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Research Article

Changes in the protein profile of beers with bilberry

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Abstract

In the present study, beers with an original extract 12°P, 14°P and 16°P were obtained. During the maturation process, on different stages, bilberries (*Vaccinium myrtillus* L.) from Bulgaria were added. Changes in the protein profile of the beers were investigated, as well as the influence of these changes on the body in the mouthfeel and the head retention of the resulting beers. The electrophoretic analysis of the proteins was performed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein concentration was determined by the Bradford method. The sensory evaluation of the beer's body and head retention was conducted by a trained five-membered testing panel. It was found that independently of the original extract, the bilberry addition led to a significant reduction of the protein concentration and the number of protein fractions. Beers with bilberry addition contained between 74% and 94% less protein, than control beers. The number of protein fractions decreased from 10 in the control beers to 4–6 in the beers with bilberry. The fractions with molecular weight 31 kDa and below 14 kDa were the most stable to the bilberry addition. Their part in the beers with bilberry remained the highest. Despite of the significant changes in the protein profiles, the beers with bilberry had very good mouthfeel body and head retention.

Keywords

beer with bilberry, protein profile, SDS-PAGE, sensory evaluation

Abbreviations

SDS-PAGE – sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA – bovine serum albumin

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Introduction

After coffee, beer probably is one of the most consumed beverages around the world. It is rich in nutrients such as carbohydrates, amino acids, proteins, minerals, all B complex vitamins and phenolic compounds. According to the Beer Purity Law from 1516, traditional German beer should be made only from barley, hop, and water, with the addition of yeast (Kunze 2004); however, in Belgium, all kind of additional ingredients can be used, especially fruits – like in Belgian lambic sourbeers (Prasad 2014; Nardini et al. 2020). This procedure of adding fruits in beer now is really popular in craft breweries all around the world. Fruits as cherry, raspberry, peach, apricot, grape and other are used.

Beer proteins originate from barley, malt and yeast and have an impact on beer quality and tasting. According to Leiper et al. (2003) three main protein groups in beer can be separated. The first one consists proline rich fragments originating from hordein, ranging in size from 15–32 kDa, which are involved in haze formation. The second is LTP1-lipid transfer protein 1 (9.7 kDa in pure form), which is involved in foam stability. The third is protein Z (40 kDa). It appears to have no direct function but may play a role in stabilizing foam once it has been formed (Steiner et al. 2011). According to other classification mentioned by Steiner et al. (2011), proteins in beer are divided into three categories by their molecular weight: high molecular weight fraction: >40 kDa, middle molecular weight fraction: 15–40 kDa and low molecular weight fraction: <15 kDa. The proteins that are foam-positive can be divided into high molecular weight proteins (35–50 kDa) and low molecular weight proteins (5–15 kDa). It is known that some of proteins, especially proline and glutamatic acid rich hordeins, are binding to polyphenols and initiate haze development in beer (Nadzeyka and Altenhofen 1979; Asano 1982; Siebert 2009; Zhao and Sun-Waterhouse 2019).

Even without adding fruits, beer contains phenolic compounds, including phenolic acids, flavonoids, condensed tannins, and non-flavonoid polyphenols. They originate from malt and hops, and depending on the raw materials and the brewing process their content can be different (Wannenmacher et al. 2018; Nedyalkov et al. 2022; Mileva et al. 2018).

Fruit addition in beer increases the antioxidant capacity and the phenolic compounds that make the beer with higher functionality (Nardini et al. 2020). Fruits can be added during the fermentation/maturation process (Pedro 2019). The presence of CO₂ and alcohol in the green beer improves the extraction of the fruits ingredients that are less or not soluble in water.

Bilberries (*Vaccinium myrtillus* L.) contain great amount of phenolic compounds – phenolic acids, flavonoids, condensed tannins and non-flavonoid polyphenols (Georgescu et al. 2022; Frum 2022). They are rich in procyanidins and anthocyanins (Georgieva et al. 2018) which easily interact with proteins.

The addition of fruit rich in phenolic compounds, such as bilberry, intensifies the protein precipitation. As a result, a reduction in the proteins, an additional haze formation and changes in mouthfeel body are expected. According to M. Kato et al. (2021) high molecular β -glucan, proteins and some maltodextrins are of particular importance for foam retention and palate fullness. These authors also reported that the chemical analysis of high molecular weight polypeptides larger than 10 kDa exhibited a stronger correlation with sensory analysis of palate fullness.

