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How maturation time affects the chemical and sensory profile of pale lager beer

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Abstract

The maturation of beer is an important production step with an impact on its quality. Changes in the chemical and sensory profile between 2 and 6 weeks of cold maturation were evaluated in pilot brews (200 l) of a pale lager of 11% and 15% original gravity. In addition to the basic analytical parameters and shelf life, volatiles, hop essential oils, fatty acids, amino acids and stale flavour aldehydes were monitored. The sensory quality of the beers was evaluated using descriptive method. The dynamics of changes in the monitored groups of analytes during maturation of the tested variants was different, but without substantial influence on the overall impression and shelf life of the beer. Under the experimental conditions, it was possible to reduce the maturation time of a common Czech-style lager to 2 weeks of cold lagering. The gradual slight improvement in the overall impression of beers brewed at original gravity indicates the need for a long maturation period to fine-tune the sensory profile of premium lagers. A short maturation period of 2 weeks appears to be beneficial for beers brewed using a high gravity brewing protocol. The findings obtained can be a useful guide for optimising the maturation of Czech-style lager in practice, although the dynamics of changes in the monitored substances and their influence on the sensory characteristics of the beer may be to some degree different when scaled up to operational practice.

Keywords: beer maturation; sensory quality; volatile compounds; essential oils; fatty acids; pale lager

1 Introduction

Many factors are involved in the final quality of the beer, from the choice of raw materials to the final operations in releasing the product to the market. The lagering and maturation of beer is a technological step in which a series of chemical and physicochemical changes of green beer takes place. Slow fermentation of carbohydrates, carbonation and its fixation, maturation of taste and aroma are caused by the change in the composition of volatile and colloidal substances and clarification of beer by the excretion of high-molecular substances, thus giving the beer a natural colloidal stability (Basařová et al., 2017).

The course of fermentation depends on many interrelated factors, the most important of which are temperature, fermentable extract content, green beer composition, yeast strain and the number of yeast cells in the medium. These factors depend on the brand (type) of beer being produced and the technology used (Basařová et al., 2017; Esslinger, 2009).

Different technological processes are known and used for conducting the fermentation of bottom-fermented beers, which Esslinger (2009) divides into three schematic groups: cold fermentation and cold maturation; warm fermentation and warm maturation; cold fermentation and warm maturation. Temperature-accelerated processes mean a reduction in the time required, but sometimes at the cost of higher production or insufficient degradation of some sensory undesirable substances. Premium brands of pale lagers are usually produced with long maturation times.

The economic and environmental need to save time and energy drives the need to intensify production. One of the possibilities is to optimise the fermentation and maturation time of beer in a conventional set-up. The aim

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of our experiment was to monitor in detail the changes in the chemical and sensory profile of a conventional all-malt pale lager, and to determine the effect of lagering time on the resulting product quality.

2 Materials and methods

2.1 Beer production

To study the effect of lagering time on beer quality, pilot 200 l brews (double decoction procedure, 11% (L1) and 15% (L2) brew) were made with commercial Bojos malt. Hopping (CO_2 extract and Saaz pellets 1:1) was conducted in three doses, 30% at the start, 50% after 30 minutes and 20% hops 10 minutes before the end of the wort boiling. After the trub separation in a whirlpool, the wort was cooled down to a fermentation temperature of 10 °C and aerated to a dissolved oxygen content of 8 ± 0.5 mg/l.

Two-phase fermentation is required in the PGI Czech Beer specification (Commission, 2008). The main fermentation was carried out in the CCT. The lager yeast strain RIBM95, which is the most widely used in domestic breweries, was used. The maximum temperature was set at 12 °C. After reaching an apparent/final attenuation difference of about 10%, the beer was cooled to a temperature of 4 °C within 24 hours, transferred to lager tanks and matured at a temperature of 1 °C and overpressure in the lager tank 1.2 bar.

After 2 weeks of lagering, aliquots of 30 litres of beer were filtered at weekly intervals with a plate filter (filter plates for a depth filtration composed of cellulose, kieselguhr and perlite), then packaged in 500-ml glass bottles, and finally pasteurized in an immersion pasteurizer. A carbon dioxide atmosphere was used for handling the beer during the whole course of the filtration and bottling. Brew with an original gravity of 15% was adjusted to the original gravity of 11% with degassed and carbonated water.

2.2 Instrumental analysis

Basic analyses of beers and determination of total polyphenols were carried out according to the EBC (Analytika EBC, 2010) and MEBAK (MEBAK, 2013) analytics by the following methods: EBC 9.4 – Original, Real and Apparent Extract and Original Gravity of Beer, EBC 9.2.6 – Alcohol in Beer by Refractometry, EBC 9.7 – Final Attenuation of Beer, EBC 9.35 – pH of Beer, EBC 9.6 – Colour of Beer, EBC 9.8 – Bitterness of Beer, EBC 9.29 – Haze in Beer, MEBAK 2.18.2. – Foam, 9.11 – Total Polyphenols in Beer by Spectrophotometry, MEBAK 2.26.1.5. – Carbon Dioxide in Beer. The shelf life of the beers was evaluated by measuring the turbidity of 3 bottles stored in the dark at room temperature at monthly intervals.

