EFFECT OF SUBSTITUTES FOR BARLEY MALT ON CHEMICAL-PHYSICAL AND SENSORIAL CHARACTERISTICS OF BEER

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Abstract
Traditionally, beer is a beverage that is made only from barley malt, hops and water. Nevertheless, apart from this, substitutes for barley malt in the form of various unmalted cereals are used. The chemical composition of wort is altered when prepared from alternative raw materials which further results in imbalance between different fermentable compounds. The yeast strain *Saccharomyces pastorianus* TUM 34/70 is one of the natural hybrids which was domesticated by man for traditional brewing based on barley malt. By changing the raw material the balance between aromatic compounds achieved by brewers over centuries could be ruined.
The results of our work showed that increasing the content of unmalted barley significantly affects all nitrogen fractions in wort and beer. The saccharides content remains comparable, except in the variant where only unmalted barley was used as a surrogate and this led to an increase in glucose and maltotriose. The increase in the content of unmalted barley also results in an extended fermentation time from six to nine days. Differences in the composition of surrogates did not affect the sensory evaluation of fresh beer and of beer after aging of three months, while the best scored beers after six months where those where we used a combination of unmalted barley and maize grist.
Key words: beer, malt surrogates, chemical composition, sensorial properties

VPLIV NADOMESTKOV JEČMENOVEGA SLADA NA KEMIJSKO-FIZIKALNE IN SENZORIČNE LASTNOSTI PIVA

Izvleček
Tradicionalno je pivo pijača, ki je izdelana samo iz ječmenovega sladu, hmelja in vode. Kljub temu se poleg osnovnih surovin pri varjenju piva uporabljajo nadomestki za ječmenov slad, v obliki različnih neslajenih žit. Izbor alternativne

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surovine pomeni spremenjeno kemijsko sestavo sladice in posledično porušeno ravnovesje med različnimi fermentativnimi spojinami. Kvasovka *Saccharomyces pastorianus* TUM 34/70 je ena izmed naravnih sevov, ki jo je človek udomačil za tradicionalno proizvodnjo piva, kjer se uporablja izključno ječmenova sladica. Ob zamenjavi osnovnega substrata, se ravnovesje aromatičnih spojin, ki so ga pivovarji dosegli skozi stoletja, poruši.
Rezultati našega dela so pokazali, da višanje vsebnosti neslajenega ječmena bistveno vpliva na dušične komponente v pivini in v pivu. Vsebnost sladkorjev ostaja primerljiva, razen v varianti, kjer je bil kot nadomestek uporabljen samo neslajeni ječmen in je to vodilo v povišanje glukoze in maltotrioze. Posledica višanja vsebnosti neslajenega ječmena je tudi podaljšan čas fermentacije iz šest na 9 dni. Razlike v sestavi nadomestkov niso vplivale na senzorično oceno svežega piva in po staranju treh mesecev, medtem ko so bila najbolje ocenjena piva po šestih mesecih tista, kjer smo uporabili mešanico neslajenega ječmena in koruznega zdroba.
Ključne besede: pivo, nadomestki slada, kemijska sestava, senzorične lastnosti

1 INTRODUCTION

Beer is one of the world's oldest prepared beverages. It is estimated that it is more than 5000 years old and is one of the world's most popular drinks. In fact, it is the third most consumed drink overall, after water and tea (Arnold, 2005).

To all known beer flavour and aroma, are the results of a fine and subtle balance between numerous flavour active compounds, which are originating from raw materials used in brewing process, together with those originating from yeasts during fermentation. A combination of taste and odour is of crucial importance for consumers' acceptance of beer (Kunze, 2010).

Standard composition of the brewing raw materials for the production of wort includes pure barley malt. However, in the breweries in addition to the malt, various surrogates in the form of unmalted cereals are used too (unmalted barley and maize grits). The problem of the use of surrogates is that they may significantly alter the chemical composition and physical properties of wort, and thus the expected metabolism of yeast during the fermentation. Consequently, the sensorial description of the final product beer may be significantly affected.

