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THE FERMENTATION KINETICS AND PHYSICO-CHEMICAL PROPERTIES OF SPECIAL BEER WITH ADDITION OF PROKUPAC GRAPE VARIETY

Article Highlights

- The mixture of wort and grape mash is a more nutritious medium for yeast growth than pure wort
- The grape beer is a better source of natural antioxidants than regular lager beer
- The rate of yeast growth in grape beer was higher compared with control beer

Abstract

Over the last decade, the market of special beers with improved healthy function and/or with new refreshing taste has significantly increased. One of the possible solutions enables mixing beer with bioactive components in grapes responsible for well-known health-promoting action of red wine. The effects of the addition of the Prokupac grape on the physicochemical properties and the fermentation kinetics of the grape beer were studied and the results were compared with a control lager beer. The effect of grape addition on the activity of yeast was also studied. Original extract, alcohol content, degree of fermentation, fermentation rate and yeast growth were significantly higher in beers with grapes as a consequence of higher concentration of simple sugars in grapes compared with pure wort. Based on the CIELab chromatic parameters the color of grape beer samples was yellow with certain proportion of redness, while the control beer was purely yellow. The increase in the concentration of grape mash affects the reduction of lightness and yellowness of beers, while the redness of samples was directly proportional with grape quantity. The phenolic content and antioxidant capacity of grape beers was remarkably higher compared to the control beer, which indicates that the grape beer is a better source of natural antioxidants than regular lager beer.

Keywords: beer, grape, phenolic compounds, antioxidants, yeast growth.

Beer is the most popular alcoholic beverage in the world, and probably one of the oldest fermented beverages, dating back more than 8000 years. Since ancient times, many different types of beer and beer-based beverages have been developed at various countries worldwide [1]. Such diversity is caused by wide variety of raw materials and technologies, which are used in their production. According to the Reinheitsgebot (beer purity law which governing commercial brewing in Germany, firstly introduced into Bava-

ria in 1516), beer could be brewed only from water, malted barley, hops and yeast. However, in other countries laws governing beer production are less stringent and brewers have more flexibility, *e.g.*, in selection of carbohydrate sources (adjuncts). Brewing adjuncts are “any carbohydrate source other than malted barley which contributes sugars to the wort”, where cereals (malted or unmalted) and sugar syrups are the most widely used, usually in conjunction with barley malt [2]. In addition, from early times different fruits have also been used in brewing as sources of fermentable extract and as flavoring agents. Furthermore, because the grains do not host naturally occurring yeast, many ancient brewers inoculated the wort by adding fruit, wine or honey [3].

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Such a wide range of raw materials that can be used in the production of beers and beer-based products provides a great opportunity for brewers to conquer new markets and to meet demands of unconventional consumer groups. In recent years, the market of special beers with improved healthy function and/or with new refreshing taste has significantly increased [4,5]. The utilization of dietary compounds and natural products as potential disease prevention agents in the form of functional foods has become an important task in current health researches [6]. A number of studies supports the hypothesis that moderate drinking of any alcoholic beverage, particularly red wine and beer, significantly reduces the risk of cardiovascular diseases [7]. Such effects can be explained by a high content of natural antioxidants, particularly phenolics compounds [8].

The antioxidants have a very important role in brewing due to their ability to delay and prevent oxidation reactions. Antioxidant capacity of beer mainly depends on the content of phenolics and Maillard compounds [9]. Beer polyphenols come from barley (malt) (70-80%) and hops (20-30%), which are basic raw materials for its production. However, besides the influence of raw materials, the total antioxidant content of beer significantly depends on the brewing process used [10,11]. Phenolic compounds, especially flavonoids and stilbenes, exhibit a number of bioactive effects, such as anti-inflammatory, antimicrobial, antiallergic, antithrombotic, anticarcinogenic, antimutagenic, antiaging and vasodilatory activities [12]. Except for a physiological role, phenolics have a significant affect to sensorial properties of beer, such as appearance, taste, mouth-feel, fragrance, astringency and bitterness [13]. In addition, various antioxidants (sulfites, ascorbic acid etc.) can be added during the brewing process to improve flavor stability of products [14]. However, minimizing the use of additives and increasing the content of antioxidants from natural sources to improve flavor stability and increase the shelf-life of products are growing trend in food and beverage industry [15,16].