Sensorially active volatile substances, alcohols (propanol, isobutanol, 2- and 3-methyl-butanol, furfuryl-alcohol, β-phenyl-alcohol, ethyl-hexanol) and esters (ethyl formate, ethyl acetate, propyl acetate, isobutyl acetate, ethyl butyrate, butyl acetate, isoamyl acetate, ethyl capronate, ethyl lactate, ethyl caprylate, ethyl caprinate, phenyl-ethyl acetate, ethyl laurate, ethyl myristate, ethyl palmitate) were determined by GC–FID analysis using the EBC 9.39. method – Dimethyl Sulphide and Other Lower Boiling Point Volatile Compounds in Beer by Gas Chromatography.

Fatty acids (isobutyric, butyric, isovaleric, valeric, caproic, caprylic, pelargonic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic) were determined by GC–FID analysis after solid phase extraction of the analytes (Horák et al., 2013). Amino acids were determined by LC–FL analysis, in-house method RIBM-K60, results are expressed in mg/l glycine.

Aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 3-methylbutan-2-one benzaldehyde, phenylacetaldehyde, hexanal, heptanal, octanal, *trans*-2-butenal, *trans*-2-octenal, *trans*-2-nonenal and furfural) were determined by GC–MS analysis (Čejka et al., 2013). Essential oils in the beer were determined by GC–MS method described previously (Mikyška et al., 2018).

α-Pinene, β-pinene, β-myrcene, limonene, β-*trans*-ocimene, *allo*-ocimene, linalool, β-caryophyllene, 4-terpineol, *trans*-β-farnesene, α-humulene, methyl geranate, α-terpineol, geranyl acetate, *cis*-geraniol, α-ionone, β-ionone, α-irone, β-caryophyllene epoxide, farnesol.

All the assays were carried out in duplicate. The results of the analyses of the wort and green beer of the L2 brew were converted to the original gravity of 11% for better comparison.

2.3 Sensorial analysis

The sensory analysis of the beers was performed by Descriptive test and Ranking test by a twelve-member trained panel, using EBC 13.10 and EBC 13.11 methods. The attribute 'balance' expresses the degree of balance of the basic parameters, sweetness, acidity and bitterness of the beer (descending scale 1–5). Overall impression, the overall assessment of the sample, with respect to the appropriateness of the all attributes present, including off-flavours, their intensities, and the unidentifiable background flavour, was rated on a descending scale (1 = excellent to 9 = inappropriate).

The bitterness of beer was evaluated using a modified procedure of the comprehensive evaluation of beer bitterness (Mikyška et al., 2015), the intensity of the bitter sensation after drinking, after 15 s (maximum), after 40 s (lingering) was recorded on

a scale of 0 (not noticeable) to 5 (very strong), and the character (pleasantness) of the bitterness on a scale of 1 (fine, pleasant) to 5 (very harsh, clinging).

3 Results and discussion

The results of the analysis of wort, green beers and beers finished during maturation are presented and discussed in terms of operational parameters (filterability, clarity, shelf-life), basic physico-chemical parameters, groups of sensory active volatile compounds and, finally, the resulting projection of chemical changes on the sensory profile of the beer.

3.1 Physico-chemical parameters of beers

The effect of maturation time on the physicochemical profile of the beer was tested on a regular 11% brew and a brew with a higher extract concentration of 15%. The L1 brew (OG=11%) had a higher final attenuation than the L2 brew (OG=15%) (78.2% and 75.2% respectively), the beers from the L2 brew were less attenuated and they had about 1 EBC higher colour, 0.3 higher pH value, 2 EBC lower bitterness, a trend towards lower foaminess (20 s/30 mm), but the total polyphenol content of the beers was identical (Table 1). These differences are due to the different original gravity of the two brews, the thicker mash slowing down the saccharolysis and promoting thermal reactions leading to the formation of coloured substances, products of the Maillard reaction (Basařová et al., 2017).

As expected, significant decreases in total polyphenols, bitterness and beer colour occurred between wort and green beer. Further marked reductions in total polyphenols, indicating clarification of the beer by the excretion of tannic-protein complexes (Steiner et al., 2010), and reductions in beer colour were found in the first two weeks of maturation, and to a lesser extent in the third week, with no further changes in values. A slight decrease in foam stability was observed during the ageing period, while analytical bitterness did not change. The reduction in foaminess was probably due to protein excretion, but the content of fatty acids impairing foaminess did not increase, as it will be discussed below.

The green beers were well-fermented, after 2 weeks of lagering the number of cells in the beer was at or below 100 thousand/ml. Between weeks 3 and 4, the beer cleared both in terms of coarse particles, yeast cells and trub (decrease in turbidity measured at angle 15 from about 10 EBC to 2–3 EBC) and fine colloidal particles (decrease in turbidity measured at angle 90 from about 3–4 EBC to 1–2 EBC) (Table 2). The filterability of the beers in terms of filtrate clarity improved slightly with ageing time for brew L2 (0.54–0.38 EBC), while no trend was evident for brew L1. Filtration rate, as assessed by the time required to filter 30 l of beer at the set input pressure on the filter, decreased for both brews, with a marked improvement observed for brew L1 after 5 weeks and for brew L2 after 3 weeks of lagering (Table 2).