Research work was primarily focused on comparison of different technological processes, where unmalted barley, maize grits and two mixtures of both in different ratios were used. Comparison of their influences on chemical composition and physical and sensorial properties of wort and beer was made. Main goals were detailed chemical characterization of wort, green beer and beer, where surrogates

for barley malt were used on industrial scale. After the production, selected parameters were monitored during the storage after 3 and 6 months to get the information on possible effects of different raw materials on beer stability and sensory.

2 MATERIALS AND METHODS

2.1 Research design

Industrial scale production was conducted in conical fermentation tanks (total volume 4400 hl, working volume 3250 hl). Strict traceability of the ingredients for wort production (malt, maize grits and unmalted barley) was assured. A conventional brewing protocol and fermentation diagram for primary and secondary fermentation was applied. Four different worts were prepared using raw materials as presented in Table 1 in proportions of particular raw material expected to be used in conventional beer production.

Table 1: *Presentation of raw materials used in particular brew in % (m/m)*

	Barley malt	Unmalted barley	Maize grist
Variant 1	70	0	30
Variant 2	70	10	20
Variant 3	70	20	10
Variant 4	75	25	0

2.2 Yeasts

The yeasts used in the brewing process for all variants was a lager yeast strain, *Saccharomyces pastorianus* (TUM 34/70), supplied by the Yeast Centre at Weihenstephan, Germany. In all cases yeast generation C was used.

2.3 Chemical analyses

All chemical analyses used for wort, green beer and beer were done according to the Analytica-EBC and MEBAK collections of analytical methods as follows:
Total nitrogen in in wort: Kjeldahl method (Analytica-EBC, 2000a), Total nitrogen in beer: Kjeldahl method (Analytica-EBC, 2000b), Free amino nitrogen in wort by spectrophotometry (Analytica-EBC, 2002), Free amino nitrogen in beer by spectrophotometry (Analytica-EBC, 2000c), Total polyphenols in wort by spectrophotometry (Analytica-EBC, 1997), Total polyphenolos in beer by spectrophotometry (Analytica-EBC, 2002), Coagulable nitrogen (MEBAK, 2013a), Nitrogen fractionations (MEBAK, 2013d), Total sulphur dioxide in beer: Distillation method (Analytica-EBC, 1997), Fermentable carbohydrates in beer by HPLC (Analytica-EBC, 1997), Free dimethyl sulphide (DMS) in wort and beer

(MEBAK, 2013b), Vicinal diketones in beer: spectrophotometric method (Analytica-EBC, 2000), Higher alcohols and esters in beer (MEBAK, 2013c), Original gravity in wort and beer (MEBAK, 2013e) and Alcohol in beer by near infrared spectroscopy (Analytica-EBC, 2008).

During fermentation, glucose, fructose, maltose (DP2) and maltotriose (DP3) contents were monitored every day of fermentation. To assess maturation effects green beers were analysed on the first day of maturation and after two weeks when the maturation was ended. Immediately after bottling a complete analysis was done on fresh bottled beer to determine possible effects of filtration on the chemical composition. After bottling beers were stored for three and six months and analysed completely to see the effects of storage on chemical composition. At the same time samples were sensory analysed by a panel consisted from six trained members to see possible effects on sensory.

3 RESULTS AND DISCUSSION

3.1 Wort

In the experiment, four different worts were prepared, which differed in the content of each surrogate (unmalted barley, or maize), as described in the previous chapter. Table 2 presents the measured values of individual parameters.

Table 2: *Results of chemical analysis of worts of all four variants*

Parameter	VAR 1	VAR 2	VAR 3	VAR 4
Original gravity (%)	11.42	11.41	11.41	11.48
Total nitrogen (mg/100mL)	72.5	74.1	81.7	98.7
High molecular weight nitrogen (mg/100mL)	21.2	22.4	25.4	28.7
Medium molecular weight nitrogen (mg/100mL)	2.8	3.4	5.9	7.3
Low molecular weight nitrogen (mg/100mL)	48.5	48.3	50.4	62.7
Coagulable nitrogen (mg/100mL)	2.5	2.6	3.2	3.5
Free alpha amino nitrogen (mg/L)	137.1	134.6	142.4	191.4
Polyphenols (mg/L)	172.4	185.6	198.1	233.9
DMS (mg/L)	0.048	0.044	0.052	0.053
Glucose [g/100mL]	0.78	0.75	0.73	1.05
Fructose [g/100mL]	0.15	0.16	0.13	0.18
Maltose (DP2) [g/100mL]	5.64	5.81	5.87	5.46
Maltotriose (DP3) [g/100mL]	1.32	1.30	1.46	1.78
sum fermentable saccharrides [g/100mL]	7.89	8.02	8.19	8.47

In Figure 1 is the graphical presentation of nitrogen fractions of the same results as presented in Table 1 for clearer outlook of the results.