In our previous work, sensorial acceptability and phenolic content of special type of beer produced by fermenting wort with different proportion of grape must were investigated [17]. Since the beer is a more flexible category than wine, such a product is usually considered a specialty beer rather than specialty wine. The obtained results indicated that special grape beers have unique sensorial profile completely acceptable for consumers and significantly higher content of phenolic compounds. Today, several craft breweries, mainly in Belgium and the United States,

produce grape beers. The most famous grape beer producers are Dogfish Head Brewery (USA), Allagash Brewing Company (USA), Cantillon Brewery (Belgium), Paeleman (Belgium), Blue Moon Brewing Company (USA), etc.

Dynamic of fermentation is one of the most important parameter in the beer production. Addition of grape in the fermenting medium has a great impact on the rate of fermentation, because it contains a higher content of fermentable sugars, mostly glucose and fructose. The main objective of this study was to investigate the influence of the addition of the Prokupac grape on the physicochemical properties and the fermentation kinetics of the grape beer. The effect of grape addition on the activity of yeast was also studied.

EXPERIMENTAL

Prokupac, Serbian autochthonous variety used for making table and top quality rose and red wines, was obtained from experimental school estate "Radmilovac" of Faculty of Agriculture, Belgrade. The all-malt wort and a bottom-fermenting industrial yeast strain *Saccharomyces pastorianus* used in this study were obtained from a local brewery collection.

Chemicals

Gallic acid, Folin-Ciocalteu's phenol reagent, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid and sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). 2,4,6-Trypyridyl-*s*-triazine (TPTZ), ferric chloride hexahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Steinheim, Germany). Ammonium hydroxide was purchased from Fisher Scientific (Loughborough, UK).

Fermentation

The grape was sorted manually, washed in cold water to remove impurities, and the clean grape was destemmed and crushed by hand. The wort and grape mash were mixed in different proportions (70:30 and 80:20) and the pH of obtained mixtures was adjusted to 5.3 with 2 vol.% solution of ammonium hydroxide. These wort:grape mash ratio was selected after pre-experimental sensorial testing of grape beers with different grape proportion (10, 20, 30, 40 and 50% of grape mash). The fermentation media (4 L) were poured into 5 L laboratory stainless steel fermenters and seeded with yeast suspension such that the concentration of cells was 17 million yeast cells per milliliter of wort. Pitching was performed at

12 °C. When the real extract was decreased to 5 mass%, the fermenting mixture was sent to a laboratory wine press. After pressing, liquid was transferred into an aging stainless steel vessel equipped with pressure control system. At the end of secondary fermentation, beer was filtered and bottled. Control beer samples were produced in the same way but without addition of grape mash.

Samples for analysis were taken in sterile condition every six hours during the primary fermentation.

Physicochemical measurements

Alcohol, original extract, real extract, degree of fermentation and calories were determined using AlcoLyzer Beer ME Analyzing System (Anton Paar GmbH, Graz, Austria). The color of beers was measured using a portable chromameter CR-410 (Minolta, Ramsey, NJ). The results were expressed in Commission Internationale d'Eclairage L^* , a^* and b^* color-space co-ordinates. These parameters defined L^* (lightness: 0 = black, 100 = white), a^* (from green to red), b^* (from blue to yellow), C^* (chroma or saturation) and h (hue angle). CIElab parameters were calculated for the CIE illuminant D_{65} . All physicochemical measurements were done in triplicate.