Table 1 *Monitoring of basic analytical parameters of beers during maturation*

Parameter	Unit	Brew L1						Brew L2					
		GB	2W	3W	4W	5W	6W	GB	2W	3W	4W	5W	6W
Apparent extract	% w	2.88	2.75	2.75	2.74	2.72	2.74	4.84	3.39	3.41	3.28	3.33	3.32
Real extract	% w	4.50	4.38	4.37	4.39	4.36	4.39	6.89	4.89	4.94	4.77	4.85	4.82
Alcohol by weight	% w	3.47	3.50	3.48	3.54	3.53	3.54	4.53	3.21	3.28	3.2	3.26	3.26
Alcohol by volume	% v	4.44	4.47	6.45	4.52	4.52	4.52	5.84	4.12	4.2	4.1	4.18	4.17
Original extract	% w	11.26	11.20	11.14	11.27	11.24	11.27	15.50	11.14	11.31	11.01	11.20	11.18
Apparent attenuation	%	74.1	75.4	75.3	75.7	75.8	75.7	68.8	69.6	69.9	70.2	70.2	70.3
Real attenuation	%	60.0	60.9	60.8	61.1	61.2	61.1	55.5	56.1	56.4	56.7	56.7	56.7
Attenuation difference	%	4.1	2.8	2.9	2.5	2.3	2.5	6.4	5.6	5.3	5.0	5.0	4.9
pH		4.54	4.7	4.75	4.55	4.51	4.64	5.4	4.95	4.97	4.88	4.82	4.96
Colour	EBC	13.7	12.7	12.9	12.4	12.6	12.4	18.8	14.6	14.8	14.2	14.6	13.9
Bitterness	IBU	32	31	31	32	31	31	42	28	28	29	29	28
Foaming (NIBEM)	s/30 mm	–	276	258	270	258	244	–	270	257	240	244	229
Total polyphenols	mg/l	208	187	172	167	168	169	223	183	173	166	171	166
Carbon dioxide	g/l	–	5.6	5.1	5.7	5.6	5.6	–	5.6	5.6	5.6	5.7	5.7

Table 2 *Changes in filterability during the maturation of beer*

Parameter	Unit	Brew L1					Brew L2				
		2W	3W	4W	5W	6W	2W	3W	4W	5W	6W
Filtration time	min	9	8	8	6	4	12	8	9	7	6
Number of cells	thous/ml	113	50	<12.5	<12.5	13	50	13	<12.5	<12.5	<12.5
Haze input 90°	EBC	5.32	3.28	0.97	1.05	1.74	4.78	4.30	1.33	1.20	2.10
Haze output 90°	EBC	0.37	0.59	0.40	0.36	0.56	0.54	0.56	0.44	0.37	0.38
Haze input 15°	EBC	13.40	10.20	2.26	2.29	3.47	11.00	11.00	3.04	2.67	3.98
Haze output 15°	EBC	0.20	0.45	0.31	0.19	0.47	0.21	0.18	0.28	0.12	0.15

Haze input – unfiltered beer; Haze output – filtered beer

The natural colloidal stability of the beer is created during the lager and maturation process. Creating a stable product at this stage saves the cost of colloidal stabilization of beers with a long shelf life guarantee. Therefore, the shelf life of prepared colloidally unstabilised beers was monitored. All samples had a shelf life of over 6 months, the turbidity after this time was below 1 EBC unit, the limit above which colloidal turbidity can no longer be observed by the consumer. From the dynamics of haze formation during storage (Figure 1), it is clear that for brew L1, a significant improvement in colloidal stability occurred only after 6 weeks of storage. Brew L2 had a slightly better stability compared to brew L1, also in this case the improvement in shelf life is only evident at maturation for 6 weeks.

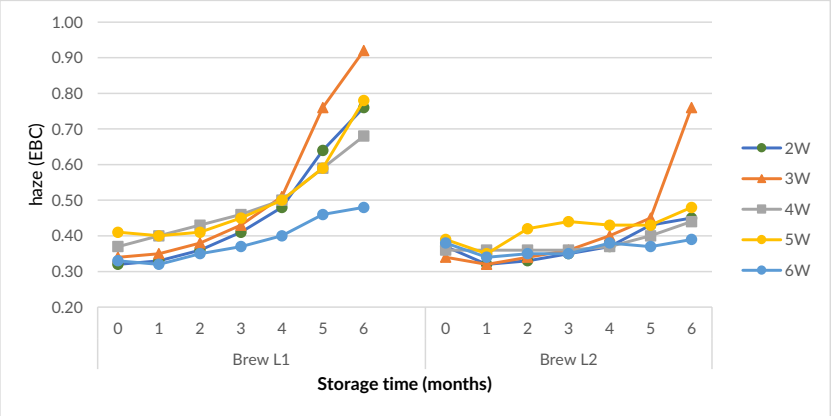

Figure 1 *Changes in shelf life during the maturation of beer.*
(2W–6W indicates the number of maturation weeks)

3.1 Sensory active substances

Sensory active volatiles, alcohols and esters are formed in yeast metabolism during fermentation depending on the precursors in the wort, yeast strain and fermentation conditions (Basařová et al., 2017; Dack et al., 2017). The contents of the discussed substances showed no clear trend in relation to the lagering time, with propanol and ethyl acetate increasing in the first two to three weeks of lagering compared to green beer, after which the values decreased again. For brew L1, an increase in the content of the sensory undesirable β-phenyl ethanol was observed in the last two weeks of lagering (Table S1, Figure 2).

