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EFFECT OF FRUIT JUICES ON TOTAL PHENOLIC CONTENT AND FLAVONOID CONTENT OF BEER

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ABSTRACT

In this study, a pale ale beer had been enriched with the juices of 10 fruits in different concentrations. The first enrichment was carried out before primary fermentation. Then, before bottling, another dose of juice (in 3 different concentrations) had also been added to the previously enriched batches and the control sample too, therefore 7 different samples were produced by each fruit, besides the control sample.
Total phenolic content (TPC) and flavonoid content of the samples were determined by spectrophotometric methods, and statistical analysis including one-way ANOVA was also performed.
Based on our results, strawberry, cherry and sour cherry juices are the richest in the analysed components – they showed outstanding results compared to the other juices. However, these results did not cause the increase of these parameters in every case. Total phenolic content of the flavoured beers was similar or lower than the control sample's result. On the other hand, flavonoid content of the beers enriched with cherry juice increased significantly, and the addition of strawberry and sour cherry juice was also efficient. Every flavored sample showed higher flavonoid content than the control samples, and the double enrichment with strawberry, cherry and sour cherry juice had the most significant impact. In case of phenolic compounds, the juice addition was more efficient before bottling than before primary fermentation.
Besides the potential health benefits, the addition of different fruit juices may also increase the popularity of these beverages, which might result in a wider range of products and consumers.

Keywords: Beer, flavoring, fruit juices, polyphenols, flavonoids

INTRODUCTION

Oxidative stress is one of the most important factors in the development of different human pathological conditions such as cancer, diabetes, pulmonary dysfunction, Alzheimer's and Parkinson's disease, etc. **(Presti *et al.*, 2017)**. Antioxidants taken from external sources may decrease the effect of oxidative stress. A healthy diet can provide several hundred milligrams of phenolic compounds daily, and specific alcoholic beverages contain other antioxidants too **(Nardini-Garaguso, 2020)**. Out of the alcoholic beverages, mostly red wines are associated with high levels of antioxidant compounds, but beer might also contain those, such as phenolic compounds, flavonoids and the products of Maillard reaction **(Polak *et al.*, 2013)**, furthermore, it might be rich in minerals, vitamins and minerals too **(Gerhäuser, 2005)**.
Polyphenols are natural compounds of fruits and vegetables, therefore they can also be found in products manufactured from those **(Zapata *et al.*, 2019)**. They are present mainly in fruits and vegetables, but beers may also contain polyphenols, derived from malt and hops. These compounds may have an effect on the stability of the beverage besides modifying organoleptic characteristics **(Gonzáles-Sanjosé *et al.*, 2017)**. These compounds protect plant organisms against stress factors such as mechanical damage, infections and radiation **(Oliveira *et al.*, 2016)**. They also have beneficial health effects on humans, e.g. anti-inflammatory, anti-oxidation, and anti-cancer effects, and they also protect the cardiovascular system **(Sotibrán *et al.*, 2011)**. Flavonoids are secondary plant metabolites, which have protective function against different conditions, namely dehydration, radiation, infections, mechanical injuries etc. **(Oliveira *et al.*, 2016)**. Based on the previously given information, enrichment of beer with different fruits may increase the concentration of its bioactive compounds besides the development of new flavour and aroma **(Ducruet *et al.*, 2017)**. This might be important in countries where beer is consumed in high volume, such as The Czech Republic, where consumption has exceeded 100 l/capita in 2021 **(Fleck, 2022)**. In case of Hungary, this value was 68 l/capita **(Medve, 2022)**. Changing consumption habits caused the transformation of the brewing industry worldwide. In Hungary, the preparation and consumption of craft beers started to increase after 2010; which was promoted by the amendment of regulations in 2012 declaring tax-free brewing under the volume of 1000 L/year, and decreased taxes of small breweries **(Magyar Köztársaság, 2011)**. The increasing popularity of craft beers represents great potentials. These products might be tempting to consumers who prefer novel, complex organoleptic characteristics **(Jaeger *et al.*, 2020)**, which is more easily provided by breweries with lower production volume **(Vecseri, 2004)**. These institutions might experiment with the proportions of the raw materials, the optionally added colouring and flavouring materials and the production technology much wider **(Donadini – Porretta, 2017)**, furthermore, the application of adjuncts might vary too, however these breweries avoid the addition of those in most of the cases **(Vecseri, 2004)**. This kind of variable use of raw materials is likely to influence the quality and nutritional parameters of beer, such as the quantity of antioxidant compounds to a great extent.