Yeast cells counting

During the fermentation, the number of yeast cells in beer samples was determined by counting the number of yeast colonies that grow on a sterile malt agar plate that was inoculated with beer sample. In order to enumerate the number of yeast cells, serial decimal dilutions in peptone saline solutions were prepared. Samples from the appropriate dilutions were inoculated on previously sterilized malt agar (1.5% peptone, 3% malt extract, 1.5% agar, *w/v*). The plated culture media were placed in a thermostat for incubation at 30 °C for 24 h. The number of yeast cells was monitored every six hours during the primary fermentation.

Determination of total phenolics

The amounts of total phenolics (TPC) in beer samples were determined according to the Folin-Ciocalteu method described by Singleton and Rossi [18]. Briefly, 0.5 mL of diluted beers were mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Two milliliters of sodium carbonate solution (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was measured. The calibration curve was prepared with gallic acid solution, and the results were expressed as mg

of gallic acid equivalents per L of sample (mg GAE/L). Triplicate measurements were performed.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of beers was estimated following the slightly modified procedure described by Kaneda *et al.* [19]. Every diluted beer sample (0.2 mL) was added to the DPPH working solution (2.8 mL; mixture of 1.86×10^{-4} mol/L DPPH in ethanol and 0.1 M acetate buffer (pH 4.3) in volume ratio 2:1). The absorbance at 525 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of inhibition of DPPH radical. The results were expressed as mmol of Trolox equivalents per L of sample (mmol TE/L). Triplicate measurements were performed.

FRAP assay

The FRAP assay was performed according to the procedure previously described by Benzie and Strain [20], with some modification. The FRAP reagent solution was made by mixing acetate buffering agent (pH 3.6), TPTZ (10 mM TPTZ solution in 40 mM HCl) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ at volume ratio 10:1:1, respectively. All samples, standards and reagents were pre-incubated at 37 °C. An aliquot of each diluted beer sample (0.1 mL) was mixed with distilled water (0.3 mL) and FRAP reagent (3 mL). After the reaction at 37 °C for 40 min, the absorbance at 593 nm was measured. The calibration curve was prepared with Trolox solution and the results were expressed as mmol of Trolox equivalents per L of sample (mmol TE/L). Measurements were done in triplicate.

Statistical analysis

All experiments were done in triplicate and obtained results are expressed as mean \pm standard deviation (SD). The experimental data were subjected to a One-way analysis of variance (ANOVA) and Tuckey's test was used to detect difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program Statistica 12 [21].

RESULTS AND DISCUSSION

Most important physicochemical characteristics of obtained beers are shown in Table 1. These parameters have a great influence on sensory quality and microbiological stability of beers: higher content of alcohol and lower pH value increase microbial stability, while the fullness of taste is mainly depends on the content of extract. The content of alcohol and pH

Table 1. Physicochemical properties of beer samples; B - control beer; P20, P30 - beer with 20 and 30% of Prokupac grape, respectively. Different letters in same row denote a significant difference according Tukey's test, $p < 0.01$

Parameter	B	P20	P30
Original extract, % Plato	11.56±0.22 ^a	12.85±0.26 ^b	13.55±0.23 ^c
Real extract, mass%	4.09±0.10 ^a	3.67±0.13 ^b	3.46±0.09 ^b
Apparent extract, mass%	2.31±0.09 ^a	1.40±0.11 ^b	1.01±0.10 ^c
Alcohol, vol.%	4.91±0.19 ^a	6.33±0.21 ^b	6.87±0.16 ^c
RDF, mass%	66.02±0.95 ^a	73.74±1.15 ^b	76.36±1.08 ^c
ADF, mass%	80.03±1.03 ^a	89.47±1.24 ^b	92.70±1.11 ^c
Calories, kJ/100 ml	174.05±8.06 ^a	200.17±12.38 ^b	209.19±13.52 ^b
pH	4.60±0.11 ^a	4.26±0.16 ^b	4.03±0.09 ^c

value are connected with sharpness, freshness and sourness of beer. In addition, a good alcohol-real extract balance is very important for beer taste.