Fatty acids are responsible for the deterioration of foaminess, long chain fatty acids are precursors of stale flavour aldehydes (Vanderhaegen et al., 2006), short chain fatty acids (C4 to C10) are also substances with sensory undesirable effects for pale lager (Basařová et al., 2017). The content of short chain fatty acids, increased compared to the green beer within 3 weeks of lagering, followed by a decrease in the L1 brew and after 6 weeks the value was at the level of the green beer (Figure 3). For brew L2, there was no decrease over the time interval studied, and after 6 weeks of lagering, L2 beer contained 34% more total short chain fatty acids compared to L1 beer. Capric and caprylic acids were the dominant compounds (Table S2). The trend for long chain fatty acids was slightly different, with a decrease in content up to 3 weeks of maturation, an increase between weeks 3 and 5, and then a decrease again.

Stale flavour aldehydes are the most important factor in the sensory ageing of beer. They are formed from precursors, amino acids and higher alcohols by the Strecker reaction (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde, phenylacetaldehyde), other aldehydes are formed from saturated fatty acids (hexanal, heptanal, octanal), unsaturated fatty acids (*trans*-2-butenal, *trans*-2-octenal, *trans*-2-nonenal) and carbohydrates (furfural) (Vanderhaegen et al., 2006).

These aldehydes are formed in significant quantities during malting and mashing due to oxidation and

thermal stress, and their content decreases during hopping, with the exception of furfural, due to the predominance of evaporation over de novo formation (Bustillo Trueba et al., 2021). During fermentation, their content is reduced by reduction in yeast metabolism (Saison et al., 2010) or by masking into complexes with sulphur dioxide (Baert et al., 2012). During beer storage, they are formed de novo by oxidation of the respective precursors or released from sulphite complexes (Baert et al., 2012). The results are presented in Table S3.

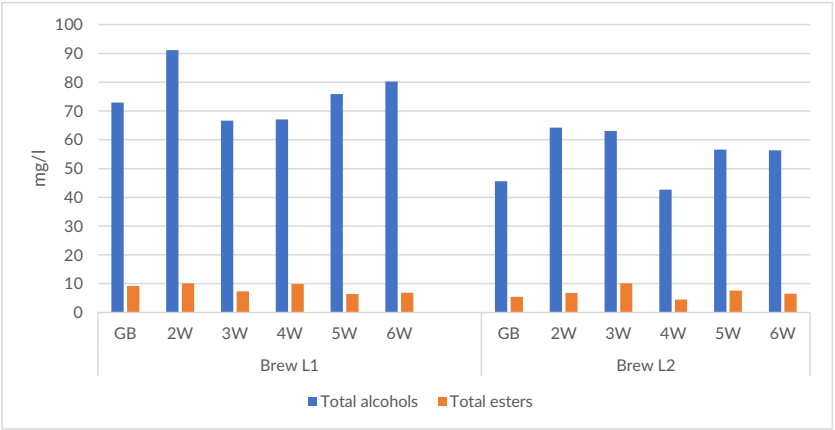

Figure 2 *Changes in volatiles during the maturation of beer.*
(GB – green beer; 2W–6W indicates the number of maturation weeks)

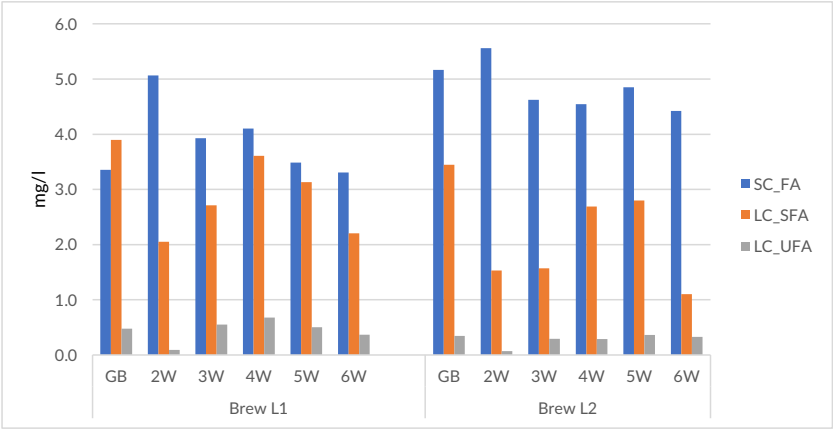

Figure 3 *Changes in short-chain and long-chain fatty acids during the maturation of beer.*
(SC_FA – short chain fatty acids; LC_SFA – long chain saturated fatty acids; LC_UFA – long chain unsaturated fatty acids; GB- green beer; 2W–6W indicates the number of maturation weeks)

Already after two weeks of lagering, both brews showed a reduction of Strecker aldehyde content by one order of magnitude compared to the green beer and a significant reduction of aldehydes originating from saturated fatty acids as well as the content of furfural (Table 3). On the other hand, a slight increase in both Strecker aldehydes and saturated fatty acid aldehydes was observed during the rest of the lagering period. The content of aldehydes from unsaturated fatty acids and furfural did not change. The concentrations of the aldehydes evaluated and the trends of their changes were comparable in the L1 and L2 brews. From the point of view of aldehyde reduction, a maturing period of 2 weeks is sufficient for Czech-style pale lager beers.