In our previous article (Nedyalkov et al. 2022), we have reported on phenolic and protein profiles of beer with bilberry. The original extract of all beers was one and the same. According to our best knowledge, the information on the influence of the technological parameters, such as an original beer extract and the time of bilberry addition on the protein profile and sensory characteristics of beer are insufficient.

The aim of the present work was to investigate the changes in the amount of proteins and the protein profile in beers with a different original extract and bilberry addition at the maturation process; also to determine if the change in protein profile affects the mouthfeel body and foam retention of beers; as well as to find suitable technological parameters (an original extract and the time of bilberry addition) in order to preserve the proteins that have a positive effect on the mouthfeel body, density and good foam retention in bilberry beers.

Materials and Methods

Raw materials. Pilsner malt (Weyermann, Germany), Perle and Cascade hops (Bulhops, Bulgaria), and dry yeast *Saccharomyces pastorianus* Saflager W 34/70 (Fermentis, France) were used. The bilberries (*Vaccinium myrtillus* L.) were obtained in a frozen state from Bulfruct Ltd., Bulgaria, and kept in a freezer until use.

Reagents. Acrylamide, suitable for electrophoresis $\geq 99\%$; n,n'-methylene-bis-acrylamide, for electrophoresis $\geq 98\%$, powder; sodium dodecyl sulfate for electrophoresis, $\geq 98.5\%$ (GC); Trizma (R) base, Primary Standard and Buffer, $\geq 99.9\%$ (titration), crystalline; ethylene diamine tetraacetic acid, free acid Sigma GR BioUltra, anhydrous, $\geq 99\%$ (titration); TEMED for molecular biology, $\geq 99\%$ (GC); Ammonium persulfate for electrophoresis, $\geq 98\%$; Glycine, for electrophoresis, $\geq 99\%$; Brilliant Blue R; Bromophenol Blue Sultone; 2-Mercaptoethanol, for molecular biology, for electrophoresis, bioreagent 99%; Sigma Marker (TM), Wide Range, mol. wt 6500–200000 Da; Brilliant Blue G. All the other used chemicals were of analytical grade and were purchased from local suppliers.

Beer production. Pilsen malt was milled using a Corona hand mill and mixed with pre-heated water in different ratio to obtain worts with an original extract of 12 °P, 14 °P and 16 °P after boiling. An infusion mashing method was applied under the following conditions: 20 minutes at 60 °C, 20 minutes at 65 °C, 25 minutes at 72 °C, and 1 minute at 78 °C. After the mashing process, the sweet wort was lautered, the spent grains were sparged and the entire amount of wort was boiled for about 60 minutes to obtain an original extract 12 °P, 14 °P and 16 °P. The bitter hops (20 g Perle – 9% α -bitter acids) were added 10 minutes after the boiling started, and the aromatic hops (17.14 g Cascade – 7% α -bitter acids) were added 7 minutes before the end of the boiling process. The total amount of α -bitter acids was 60 mg.L⁻¹. After removal from the hot trub, the wort was cooled to 14 °C. All processes were conducted in a Home Brew 50 all-in-one 50 dm³ brewing system (TM INOX, Bulgaria). The wort was aerated and placed in a stainless steel cylindroconical fermenter (TM INOX, Bulgaria). The yeast was sprinkled into the wort, according to the manufacturer's instructions. The fermentation

temperature was 14 °C. The main fermentation was monitored hydrometrically. For maturation, the “green beer” was transferred to small stainless-steel fermenters when 60 % of the original wort extract had been fermented. Beer maturation continued for 14 days at 14 °C, and beer lagering for 5 days at 2 °C. Both were conducted under pressure. The bilberries were pasteurised right before being added to a water bath for 10 minutes at 70 °C and cooled to 14 °C after that. They were added at a concentration of 167 g.L⁻¹, in an empty small fermenter, which had previously been flushed with CO₂. At the beginning of maturation, the “green beer” was slowly transferred from the stainless steel cylindroconical fermenter to the small fermenter with bilberries using a hosepipe, which reached the bottom of the small fermenter. After the transfer, a pressure of 0.5 bar was reached in the fermenter using CO₂. On the third and a half or seventh day of maturation, the “green beer” was transferred under pressure from one small fermenter to other one with bilberries. The presence of CO₂ kept the bilberries and “green beer” away from oxidation. All variants were carried out in duplicate. After lagering, the beer was bottled using a “beer gun” (Blichmann Engineering, USA).