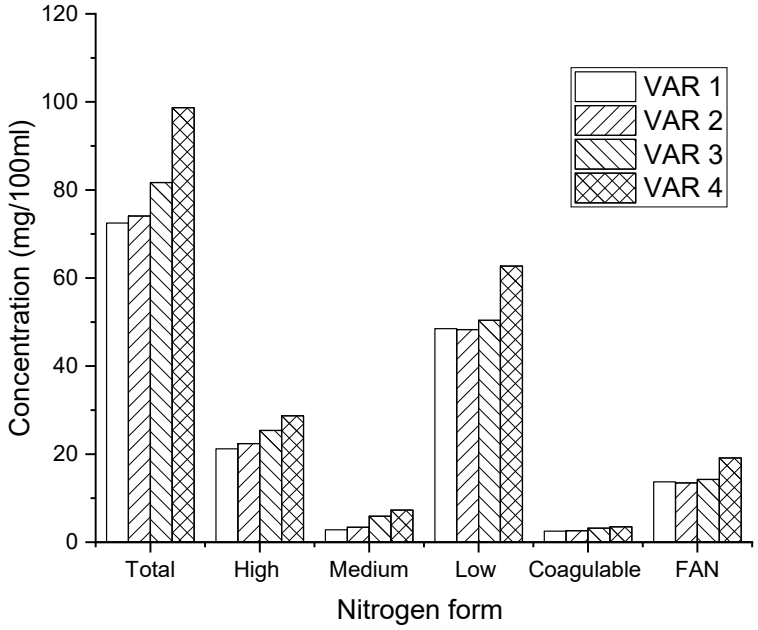

Figure 1: *Graphical presentation of nitrogen fractions in worts.*

The results show that increasing the proportion of unmalted barley significantly increases all forms of nitrogen that were included in the analyses. The same trend can be observed in the contents of the polyphenols as in the case of nitrogen fractions, as the increase in polyphenols is as much as 14.9 %.In the case of fermentable saccharides, it could be seen that there are no significant differences between the first three variants, either in the individual type of saccharide or in the total sum of them. A larger deviation is observed in the fourth variant, where the content of glucose and maltotriose (DP3) is increased, and the content of maltose (DP2) is reduced. Fructose remains comparable in all variants. The content of DMS did not differ significantly in all three variants.

3.2 Fermentation

Fermentation took place in all four variants with the same yeast generation (C generation) and under the same conditions. In Variant 1, the fermentation was

completed within five days and in Variant 2 within six days. In Variant 3, the fermentation took 10 days, and in Variant 4, 9 days. Table 3 collects data on the concentrations of individual fermentable sugars during the course of fermentation.

The dynamics of consumption of individual saccharides during fermentation shows that the change of surrogate does not affect significantly the consumption of mono saccharides glucose and fructose, as their concentration falls below 0.1 g/100mL after two or three days of fermentation. However, increasing the unmalted barley surrogate begins to significantly increase the consumption time of DP2 and DP3. In variants 3 and 4, the fermentation took place for 10 or 9 days.

