MIRCOBIOLOGICAL COMPOSITION ASSESSMENT OF BREAD KVASS

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Abstract
Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast; alcohol content in kvass must be less than 1.2% alcohol by volume. Microbiological safety of kvass is an important issue because European Regulation No 2073/2005 on microbiological criteria for foodstuffs does not provide microbiological criteria for kvass production. Microbiological safety of kvass depends on raw materials, personal hygiene, environment, kvass blending and filtration. Experiments were carried out at the Latvia University of Agriculture Department of Food Technology from November 2013 to January 2014. The aim of this work was to assess the microbiological environment changes during kvass production process and shelf-life. Understanding the development of dynamic of microbiological environment provides a better management for kvass production processes. Samples of bread kvass were analysed during production (12 and 13 h) and storage (36, 60, 84, 132, 136 h) at 3 ± 1 °C to determine kvass quality. Yeasts (LVS EN ISO 21527 - 2: 2008), lactic acid bacteria (ISO 9332:2003) and total plate count (LVS EN ISO 4833:2003) were determined in kvass samples. Microorganisms in kvass were identified using API identification system; the dominating microflora in kvass was *Saccharomyces cerevisiae* and *Leuconostoc mesentericus*. Changes of total plate count during fermentation and maturation were not significant ($p>0.05$).
Key words: kvass, yeast, microbiological composition.

Introduction

Production of non-alcoholic beverages has an important part in the Latvian food industry. One of the best known non-alcoholic beverages in Latvia is naturally fermented kvass. Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast. The alcohol content in kvass must be less than 1.2% alcohol by volume. Nowadays most of the commercially available beverages sold as kvass are kvass drinks and malt extract drinks. They are made by diluting grain extract concentrates with water and adding colourings and different flavours (Klosse, 2013) and artificial sweeteners.

The rapid segment production of naturally fermented kvass can be explained by high quality taste, tonic effect and beneficial effects on digestion and on pancreas (Feik et al., 2007). Many consumers choose naturally fermented kvass over kvass drinks. It is technologically more complicated to produce naturally fermented kvass with good quality than kvass drinks from grain extract concentrates.

Naturally fermented kvass is made from dried rye bread by soaking it in hot water for a few hours. After separating water-bread extract from soaked bread, it is fermented by adding bread yeast. Fermented kvass production process must be monitored carefully in order not to exceed the permitted amount of alcohol, as well as hygiene standards must be strictly abided to avoid the product contamination with undesirable microflora, which may result in an adverse effect on product quality. An increased count of microorganisms indicates the general norms of hygiene have been disregarded in a food company, leading to food contamination during processing and manufacturing. It can also lead to high contamination levels during improper storage (Wong et al., 2005). Therefore, it is important to investigate and to determine microbiological environment and possible risk factors during kvass production; bread rusks are one of the possible risk factors. It is essential for any manufacturer to obtain high quality product with a longer shelf life, retaining its characteristic properties during storage.

Microbiological criteria for kvass are not defined by European Regulation No 2073/2005 on microbiological criteria for foodstuffs. Therefore, it is important to study microbiological environment of kvass and existing microorganisms that can cause product spoilage. Moulds, yeasts and bacteria cause food spoilage (Da Silva et al., 2013). There are various microorganisms that can cause kvass spoilage: *Pseudomonas* spp. (42 ± 1 °C, pH 6.6-7.0), *Acinotobacter* spp. (35 ± 1 °C, pH 6.5-7.5) and *Moraxella* spp. (36 ± 1 °C, pH 7.0-9.0) spoil food products by producing substances that give undesired taste and odour (Wilson, 2005; Jeyalakshmi and Kanmani, 2008; UK Standards..., 2011). *Clostridium perfringens* and *Clostridium botulinum* produce toxins dangerous to humans. Facultative anaerobic bacteria which can grow in kvass environments and spoil it are *E. coli*, *Staphylococus aureus* and *Salmonella* species. Using spoiled foodstuff in human nutrition can cause various toxicosis and intoxications (Krämer, 1992). Optimal environment for mould growth is pH 4.0-6.0 at 27 ± 3 °C, and wild yeasts proliferate at 25 ± 1 °C (Baumgart, 1993).

Research on microbiological environment of bread kvass made from rye bread rusks has not been done in Latvia yet. The aim of this work was to assess

the microbiological changes during kvass production process and shelf-life.

Materials and Methods

Experiments were carried out at the Latvia University of Agriculture, Faculty of Food Technology Microbiology research laboratory from November 2013 to January 2014.

