Supplementary information

Figures

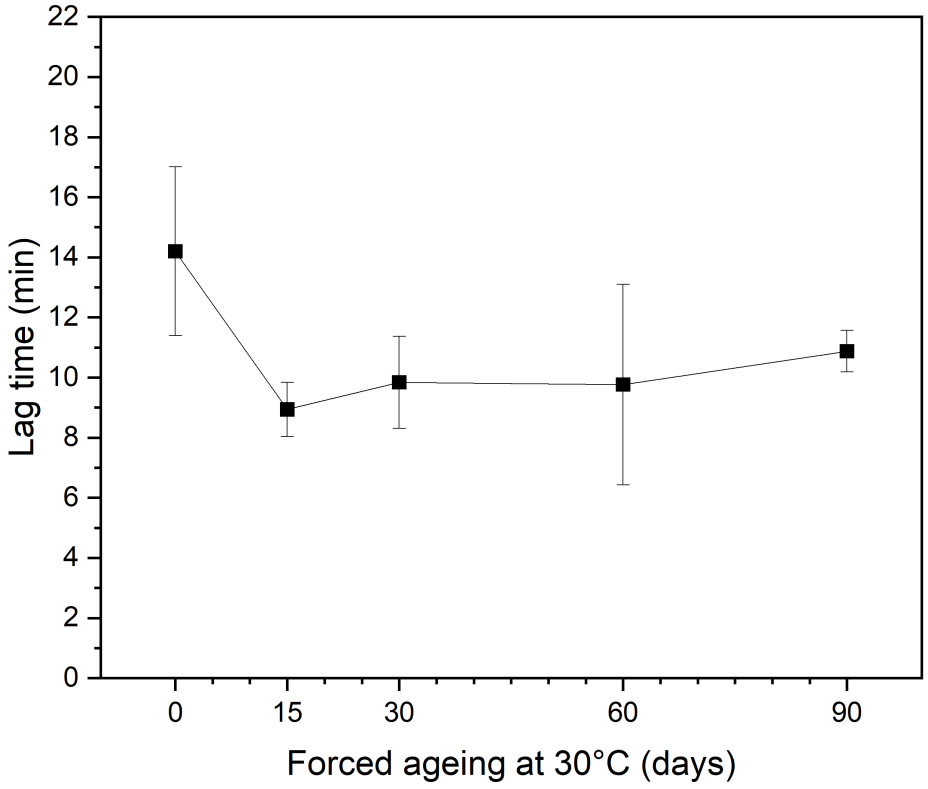

Figure S1: The ESR lag time in the produced beers as a function of storage time at 30°C. The data represent mean values and standard deviations.

