



Food Science and Applied Biotechnology

e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
<https://doi.org/10.30721/fsab2021.v4.i2>



Research Article

Effect of blueberries addition during beer maturation on yeast metabolism

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Abstract

In recent years, there has been a significant interest in beverages with increased biological value, such as beer with blueberries. In this study, blueberries were added at the beginning of maturation of lager beer with an initial extract of 12, 14 and 16°P. The effect of blueberries addition on yeast metabolism was investigated as concentration of ethanol, higher alcohols, esters, aldehydes, and vicinal diketones in the final beer were measured and compared to control samples without blueberries. The results showed that blueberries affected positively ethanol formation only when wort with initial extract of 12°P was used and had no significant effect when wort with higher extract was used. In regard to secondary metabolites, blueberries addition led to a decrease in higher alcohols concentration and an increase in esters amounts. All the carbonyl compounds (aldehydes and vicinal diketones) were higher in beer with blueberries.

Keywords: beer, blueberries, ethanol, esters, higher alcohols, aldehydes, vicinal diketones

Abbreviations:

VDK – vicinal diketones

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Article history:

Received 14 December 2020

Reviewed 1 July 2021

Accepted 30 August 2021

Available on-line 10 October 2021

<https://doi.org/10.30721/fsab2021.v4.i2.134>

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Introduction

Fruit beers are a special type of beers made with fruit addition in the production process. They are becoming popular especially in the field of craft breweries (Fanari et al. 2019). Fruit addition gives beer different taste and flavour or increases its biological value, when a fruit with high antioxidant capacity as blueberry is used.

Blueberries are fruit with intensive colour (dark-blue), firmness and pleasant sweet taste. According to Skupien (2006) 100 g of fresh fruit contains 83.4 g of water, 0.6 g of protein, 0.6 g of fat, 15.0 g of sugars, and 0.3 g of ash. Moreover, 0.02 mg of vitamin B1, 0.02 mg of vitamin B2, 0.3 mg of vitamin PP, 16 mg of vitamin C, 289 I.E. (International Equivalents) of vitamin A, 16.0 mg of calcium, 13.0 mg of phosphorus and 0.8 mg of iron can be found in blueberries. The blueberries are rich in phenolic compounds and flavonoids such as quercetin, resveratrol, myricetin and kaempferol (Barnes et al. 2009). Unfortunately, phenolic compounds such as anthocyanins are sensitive to temperatures above 60°C, producing losses of about 20–50% in weight of the fresh fruit (Wang et al. 2017). Therefore, in beer production blueberries should be added during main fermentation or maturation.

The results for chemical composition of blueberries showed that blueberries contain sugars and amino acids and therefore, they can affect brewer's yeast metabolism. Brewer's yeast produce big spectrum of secondary metabolites, which form beer flavour profile. Some of them as higher alcohols and esters are desirable volatile constituents of beer but others as VDK and aldehydes contribute to flavour defects of beer (Pires et al. 2014).

The aim of this study is to investigate the effect of blueberries addition at the beginning of maturation of beer with initial extract of 12, 14 and 16°P. The effect of blueberries addition on yeast metabolism was investigated as concentration of ethanol; higher alcohols, esters, aldehydes, and VDK in the final beer were measured and compared to control samples without blueberries.

Materials and Methods

Yeast strain. Beer fermentation was carried out using a bottom-fermenting dry yeast *Saccharomyces pastorianus* (carlsbergensis)

Saflager W-34/70 (Fermentis, France). They were pitched according to the manufacturer's instruction.

Blueberries. Blueberries were obtained at a frozen condition from Bulfruct Ltd., Bulgaria. They were kept in a freezer until use. Blueberries were pasteurized and added to "green" beer in concentration 167 g/L.

Wort. All malt wort with original extract (OE) 12±0.5°P, 14±0.5°P, and 16±0.5°P were produced in 50 L laboratory scale brewery (TM INOX, Bulgaria). Malt was milled coarsely with Corona hand mill and mixed with previously heated to 63°C water in proper ratio. Mashing was conducted by increasing the temperature by 1°C/min and by maintaining the following temperature rests: 20 min at 60°C, 20 min at 65°C, 25 min at 72°C, and 1 min at 78°C. Lautering and boiling were also conducted in the same laboratory brewery. Boiling duration was approximately 1 h and bitter Perle and aromatic Cascade hop granules in ratio 60/40 were added to the wort to obtain 60 mg α-hop bitter acids/L. After the hot trub removal, the wort was cooled to 14°C.