Table 3: *Concentrations of fermentable saccharides during fermentation*

Saccharide Variant and fermentation day	DP3 [g/100mL]	DP2 [g/100mL]	Glucose [g/100mL]	Fructose [g/100mL]
VAR 1/1	1.21	4.92	0.04	0.16
VAR 1/2	0.91	3.41	0.00	0.03
VAR 1/3	0.41	1.14	0.01	0.04
VAR 1/4	0.32	0.67	0.04	0.05
VAR 1/5	0.24	0.27	0.00	0.01
VAR 2/1	1.00	4.05	0.10	0.08
VAR 2/2	0.87	3.12	0.02	0.02
VAR 2/3	0.69	2.65	0.04	0.04
VAR 2/4	0.50	1.66	0.00	0.01
VAR 2/5	0.38	0.77	0.07	0.01
VAR 2/6	0.36	0.61	0.08	0.02
VAR 3/1	1.39	5.57	0.72	0.09
VAR 3/3	0.99	3.70	0.09	0.02
VAR 3/5	0.82	2.55	0.01	0.03
VAR 3/7	0.50	1.40	0.03	0.01
VAR 3/9	0.46	0.74	0.00	0.01
VAR 3/10	0.32	0.49	0.01	0.02
VAR 4/1	1.84	5.55	0.49	0.33
VAR 4/3	1.41	3.26	0.25	0.02
VAR 4/5	0.69	1.93	0.04	0.01
VAR 4/7	0.53	0.98	0.02	0.02
VAR 4/9	0.48	0.80	0.03	0.03

3.3 Maturation

For the purpose of maturation monitoring of the green beer, we performed a chemical analysis on the first day of maturation and the last day of maturation before beer filtration and bottling. We determined the parameters listed in Table 4.

***Table 4**: Results of chemical analysis at the beginning and end of maturation*

	Start of maturation				End of maturation			
	VAR 1/6	VAR 2/7	VAR 3/11	VAR 4/10	VAR 1/7	VAR 2/8	VAR 3/12	VAR 4/11
Total nitrogen (mg/100mL)	53.8	58.5	67.9	85.4	53.2	57.5	68.3	85.0
High molecular weight nitrogen (mg/100mL)	11.0	14.1	17.1	18.2	11.3	12.9	15.7	20.6
Medium molecular weight nitrogen (mg/100mL)	11.0	11.1	11.2	17.9	10.9	11.8	12.9	14.4
Low molecular weight nitrogen (mg/100mL)	31.8	33.2	39.6	49.3	31.0	32.8	39.7	50.0
Coagulable nitrogen (mg/100mL)	1.4	1.3	1.5	1.9	1.6	1.8	2.0	2.5
Free alpha amino nitrogen (mg/L)	63.3	72.5	103.8	155.1	65.5	75.9	111.4	167.4
SO_2 (mg/L)	0.6	2.7	4.1	6.1	0.8	1.9	1.6	3.3
polifenoli (mg/L)	152.7	164.5	184.1	202.0	148.4	161.2	177.3	189.4
Acetaldehyde (mg/L)	8.63	6.13	8.08	13.01	2.81	3.48	4.65	8.74
Ethylacetate (mg/L)	14.49	17.99	17.28	18.64	16.30	17.55	16.63	17.68
Methanol (mg/L)	2.08	1.90	2.81	2.68	2.27	2.82	2.13	2.37
1-propanol (mg/L)	13.64	14.69	14.29	15.13	14.20	15.23	14.25	15.16
Iso-buthanol (mg/L)	10.26	2.30	7.43	8.74	10.20	9.50	7.15	8.63
Iso-amyl acetate (mg/L)	1.32	1.03	0.74	1.08	1.29	1.10	0.80	0.97
2-methylbutanol-1 (mg/L)	12.04	9.65	11.74	13.40	12.09	12.53	10.62	13.02
3-methylbutanol-1 (mg/L)	37.43	30.17	31.09	33.98	36.12	38.04	31.32	34.21
2-phenylethyl acetate (mg/L)	0.44	0.16	0.17	0.20	0.45	0.46	0.20	0.29
2-phenylethanol (mg/L)	28.66	12.77	14.10	13.13	29.93	25.82	12.93	12.69
DMS (mg/L)	0.05	0.07	0.07	0.06	0.10	0.10	0.04	0.04
diacetyl (mg/L)	0.09	0.09	0.04	0.04	0.05	0.05	0.05	0.06
DP3 [g/100mL]	0.18	0.25	0.18	0.15	0.33	0.46	0.54	0.40
DP2 [g/100mL]	0.13	0.21	0.11	0.17	0.23	0.26	0.31	0.19
Glc [g/100mL]	0.00	0.04	0.02	0.05	0.03	0.03	0.04	0.06
Fru [g/100mL]	0.02	0.01	0.02	0.01	0.00	0.00	0.00	0.00