Many hop essential oils are known to undergo changes during the fermentation process as a result of biotransformation by yeasts or by sorption on the yeast cells (Haslbeck et al., 2017; Praet et al., 2012; Rettberg et al., 2006). After the main fermentation, a higher content of the sesquiterpenes β-caryophyllene, β-farnesene, α-humulene, the terpenic alcohol farnesol and the ester geranyl acetate were found. On the other hand, the myrcene and caryophyllene oxide contents decreased to about one-half and one-third of the wort value, respectively (Table S4).

Table 3 *Changes in grouped aldehydes from wort to 6 weeks of beer maturation*

Compounds	WO	GB	2W	3W	4W	5W	6W
	Brew L1						
STR	1317	1041	34	44	42	53	63
FA1	26	10	1.7	5.5	10.6	10.6	8.2
FA2	1.49	2.87	0.53	0.28	0.38	0.28	0.53
FUR	295	8.23	3.65	1.98	2.26	2.02	2.54
	Brew L2						
STR	1724	445	38	49	59	58	103
FA1	17.3	11.4	1.5	5.4	12.5	7.9	8.4
FA2	1.3	1.78	0.57	0.3	0.55	0.39	0.81
FUR	311	5.82	3.67	2.08	2.2	2.33	3.14

STR – Strecker aldehydes; FA1 – aldehydes from saturated fatty acids; FA2 – aldehydes from unsaturated fatty acids; FUR – furfural

During the maturation of the beers, a loss of about 40 to 95% of the essential oils compared to the green beer was found. Relatively low losses of 50–60% were observed for the monoterpenic alcohols linalool, α-terpineol, 4-terpineol, *cis*-geraniol and its derivatives methylgerenate and geranyl acetate. High losses, 80–95%, were observed for the sesquiterpenes β-caryophyllene, β-farnesene, α-humulene and β-caryophyllene epoxide. A major loss of sesquiterpenes during beer maturation occurred in 2 weeks of lagering, while the content of monoterpenic hydrocarbons and alcohols decreased gradually with lagering time (Figure 4).

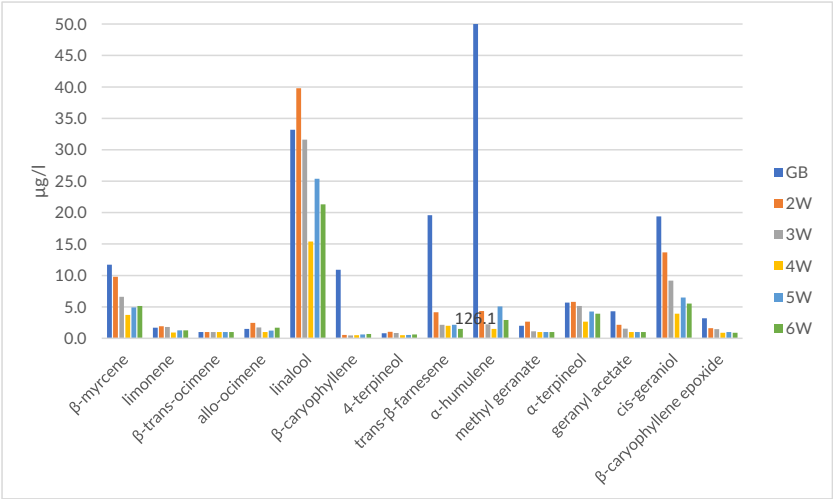

Figure 4 *Changes in selected essential oils during the maturation of beer*
(GB – green beer; 2W–6W indicates the number of maturation weeks)

Free amino acids in beer can be sensory active (Kabelova et al., 2008), some (alanine, phenylalanine tyrosine, valine, isoleucine, leucine) are precursors of Strecker's stale flavour aldehydes (Vanderhaegen et al., 2006). Some authors report (Moll, 1994) a several-fold increase in free amino acid content during fermentation and maturation of beer in the presence of yeasts. During the lagering of the beers of our experimental brews, the content of most free amino acids decreased compared to the green beer after two weeks of lagering and showed no further trend of increase or decrease (Table S5).

3.2 Sensorial analysis

The results of the sensory evaluation of the beers (Table 4) show a trend towards an increase in the value of the bitterness culmination, a slower decline in the bitterness, a less gentle bitterness character and also an increase in astringency between the 2nd and 4th week