The original extract and alcohol content were higher in beers produced with the addition of Prokupac grapes compared with control beer, which is a consequence of higher amount of sugars in grapes in comparison with pure wort (Table 1). The pH value was decreasing with increasing of grape proportion in the fermenting medium, and in sample P30 pH was closer to the pH of wine than the pH of beer. Degree of fermentation was significantly higher in beers with grapes with the highest value founded in sample P30, which could be explained by the higher concentration of simple sugars (mainly glucose and fructose) originating from grapes in the initial medium for fermentation. In all beer samples high content of extract was left in order to achieve greater fullness of beers.

Figure 1 illustrates the dynamics of gravity decreasing during the primary fermentation of beers. Sugar uptake and utilization is of the greatest importance in brewery fermentation, and its kinetics determines the time needed for primary fermentation. In commercial worts, maltose is by far the most abundant fermentable sugar, about 40-60% of the total car-

bohydrates. Carbohydrate concentrations and profiles of the wort depend on the composition of grist, mashing procedures and adjuncts. All-malt wort contains approximately 9% hexose (glucose and fructose), 6% sucrose, 41% maltose, 14% maltotriose, 6% maltotetraose and 22% dextrans [22]. Compared with all-malt wort, sugar profiles of fermenting medium containing mixture of wort and grape mash is significantly different, with higher concentration of simple sugars, mainly glucose and fructose, originating from grapes [22,23]. Consequently, the slope of fermentation curves of grape beers was significantly higher compared with control beer, which means higher rate of fermentation (percentage of utilized extract per hour, °P/h, Figure 1). After 80 h, the fermentation rate of the grape beers was similar to the fermentation rate of control beer. It is well established that glucose is preferentially utilized during brewing fermentation, followed by fructose and then maltose [24]. This is the reason why the gravity during grape beer fermentation was more rapidly decreased.

The color is one of the most easily recognizable characteristics of beers and has the most important impact on their appearance. Also, the visual characteristics can provide useful information about quality

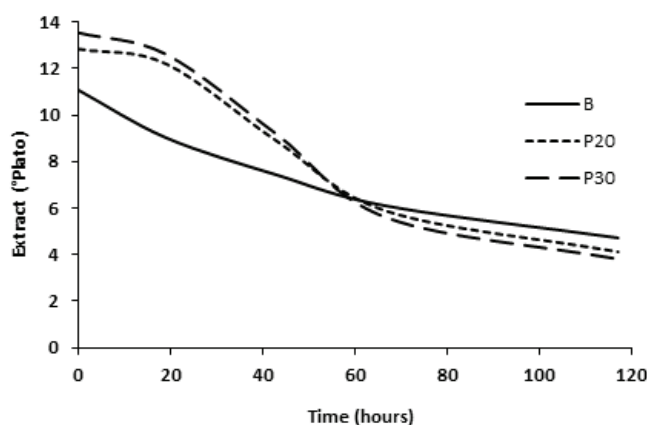


Figure 1. Fermentation profiles (gravity vs. time).

and style of beer. CIELab chromatic parameters of beer samples are presented in Table 2.

Based on the parameters a^* , b^* and the hue angle, it can be concluded that color of grape beer samples was yellow with certain proportion of redness, while the control beer was purely yellow. The beer P30 had the lowest value for lightness (L^*) and the highest values for a^* and C^* , which indicates that this sample was the darkest with the highest proportion of red color. The increase in the concentration of grape mash in the initial fermenting medium affects the reduction of lightness and yellowness of beers, while the redness of samples was directly proportional with grape quantity.