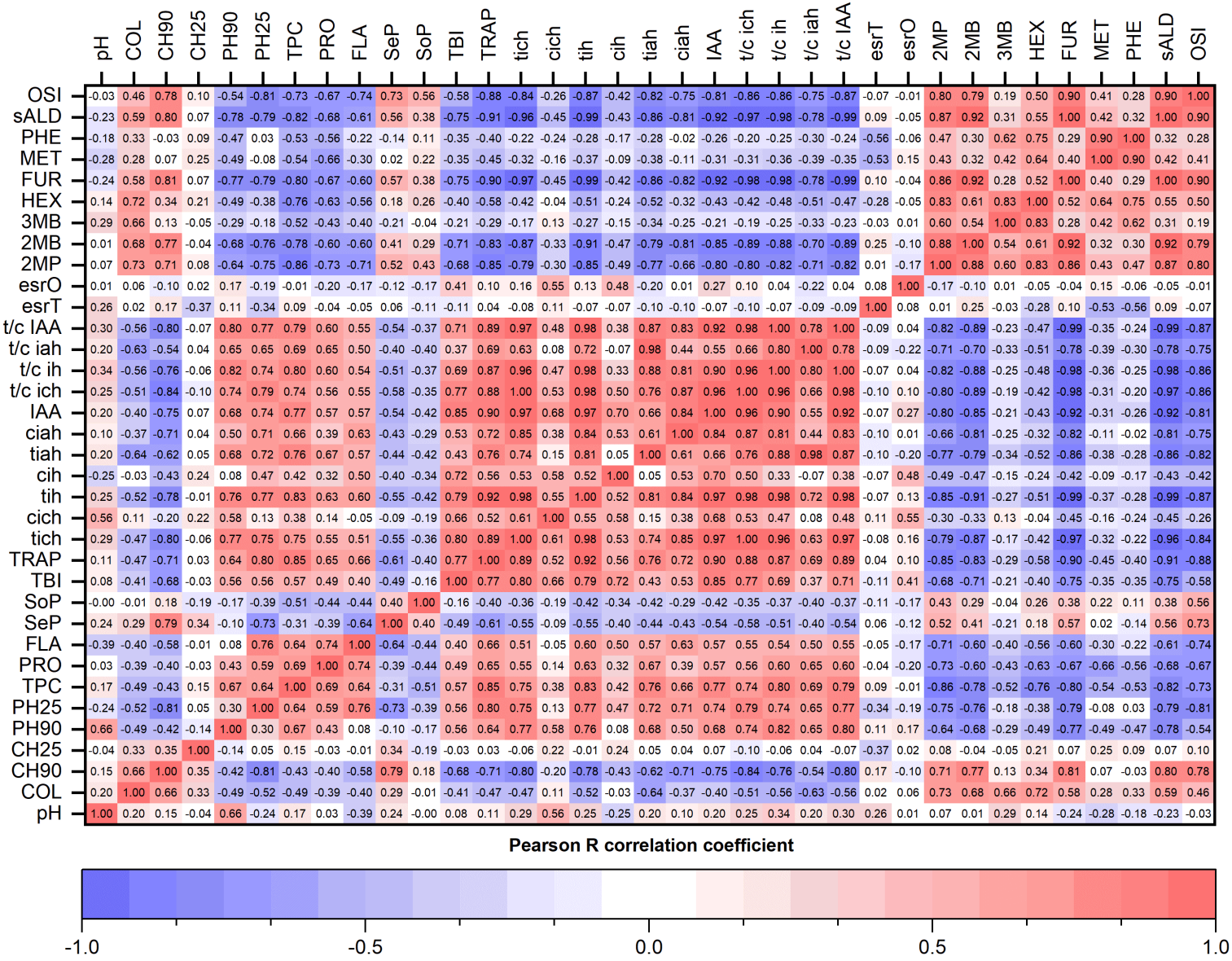
pH COL CH90 CH25 PH90 PH25 TPC PRO FLA SeP SoP TBI TRAP tich cich tih cih tiah ciah IAA t/c ich t/c ih t/c iah t/c IAA esrT esrO 2MP 2MB 3MB HEX FUR MET PHE sALD OSI
OSI -0.03 0.46 0.78 0.10 -0.54 -0.81 -0.73 -0.67 -0.74 0.73 0.56 -0.58 -0.88 -0.84 -0.26 -0.87 -0.42 -0.82 -0.75 -0.81 -0.86 -0.86 -0.75 -0.87 -0.07 -0.01 0.80 0.79 0.19 0.50 0.90 0.41 0.28 0.90 1.00
sALD -0.23 0.59 0.80 0.07 -0.78 -0.79 -0.82 -0.68 -0.61 0.56 0.38 -0.75 -0.91 -0.96 -0.45 -0.99 -0.43 -0.86 -0.81 -0.92 -0.97 -0.98 -0.78 -0.99 0.09 -0.05 0.87 0.92 0.31 0.55 1.00 0.42 0.32 1.00 0.90
PHE -0.18 0.33 -0.03 0.09 -0.47 0.03 -0.53 -0.56 -0.22 -0.14 0.11 -0.35 -0.40 -0.22 -0.24 -0.28 -0.17 -0.28 -0.02 -0.26 -0.20 -0.25 -0.30 -0.24 -0.56 -0.06 0.47 0.30 0.62 0.75 0.29 0.90 1.00 0.32 0.28
MET -0.28 0.28 0.07 0.25 -0.49 -0.08 -0.54 -0.66 -0.30 0.02 0.22 -0.35 -0.45 -0.32 -0.16 -0.37 -0.09 -0.38 -0.11 -0.31 -0.31 -0.36 -0.39 -0.35 -0.53 0.15 0.43 0.32 0.42 0.64 0.40 1.00 0.90 0.42 0.41
FUR -0.24 0.58 0.81 0.07 -0.77 -0.79 -0.80 -0.67 -0.60 0.57 0.38 -0.75 -0.90 -0.97 -0.45 -0.99 -0.42 -0.86 -0.82 -0.92 -0.98 -0.98 -0.78 -0.99 0.10 -0.04 0.86 0.92 0.28 0.52 1.00 0.40 0.29 1.00 0.90
HEX 0.14 0.72 0.34 0.21 -0.49 -0.38 -0.76 -0.63 -0.56 0.18 0.26 -0.40 -0.58 -0.42 -0.04 -0.51 -0.24 -0.52 -0.32 -0.43 -0.42 -0.48 -0.51 -0.47 -0.28 -0.05 0.83 0.61 0.83 1.00 0.52 0.64 0.75 0.55 0.50
3MB 0.29 0.66 0.13 -0.05 -0.29 -0.18 -0.52 -0.43 -0.40 -0.21 -0.04 -0.21 -0.29 -0.17 0.13 -0.27 -0.15 -0.34 -0.25 -0.21 -0.19 -0.25 -0.33 -0.23 -0.03 0.01 0.60 0.54 1.00 0.83 0.28 0.42 0.62 0.31 0.19
2MB 0.01 0.68 0.77 -0.04 -0.68 -0.76 -0.78 -0.60 -0.60 0.41 0.29 -0.71 -0.83 -0.87 -0.33 -0.91 -0.47 -0.79 -0.81 -0.85 -0.89 -0.88 -0.70 -0.89 0.25 -0.10 0.88 1.00 0.54 0.61 0.92 0.32 0.30 0.92 0.79