MATERIAL AND METHODS

Production and analysis of the samples were carried out in the Institute of Food Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. Malts, hops, yeasts and other material and equipment required for the brewing were purchased in a brewing specialty shop in Budapest. Fruits were collected in local markets right before the brewing process.

Brewing

Recipe of the control sample is the following: Malts: pale ale malt (4.0 kg), wheat malt (0.5 kg), caramel malt (0.2 kg); Adjuncts: -; Mashing water: 15 L; Mashing: protease step – 50 °C 20 min, amylase step – 67 °C 75 min; Sparging water: 11 L; Hop boiling: 60 min; Hop addition: Citra hop pellets (alpha-acid: 13%): 40 g, 0. min, Citra hop pellets (alpha-acid: 13%): 10 g, 50. min; Yeast type: Fermentis S-05 (dried ale yeast); Fermentation: 20 ºC, 14 days.
Fruits applied for the flavoring were collected on the day of the brewing process from local markets, namely strawberry, sour cherry, plum, cherry, watermelon, melon, peach, apricot, nectarine and red grape. Their juice was extracted by single pressing. The addition of the juices happened in three different ways, in a fresh form, without any further treatment.
Type 1.: Juices were added before the fermentation process, with the volume of 7.5% of the total fermentation volume (1725 ml in 23 L). Henceforward: fruity ales.

Type 2.: Fermentation was carried out without containing any fruit juice. Juices were added to the samples before bottling with different volumes: proportion of the juice was 2.5% (12.5 ml), 5% (25 ml) and 7.5% (37.5 ml) of the bottling volume (500 ml). Henceforward: simple fruity ales.
Type 3.: Combining the two above-mentioned methods, I have added extra quantities of fruit juices to the fruity ale samples with the previously described proportions (2.5; 5 and 7.5%), therefore the samples prepared by fermentation with the juices were enriched again before bottling. Henceforward: double fruity ales.
The analytical measurements were carried out within all types of beers made by one fruit variety at the same time, which was 4 weeks after bottling.

Analytical methods

Determination of total phenolic content (TPC)

The principle of the method is that phosphotungstic and phosphomolybdic acid found in Folin-Ciocalteu reagent oxidize phenolic compounds, resulting in a blue-coloured solution. Colour intensity is proportionate to the concentration of phenolic compounds, therefore the absorbance of the mixtures is measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at a wavelength of 760 nm, against the mixture of methanol and distilled water (80:20).
To prepare the calibration solutions, a gallic acid stock solution is used. Applied chemicals: 3,4,5-trihydroxybenzoic acid (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany), sodium carbonate (Sigma-Aldrich Chemie GmbH, Germany), methanol (Scharlab S.L., Spain), Folin-Ciocalteu reagent (VWR International S.A.S., France). Results were expressed in mg GAE/100 g (or ml) (mg gallic acid equivalent/100 g) **(Singleton *et al.*, 1999)**.

Determination of flavonoid content

The determination of flavonoid content was also carried out by a spectrophotometric method. Absorbance of the rose-coloured complex created during the analysis was measured at a wavelength of 510 nm by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) against a blank solution. To prepare the calibration solutions, a catechin stock solution was used. Applied chemicals: catechin (Cayman Chemical Company, USA), aluminium chloride (Scharlab S.L., Spain), sodium nitrite (Scharlau Chemie S.A., Spain), sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany), methanol (Scharlab S.L., Spain). Results were expressed in mg CE/100 g (or ml) (mg catechin equivalent/100 g) **(Kim *et al.*, 2003)**.