Table 4 *Sensory quality of beers with different maturation times*

Parameter	Brew L1					Brew L2				
	2W	3W	4W	5W	6W	2W	3W	4W	5W	6W
Carbonation	2.6	2.6	1.9	2.2	2.6	2.4	2.6	1.9	1.9	2.1
Palate-fulness	2.6	2.5	2.9	2.8	2.4	2.8	2.8	2.9	2.8	2.4
Bitterness	2.4	2.5	2.6	2.1	2.3	2.4	2.4	2.5	1.9	2.0
Bitterness – culmination	3.1	3.4	3.6	2.9	3.2	3.1	3.1	3.4	2.6	2.9
Bitterness-aftertaste	2.3	2.3	2.6	1.8	1.9	2.0	1.9	2.6	1.8	1.7
Bitterness-character	2.6	3.1	3.0	2.3	2.4	2.6	2.7	2.6	2.7	2.3
Astringent	1.5	1.7	1.7	1.3	1.2	1.3	1.5	1.7	1.2	1.5
Sweet	1.5	1.6	1.6	1.5	1.4	1.8	1.7	1.9	2.0	1.7
Sour	2.3	2.1	2.2	1.9	1.4	1.8	1.6	2.0	1.6	1.6
Hoppy	1.7	1.7	2.0	1.2	1.7	1.9	1.6	2.2	1.3	1.7
Fruity/esteric	1.4	1.4	1.9	1.6	1.5	1.6	1.8	1.7	1.7	1.5
Yeasty	0.8	0.9	1.1	1.2	1.1	0.8	0.7	1.0	1.1	1.1
Balance	2.3	2.3	2.1	2.1	2.1	2.1	2.1	2.2	2.2	2.3
Acceptance	4.4	4.5	3.9	4.0	4.0	3.3	3.6	3.8	4.2	4.4
Overall impression	4.1	4.0	4.1	3.9	3.6	3.6	3.9	4.1	4.1	4.0

Descriptors – scale 0–5; Bala ce, Acceptance, Overall impression – descending scale 1–9

of ageing, followed by a decline again between the 4th and 6th week of maturation. The hop flavour intensity of the beers did not show any trend in relation to the decrease in the concentration of essential oils during the lagering period.

The balance, acceptability and overall impression of the beers improved slightly with lagering time in the L1 brew, while the trend was the opposite in the L2 brew. The sensory quality of the beers, as assessed by the overall impression, was fairly similar for both brews and ageing times (score 3.6–4.1 on a descending eight-point scale). In brew L1, the beer after 6 weeks of lagering was the best evaluated, while in brew L2 the beer after 2 weeks of lagering it was the best evaluated (Table 4). However, the ranking test did not show significant differences between the beers in relation to the duration of the lagering period.

4 Conclusion

The results of the study showed that, under certain conditions, the maturation time of common Czech-style lagers can be shortened by up to two weeks of cold lagering without significantly affecting the sensory quality and shelf life of the beer, but at the risk of poorer filterability. An improvement in shelf life, the natural colloidal stability, can be expected after six weeks or more of maturation. The sensory quality of beers brewed to original gravity improved slightly over six weeks of maturation, supporting the generally accepted view that a long maturation period is necessary to fine-tune the sensory profile of premium lagers. In contrast, a short two weeks maturation period appears to be beneficial for high gravity brewing. The results were obtained from relatively small pilot scale trials using common raw material types. On an operational scale in large volume vessels, the dynamics of changes in the monitored substances may be somewhat different, but the knowledge gained may still be a useful guide for optimising the maturation of Czech-style lager in production practice. This study provides some guidance on how a brewery should proceed in optimising the lagering time, what parameters to monitor and what negative consequences this could have.

5 Acknowledgement

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7 Supplements

Table S1 *Changes in the concentration of sensory active alcohols and esters during maturation (mg/l)*

Compound	Brew L1						Brew L2					
	GB	2W	3W	4W	5W	6W	GB	2W	3W	4W	5W	6W
propanol	8.08	7.02	9.9	7.48	7.96	7.77	4.17	6.8	6.87	3.6	5.71	5.79
isobutanol	6.83	9.94	6.3	6.57	6.4	6.9	4.29	7.19	6.54	3.7	5.6	5.57
2- and 3-methylbutanol	45.08	60.44	38.4	41.9	44.35	45.36	29.87	42.47	40.28	24.77	37.16	37.3
furfuryl alcohol	0.58	0.02	0.04	0.05	0.02	0.03	0.04	0.07	0.06	0.03	0.01	0.03
β-phenyl alcohol	12.38	13.69	12.01	11.05	16.99	19.98	7.14	7.56	9.24	10.47	8.1	7.55
ethyl hexanol	0.03	0.07	0.01	0.05	0.19	0.17	0.05	0.12	0.03	0.06	0.01	0.06
ethyl formate	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00
ethyl acetate	7.3	7.7	5.2	7.7	4.8	5.32	4.4	5.3	7.7	3.5	6.8	5.1
propyl acetate	0.02	0.013	0.012	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.006
isobutyl acetate	0.03	0.06	0.03	0.04	0.02	0.03	0.02	0.01	0.04	0.02	0.03	0.02
ethyl butyrate	0.07	0.19	0.06	0.07	0.05	0.06	0.04	0.12	0.08	0.04	0.06	0.05
butyl acetate	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.01
isoamyl acetate	0.74	1.3	0.76	0.78	0.47	0.36	0.45	0.39	0.97	0.44	0.058	0.45
ethyl caproate	0.26	0.32	0.24	0.25	0.14	0.17	0.15	0.15	0.22	0.07	0.1	0.1
ethyl lactate	0.08	0.12	0.05	0.03	0.044	0.06	0.07	0.16	0.06	0.05	0.05	0.05
ethyl caprylate	0.53	0.36	1.00	0.93	0.82	0.83	0.26	0.32	0.95	0.32	0.48	0.65
phenyl acetate	0.01	0.01	0.01	0.01	0.01	0.01	0.001	0.01	0.01	0.02	0.01	0.01
ethyl caprinate	0.01	0.01	0.01	0.01	0.01	0.01	0.001	0.01	0.01	0.01	0.01	0.01
phenylethyl acetate	0.086	0.03	0.011	0.02	0.001	0.003	0.04	0.21	0.06	0.01	0.01	0.002
ethyl laurate	0.001	0.01	0.001	0.001	0.01	0.01	0.001	0.01	0.001	0.001	0.001	0.01
ethyl myristate	0.007	0.01	0.001	0.001	0.001	0.001	0.001	0.01	0.003	0.001	0.001	0.005
ethyl palmitate	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.002	0.001	0.001	0.001