2MP 0.07 0.73 0.71 0.08 -0.64 -0.75 -0.86 -0.73 -0.71 0.52 0.43 -0.68 -0.85 -0.79 -0.30 -0.85 -0.49 -0.77 -0.66 -0.80 -0.80 -0.82 -0.71 -0.82 0.01 -0.17 1.00 0.88 0.60 0.83 0.86 0.43 0.47 0.87 0.80
esrO 0.01 0.06 -0.10 0.02 0.17 -0.19 -0.01 -0.20 -0.17 -0.12 -0.17 0.41 0.10 0.16 0.55 0.13 0.48 -0.20 0.01 0.27 0.10 0.04 -0.22 0.04 0.08 1.00 -0.17 -0.10 0.01 -0.05 -0.04 0.15 -0.06 -0.05 -0.01
esrT 0.26 0.02 0.17 -0.37 0.11 -0.34 0.09 -0.04 -0.05 0.06 -0.11 -0.11 0.04 -0.08 0.11 -0.07 -0.07 -0.10 -0.10 -0.07 -0.10 -0.07 -0.09 -0.09 1.00 0.08 0.01 0.25 -0.03 -0.28 0.10 -0.53 -0.56 0.09 -0.07
t/c IAA 0.30 -0.56 -0.80 -0.07 0.80 0.77 0.79 0.60 0.55 -0.54 -0.37 0.71 0.89 0.97 0.48 0.98 0.38 0.87 0.83 0.92 0.98 1.00 0.78 1.00 -0.09 0.04 -0.82 -0.89 -0.23 -0.47 -0.99 -0.35 -0.24 -0.99 -0.87
t/c iah 0.20 -0.63 -0.54 0.04 0.65 0.65 0.69 0.65 0.50 -0.40 -0.40 0.37 0.69 0.63 0.08 0.72 -0.07 0.98 0.44 0.55 0.66 0.80 1.00 0.78 -0.09 -0.22 -0.71 -0.70 -0.33 -0.51 -0.78 -0.39 -0.30 -0.78 -0.75
t/c ih 0.34 -0.56 -0.76 -0.06 0.82 0.74 0.80 0.60 0.54 -0.51 -0.37 0.69 0.87 0.96 0.47 0.98 0.33 0.88 0.81 0.90 0.96 1.00 0.80 1.00 -0.07 0.04 -0.82 -0.88 -0.25 -0.48 -0.98 -0.36 -0.25 -0.98 -0.86
t/c ich 0.25 -0.51 -0.84 -0.10 0.74 0.79 0.74 0.56 0.55 -0.58 -0.35 0.77 0.88 1.00 0.53 0.98 0.50 0.76 0.87 0.96 1.00 0.96 0.66 0.98 -0.10 0.10 -0.80 -0.89 -0.19 -0.42 -0.98 -0.31 -0.20 -0.97 -0.86
IAA 0.20 -0.40 -0.75 0.07 0.68 0.74 0.77 0.57 0.57 -0.54 -0.42 0.85 0.90 0.97 0.68 0.97 0.70 0.66 0.84 1.00 0.96 0.90 0.55 0.92 -0.07 0.27 -0.80 -0.85 -0.21 -0.43 -0.92 -0.31 -0.26 -0.92 -0.81
ciah 0.10 -0.37 -0.71 0.04 0.50 0.71 0.66 0.39 0.63 -0.43 -0.29 0.53 0.72 0.85 0.38 0.84 0.53 0.61 1.00 0.84 0.87 0.81 0.44 0.83 -0.10 0.01 -0.66 -0.81 -0.25 -0.32 -0.82 -0.11 -0.02 -0.81 -0.75
