Influence of physicochemical parameters of water on the amino acid composition of bread kvass

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Abstract

Introduction. The influence of drinking water, which was prepared with the help of clinoptilolite, rock crystal and activated carbon, on the amino acid composition of wort and kvass was determined.

Materials and methods. Water was prepared by treating it with clinoptilolite, activated carbon and rock crystal. The fermented wort was obtained by fermentation of rye-based wort with yeast *Saccharomyces cerevisiae* MP-10. The amino acid composition of wort and kvass was determined by ion exchange chromatography, the dynamics of kvass wort fermentation was determined by fermentation test.

Results and discussion. As a result of water treatment with clinoptilolite, activated carbon and rock crystal, the total hardness was reduced from 4.5 to 1.1 mmol/dm³, the permanganate oxidation was reduced from 4.0 to 0.5 mg O₂/dm³, iron and residual chlorine were removed completely.

The change in the mineral composition of water significantly affected the course of the technological process, quantitative and qualitative amino acid composition of wort and kvass. The use of prepared water improved the qualitative amino acid composition, increased their total content in the initial wort from 3.3 to 8.87 mg/100 g, in particular amount of essential amino acids improved from 0.44 to 3.31 mg/100 g, which reduced their resynthesis, re- and deamination by yeast cells. The total amount of amino acids in the fermented wort with using prepared water was 9.88 mg/100 g, in the control it was 5.57 mg/100 g.

The ratio of amino acids in the wort had significant differences, in particular the content of proline, which is difficult to digest by the yeast cell when using prepared water, was 14%, and for the control sample it was 30%.

Intensification of the process of yeast cells culturing increased their concentration in the culture fluid for 24 hours from 96.8 to 85.1 million/cm³, as well as reduced the duration of fermentation of kvass wort from 15 to 13 hours.

The amino acid score of kvass, which was made with using prepared water in the stages of yeast cultivation and fermentation of yeast wort, was more acceptable compared to the method with untreated water.

Conclusions. The use of prepared water with clinoptilolite, rock crystal and activated carbon in kvass technology improves the qualitative amino acid composition and increases their total content in the initial and fermented wort.

Introduction

The chemical composition of most soft drinks is unbalanced, which is due to the use of mainly sucrose and dietary supplements with low or no protein, dietary fiber, vitamins, minerals and other biologically active substances [1, 2].

Bread kvass, which is prepared on the basis of rye raw materials, is a traditional Slavic drink and contains a wide range of biologically active substances [3, 4].

The drink is obtained by incomplete combined alcohol and lactic acid fermentation [4, 5]. In this case, due to the activity of yeast and lactic acid bacteria, a complex of biologically active substances is formed. However, to simplify the technology, often only yeast is used, and the required acidity is achieved by adding lactic acid [3, 6, 7].

In the process of fermentation, the caloric content of the beverage decreases, the amount of digestible nutrients increases, and the biological value increases [3, 8]. However, the search for ways to increase the biological value of kvass remains relevant.

Quantitative and qualitative amino acid composition of most soft drinks is very limited [9]. Therefore, in the production of kvass it is important to preserve and accumulate in the fermentation process the maximum possible and balanced amount of amino acids, mainly essential, which determines the usefulness of the drink, affects the process and indicators of the finished product [1, 10].

It is known that the yeast *Saccharomyces cerevisiae* absorbs inorganic nitrogen, in particular ammonium sulfate, urea, ammonia salts of acetic, lactic, malic and succinic acids, as well as most amino acids [11]. However, some of the amino acids (proline, histidine, glycine, cystine) are difficult for cells to digest [12]. Therefore, it was important to conduct research to determine the effect of the amino acid composition of kvass wort on the physiological activity of yeast.

For the production of fermented beverages water from centralized water supply networks or from artesian wells is used [13]. In the first case, the water is already reduced to drinking condition, and in the second, which is more common [3], may not meet such requirements. There are additional requirements for process water in non-alcoholic production [14], but they are absent for kvass production. In many cases, water-softened Na-cationite filters are used, but such treatment only replaces calcium and magnesium ions with sodium ions [15]. Therefore, it is important to improve the technology of water treatment with the use of effective and practically convenient natural minerals of various types during mechanical filtration.

