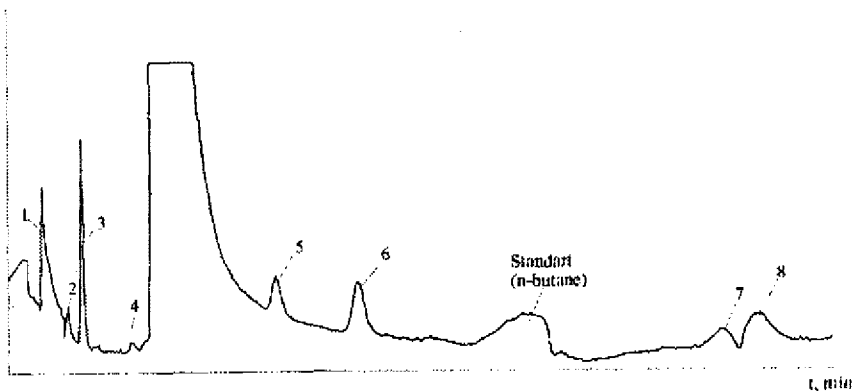


Fig. 2. Characteristic chromatograms of definition of the by- products of fermentation in distillates of whey and malt worts fermented with *Saccharomyces lactis* 95:1- acetaldehyde, 2 - methylacetate, 3- ethylacetate, 4 -methanol, 5 - n-propane, 6 - isobutane., 7 - 2-methyl-1-butanol, 8 - 3-methyl-1-butanol.

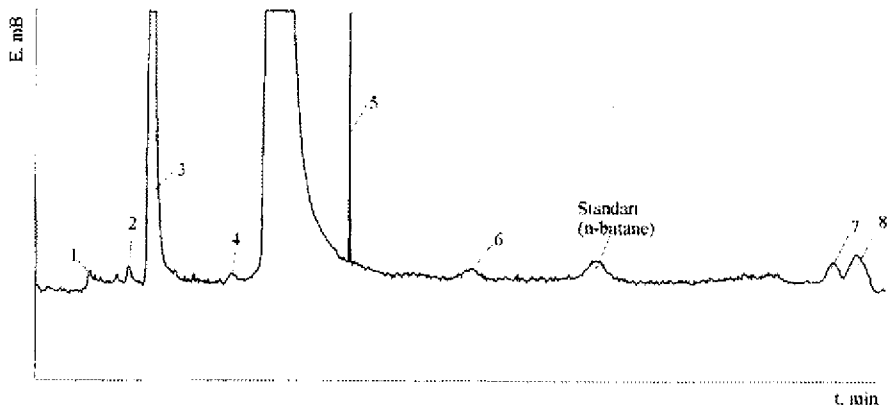


Fig. 3. Characteristic chromatograms of definition of the by-products of fermentation in distillates of whey and malt worts fermented with *Kluyveromyces lactis* 469: 1- acetaldehyde, 2 - methylacetate, 3- ethylacetate, 4 -methanol, 5 - n-propane, 6 - isobutane,, 7 - 2-methyl-1-butanol, 8 - 3-methyl-1-butanol.

With regard of Table 1 data and indicated threshold concentrations of methylacetate and ethylacetate, the following can be stated: concentrations of methylacetate (10,61 mg/dm³) and ethylacetate (85,11 mg/dm³) in wort, fermented with *Zygosaccharomyces lactis* 868K yeast are optimal for forming a harmonious fermented beverage flavor. In worts fermented with *Saccharomyces lactis* 95 and *Kluyveromyces lactis* 469 yeast, there are observed low concentrations of methylacetate (6,69 mg/dm³ and 8,06 mg/dm³, respectively) in comparison with threshold concentration, fruit and flower flavor of which will be very mild and, vice versa, concentrations of ethylacetate will be much higher in comparison with threshold concentration which adds sharp flavor of an artificial fruit essence to a beverage.

So, by content of higher alcohols and aldehydes, the best wort is deemed to be wort fermented with *Zygosaccharomyces lactis* 868K yeast which is characterized by low concentrations of n-propane (1,84 mg/dm³), isobutane (29,30 mg/dm³), acetaldehyde (27 mg/dm³) and high concentrations of 2-methyl-1-butanol (58,59 mg/dm³) and 3-methyl-1-butanol (211,11 mg/dm³). In worts fermented with *Saccharomyces lactis* 95 and *Kluyveromyces lactis* 469 yeast, there are accumulated both high concentrations of n-propane (157, 53 mg/dm³ and 33,29 mg/dm³, respectively), isobutane (261,80 mg/dm³ and 32,27 mg/dm³, respectively), acetaldehyde (229,04 mg/dm³ and 172,48 mg/dm³, respectively), and low concentrations of 2-methyl-1-butanol (20,78mg/dm³ and 173,52mg/dm³, respectively) and 3-methyl-1-butanol (6,24 mg/dm³ and 17,56 mg/dm³) influencing on forming general flavor of a fermented beverage.

Sensory properties of worts under investigation fermented with different yeast races are shown in Table 2.

Table 2 Sensory properties of whey and malt worts fermented with different yeast races

Indicator name	Whey and malt wort fermented with yeast		
	<i>Zygosaccharomyces lactis</i> 868-K	<i>Saccharomyces lactis</i> 95	<i>Kluyveromyces lactis</i> 469
Appearance	Opaque thick liquid with yeast deposit		
Color	Dark brown		
Taste	Soar and sweet malt without strong bitterness	Soar and sweet malt with mild bitterness	Soar and sweet malt with mild bitterness
Flavor	Harmonious malt flavor with fruit and flower tones	Malt flavor with light fruit and sharp soar tones	Malt flavor with sharp soar tones and rough fruit essence tones

Data of Table 2 point at the advantage of sensory indicators of whey and malt wort fermented with *Zygosaccharomyces lactis* 868-K yeast.

Conclusions

Results of gas chromatographic studies for definition of by-products of fermentation of whey and malt worts enabled evaluate advantages *Zygosaccharomyces lactis* 868-K yeast race objectively. Presence of the given yeast race in nutritional medium has a positive effect on producer's metabolism, thus stimulating biosynthesis or transformation of nutritional medium aromatic substances.

The use of *Zygosaccharomyces lactis* 868-K lactose fermenting yeast race in production of whey and malt beverages points at objective possibilities for wide implementation of release of this kind of a fermented beverage both at milk processing and at alcohol free enterprises which will enable providing processing secondary raw materials in milk industry.

LITERATURE

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