Basic characteristics of beer. The original extract (method 8.2.1) and alcohol concentration (method 9.2.1) were measured according to EBC standard methods (EBC 2018).

Quantitative analysis of proteins. The protein concentration was determined by the Bradford assay (Bradford 1976), with Stoscheck's modification (Stoscheck 1990). The results were expressed in mg/dm³ bovine serum albumin (BSA).

Electrophoretic analysis of proteins. The proteins were isolated from the beer by precipitation using trichloroacetic acid (TCA) (Tattersall et al. 1997). An Eppendorf microcentrifuge was used. Electrophoretic analysis was performed by SDS-PAGE according to Laemmli at 15 % acrylamide concentration in gel (Laemmli 1970; Lyutskanov et al. 1994). The quantity and percentage of each fraction in the total quantity and the protein molecular weight were determined by markers, SigmaMarker™ 6500-200000 Da, and TotalLab specialised software, trial version, for image analysis of electrophoretic gels as described early (Bakardzhiyski 2022).

Sensory analysis. Sensory evaluation of the beverages was performed by a trained 5-member tasting panel. The samples were evaluated using the descriptive method (EBC 2018).

Statistics. The results reported in the present study are the mean values of at least three analytical determinations, and the coefficients of variation expressed as the percentage ratios between the standard deviations and the mean values were found to be <5 % in all cases. The means were calculated with Microsoft Excel™ at a 95 % confidence level. The correlation coefficients were obtained by Excel's functions. One-way ANOVA and Scheffe's multiple range test at $p < 0.05$ as described by Donchev et al. (2007) were used, too.

Results and Discussion

Beer characteristics

Basic characteristics – alcohol content and real extract. The beers produced for the experiment were with original extract 12 °P, 14 °P, 16 °P. The values of alcohol content and the real extract of all beers, with and without bilberry (table 1), were normal for these original extracts and the attenuation of the lager type yeast that was used.

Table 1. Alcohol content and real extract of beers

Beer sample	Alcohol content, % (v/v)	Real extract, % (w/w)
CB(12)	4.15±0.32 ^{ab}	4.64±0.19
BB(12)0	3.71±0.28 ^{ab}	4.35±0.07
BB(12)3.5	4.44±0.34 ^a	4.56±0.10
BB(12)7	4.04±0.14 ^{ab}	4.46±0.00
CB(14)	5.45±0.19	4.62±0.16
BB(14)0	4.79±0.12 ^d	4.36±0.12
BB(14)3.5	5.13±0.00 ^{cd}	4.41±0.04
BB(14)7	5.13±0.41 ^{cd}	4.45±0.20
CB(16)	6.71±0.16	5.12±0.05
BB(16)0	6.10±0.00 ^e	5.19±0.05
BB(16)3.5	5.48±0.35 ^f	4.94±0.45
BB(16)7	6.06±0.52 ^{ef}	4.95±0.04

The same letter for a given sample means no significant differences (95% confidence level)

Quite normal, the alcohol content of the beer was higher when the original extract of wort had been increased. The bilberry addition did not change that. No definite relationship was found between the time of adding the bilberry and the alcohol content of the beers.

Sensory evaluation by description analysis. The sensory evaluation by description analysis of the beers showed that with the increase of the original extract of the control beers, the body of the beer became fuller and thicker (table 2).

Table 2. Descriptive sensory test of beers

Beer sample	Mouthfeel body
CB(12)	3.2
BB(12)0	2.1
BB(12)3.5	2.4
BB(12)7	2.5
CB(14)	3.5
BB(14)0	3.3
BB(14)3.5	2.3
BB(14)7	3.3
CB(16)	4.3
BB(16)0	3.4
BB(16)3.5	4.3
BB(16)7	3.4

0 – absent; 5 – very strong

The bilberry addition in beers with all of the researched original extracts led to the decrease of mouthfeel body; however, in the beers with original extract 12 °P, it manifested the biggest decrease. The beer with the original extract 12 °P and bilberry addition at the 7th day had the best body in the mouthfeel. This is probably because the time of contact between the beer proteins and the phenolic compounds from the bilberry was less than the other samples. Not the same tendency was observed in the beers with bilberry addition and the original extract 14 °P. The sample with bilberry addition at the 3.5th days was with the most watery body; therefore, here the contact time did not work in the same way. The beer with the 16 °P original extract and bilberry addition at the 3.5th day showed the best results. It had the same full body as the control beer with the same original extract. It can be suggested that despite the proteins precipitation, the higher original extract keep beer body full and tick.