Water treatment using mechanical filters with natural mineral backfill (quartz sand, gravel) does not fully ensure the quality of prepared water by organoleptic, physicochemical and microbiological parameters [5]. Therefore, it is important to improve the mechanical filtration of water with the use of new effective natural materials, including minerals. Due to strictly defined pore sizes and internal cavities, they are effective sorbents of organic and inorganic substances [16]. Such materials have the ability to improve the organoleptic and physicochemical parameters of water, to ensure its structuring, which increases the health effects of the finished drink [3, 13].

The use of minerals can solve various problems [4]. Sorption processes can be provided not only by the use of activated carbon and its analogues (graphite-mineral sorbents) [15]. For these purposes, as well as a filter material, it is advisable to use zeolites, in particular clinoptilolite [17].

Due to the specific structure of clinoptilolite, which is a tetrahedron with octagonal rings on the tops containing alkaline and alkaline earth metals inside the cations and a significant

number of entrance windows ($3.5\text{--}4.8\text{ \AA}^0$) and channels, its use as a molecular sieve with ion exchange properties is ensured [15].

The aim of the work is to study the effect of clinoptilolite, rock crystal and activated carbon in the preparation of water on the amino acid content in the original and fermented wort to improve the viability of yeast and obtain the finished drink with balanced amino acid composition.

Materials and methods

Water treatment using clinoptilolite, activated carbon and rock crystal

Tap water treatment was performed with clinoptilolite of Sokyrnytsia deposit (Ukraine), activated carbon and rock crystal at a speed of 8–15 m/h. Water was prepared in a cyclic mode with the following sequential operations:

- Preparation of materials;
- Water filtration to reach the limit values: clinoptilolite for hardness; rock crystal for transparency; activated carbon for transparency, color and permanganate oxidation;
- Padding of a layer of materials with a stream of untreated water for prevention of caking and removal of dirt from their surface;
- Regeneration of clinoptilolite.

The use of clinopylolite is due to the fact that it reduces the hardness of water and removes heavy metals [17].

Activated carbon was used to reduce the content of organic impurities and improve the organoleptic characteristics of water [3].

Rock crystal was used to structure the water, adjust the redox potential and remove foreign microflora [15].

In the prepared water the content of total iron was 0.01 mg/dm^3 , total hardness 1.1 mmol/dm^3 , permanganate oxidation $0.5\text{ mg O}_2/\text{dm}^3$. Untreated water with a total iron content of 0.05 mg/dm^3 , a total hardness of 4.5 mmol/dm^3 , and a permanganate oxidation of $4.0\text{ mg O}_2/\text{dm}^3$ was used as a control.

Preparation of kvass wort and kvass

For the cultivation of yeast, wort was prepared from a concentrate of kvass wort, sugar syrup and water with concentration of 7.0–8.0% of dry matter [13]. The wort for kvass preparation was prepared to a dry matter concentration of 3.4–3.6%, it was fermented with a pure culture of yeast *Saccharomyces cerevisiae* MP-10, which was selected from acidic concentrate of kvass wort (patent № 134310 Yeast strain *Saccharomyces cerevisiae* MP-10 using for fermentation in the production of fermented beverages) with the initial yeast cells concentration of 0.6 million/cm^3 at a temperature of $34\text{ }^\circ\text{C}$ to reduce the dry matter content by 0.8–1.0%. The fermented wort released from the yeast sludge was blended to a dry matter content of 5.4–5.6% [4].

Two samples of kvass wort were prepared:

- Control – wort with untreated water;
- Experiment – wort with prepared water.

Determination of yeast fermentation activity

The concentration of yeast cells in the culture fluid was determined using the Goryaev chamber [18], the dynamics of fermentation of yeast wort was determined by biological method.