tiah 0.20 -0.64 -0.62 0.05 0.68 0.72 0.76 0.67 0.57 -0.44 -0.42 0.43 0.76 0.74 0.15 0.81 0.05 1.00 0.61 0.66 0.76 0.88 0.98 0.87 -0.10 -0.20 -0.77 -0.79 -0.34 -0.52 -0.86 -0.38 -0.28 -0.86 -0.82
cih -0.25 -0.03 -0.43 0.24 0.08 0.47 0.42 0.32 0.50 -0.40 -0.34 0.72 0.56 0.53 0.58 0.52 1.00 0.05 0.53 0.70 0.50 0.33 -0.07 0.38 -0.07 0.48 -0.49 -0.47 -0.15 -0.24 -0.42 -0.09 -0.17 -0.43 -0.42
tih 0.25 -0.52 -0.78 -0.01 0.76 0.77 0.83 0.63 0.60 -0.55 -0.42 0.79 0.92 0.98 0.55 1.00 0.52 0.81 0.84 0.97 0.98 0.98 0.72 0.98 -0.07 0.13 -0.85 -0.91 -0.27 -0.51 -0.99 -0.37 -0.28 -0.99 -0.87
cich 0.56 0.11 -0.20 0.22 0.58 0.13 0.38 0.14 -0.05 -0.09 -0.19 0.66 0.52 0.61 1.00 0.55 0.58 0.15 0.38 0.68 0.53 0.47 0.08 0.48 0.11 0.55 -0.30 -0.33 0.13 -0.04 -0.45 -0.16 -0.24 -0.45 -0.26
tich 0.29 -0.47 -0.80 -0.06 0.77 0.75 0.75 0.55 0.51 -0.55 -0.36 0.80 0.89 1.00 0.61 0.98 0.53 0.74 0.85 0.97 1.00 0.96 0.63 0.97 -0.08 0.16 -0.79 -0.87 -0.17 -0.42 -0.97 -0.32 -0.22 -0.96 -0.84
TRAP 0.11 -0.47 -0.71 0.03 0.64 0.80 0.85 0.65 0.66 -0.61 -0.40 0.77 1.00 0.89 0.52 0.92 0.56 0.76 0.72 0.90 0.88 0.87 0.69 0.89 0.04 0.10 -0.85 -0.83 -0.29 -0.58 -0.90 -0.45 -0.40 -0.91 -0.88
TBI 0.08 -0.41 -0.68 -0.03 0.56 0.56 0.57 0.49 0.40 -0.49 -0.16 1.00 0.77 0.80 0.66 0.79 0.72 0.43 0.53 0.85 0.77 0.69 0.37 0.71 -0.11 0.41 -0.68 -0.71 -0.21 -0.40 -0.75 -0.35 -0.35 -0.75 -0.58
SoP -0.00 -0.01 0.18 -0.19 -0.17 -0.39 -0.51 -0.44 -0.44 0.40 1.00 -0.16 -0.40 -0.36 -0.19 -0.42 -0.34 -0.42 -0.29 -0.42 -0.35 -0.37 -0.40 -0.37 -0.11 -0.17 0.43 0.29 -0.04 0.26 0.38 0.22 0.11 0.38 0.56
SeP 0.24 0.29 0.79 0.34 -0.10 -0.73 -0.31 -0.39 -0.64 1.00 0.40 -0.49 -0.61 -0.55 -0.09 -0.55 -0.40 -0.44 -0.43 -0.54 -0.58 -0.51 -0.40 -0.54 0.06 -0.12 0.52 0.41 -0.21 0.18 0.57 0.02 -0.14 0.56 0.73