Table S2 *Changes in the concentration of fatty acids during maturation (mg/l)*

Fatty acid	Brew L1						Brew L2					
	GB	2W	3W	4W	5W	6W	GB	2W	3W	4W	5W	6W
isobutyric acid	0.08	0.30	0.09	0.08	0.09	0.08	0.12	0.57	0.11	0.13	0.13	0.12
butyric acid	0.06	0.30	0.09	0.08	0.09	0.11	0.09	0.39	0.10	0.10	0.09	0.15
isovaleric acid	0.22	0.64	0.26	0.27	0.27	0.24	0.37	0.30	0.34	0.38	0.40	0.38
valeric acid	0.01	0.04	0.01	0.01	0.01	0.02	0.01	0.06	0.02	0.02	0.02	0.02
caproic acid	1.08	2.60	1.24	1.27	1.12	1.11	1.56	1.93	1.39	1.39	1.46	1.38
caprylic acid	1.54	0.48	1.93	2.05	1.67	1.61	2.32	2.10	2.22	2.15	2.31	2.09
pelargonic acid	0.02	0.01	0.03	0.02	0.02	0.03	0.03	0.01	0.05	0.05	0.06	0.06
capric acid	0.34	0.70	0.28	0.32	0.22	0.12	0.67	0.20	0.39	0.33	0.38	0.23
lauric acid	0.96	0.47	0.26	0.36	0.23	0.11	1.00	0.42	0.31	0.27	0.34	0.15
myristic acid	0.09	0.11	0.06	0.09	0.01	0.10	0.07	0.10	0.06	0.11	0.13	0.07
palmitic acid	1.46	1.02	1.20	1.51	2.88	1.33	1.23	0.70	1.20	2.30	2.33	0.88
stearic acid	1.38	0.45	1.20	1.65	0.01	0.67	1.15	0.31	0.01	0.00	0.00	0.00
oleic acid	0.05	0.05	0.05	0.08	0.11	0.03	0.05	0.02	0.03	0.05	0.09	0.03
linoleic acid	0.12	0.01	0.14	0.10	0.18	0.06	0.10	0.01	0.09	0.10	0.12	0.15
linolenic acid	0.31	0.03	0.36	0.50	0.22	0.28	0.20	0.04	0.17	0.14	0.15	0.14

Table S3 *Changes in the concentration of aldehydes during maturation (µg/l)*

Aldehyde	Brew L1							Brew L2						
	WO	GB	2W	3W	4W	5W	6W	WO	GB	2W	3W	4W	5W	6W
2-methylpropanal	282	256	7.11	7.50	7.79	9.39	12.6	454	87	7.44	7.63	11.00	9.88	18.0
3-methylbutan-2-on	9.86	9.66	2.74	1.06	3.90	1.02	3.22	9.46	8.07	4.49	2.06	4.38	2.19	3.61
2-methylbutanal	179	287	3.70	5.56	6.29	6.55	8.16	264	210	4.06	5.53	7.20	7.21	12.50
3-methylbutanal	431	334	12.5	15.4	11.3	17.5	18.9	675	72	13.0	16.1	15.3	18.6	26.5
trans-2-butenal	1.40	2.84	0.49	0.25	0.37	0.26	0.52	1.22	1.75	0.52	0.27	0.53	0.33	0.79
hexanal	16.7	4.79	0.88	0.91	5.81	2.68	4.27	10.2	4.6	0.83	1.09	4.76	2.28	5.92
heptanal	3.95	1.93	0.34	0.44	0.71	0.77	0.66	2.65	2.53	0.20	0.52	0.81	0.75	0.70
octanal	5.37	3.24	0.44	4.14	4.06	7.12	3.26	4.42	4.25	0.48	3.81	6.94	4.90	1.77
furfural	295	8.23	3.65	1.98	2.26	2.02	2.54	311	5.82	3.67	2.08	2.20	2.33	3.14
trans-2-octenal	0.06	0.02	0.02	0.01	<0.01	0.01	<0.01	0.04	0.02	0.02	0.02	0.01	0.04	0.01
trans-2-nonenal	0.03	0.01	0.02	0.01	0.01	<0.01	0.01	0.04	0.01	0.02	0.01	0.02	0.02	0.01
benzaldehyde	14.6	4.30	3.20	2.85	4.18	3.47	3.74	13.4	6.5	3.17	3.63	6.48	3.43	5.61
phenylacetaldehyde	410	160	7.75	12.8	12.4	16.4	19.7	318	69	10.20	16.1	18.9	19.0	40.7

Table S4 *Changes in the concentration of essential oils during maturation (µg/l)*