Fermentation. The wort was aerated with 6 mg/L oxygen and placed in cylindrical-conical tank. Afterwards, yeast was added according to manufacturer instructions at concentration of 0.9 g/L. The fermentation temperature was 14°C. The main fermentation continued until the difference between attenuation limit and apparent attenuation became approximately 20%. The "green beer" was divided into 2 kegs: one as a control sample and one for blueberries addition. Beer maturation continued 14 days at 14°C and beer lagering - 5 days at 2°C. All the fermentations were carried out in duplicate.

Analytical methods and procedures. Wort and beer extracts were measured by pycnometer using Methods 8.3 and 9.4 (Analytica-EBC 2005). Attenuation limit and apparent attenuation were determined by Method 9.5 (Analytica-EBC 2005). Alcohol content was measured after simple beer distillation according to Method 9.2.1 (Analytica-EBC 2005). VDK were determined after steam distillation according to Method 9.24.1 (Analytica-EBC 2005). The ester concentration was determined by ester saponification with NaOH after simple distillation of the beer (Marinov 2010). The aldehyde concentration was determined according to the bisulphite method after simple distillation of the beer (Marinov 2010). Higher alcohols in beer

were quantified according to the Komarovskiy-Felenberg method after simple distillation of the beer (Marinov 2010).

Statistical analysis. The results of all analysis were expressed as the mean $\pm$ the standard deviation of three replicates.

Results and Discussion

The effect of blueberries addition on ethanol concentration is shown on Figure 1. The results showed that blueberries addition affected negatively ethanol production at wort original gravity of 14 and 16°P. Beer with blueberries contained more alcohol than control sample, when wort with 12°P was fermented. The results were very interesting, because blueberries contained approximately 15% (w/w) sugars (Skupien 2006) and therefore it was expected that alcohol concentration would be higher in all beers with blueberries. Moreover, the dominant sugars in blueberries are glucose, fructose, and galactose (Fotirić Akšić et al. 2019) which are easily utilized by brewer's yeast.

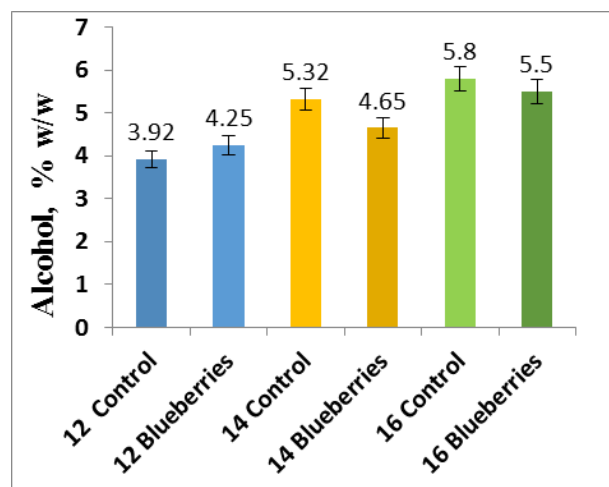


Figure1. Effect of blueberries addition on ethanol production during fermentation of wort with 12°P, 14°P and 16°P original gravity

As ethanol production is a result of extract consumption, the results for extract consumption are presented on Figure 2. The data confirmed the results for ethanol production because more real extract in beer meant less ethanol production. It can be hypothesized that blueberries addition at higher extracts led to osmotic stress to yeast and therefore, they were not able to utilize all sugars in blueberries. The presence of high ethanol concentration in "green" beer additionally hindered yeast main

metabolism, when blueberries were added at maturation of wort with 14°P and 16°P original gravity.

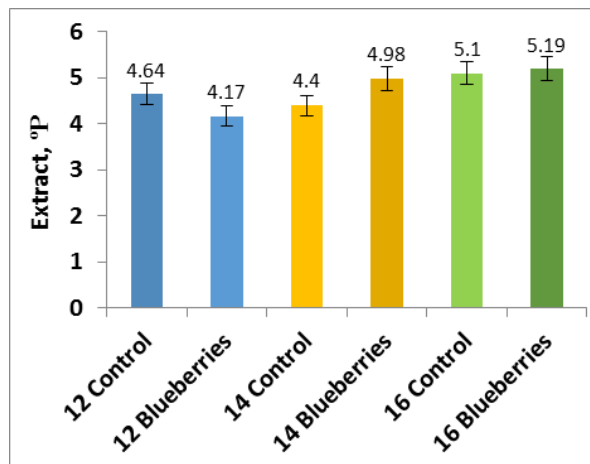


Figure2. Effect of blueberries addition on residual extract during fermentation of wort with 12°P, 14°P and 16°P original gravity