In the case of nitrogen fractions, elevated concentrations of practically all forms are maintained even after fermentation and the end of maturation, depending on the increase in the surrogate of unmalted barley. The difference after the end of maturation in most cases are no longer as obvious as in the case of worts, but are fairly even. High-molecular and alpha amino nitrogen forms stand out, which are significantly higher in variants 3 and 4. They are also significantly higher compared to variant 2 (malt/maize/unmalted barley). Polyphenols levels are no longer as high after maturation as in worts, but the difference between variants 1, 3 and 4 remains high. The contents of SO_2, DMS and diacetyl do not differ significantly between the individual variants. The concentration of diacetyl drops as expected towards the end of maturation. In the field of higher esters and alcohols, the largest differences are observed in lower concentrations of iso-butanol, iso-amyl acetate, 2- and 3-methylbutanol-1, 2-phenyl acetate and 2-phenylethanol, which are consistent with the increase in the proportion of unmalted barley surrogate.

3.4 Beer storage

Table 5 presents the results of measurements in beer at bottling and after three or six months of aging. After filtration, the same set of parameters was analysed in the beer as after the end of maturation, since we wanted to see also the influence of filtration on individual parameters. The same set of parameters was then determined also after storage for three and six months. The results of the measurements show that the filtration process does not have a significant effect on individual nitrogen forms. The greatest impact in terms of concentration reduction is detectable in the case of coagulable nitrogen. Also during aging, the ratios between the individual forms depending on the surrogate used remain constant. Filtration significantly reduces the concentration of polyphenols. Reduction is more than 50 % for all variants. As beer is aged, their concentrations no longer change significantly. The concentration of DMS, SO_2 and diacetyl is not expected to be affected by filtration and their concentrations do not change significantly during aging. In all variants, the concentrations of those parameters are low. The concentrations of higher esters and alcohols are not significantly affected by filtration. The data for iso-butanol differ, but we don’t have the explanation. The ratios between the individual alcohols and esters also remain comparable during aging and their concentration depends on the surrogate used, however, there are no major differences between the individual variants. Among some, a trend of increasing concentration of 2-phanylethanol and 2-phenyl acetate and decreasing concentration of iso-amyl acetate is observed during aging. Concentrations of other alcohols and esters do not change significantly. Table 5 presents the results of measurements in beer at bottling and after three or six months of aging.

Table 5: *Results of measurements in bottled beer at bottling and after three and six months of aging*

	Beer at filling				Beer after 3 months				Beer after 6 months			
	VAR 1	VAR 2	VAR 3	VAR 4	VAR 1	VAR 2	VAR 3	VAR 4	VAR 1	VAR 2	VAR 3	VAR 4
Total nitrogen (mg/100mL)	52.5	57.1	70.7	79.0	54.6	58.1	70.7	85.4	52.4	56.8	67.9	78.8
High molecular weight nitrogen (mg/100mL)	10.2	11.5	14.6	14.6	10.6	11.2	13.6	14.2	8.5	8.9	10.5	12.0
Medium molecular weight nitrogen (mg/100mL)	11.5	13.6	20.5	23.6	11.5	13.8	16.7	19.9	13.1	14.8	18.2	20.6
Low molecular weight nitrogen (mg/100mL)	30.9	32.0	35.6	40.7	32.5	33.1	40.4	51.3	30.8	33.2	39.2	46.2
Coagulable nitrogen (mg/100mL)	0.8	0.9	0.9	1.0	1.3	1.2	1.6	1.8	1.6	1.5	1.8	2.4
Free alpha amino nitrogen (mg/L)	64.9	75.8	125.4	150.2	60.7	79.5	123.8	142.9	60.9	70.8	110.5	162.8
SO_2 (mg/L)	1.4	1.6	1.9	2.4	1.0	1.4	1.5	1.8	0.1	0.2	0.9	1.3
Acetaldehyde (mg/L)	2.4	2.2	3.3	3.5	-	-	-	-	-	-	-	-
Ethylacetate (mg/L)	17.5	19.5	18.1	19.3	17.9	23.5	17.8	15.0	13.0	17.0	15.0	13.0
1-propanol (mg/L)	14.0	15.0	14.9	14.9	18.9	19.0	17.1	14.3	14.0	11.0	15.0	15.0
Iso-buthanol (mg/L)	2.6	2.5	2.3	2.3	2.4	2.3	1.9	1.9	1.6	1.5	1.2	1.2
Iso-amyl acetate (mg/L)	1.1	1.1	0.8	0.9	0.5	0.6	0.3	0.9	0.2	1.3	0.2	1.3
(2+3)-methylbutanol-1 (mg/L)	42.1	42.8	40.7	40.9	44.3	42.7	41.9	42.0	48.0	41.0	43.0	43.0
2-phenylethyl acetate (mg/L)	0.2	0.2	0.1	0.1	1.6	0.7	0.9	1.4	2.5	2.3	0.7	0.4
2-phenylethanol (mg/L)	17.8	13.8	7.7	8.0	30.2	25.9	12.1	10.7	42.0	38.0	17.0	13.0
DMS (mg/L)	0.038	0.039	0.045	0.044	0.000	0.040	0.046	0.045	0.040	0.040	0.049	0.048
diacetyl (mg/L)	0.10	0.12	0.08	0.06	0.11	0.11	0.09	0.12	0.12	0.10	0.11	0.08
polyphenols (mg/L)	85.4	82.4	75.6	66.6	83.4	90.0	100.8	106.2	87.7	81.2	103.4	102.1