Determination of amino acid content

The amino acid composition of wort and kvass was determined by ion exchange chromatography on Biotronik Amino Acid Analyzer LC2000 equipment with amino acid distribution using ion exchange resin and tribuffer system. Identification was performed by reaction with ninhydrin and detection with photocolormetry and electrophoresis methods using the system «Drops-105/105M», which involves acid hydrolysis with the transition of amino acids into free forms with their subsequent separation and quantification by capillary electrophoresis. Detection was performed in the UV region of the spectrum at a wavelength of 254 nm [4].

Determination of amino acid score

To characterize the biological value of kvass, we compared their amino acid composition with the ideal scale of amino acids [19, 20]. Amino acid score was calculated by the formula:

$$C = (A / H) \cdot 100,$$

where:

C is score, %;

A is amino acid content in kvass, mg/g protein;

H is the amino acid content in an ideal protein, mg/g protein.

Results and discussion

Influence of filter materials on physicochemical parameters of water

Water used for the production of fermented beverages must be epidemiologically safe, harmless in chemical composition and have acceptable organoleptic properties [13, 21].

Physicochemical parameters of control and experimental water samples are given in Table 1.

It is established that the joint use of the studied rawmaterials provided the necessary physicochemical parameters of process water. The total hardness was reduced by 82%, the permanganate oxidation was reduced by 3.6 times, iron and residual chlorine were removed completely.

In the first stage, the water was treated with clinoptilolite to reduce stiffness and remove mechanical impurities [15]. This reduces the load on the carbon filter, which increases its service life [4]. Treatment of water with activated carbon in the second stage provided sorption of impurities, stabilization of the redox potential and improvement of organoleptic characteristics [3]. The third stage involves the treatment of water with rock crystal for its structuring and design of organoleptic characteristics [13].

Table 1

Influence of filter materials on physicochemical parameters of water

Sample name	Dry residue, mg/dm ³	Total stiffness, mmol/dm ³	Total iron, mg/dm ³
Control	251.0	4.5	0.05
Experiment	205	1.1	less than 0.01

Sample name	Cl ⁻ , mg/dm ³	NO ₃ ⁻ , mg/dm ³	SO ₄ ²⁻ , mg/dm ³	pH	Permanganate oxidation, mg O ₂ /dm ³
Control	34	4.6	37	7.39	4.0
Experiment	15	less than 0.05	15	8.10	0.5

Effect of water on the amino acid composition of the original kvass wort

The main amine nutrition for yeast in kvass wort is free amino acids [22], which directly affect their physiological activity [23, 24]. Table 2 shows the amino acid content in the studied samples of kvass wort.

Table 2

Amino acid composition of kvass wort

Amino acids	The content of amino acids in the wort, mg / 100 g	
	Control	Experiment
Aspartic acid	1.65	2.85
Threonine	0	0.42
Serine	0.16	0.39
Glutamic acid	0	0.05
Proline	0.97	1.25
Glycine	0	0.05
Alanine	0	0.74
Valine	0.05	0.34
Methionine	0	0.06
Isoleucine	0.02	0.33
Leucine	0.04	0.45
Tyrosine	0	0.29
Phenylalanine	0	0.94
Histidine	0.33	0.45
Arginine	0	0
Lysine	0	0.03
Essential	0.44	3.31
Non-essential	2.86	5.56
Total	3.30	8.87

The obtained data indicate the presence of seventeen amino acids in the kvass wort, in particular 9 essential ones. When using the prepared water, the total amino acid content in the kvass wort was higher by 63%, in particular essential ones by 87%. The content of inorganic nitrogen (ammonium salt) in the test sample increased almost threefold (from 0,08 to 0,23 mg/100 g). The significant difference in the content of amino acids in the studied samples can be explained by the mineral composition of the untreated water, in particular the increased amount of hardness salts, which while interacting with organic acids change the acidity of the wort.

Amino acids in solutions exhibit the properties of amphoteric electrolytes. Depending on the pH of the medium, they can have acidic or alkaline properties. In an acidic environment, the dissociation of the carboxyl group is inhibited, the amino acid molecule thus acquires a positive charge and reacts as a cation:

In an alkaline environment, under the action of OH⁻ ions, the ionization of amino groups is inhibited, the amino acid molecule acquires a negative charge and reacts as an anion:

The pH value of the medium at which the total charge of the amino acid is zero is called the isoelectric point [25–27]. Therefore, the pH of the wort has a significant effect on the process. Table 3 shows the acidity of the kvass wort of the test samples.