Statistical analysis

The analytical measurements were carried out in triplicate. For the statistical analysis, SPSS (version 13, SPSS Inc. Chicago, Illinois, USA) was applied, thus minimum and maximum values, average calues and standard deviations were determined. To compare the samples within one group, One-Way ANOVA was applied, for which a homogeneity test was carried out first at the level of 5% of significance. Since all of the variables were homogeneous, Dunett's T3 test was carried out.

RESULTS AND DISCUSSION

Juices

Figure 1. illustrates total phenolic content and flavonoid content of the fruit juices.

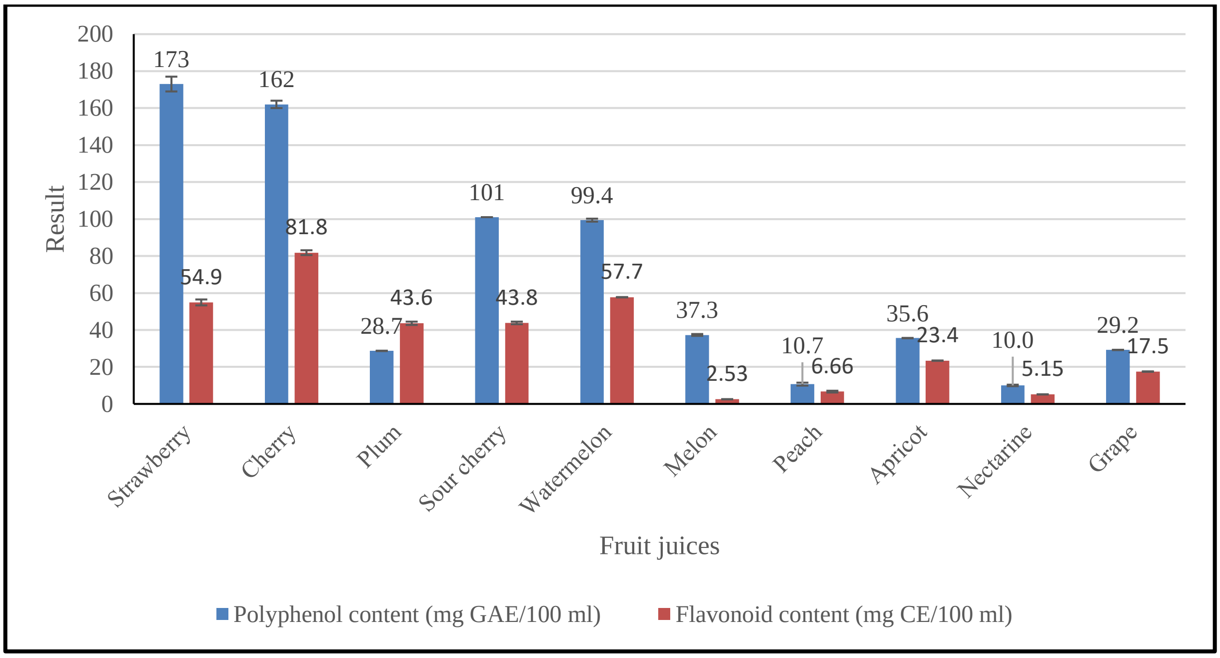

Figure 1 Total phenolic content and flavonoid content of the fruit juices

Total phenolic content of the samples ranged between 10.0 and 173 mg GAE 100 ml^{-1}. Polyphenol content of strawberry and cherry juices were significantly higher than the other juices', strawberry juice showed more than 17 times higher phenolic content than nectarine juice with the lowest concentration. These two were followed by sour cherry and watermelon juice, which showed similar phenolic content. Strawberry and cherry juices showed a statistically verified difference from every other sample, but no statistically verified difference could be observed between those two (p = 0.256), and there was no significant difference between sour cherry and watermelon juice either (p = 0.269).
Flavonoid content of the samples ranged between 2.53 and 81.8 mg CE 100 ml^{-1}. Cherry juice showed much higher flavonoid content than every other sample, which could be statistically verified in every case. Strawberry and watermelon (p = 0.494); and plum and sour cherry (p = 1.00) juices showed similar flavonoid contents. The lowest flavonoid content was measured in melon juice. High antioxidant activity of cherry and sour cherry is already proven by previous studies. Cherry is rich in anthocyanin and other bioactive compounds, thus it protects the cardiovascular system **(Moosavian *et al.*, 2022)**. **Kerekes (2018)** measured a polyphenol content of 670-764 mg GAE 100 ml^{-1}, and a flavonoid content of 145-383 mg CE 100 ml^{-1} in three Hungarian sour cherry species. However, a great amount of these compounds can be found in the peel of fruits, therefore we have measured much lower concentrations in the fruit juices, but we could also verify that cherry and sour cherry are remarkably rich in these components.