The head retention of all beers was quite good; regardless of whether they were control beers or beers with bilberry addition. Some proteins of importance for the foam stability probably remained unaffected by the interactions with phenolic compounds from bilberry. The control beer was with goldish colour and the bilberry beers were with intense ruby colour. (fig. 1.).



Figure 1. Beer with bilberry added on the seventh day of maturation (on the left) and control beer (on the right)

The colour of the control beer was due to the malts that were used and the colour of the bilberry beers was due to the presence of anthocyanins extracted from the bilberry.

Proteins. In the control beers without bilberry, the highest protein concentration (table 3) was observed in the variant with original extract 12 °P. The control beers with 14 °P and 16 °P original extract contained comparable amount of proteins, but about 20 % lower compared to the control with 12 °P original extract. Although higher original extract supposes more protein extraction in the wort, it can be speculated that there was more precipitation and

protein loss during the boiling process of the more extractable worts, because of more phenolic compounds extraction in the mashing process, as well.

The greatest protein loss in bilberry beers was observed when the bilberries were added on the third and a half day from the start of the maturation process for the samples with 12 °P and 16 °P, as well as in all the variants with original extract 14 °P. This irregularity indicates that the protein concentration depends on both the original beer extract and the time of the bilberry addition. Obviously, the dependence was non-linear; there were distinct minimums. It is interesting to understand what caused the observed trends; this requires further research. We can only guess at the moment. Probably, the change in the concentration of extracted proteins during mashing and the amount of precipitated proteins during boiling are not directly proportional when the wort extract is increased.

A total of 19 protein fractions with molecular weight below 222 kDa were identified (table 4), but not all were found in the beers with different original extract. Their total number in the control beers was 10 – in the variants with original extract 12 °P and 14 °P, and 8 in the beer with original extract 16 °P. Fractions with molecular weight 31 kDa, 27 kDa and 26 kDa were present in all control beers. The fraction with molecular weight 31 kDa had the biggest share among the protein fractions. It makes the impression, that the medium molecular fractions (15–40 kDa) predominated in the control beers. This is very good for the head retention and the body mouthfeel of the beers. A number of authors report such a positive correlation (Kunze 2004; Steiner et al. 2011; Kabzev and Ignatov 2011).

Table 3. Protein concentration in beers

Original beer extract, °P	Control beer sample, mg.L ⁻¹	Beer with added bilberry		
		at the beginning of maturation, mg.L ⁻¹	on the third and a half day of maturation, mg.L ⁻¹	on the seventh day of maturation, mg.L ⁻¹
12	103.7±7.6	27.1±1.6	9.3±0.1	23.3±3.0
14	83.7±1	3.7±0.9	4.0±0.0	5.5±0.8
16	87.8±13	14.5±1.0	12.2±0.0	15.3±1.9

Results are expressed as bovine serum albumin in mg per liter beer, means ± SD, n=3