Table 3

Acidity of kvass wort

Sample name	Titrated acidity, cm ³ of NaOH solution conc. 1 mmol/dm ³ per 100 cm ³ of wort	pH
Control	0.9	5.94
Experiment	1.2	5,45

It is known that the isoelectric point for monoaminomonocarboxylic acids corresponds to pH 6. In isoelectric points of amino acids are the least stable and precipitate directly and in the form of salts. According to the data, the active acidity of the wort of the control sample in contrast to the experimental was close to this value. Therefore, the content of monoaminomonocarboxylic acids in the test sample was 2.78 mg/100 g, which is 10 times higher than in the control (see Table 2).

Yeast assimilates amino acids in different sequences [26]. Table 4 shows the distribution of amino acids by their digestibility.

Table 4

Distribution of amino acids by digestibility by yeast species *Saccharomyces cerevisiae*

Class 1	Class 2	Class 3	Class 4
Arginine	Histidine	Alanine	Proline
Aspartic acid	Isoleucine	Glycine	-
Glutamic acid	Leucine	Phenylalanine	-
Lysine	Methionine	Tryptophan	-
Serine	Valine	Tyrosine	-
Threonine	-	-	-

Figures 1 and 2 show the ratio of the above classes of amino acids in the studied samples of wort.

These data indicate that the test sample contained a larger range of amino acids compared to the control, which promotes their assimilation by yeast in the first stage of reproduction with little resynthesis, re- and deamination [26]. In this case, amino acids are used by cells for protein synthesis directly, and therefore their assimilation is more efficient [4]. The content of proline, which is difficult to digest by the yeast cell was 2 times less than in the control. It should also be noted that the test sample, in contrast to the control, contained methionine, which is a partial source of phosphorus nutrition for yeast [19].

The assimilation of amino acids by yeast occurs in two ways, one of which is associated with deamination and the other with decarboxylation [27, 28]. Decarboxylation occurs according to the equation:

The deamination reaction is associated with the release of ammonia from the amino acid by the equation:

The deamination process is associated with reamination reactions. These two processes allow cells to synthesize amino acids most intensively [29].

Influence of water treatment on the process of yeast cultivation and kvass wort fermentation

Qualitative and quantitative composition of amino acids of wort determines the biosynthetic processes of yeast cells and directly affects the rate of their growth [3, 13]. Tables 5, 6 show a comparative characteristic of the concentration of yeast cells and their specific growth rate during cultivation.

Figure 1. The ratio of amino acids in the wort when using untreated water (control)

Figure 2. The ratio of amino acids in the wort when using prepared water (experiment)

Table 5

Influence of cultivation duration on yeast cell accumulation

Duration, hours	Yeast cell concentration, mln/cm ³	
	Control	Experiment
0	0.5	0.5
6	3.7	8.1
12	54.1	61.5
18	78.2	89.0
24	85.1	96.8

Throughout the cultivation process, the content of yeast cells in the test sample was higher than in the control: after 6 hours – 54%, the next – an average of 12%, which can be explained by the increased content of amino acids in the wort used as a source of amine nutrition for yeast. These data indicate a positive effect of a more balanced nitrogen nutrition of the experimental sample of yeast wort on the accumulation of yeast cells.

Table 6

Effect of cultivation duration on the specific growth rate of yeast

Duration, hours	Specific growth rate (μ), h ⁻¹	
	Control	Control
6	0.333	0.464
12	0.447	0.337
18	0.061	0.062
24	0.014	0.014

When comparing the studied samples, it was found that the maximum specific growth rate of the experimental sample was 3,6% higher for the experimental sample compared to the control. Achieving high growth rate by the sixth hour of growth for this sample (28% more than in the control) indicates a reduction in the lag phase and accelerating the beginning of the phase of exponential development of yeast [9]. Subsequently, the specific growth rate of yeast in the experimental and control samples did not differ. The obtained results can be explained by the expanded qualitative composition of amino acids in the experimental sample, which are not close to the isoelectric point and do not precipitate. After 18 hours in the culture medium there was a phase of cell death, which was accompanied by a decrease in the specific growth rate.