Total polyphenol content of the beers are presented in Table 1. Despite the high polyphenol content of strawberry, cherry, sour cherry and watermelon juices, every fruity ale showed lower phenolic content than the control sample. Polyphenol content of fruity ales was lower than the one measured in the control sample in every case, which was statistically verified except for apricot (p = 1.000) and nectarine (p =0.365) ales. Further addition of fruit juices did not increase the concentration either in case of watermelon, melon, peach and double nectarine ales, which could happen due to the more intensive fermentation caused by the increased sugar content, which might decrease polyphenol content of beers **(Yang *et al.*, 2022)**, but polyphenol content of the above-mentioned juices was not high either. The highest phenolic content was measured in 2.5% simple peach ale, followed by 2.5% simple apricot, 7.5% simple nectarine and 7.5% double cherry ale, while the lowest was found in 2.5% double plum ale. A continuous increase can be observed by the increasing juice content in case of every strawberry, cherry and sour cherry ale, and simple nectarine ales. Comparing simple and double fruity ales, simple enrichment caused higher polyphenol content in case of strawberry, plum, sour cherry, watermelon, melon, peach and grape ales, and 5 and 7.5% nectarine ales, which shows that the enrichment following primary fermentation increases polyphenol content more significantly. In most of the cases, the increase in the phenolic content in case of simple fruity ales is proportionate to the quantity of the juice added. Simple and double cherry and apricot ales showed similar polyphenol contents, which was also statistically verified (p = 1.00; p = 0.987).

Table 1 Total phenolic content of the beers

Fruit	Type	Juice content (%)	TPC (mg GAE/100 ml)	Juice content (%)	TPC (mg GAE/100 ml)	Juice content (%)	TPC (mg GAE/100 ml)
Control sample		45.4±0.4					
Strawbery	***ale***	34.9±2.0					
	simple	2.5	42.6±1.2	5	43.1±2.3	7.5	46.1±1.9
	double	7.5+2.5	36.9±1.8	7.5+5	38.2±0.9	7.5+7.5	40.9±0.5
Cherry	***ale***	38.6±0,5					
	simple	2.5	41.6±0.2	5	44.1±1.5	7.5	45.8±2.2
	double	7.5+2.5	42.0±1.4	7.5+5	42.6±0.2	7.5+7.5	47.5±0.6
Plum	***ale***	26.8±0,7					
	simple	2.5	41.5±4.1	5	42.5±0.4	7.5	41.4±1.0
	double	7.5+2.5	25.3±1.3	7.5+5	26.2±0.4	7.5+7.5	26.1±0.3
Sour cherry	***ale***	28.1±0,6					
	simple	2.5	40.4±0.7	5	41.3±2.0	7.5	43.0±2.6
	double	7.5+2.5	27.9±0.8	7.5+5	28.5±0.2	7.5+7.5	30.2±0.6
Watermelon	***ale***	28.1±1,2					
	simple	2.5	40.6±1.1	5	44.8±1.2	7.5	42.9±1.3
	double	7.5+2.5	25.5±0.5	7.5+5	26.5±0.8	7.5+7.5	23.7±1.6
Melon	***ale***	37.4±1,5					
	simple	2.5	44.8±1.0	5	42.9±0.3	7.5	45.4±0.3
	double	7.5+2.5	39.7±0.7	7.5+5	39.8±1.0	7.5+7.5	39.7±1.0
Peach	***ale***	38.1±2,3					
	simple	2.5	49.8±0.3	5	45.9±1.0	7.5	40.6±1.6
	double	7.5+2.5	37.2±1.7	7.5+5	37.1±2.0	7.5+7.5	37.5±0.3
Apricot	***ale***	44.7±1,4					
	simple	2.5	48.9±1.6	5	45.3±1.2	7.5	45.3±1.5
	double	7.5+2.5	46.0±1.7	7.5+5	44.2±1.1	7.5+7.5	46.4±0.8
Nectarine	***ale***	42.5±1,9					
	simple	2.5	43.4±0.2	5	45.6±1.0	7.5	48.2±1.0
	double	7.5+2.5	47.1±2.0	7.5+5	39.7±0.4	7.5+7.5	42.4±2.0
Red grape	***ale***	41.0±1,7					
	simple	2.5	45.4±1.5	5	44.0±2.3	7.5	44.8±1.2
	double	7.5+2.5	39.4±3.1	7.5+5	35.9±3.3	7.5+7.5	38.5±3.1