FLA -0.39 -0.40 -0.58 -0.01 0.08 0.76 0.64 0.74 1.00 -0.64 -0.44 0.40 0.66 0.51 -0.05 0.60 0.50 0.57 0.63 0.57 0.55 0.54 0.50 0.55 -0.05 -0.17 -0.71 -0.60 -0.40 -0.56 -0.60 -0.30 -0.22 -0.61 -0.74
PRO 0.03 -0.39 -0.40 -0.03 0.43 0.59 0.69 1.00 0.74 -0.39 -0.44 0.49 0.65 0.55 0.14 0.63 0.32 0.67 0.39 0.57 0.56 0.60 0.65 0.60 -0.04 -0.20 -0.73 -0.60 -0.43 -0.63 -0.67 -0.66 -0.56 -0.68 -0.67
TPC 0.17 -0.49 -0.43 0.15 0.67 0.64 1.00 0.69 0.64 -0.31 -0.51 0.57 0.85 0.75 0.38 0.83 0.42 0.76 0.66 0.77 0.74 0.80 0.69 0.79 0.09 -0.01 -0.86 -0.78 -0.52 -0.76 -0.80 -0.54 -0.53 -0.82 -0.73
PH25 -0.24 -0.52 -0.81 0.05 0.30 1.00 0.64 0.59 0.76 -0.73 -0.39 0.56 0.80 0.75 0.13 0.77 0.47 0.72 0.71 0.74 0.79 0.74 0.65 0.77 -0.34 -0.19 -0.75 -0.76 -0.18 -0.38 -0.79 -0.08 0.03 -0.79 -0.81
PH90 0.66 -0.49 -0.42 -0.14 1.00 0.30 0.67 0.43 0.08 -0.10 -0.17 0.56 0.64 0.77 0.58 0.76 0.08 0.68 0.50 0.68 0.74 0.82 0.65 0.80 0.11 0.17 -0.64 -0.68 -0.29 -0.49 -0.77 -0.49 -0.47 -0.78 -0.54
CH25 -0.04 0.33 0.35 1.00 -0.14 0.05 0.15 -0.03 -0.01 0.34 -0.19 -0.03 0.03 -0.06 0.22 -0.01 0.24 0.05 0.04 0.07 -0.10 -0.06 0.04 -0.07 -0.37 0.02 0.08 -0.04 -0.05 0.21 0.07 0.25 0.09 0.07 0.10
CH90 0.15 0.66 1.00 0.35 -0.42 -0.81 -0.43 -0.40 -0.58 0.79 0.18 -0.68 -0.71 -0.80 -0.20 -0.78 -0.43 -0.62 -0.71 -0.75 -0.84 -0.76 -0.54 -0.80 0.17 -0.10 0.71 0.77 0.13 0.34 0.81 0.07 -0.03 0.80 0.78
COL 0.20 1.00 0.66 0.33 -0.49 -0.52 -0.49 -0.39 -0.40 0.29 -0.01 -0.41 -0.47 -0.47 0.11 -0.52 -0.03 -0.64 -0.37 -0.40 -0.51 -0.56 -0.63 -0.56 0.02 0.06 0.73 0.68 0.66 0.72 0.58 0.28 0.33 0.59 0.46
pH 1.00 0.20 0.15 -0.04 0.66 -0.24 0.17 0.03 -0.39 0.24 -0.00 0.08 0.11 0.29 0.56 0.25 -0.25 0.20 0.10 0.20 0.25 0.34 0.20 0.30 0.26 0.01 0.07 0.01 0.29 0.14 -0.24 -0.28 -0.18 -0.23 -0.03
Pearson R correlation coefficient
-1.0 -0.5 0.0 0.5 1.0