Table 4. Protein fractions in beers

Fraction number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Molecular weight, kDa	222	220	217	110	101	90	71	65	64	61	51	49	37	35	31	28	27	26	below 14
Part of total protein fractions, %																			
CB(12)	nd	nd	nd	9.1	nd	nd	3.1	nd	2.8	nd	4.8	nd	9.4	nd	36.0	6.7	4.4	4.5	19.2
BB(12)0	nd	nd	nd	nd	nd	nd	nd	nd	9.4	nd	6.9	nd	nd	nd	64.4	nd	nd	nd	19.4
BB(12)3.5	2.1	nd	nd	4.1	nd	nd	1.9	nd	nd	nd	nd	nd	17.0	nd	38.2	16.0	3.3	nd	17.4
BB(12)7	1.9	nd	nd	4.7	nd	nd	2.2	nd	2.2	nd	nd	nd	15.3	nd	37.5	13.1	4.0	nd	19.2
CB(14)	nd	1.2	nd	nd	9.0	nd	nd	3.3	nd	nd	nd	4.7	11.3	nd	35.5	13.3	3.0	4.6	14.2
BB(14)0	nd	8.5	nd	nd	nd	nd	nd	6.4	nd	nd	nd	nd	nd	nd	58.2	7.3	nd	nd	19.6
BB(14)3.5	nd	8.1	nd	nd	nd	nd	nd	16.4	nd	nd	nd	3.2	nd	nd	47.7	nd	nd	nd	24.7
BB(14)7	nd	5.9	nd	nd	nd	nd	nd	15.0	nd	nd	nd	8.5	nd	nd	50.1	nd	nd	nd	20.5
CB(16)	nd	nd	2.3	nd	nd	3.9	nd	nd	nd	9.3	nd	nd	nd	11.6	43.5	nd	10.7	4.0	14.7
BB(16)0	nd	nd	7.9	nd	nd	nd	nd	nd	nd	10.6	7.4	nd	nd	nd	52.9	nd	nd	nd	21.2
BB(16)3.5	nd	nd	5.8	nd	nd	nd	nd	nd	nd	19.9	11.8	nd	nd	nd	39.7	nd	nd	nd	22.9
BB(16)7	nd	nd	4.6	nd	nd	nd	nd	nd	nd	11.5	8.1	nd	nd	nd	43.5	nd	nd	12.8	19.5
Degree of protein reduction compared to the control samples, %																			
Comparison to the sample CB(12)																			
BB(12)0				-100			-100		-15		-64		-100		-55	-100	-100	-100	-75
BB(12)3.5	+100			-74			-64		-100		-100		0		-38	+ 39	-56	-100	-47
BB(12)7	+100			-68			-56		-50		-100		0		-36	+ 19	-45	-100	-39
Comparison to the sample CB(14)																			
BB(14)0		0			-100			-58				-100	-100		-80	-83	-100	-100	-80
BB(14)3.5		-2			-100			-24				-69	-100		-77	-100	-100	-100	-72
BB(14)7		-21			-100			-30				-72	-100		-78	-100	-100	-100	-78
Comparison to the sample CB(16)																			
BB(16)0			-22			-100				-74	+100			-100	-72		-100	-100	-67
BB(16)3.5			-47			-100				-52	+100			-100	-80		-100	-100	-64
BB(16)7			-33			-100				-58	+100			-100	-66		0	-100	-53

CB – control beer; BB – beer with bilberry; (12), (14), (16) – an original extract, °P; 0;3.5;7 – time of bilberry addition, day of maturation

The bilberry addition at the maturation process was accompanied by a significant decrease in the amount of individual protein fractions and their number. This was expected and normal. As mentioned, bilberries contain significant amounts of procyanidins. It is known that they are one of the groups of phenolic compounds that induce protein precipitation in beer (Zhao and Sun-Waterhouse 2019). The protein fractions with molecular weight 26, 35, 37, 90 and 101 kDa were the most unstable. They disappeared completely from bilberry beers regardless of the time of the bilberry addition. It could be assumed that they are proline-rich proteins. The proline residues are involved in the site where the polyphenols are attached to the protein, and adjacent proline and glutamine tend to enable strong binding to polyphenols (Siebert 2009; Zhao and Sun-Waterhouse 2019). It has been proved that proline and glutamic acid rich hordeins with molecular weight of 10–30 kDa according to some researchers (Nadzeyka and Altenhofen 1979), or 1–40 kDa according to others (Asano et al. 1982), are the main initiators of haze development in beer (Zhao and Sun-Waterhouse 2019).

Interestingly, the 27 kDa molecular weight fraction was completely lost in the 14 °P and 16 °P bilberry beers, but was retained in the 12 °P beers, even though in smaller amounts compared to the control. Probably, the amount of this protein fraction in more extractive beers increases and reaches critical values that lead to its complete precipitation with the bilberry phenols.

Protein fractions, not present in the control beers, were identified in some of the beers samples with bilberry. In particular, these were the fractions with molecular weight 222 and 51 kDa. An increase in the fraction with a molecular weight 28 kDa was also observed in the beers with the original extract 12 °P with bilberry addition at 3.5 and 7 days of maturation. It was quite possible that these fractions were present in the corresponding control beers, but due to their low concentrations and highly saturated background of the electrophoretic gels, they remained masked and therefore not measured. It can be speculated that these were protein fractions separated from yeast cells. However, confirmation of this hypothesis requires further research.