Flavonoid content of the samples are shown by Table 2. As opposed to the total phenolic content, higher concentrations could be measured in every fruity ale than in the control sample, which was statistically verified except for nectarine ale (p = 0.213). Out of the fruity ales, cherry and sour cherry ales showed the highest flavonoid contents, which is explicable by the high flavonoid content of the juices added. Cherry ale showed 2.5 times higher, while sour cherry ale showed twice as high flavonoid content as the control sample.

Table 2 Flavonoid content of the beers

Fruit	Type	Juice content (%)	TPC (mg GAE/100 ml)	Juice content (%)	TPC (mg GAE/100 ml)	Juice content (%)	TPC (mg GAE/100 ml)
Control sample		6.78±0.27					
Strawbery	***ale***	10.5±0.8					
	simple	2.5	12.5±0.4	5	12.3±0.6	7.5	13.0±0.3
	double	7.5+2.5	10.1±0.2	7.5+5	10.3±0.4	7.5+7.5	12.3±0.9
Cherry	***ale***	17.8±0.7					
	simple	2.5	13.3±0.3	5	15.2±1.4	7.5	16.2±0.4
	double	7.5+2.5	19.8±0.6	7.5+5	20.5±0.2	7.5+7.5	24.4±0.7
Plum	***ale***	9.43±0.73					
	simple	2.5	7.79±0.49	5	9.42±0.82	7.5	10.6±0.1
	double	7.5+2.5	9.63±0.52	7.5+5	8.06±0.06	7.5+7.5	8.61±0.83
Sour cherry	***ale***	13.3±0.5					
	simple	2.5	11.6±0.3	5	12.1±0.5	7.5	13.1±0.4
	double	7.5+2.5	13.8±0.8	7.5+5	13.7±0.4	7.5+7.5	14.6±1.2
Watermelon	***ale***	11.0±0.3					
	simple	2.5	7.22±0.31	5	5.69±0.23	7.5	7.63±0.08
	double	7.5+2.5	13.3±0.9	7.5+5	10.5±0.5	7.5+7.5	11.0±0.2
Melon	***ale***	8.90±0.13					
	simple	2.5	7.94±0.10	5	7.92±0.72	7.5	8.21±0.15
	double	7.5+2.5	8.57±0.34	7.5+5	8.65±0.23	7.5+7.5	7.42±0.29
Peach	***ale***	8.60±0.65					
	simple	2.5	11.0±0.3	5	8.10±0.24	7.5	8.04±0.25
	double	7.5+2.5	8.44±0.10	7.5+5	8.86±0.35	7.5+7.5	9.08±0.08
Apricot	***ale***	9.25±0.13					
	simple	2.5	7.99±0.10	5	6.72±0.10	7.5	7.21±0.18
	double	7.5+2.5	8.96±1.00	7.5+5	7.51±0.27	7.5+7.5	8.06±0.52
Nectarine	***ale***	7.92±0.40					
	simple	2.5	8.08±0.07	5	9.86±0.23	7.5	9.86±0.40
	double	7.5+2.5	6.36±0.17	7.5+5	6.86±0.28	7.5+7.5	6.96±0.72
Red grape	***ale***	8.74±0.23					
	simple	2.5	6.94±0.23	5	6.86±0.21	7.5	7.24±0.27
	double	7.5+2.5	8.14±0.98	7.5+5	7.10±0.40	7.5+7.5	8.14±0.81