Essential oil	Brew L1							Brew L2						
	WO	GB	2W	3W	4W	5W	6W	WO	GB	2W	3W	4W	5W	6W
α-pinene	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
β-pinene	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.34	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
β-myrcene	20.6	9.65	8.75	7.15	3.10	4.64	5.92	25.4	10.16	10.83	6.04	4.33	5.20	4.33
limonene	2.61	2.00	1.72	2.20	0.82	1.08	0.94	2.51	0.96	2.08	1.40	1.02	1.47	1.59
β-*trans*-ocimene	1.39	<1	<1	<1	<1	<1	<1	1.39	<1	1.01	<1	<1	<1	<1
allo-ocimene	1.79	1.36	1.69	1.44	<1	<1	1.68	2.53	1.22	3.21	2.03	<1	1.46	1.72
linalool	30.8	27.1	31.5	26.8	10.6	18.7	21.3	36.9	28.8	48.1	36.4	20.3	32.0	21.3
β-caryophyllene	4.68	11.39	0.50	<0.5	<0.5	0.69	<0.5	3.81	7.60	0.57	<0.5	<0.5	<0.5	0.84
4-terpineol	0.68	0.81	0.94	0.89	<0.5	<0.5	0.71	0.79	0.65	1.15	0.77	<0.5	0.54	<0.5
trans-β-farnesene	5.92	19.43	4.15	2.27	<2	2.29	<2	5.06	14.51	4.13	<2	<2	<2	<2
α-humulene	56.3	122	4.49	2.63	1.35	8.71	2.66	56.9	95	4.15	1.82	1.61	1.41	3.20
methyl geranate	1.02	1.36	2.25	1.21	<1	<1	<1	2.12	1.90	3.00	<1	<1	<1	<1
α-terpineol	6.86	4.76	5.18	5.09	2.12	3.69	3.94	8.13	4.94	6.45	5.22	3.18	4.88	3.90
geranyl acetate	<1	3.48	1.90	2.07	<1	<1	<1	<1	3.71	2.37	<1	<1	<1	<1
cis-geraniol	13.9	15.1	12.29	10.1	3.03	6.27	6.76	13.6	17.3	15.05	8.3	4.81	6.68	4.27
α-ionone	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<1	0.58	<0.5	<0.5	<0.5	<0.5	<0.5
β-ionone	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
α-irone	0.74	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	1.30	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
β-caryophyllene epoxide	12.4	3.85	1.40	1.56	0.75	0.90	0.87	10.1	1.84	1.81	1.31	1.00	1.07	0.94
farnesol	25.97	48.46	35.8	25.37	<20	<20	<20	22.50	53.10	33.0	<20	<20	<20	<20

Table S5 *Changes in the concentration of amino acids during maturation (mg/l)*

Amino acid	Brew L1							Brew L2						
	WO	GB	2W	3W	4W	5W	6W	WO	GB	2W	3W	4W	5W	6W
aspartic acid	86.3	47.1	39.5	41.3	50.4	50.8	44.0	83.9	59.5	70.0	60.1	46.4	61.9	51.4
glutamic acid	53.6	37.8	28.2	32.5	33.5	38.8	34.4	52.1	41.6	52.6	42.6	31.3	43.4	36.4
serine	114.9	10.1	12.1	10.8	31.1	12.2	10.8	98.9	26.7	29.0	29.7	23.9	28.7	24.3
glycine	68.6	40.0	45.3	45.8	48.7	47.2	45.9	59.9	52.0	55.5	57.0	45.2	53.5	34.5
histidine	58.9	33.8	38.4	38.6	44.0	38.8	39.1	62.3	39.4	40.4	41.8	33.5	39.7	33.9
threonine	92.9	49.2	46.3	48.7	50.0	50.7	49.3	87.0	56.4	56.2	56.4	45.7	56.9	48.6
arginine	420	382	349	356	367	367	364	452	381	399	401	315	382	323
alanine	280.3	69.0	81.1	77.7	122.0	82.6	111.5	200.5	90.0	97.7	99.8	103.9	96.0	101.7
proline	314.5	42.1	68.2	71.9	71.6	73.2	72.7	202.2	63.3	72.0	74.7	60.6	71.3	62.6
tyrosine	107.1	72.7	73.6	77.9	81.8	77.8	75.9	105.9	81.1	82.8	84.7	67.9	82.2	69.7
cysteine	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.1	0.0
valine	135.8	75.9	72.0	73.0	76.3	74.4	73.7	139.8	82.7	93.5	95.4	75.2	90.8	76.3
methionine	29.0	7.0	6.7	5.0	6.1	5.7	7.3	40.4	11.9	12.1	10.7	8.8	12.5	10.8
isoleucine	87.5	28.2	28.0	28.5	30.0	29.3	28.9	85.1	45.1	45.6	46.2	36.3	46.6	39.1
leucine	169.4	42.6	43.4	44.3	45.7	45.2	44.6	172.7	73.9	77.7	78.7	61.9	75.6	63.3
lysine	101.5	22.5	20.2	20.2	23.2	22.5	21.1	102.2	40.3	39.2	39.1	31.2	37.8	32.3
phenylalanine	144.8	65.3	71.2	74.2	75.5	73.3	72.6	138.6	88.6	92.3	94.0	74.8	90.4	76.6