3.5 Sensorial assesment during beer aging

All beer samples were sensorial assessed at bottling time and after 3 and 6 months of aging. In sensorial assessment performed with the panel consisted of 6 trained members three parameters were assessed, namely odour, taste and bitterness, each scored from 1 to 5, where 1 mean the lowest and 5 the highest score. Final average scores are presented in Table 6.

Table 6: *Average scores for sensory assessment*

Sample	Odour (months)			Taste (months)			Bitterness(months)			Overall(months)		
	0	3	6	0	3	6	0	3	6	0	3	6
Variant 1	3.50	3.33	2.75	3.33	3.58	2.58	3.50	3.50	2.67	3.44	3.47	2.67
Variant 2	3.42	3.67	3.08	3.33	3.58	3.17	3.33	3.42	3.17	3.36	3.56	3.14
Variant 3	3.75	3.00	3.00	3.33	3.17	3.00	3.33	3.00	3.00	3.47	3.00	3.00
Variant 4	3.58	3.58	2.50	3.42	3.58	2.58	3.39	3.42	2.58	3.39	3.42	2.58

At the bottling time six panellist did not rate negatively none of the variants and they did not notice significant differences. No major changes were observed between the different ratios of unmalted barley as a surrogate after 3 months of aging. They did not recognise oxidation of beer and all samples are sensory stable. Among variants, the best scored sample was Variant 2 (10% of unmalted barley, 20% maize), followed by Variant 4 (25 % of unmalted barley). Still the panel concluded that there were no major differences between samples. After 6 months in all beers panel recognised storage impact on quality. However the best rated samples were those produced from the combination of unmalted barley and maize (Variants 2 and 3).

4 CONCLUSION

Examining the results of beer testing, monitoring fermentation and maturation and aging of bottled beer produced from different combinations of surrogates, we can see that at all technological stages there are significantly increased contents of nitrogen forms depending on the increase in the proportion of unmalted barley used. This could cause problems with the turbidity of the beer or the formation of unwanted aroma components. Increasing the proportion of unmalted barley significantly increases the content of polyphenols, which could also easily cause problems with the turbidity of beer. As can be seen from the results before and after filtration, their content is reduced by about 50 % by filtration. Although their content is still comparatively higher in the case of the use of unmalted barley, the absolute values are probably no longer so high as to cause problems during aging. It is interesting to note that the prolongation of the fermentation time by increasing the proportion of unmalted barley is mainly due to the slower consumption of DP2 and DP3. If the actual cause of the prolonged fermentation was a higher proportion of unmaletd barley as it could be concluded from our results, this can be

problematic from the point of view of increased production costs. However, research shows that yeasts can adapt to a new environment and, in subsequent successive uses, the negative impact of the environment on their viability declines. The measured parameters do not show significant deviations between individual variants in the case of volatile components of aroma. This may indicate that there are no increased precursors for the formation of negative components such as DMS, SO_2 in any surrogate, or that different surrogates do not significantly affect the ability to excrete secondary yeast metabolites.

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