Higher alcohols can be synthesized via two routes: de novo from wort carbohydrates (the anabolic route) or as by-products of amino acid assimilation (the catabolic route). The contribution of the two routes is influenced by a number of factors, but generally, when low levels of amino acids are available, the anabolic route predominates and when high concentrations of amino acids are present the catabolic pathway is favoured (Russell 2018). It can be hypothesized that if blueberries addition affects higher alcohol synthesis, it will be by using anabolic route because blueberries contain more sugars than proteins. The results for higher alcohol concentrations in beers are presented on Figure 3. The data show that higher alcohols concentrations in blueberry beers were approximately 5 mg/L (for wort with original gravity 12°P and 14°P) and 10 mg/L (for wort with original gravity 16°P) lower than the concentrations in control samples (Fig.3). Therefore, it can be concluded that blueberries addition did not affect higher alcohol synthesis significantly. This is due to the fact that over 90% of the higher alcohols have been built at the end of main fermentation, the rest arise during the maturation process (Tenge 2009). Moreover, the decrease in higher alcohols in blueberry beers may be due to their use as a substrate for ester synthesis.

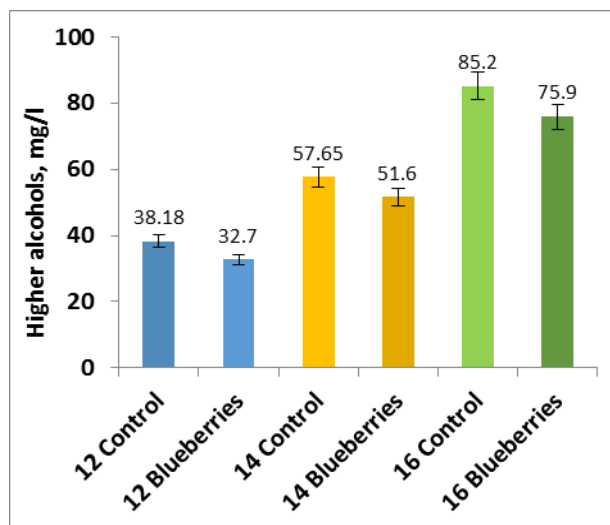


Figure 3. Effect of blueberries addition on higher alcohol synthesis during fermentation of wort with 12°P, 14°P and 16°P original gravity

Esters represent the largest and most important group of the flavour-active substances produced by yeast. They are responsible for the highly desired fruity/floral character of beer. Esters are formed intracellularly by an enzyme-catalysed condensation reaction between two co-substrates, a higher alcohol and an activated acyl-coenzyme A (acyl-CoA) molecule (Russell 2018). The data for ester concentrations in beers are presented on Figure 4.

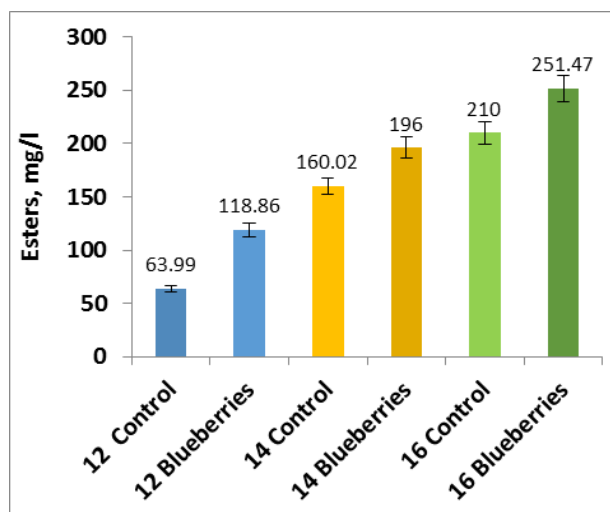


Figure 4. Effect of blueberries addition on esters concentration during fermentation of wort with 12°P, 14°P and 16°P original gravity

Blueberry addition led to an increase in ester concentrations for all beers. This may be due to the

fact that blueberries contains significant amount of glucose and fructose. According to Verstrepn et al. (2003) wort containing higher levels of glucose and fructose produce more esters than wort with high maltose contents. Moreover, unlike higher alcohols, 40% of esters in beer are formed during maturation decreasing (Russell 2018). In addition, blueberries contain various organic acids such as: citric, malic, quinic, acetic, caffeic, p-coumaric, ferulic and shikimic acid (Kalt and McDonald 1996). These acids can react with ethanol and higher alcohols for ester formations.