Figure 3 shows the dynamics of the decrease in dry matter content during the fermentation of wort by yeast at the initial cells content in the inoculum of 61 million/cm³ in the phase of their exponential growth.

Figure 3. Dynamics of dry matter content during fermentation of kvass wort

The general nature of the fermentation process in the studied samples was similar. However, in the experimental sample the beginning of intensive fermentation was observed from the second hour, and in the control – from the third, which can be explained by more active physiological state of yeast, reducing the duration of their adaptation to environmental conditions [26], increased initial amine nitrogen content. In addition, calcium, magnesium, and sodium ions, which reduce the permeability of the yeast membrane, had a negative effect on the control sample [29]. The required decrease in dry matter content (by 0.8–1.0%) for the test sample occurred on average by 2 hours faster than control.

Effect of water on the amino acid composition of fermented kvass wort

It was found that the treated water allowed not only to preserve most of the amino acids of the kvass wort concentrate, but also to optimize their quality ratio (see Table 2). At the same time, full nutrition of yeast was provided and fermentation was accelerated, the influence of resynthesis, re- and deamination processes was significantly reduced (Figures 1, 2). It should be kept in mind that amino acids are the component not only of cell organelles, but also of enzymes and vitamins [26]. Alanine is part of pantothenic acid, which plays an important role in the life of yeast [9]. Glutamic acid is involved in the construction of enzymes that catalyze the synthesis of purine bases and peptides [19]. In addition, the qualitative composition of amino acids used for protein synthesis directly affects the stability of RNA [28, 29].

Figures 4 and 5 show the comparative characteristics of the amino acid composition of the original and fermented wort using the yeast *Saccharomyces cerevisiae* MP-10.

Figure 4. Amino acid composition of the wort when using untreated water (control)

Figure 5. Amino acid composition of the wort when using prepared water (experiment)

During fermentation, the content of amino acids changed significantly at their relatively constant ratio – 40 and 10% for experimental and control samples, respectively. The total amount of amino acids in the experimental sample of fermented wort was 9,88 mg/100 g, in the control 5.57 mg/100 g, respectively .

The possibility of protein utilization by the human body is due to the minimum score of one of the essential amino acids [14]. The main one is not the quantitative content of biologically active substances, but their adequacy in accordance with the needs of the human body [2, 7, 19]. Therefore, to characterize the biological value of kvass obtained from the studied samples, we compared their amino acid composition with the ideal scale of amino acids. The amino acid score of the kvass samples is shown in table 7.

Table 7

Amino acid score of kvass

Sample	Amino acid					
	Valine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine + tyrosine
Control	12	334	15	16	5	0
Experiment	188	421	30	29	22	170

In the studied kvass samples, the amino acid score did not meet the requirements of an ideal protein, which is typical for most foods [4, 7, 20]. However, the amino acid score of experimental sample was closer to the ideal protein than the test sample (leucine, isoleucine and lysine). Lysine was determined as the limiting amino acid for both samples. Phenylalanine and tyrosine were absent in the control sample.

Therefore, kvass prepared using clinoptilolite, activated carbon and rock crystal in water treatment had increased biological value.

Conclusions

1. The use of prepared water improves the qualitative amino acid composition, increases their total content in the initial wort by 63%, including essential by 87%, which reduces their resynthesis, re- and deamination by the yeast cell. The content of monoaminomonocarboxylic acids in the test sample in comparison with the control increases by 10 times.
2. Water treatment with clinoptilolite, activated carbon and rock crystal intensifies the process of yeast cultivation and increases its concentration in the culture fluid by 12%. During the fermentation of kvass wort, the normative decrease in the dry matter content for the experimental sample occurred on average 2 hours faster compared to the control.
3. The amino acid score of kvass which was made by using prepared water is more acceptable compared to the use of untreated water. Prepared water provides an increase in the quantity and improvement of the qualitative amino acid composition of bread kvass.

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