Every beverage containing strawberry juice showed higher flavonoid content than the control sample, however this was not statistically verified in case of the strawberry ale ($p = 0.069$). Juice addition before bottling caused noticeable, continuous increase in the flavonoid content only in case of simple cherry ales. Simple fruity ales showed higher flavonoid content than double fruity ales in case of strawberry and nectarine ales, however double cherry and watermelon ales had significantly higher flavonoid contents than their single-enriched pairs.
Although the enrichment after fermentation increases flavonoid content more efficiently, the flavonoid content of every final product could be considered higher than the control sample's.
Fruit species, and the time and proportion of juice addition influenced the concentration of phenolic compounds and flavonoids in the final products. The application of cherry juice increased flavonoid content the most. Out of the double fruity ales, cherry was the most efficient in case of 7.5% juice content, but the addition of cherry juice also resulted in the second highest flavonoid content in case of the addition in 2.5 and 5%. Flavonoid contents reached by this method were higher than the control sample's in every case. On the other hand, only 7.5% double cherry ale showed higher phenolic content than the control sample. Besides cherry juice, flavonoid content was increased mostly by the addition of sour cherry and strawberry juice, while we could observe a significantly higher polyphenol content in case of 2.5% simple apricot juice compared to the control sample. In case of the other methods of addition, apricot juice was the most efficient, however these results were not, or was just minimally higher than the control sample's polyphenol content. The continuous increasing in the concentrations proportionate to the juice content can be explained by high polyphenol and flavonoid content of the juices. On the other hand, the time of addition also influences the concentration of these compounds in the final product, since their quantity could decrease due to the more intensive fermentation process which is caused by the increased sugar content after the addition of the juices.

CONCLUSION

In this study, pale ale beers were produced and flavored by 10 fruit juices by different methods and different concentrations. A total number of 71 samples were prepared, which contained the control sample, 10 fruity ales, 30 simple enriched and 30 double enriched beers with the juice content of 2.5; 5.0 and 7.5%. Total phenolic content and flavonoid content of the juices and the beers were determined, and statistically verified differences were analyzed by one-way ANOVA.
Based on the results, the highest polyphenol and flavonoid contents were shown by strawberry and cherry juices, however sour cherry and watermelon juices were also rich in these compounds. The lowest concentrations were measured in peach, nectarine and melon juices. Despite the high polyphenol content of the above-mentioned juices, none of the fruity ales had higher phenolic content than the control sample. On the other hand, flavonoid content of fruity ales was higher than the control sample's in every case. Simple enrichment increased polyphenol content of the samples more efficiently than double enrichments in most of the cases, however the addition of another 7.5% of juice to the cherry ale increased its concentration by 23.1%, which resulted in a higher polyphenol content than the control sample's. As opposed to the previous observations, fruity ales showed significantly higher flavonoid content than the control sample, which was statistically verified except for nectarine ale. The increase in polyphenol and flavonoid content was proportionate to the volume of the juice added in most of the cases.
To sum up, the concentration of antioxidant compounds can be increased efficiently by the addition of fruit juices, however it is important that enrichment before bottling has a more significant impact, since the intensity of fermentation and the volume of alcohol created might decrease polyphenol content of beer.

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