The behaviour of the fraction with 28 kDa molecular weight in the beers with the original

extract 14 °P was interesting. It was absent from the electrophoretic profiles of the beers with the bilberry addition at a later stage, but was present in the beer with fruit added at the beginning of maturation. It is possible the reversible coagulation or other precipitating factors in samples with fruit addition at the 3.5 and 7 days of maturation process intervened.

The reduction degree of the individual fractions was quite heterogeneous in the different variants compared to the corresponding controls (table 4). It was difficult to formulate a certain trend.

Correlation between protein fractions and mouthfeel body of beers. Since the fractions with a molecular weight of 31 kDa and below 14 kDa were present in all tested variants and had the largest share of the total protein profile, it was interesting to see what their influence was on the organoleptic properties of the beer and on the body and foam retention, in particular. In that in mind, correlation coefficients (table 5) between the sensory evaluations for the body of the samples (table 2) and the content of these two fractions, determined by the electrophoregrams (fig. 2) using specialized Totallab image analysis software, were founded by ANOVA. It is known that at values of the correlation coefficient greater than or equal to 0.85 the correlation is strong and below 0.5 it is weak (Donchev et al. 2007).

Table 5. Correlation coefficients between body in the mouthfeel of beers and the concentration of the protein fractions

An original extract of beers, °P	Fraction molecular weight, kDa	
	31	below 14
12	0.998782	0.998936
14	0.417924	0.362851
16	0.383806	0.478715

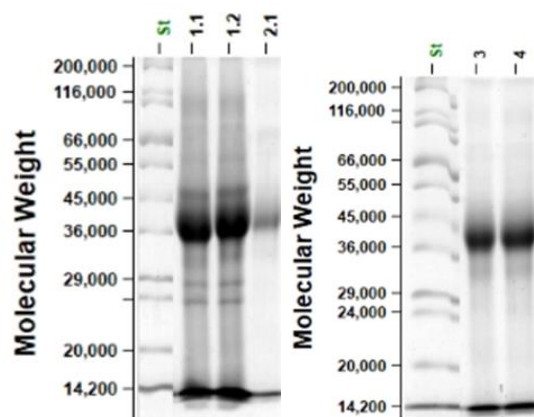
As could be seen from the table 5, in the series of beers with an original extract 12 °P, a strong correlation was observed between the concentration of the protein fractions with molecular weight 31 kDa and below 14 kDa and the sensory evaluation for the body of the samples. This is in agreement

with established by Kato et al. (2021) strong correlation between high molecular weight polypeptides larger than 10 kDa and sensory analysis of palate fullness.

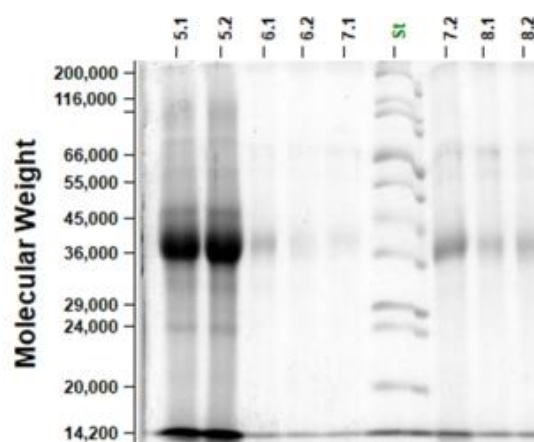
The correlation in the series of beers with the original extract 14 °P and 16 °P was weak. The correlation coefficient was below 0.5 (table 5). It could be assumed that the original extract of the beer had a significant influence on the role of the two protein fractions in forming the fullness of the taste. Probably, in beers with a higher original extract, other substances (β -glucans, dextrins, glycerol) that give fullness to the taste were in greater concentrations. Because of this, the variation in the concentration of the protein fractions (31 and below 14 kDa) did not reflect on the sensation of body and fullness of taste. The influence of high molecular weight β -glucans, dextrins, proteins and other components of beer on its body was discussed by Langstaf and Lewis (1993) and Kato et al. (2021).

Based on the correlation coefficients, it should be pointed out that the variation in the concentration of the protein fractions with molecular weight 31 kDa and below 14 kDa affects the fullness of the taste of beers with a lower original extract. Since the correlation coefficients had a positive sign, the dependence was directly proportional. The increase in the concentrations of the two protein fractions led to an increase in the feeling of fullness of the taste of beers with the original extract 12 °P and bilberry addition.