Preparation of kvass

For bread kvass production the following materials were used: 'Liepkalni Ltd' rye bread rusks, baker's yeast *Saccharomyces cerevisiae*, lactic acid bacteria *Leuconostoc mesentericus*, 'Ltd Dansukker' beet sugar and 'Liepkalni Ltd' dark rye-barley malt. For preparing of 1 L kvass mash, 200 g of rye bread rusks and 2 g dark malt are soaked in 2 L of hot water (78 ± 2 °C). Bread rusks are left soaking for 3 h, then the water-bread rusk suspension is filtered (300 microns) and the liquid fraction is cooled down and used in further kvass production stages. 1 g baker's yeast, 2 units of lactic acid bacteria starter and 1/3 of the estimated quantity of sugar are added to 1 L of kvass mash. The total quantity of sugar for kvass production is 30 g; 10 g of sugar are added prior to fermentation. The fermentation of kvass mash takes 9 h at 27 ± 1 °C. After fermentation kvass is placed in a refrigeration chamber to cool down at 3 ± 1 °C. After cooling, the yeasts are filtered (5 microns) and the remaining sugar is added. Kvass is maturated for 12 h at 6 ± 1 °C and then it is ready for drinking (total production process of 25 h). Kvass was filled in 0.5 L PET bottles and stored for 156 h in total in order to complete the microbiological analysis. The stages of kvass production process are shown in Table 1.

Table 1

Stages of kvass production process

Stage	Materials and technological process	Time, h
S_0	Rye bread rusks, before soaking	0
S_1	Kvass after fermentation	12
S_2	Kvass after blending and the start of maturation	13
	End of kvass production process	*25*
S_3	Kvass during storage	36
S_4	Kvass during storage	60
S_5	Kvass during storage	84
S_6	Kvass during storage	132
S_7	Kvass during storage	156

Microbiological analyses

Preparation of test samples, initial suspensions and decimal dilutions for microbiological examination was completed according to LVS EN ISO 6887-5:2011. The total plate count (TPC) was determined according to the standard LVS EN ISO 4833-1:2014, moulds and yeasts were determined according to the standard ISO 21527-2: 2008 and lactic acid bacteria were determined according to the standard ISO 9332:2003.

Microorganisms in kvass were identified using API (analytical profile index) identification system: lactic acid bacteria – ID 50 CHL and yeasts – ID 32C. API test was completed with ready-to-use kvass samples after storage for 10 h and 20 h. Photos of identified microorganisms were taken with Axioscop 40.

Data analyses

The obtained data processing was performed with Microsoft Excel 13 for Windows; mean values and standard deviations were calculated. For data cross-comparison ANOVA and correlation were used. Both t-test and F-test were used in order to assess the significance of changes and inter-comparison of the obtained data. For the interpretation of the results it is assumed that α=0.05 with 95% confidence.

Results and Discussion

Microbiological analyses were carried out during all production stages of laboratory produced kvass. The initial total plate count (TPC) in bread rusks was 4.16 log cfu g^{-1} (Figure 1). The first stage (0 hours) of naturally fermented kvass production was TPC determination in the main raw material – rye bread rusks. TPC during kvass mash fermentation was unstable. During the first 13 hours the increase in TPC was low, compared to other dominant organisms in the environment.

Yeasts and lactic acid bacteria are the main microorganisms in kvass fermentation process (Salovaara and Gänzle, 2011), both of which form substances that give kvass its specific taste and aroma. During fermentation the alcohol and lactic acid are produced which are considered as natural preservatives that partially protect kvass from undesirable development of microorganisms.

During the first 12 h of naturally fermented kvass production (9 of which was fermentation), yeast concentration increased from 4.55 log cfu g^{-1} (dry bread rusks at 0 hours) to 6.06 log cfu g^{-1} which is between the growth rate of TPC and lactic acid bacteria. This indicated that the raw material already contained a certain amount of yeast cells. No moulds were detected during kvass production process.

A slightly lower amount of lactic acid bacteria was found in bread rusks - 3.34 log cfu g^{-1}. The initial line segment for the growth of lactic acid bacteria is steeper than the other two groups, suggesting a higher growth rate. During the first 12 h of naturally

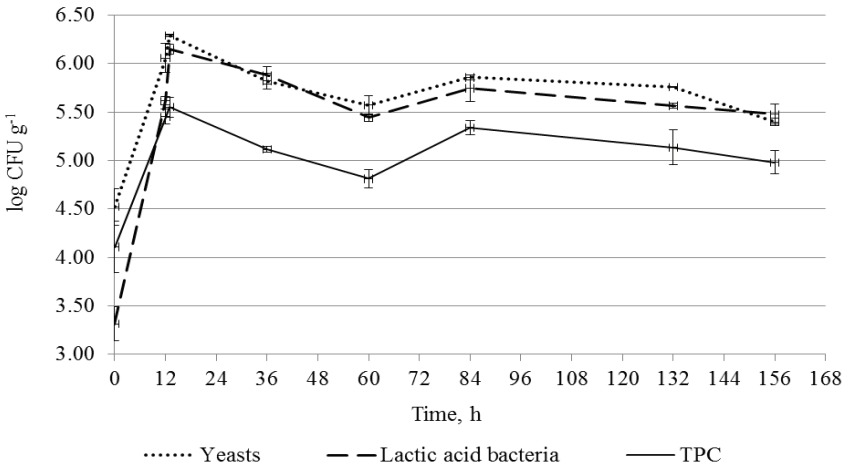

Figure 1. Changes of total plate count, lactic acid bacteria and yeast count during kvass production and storage.