Table 2. CIELab chromatic parameters (values represent means of triplicate determinations \pm standard deviation. Different letters in same column denote a significant difference according Tukey's test, $p < 0.01$) of beer samples; B - control beer; P20, P30 - beer with 20 and 30% of Prokupac grape, respectively

Sample	$L^*(D65)$	$a^*(D65)$	$b^*(D65)$	$C^*(D65)$	$h(D65)$	Dominant wavelength (D65), nm
B	52.89 \pm 0.01 ^a	-0.06 \pm 0.01 ^a	23.52 \pm 0.01 ^a	23.52 \pm 0.01 ^a	90.15 \pm 0.01 ^a	576.15 \pm 0.01 ^a
P20	46.52 \pm 0.01 ^b	8.12 \pm 0.02 ^b	22.83 \pm 0.01 ^b	24.24 \pm 0.02 ^b	70.43 \pm 0.04 ^b	582.54 \pm 0.02 ^b
P30	42.19 \pm 0.01 ^c	13.74 \pm 0.02 ^c	20.37 \pm 0.01 ^c	24.57 \pm 0.01 ^c	56.00 \pm 0.03 ^c	587.87 \pm 0.01 ^c

Yeast growth monitoring is a direct method of estimating fermentation progress and overall performance. However, yeast concentration monitoring is not usual during commercial fermentation, but it is common practice in laboratory or pilot scale fermentations. The main reason for that is heterogeneity of vessel contents in a production scale fermenters [25].

The curves of yeast growth are given in Figure 2. All samples were seeded with same concentration of yeast cells at the same temperature (17 million yeast cells per milliliter of fermenting medium at 12 °C). The curves showed that duration of lag phase was significantly longer in control beer fermentation, while in the case of the grape beers yeast growth was

began almost immediately after pitching. The growth rate (speed of yeast reproduction, % of cell number increase/h) was the highest in sample P30, but the maximum yeast concentration was the same in both grape beers. Compared with grape beers, growth rate and maximum concentration of yeast in control beer were significantly lower. Also, yeast was firstly entered stationary phase in control beer. These results indicate that mixture of wort and grape mash is a more nutritious medium for yeast growth than pure wort. Consequently, yeast growth was more rapid in the case of grape beer fermentation. However, at the beginning of fermentation, yeast in control beer used sugar mainly for alcohol production but not for growth

(lag phase). In contrast to this, yeast in grape beer was immediately started to growth and used nutrients firstly for growth and after that for alcohol production. For this reason slope of control beer fermentation curve was higher at the beginning compared with grape beers (Figure 1).

The total phenolic content and antioxidant activity of samples are presented in Table 3. Phenolic compounds are generally considered as one of the main antioxidant sources in beer, and beer antioxidant capacity is highly correlated with the total phenolic content. The amounts of phenolic compounds vary markedly in different types of beers, depending on the raw materials and brewing procedure. The

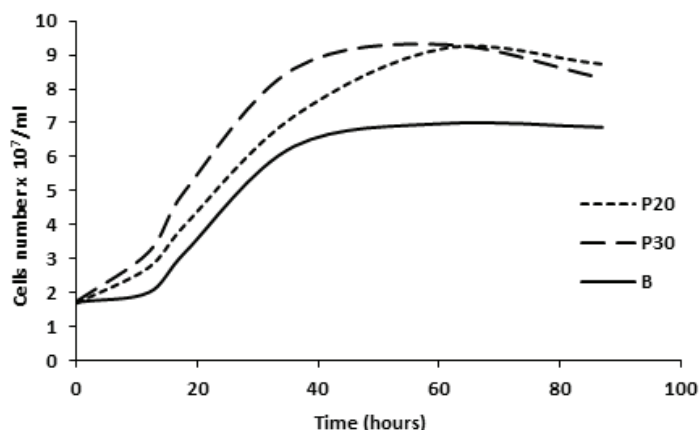


Figure 2. Number of yeast cells during the primary fermentation.