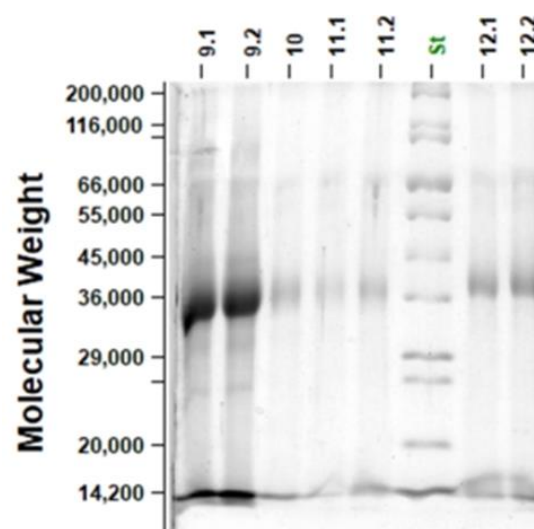
In order to preserve the taste's fullness of the beers with the original extract 12 °P, it was important to establish the influence of the time of bilberry addition at the maturation process on the concentration of the two protein fractions (31 kDa and below 14 kDa). The results of the single factor analysis, carried out using ANOVA, showed that the time of bilberry addition affected the amount of both fractions in beers with original extract 12 °P. The p-values were 0.000194 and 0.009959, respectively, for the fractions with a molecular weight 31 kDa and below 14 kDa. The results of the multiple comparisons, carried out according to Scheffe's multiple range test, are present in the table 6. It could be seen that the bilberry addition on the third and a half day or on the seventh day of maturation did not lead to a significant difference in the concentration of the two protein fractions.



a) Beers with an original extract 12 °P



b) Beers with an original extract 14 °P



c) Beers with an original extract 16 °P

Figure 2. Electrophoregrams of beers

Table 6.

Comparison of the protein fractions concentration according to Scheffe's multiple range test

Sample	Fraction 31 kDa	Fraction below 14 kDa
BB(12)0	a	a
BB(12)3.5	b	b
BB(12)7	b	b

The same letter for a given sample means no significant differences (95 % confidence level)

However, there was a significant difference if the bilberries were added at the beginning of the maturation. The degree of reduction in the amount of both protein fractions compared to the control (table 4) was the greatest when bilberries were added at the initial stage of maturation. Based on these results, for beers with an original extract 12 °P, it could be recommended that the bilberry addition should not be done at the initial stage of maturation, as this led to a greater loss of proteins with molecular weight 31 kDa and below 14 kDa. The loss of these proteins had a negative impact on the fullness of the beer's taste, as already mentioned.

Conclusions

This study provides new information related to the influence of technological parameters, such as the original extract of beer and time of bilberry addition during maturation of beer, on protein concentration and protein profile of beers with bilberry, as well as the correlation between the protein profile and the mouthfeel body of beer. It was found great loss of protein concentration in beers with bilberry, which depends on both – the original beer extract and the time of bilberry addition. The dependence was not linear. The bilberry addition led to a significant reduction in number of protein fractions, too. The protein fractions with molecular weight 26, 35, 37, 90 and 101 kDa were the most unstable. They disappear completely from the bilberry beers. The fraction with molecular weight 31 kDa had the biggest share among the protein fractions. This fraction and the fractions with molecular weight below 14 kDa were the most stable to the bilberry addition. They remained in all beer samples with

added bilberry and had the highest part among protein fractions.

Despite the strong protein reduction, the beers with bilberry had good head retention and body. The strong positive correlation between the content of the protein fractions with molecular weight 31 kDa and below 14 kDa and the body in the mouthfeel of beers with 12 °P original extract was found. This correlation was weak in beers with the original extract 14 °P and 16 °P.

As a result of the present research, for beers with an original extract 12 °P, it could be recommended that the bilberry addition should not be done at the initial stage of maturation in order to preserve protein fractions with molecular weight 31 kDa and below 14 kDa as well as the taste's fullness of the beers. The body in the mouthfeel of beers with the original extract 14 °P and 16 °P was not affected by the changes in the protein fractions and concentrations after the bilberry addition

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