More than 200 carbonyl compounds were found in beer, but most important of them are: aldehydes and VDK. Acetaldehyde is of special interest as an immediate precursor of ethanol. The concentration of acetaldehyde varies during fermentation and aging/conditioning, reaching a maximum during the main fermentation and then decreasing (Russell 2018). The data in Figure 5 shows that blueberry addition led to an increase in aldehyde concentrations for all the beers.

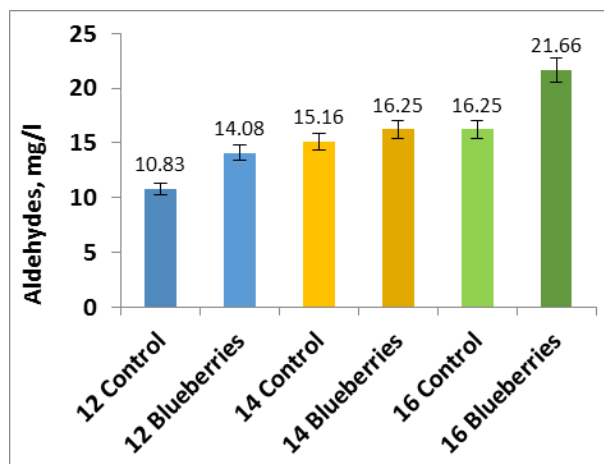


Figure 5. Effect of blueberries addition on aldehydes concentration during fermentation of wort with 12°P, 14°P and 16°P original gravity

The differences between the control samples and beers with blueberries in all cases did not exceed 6 mg/l, therefore it can be concluded that blueberries did not affected significantly aldehydes synthesis and reduction. The increase in aldehyde concentration in beers with blueberries can be ascribed to the fact that yeast does not have enough time for aldehydes reduction due to late period of blueberries addition.

VDK includes diacetyl and 2,3-pentanedione and their precursors. Both VDK are formed from intermediates of the amino acid biosynthesis. Diacetyl relates to valine and 2,3-pentanedione relates to isoleucine. The first intermediates in this metabolism are α -acetolactate and α -acetoxybutyrate. These components are discharged from the cell and undergo an oxidative decarboxylation to form diacetyl and 2,3-pentanedione. Yeast takes in these substances again and reduces them to 2,3-butanediol and 2,3-pentanedione, respectively (Tenge, 2009). Results for VDKs are presented in Figure 6.

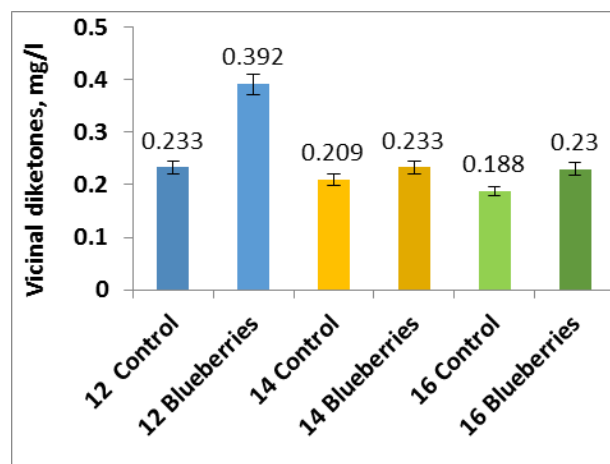


Figure 6. Effect of blueberries addition on VDK concentration during fermentation of wort with 12°P, 14°P and 16°P original gravity

Blueberry addition led to an increase VDKs concentration for all beers. It can be explained with amino acids content of blueberries and free amino nitrogen depletion in “green” beer. According to Zhang et al. (2014) blueberries contained significant amount of valine and no lysine and isoleucine. They contained also amino acids from group A - asparagine, glutamate, lysine, serine and threonine, which are faster utilized than valine, which is from group B. Blueberry addition would cause greater diacetyl formation, because yeast could not start valine utilization until there were amino acids from group A in “green” beer. VDKs reduction would not be efficient because of late period of blueberries addition.

Conclusions

A study for effect of blueberry addition at the beginning of maturation on brewer's yeast metabolism was carried out. The additions of blueberries had positive effect on ester formation of all beers studied. Blueberries addition affected other yeast metabolites synthesis differently depending on yeast by-product type and wort original gravity. These results will be used for optimisation of the production of beer with blueberry addition

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