Table 3. Total phenolic content and antioxidant capacity of beer samples; values represent means of triplicate determinations \pm standard deviation. Different letters in same column denote a significant difference according Tukey's test, $p < 0.05$; B - control beer; P20, P30 - beer with 20 and 30% of Prokupac grape, respectively

Sample	TPC, mg GAE/L	DPPH, mM TE	FRAP, mM TE
B	467.78 \pm 6.19 ^a	0.73 \pm 0.03 ^a	1.28 \pm 0.07 ^a
P20	550.00 \pm 15.00 ^b	1.02 \pm 0.15 ^b	2.64 \pm 0.02 ^b
P30	569.63 \pm 4.44 ^c	1.05 \pm 0.01 ^c	2.65 \pm 0.03 ^c

grape beers had a significantly higher content of total phenolic compounds compared with control beer. These results were expected considering that the grapes are one of the major sources of phenolic compounds among different fruits [26].

A number of different assays are developed for the measurement of antioxidant capacity, so there is no standardized method. Because of that, two more frequently used methods (DPPH and FRAP) were selected to analyze antioxidant capacity of beer samples. Antioxidant capacity of samples was strongly and statistically significantly correlated with total phenolic content, so the antioxidant capacity of grape beers was remarkably higher compared with control beer. Consequently, it can be concluded that the grape beer is a better source of natural antioxidants than regular lager beer.

CONCLUSION

the obtained results suggest that the original extract, alcohol content, degree of fermentation, fermentation rate and yeast growth were significantly higher in beers with grapes compared with the control beer as a consequence of higher concentration of simple sugars in grapes in comparison with pure wort. The increase in the concentration of grape mash in the initial fermenting medium affects the reduction of lightness and yellowness of beers, while the redness of samples was directly proportional with grape quantity. The rate of yeast growth and maximum concentration of yeast in control beer were significantly lower, which indicate that mixture of wort and grape mash is a more nutritious medium for yeast growth than pure wort. Also, the grape beer is a better source of natural antioxidants than regular lager beer. In addition, our previous research showed that beers enriched with grape have specific pleasant freshness, color and aroma and could be very interesting for consumers [17].

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NAUČNI RAD

KINETIKA FERMENTACIJE I FIZIČKOHEMIJSKE KARAKTERISTIKE SPECIJALNIH PIVA SA DODATKOM GROŽĐA SORTE PROKUPAC

Poslednjih godina, tržište specijalnih vrsta piva koja se odlikuju poboljšanom zdravstvenom funkcijom i/ili novim osvežavajućim ukusom se značajno povećalo. Jedna od mogućnosti je i obogaćivanje piva sa bioaktivnim jedinjenjima grožđa koja su odgovorna za dobro poznato blagotvorno dejstvo crvenih vina na zdravlje. U radu je ispitivan uticaj dodatka grožđa sorte Prokupac na fizičko-hemijske karakteristike i kinetiku fermentacije specijalnih piva sa dodatkom grožđa, pri čemu su dobijeni rezultati poređeni sa kontrolnim lager pivom. Uticaj dodatka grožđa na aktivnost kvasca je takođe ispitivan. Početni ekstrakt, sadržaj alkohola, stepen prevrelosti, brzina fermentacije i razmnožavanje kvasca je bilo značajno veće kod piva sa dodatkom grožđa, zbog većeg sadržaja prostih šećera u grožđu u poređenju sa čistom sladovinom. Prema CIELab parametrima boje, boja piva sa dodatkom grožđa je bila žuta sa određenim udelom crvene, dok je kontrolno pivo bilo čiste žute boje. Povećanjem udela grožđa boja piva postaje tamnija i sa manjim udelom žute boje, dok je udeo crvene boje direktno proporcionalan sadržaju grožđa. Sadržaj fenolnih jedinjenja i antioksidativni kapacitet piva sa dodatkom grožđa je bio značajno veći u odnosu na kontrolno pivo, što znači da je pivo sa grožđem bolji izvor prirodnih antioksidanasa nego komercijalno lager pivo.

Ključne reči: pivo, grožđe, fenolna jedinjenja, antioksidansi, kvasac.