fermented kvass production, the changes in growth of lactic acid bacteria were greater compared to TPC and yeasts ($p<0.05$).

The literature indicates that yeasts and lactic acid bacteria are symbiotic microorganisms (Ramos et al., 2011). Lactic acid bacteria create an acidic environment that is optimal for yeasts, while yeasts produce amino acids and vitamins that are vital for microorganisms. Lactic acid bacteria and yeasts compete for nutrients at the same time. Reduction of dry matter and increase in acidity create more favourable conditions for lactic acid bacteria growth. Excessive environment acidity suppresses both yeasts and lactic acid bacteria; therefore it can promote the growth of undesirable microorganisms (Помозова, 2006). During kvass fermentation, the count of lactic acid bacteria and yeasts was slightly superior, but the growth of aerobic colonies decreased more rapidly than the changes in other two groups of microorganisms.

There are some similarities in the development of growth dynamics in all tested groups of microorganisms, which indicatesthat kvass is a beneficial and suitable environment for a variety of microorganisms. The added yeast and lactic acid bacteria starterhave certain growth advantages because they are dominant due to the high microorganism count in this particular environment. The changes of pH in kvass, due to the increase of lactic acid concentration, promote the growth of yeasts and lactic acid bacteria while preventing the growth of saprophytic bacteria (Lidums, 2011).

The changes in the determined microorganisms during the technological process of kvass production can be explained partially by the changes in temperature. The temperature during fermentation (27 ± 1 °C for 9 h) is optimal for yeast and lactic acid bacteria growth; however, during kvass maturation (6 ± 1 °C for 12 h) it is significantly below the optimum levels.

During the fourth stage of kvass production process active bacteria and yeast count decreased; the next stage showed a repeated bacteria and yeast cell growth. The increase in cell count can be explained by the end of cellular adaptation to the reduced temperature conditions and the added sugar prior to fermentation. At this stage a trend was observed, - a higher growth rate was found in TPC group ($p<0.05$), while the increase was equally slower for lactic acid bacteria and yeasts.

The total kvass storage time was 130 h (the total production time 168 h); there were practically no nutrients available for microorganisms in kvass environment; ethanol and lactic acid were created, and a gradual decrease in the number of microorganisms began with a similar negative rate in all groups.

Correlation of changes in detected microorganism count in kvass

Based on correlation coefficients of microorganisms, the growth of yeasts, TPC and lactic acid bacteria proceeded similarly (Table 2). The changes in microorganism count during kvass maturation had a strong positive correlation.

There were significant differences in the initial count of microorganisms between groups ($p<0.01$), and yeast count was greater than the count of lactic acid bacteria. During the first 12 h of kvass fermentation, yeasts dominated over other groups ($p<0.001$), the lactic acid bacteria and TPC developed equally, with a small but significant superiority of TPC ($p<0.05$). After adding the additional dose of sugar, a significant increase in the number of yeasts was observed.

In the next stages of kvass production process (S_2 to S_4), the number of microorganisms decreased; however, lactic acid bacteria and yeasts

Table 2

The values of correlation coefficients (r) between TPC, lactic acid bacteria and yeasts during kvass maturation (n=30)

Group	Yeasts	Lactic acid bacteria	TPC
Yeasts	1	0.94	0.97
Lactic acid bacteria	0.94	1	0.92
TPC	0.97	0.92	1

continued to dominate over TPC group ($p<0.001$). Microbiologically detectable decrease in the count of lactic acid bacteria and yeasts did not differ significantly ($p>0.05$). During storage (60 to 84 h), a repeated increase in microorganism growth was observed; this period is characterized by the temperature raise of approximately 1.5 °C.

Identification of microorganisms

API identification system was used to isolate yeasts and lactic acid bacteria. Two types of microorganisms were isolated and identified in naturally fermented bread kvass – *Saccharomyces cerevisiae* and *Leuconostoc mesentericus spp. cremoris.*

Conclusions

1. Lactic acid bacteria and yeast count increase during production process of bread kvass.
2. The dominating microflora in kvass was *Saccharomyces cerevisiae* and *Leuconostoc mesentericus*.

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