Figure S2: Pearson R correlation matrix of the investigated parameters colour (COL), cold haze at 90° (CH90), cold haze at 25° (CH25), permanent haze at 90° (PH90), permanent haze at 25° (PH25), total polyphenol content (TPC), proanthocyanidins (PRO), flavanoids (FLA), sensitive proteins (SeP), soluble proteins (SoP), thiobarbituric acid index (TBI), total radical-trapping antioxidant potential (TRAP), *trans*-isocohumulone (tich), *cis*-isocohumulone (cich), *trans*-isohumulone (tih), *cis*-isohumulone (cih); *trans*-isoadhumulone (tiah), *cis*-isoadhumulone (ciah), total iso-α-acids (IAA), *trans*/*cis*-iso-cohumulone (t/c ich), *trans*/*cis*-iso-humulone (t/c ih), *trans*/*cis*-iso-adhumulone (t/c iah), *trans*/*cis*-iso-α-acids (t/c IAA), ESR lag time (esrT),ESR rate of radical formation (esrO), 2-methylpropanal (2MP) 2-methylbutanal (2MB), 3-methylbutanal (3MB), hexanal (HEX), furfural (FUR), methional (MET), phenylacetaldehyde (PHE) sum of volatile aldehydes (sALD), sensory evaluated staleness intensity (OSI)**.**

Tables

Table S1: Ranking of the beer samples (n=49) representing ageing categories according to the decreasing intensity of overall staleness. Statistical significance was evaluated by Friedman's nonparametric test followed by Wilcoxon pairwise comparison test.

Aging time	Mean rank*	Group**
Fresh	1.50	A
15 days	1.39	A
30 days	1.80	A
60 days	2.67	B
90 days	2.61	B

* Friedman's test statistics: chi^2 38.27, degrees of freedom 4, asymptotical sign. 0.000

**Wilcoxon test; asymptotical sign. according to Bonferroni correction ($P<0.05$)

Table S2: Relative change (%) in the determined beer parameters from fresh to 90 days at 30°C. Statistical significance was evaluated by ANOVA test $p<0.05$. Parameters for which no statistical differences were identified were marked as [N.S.]. Parameters, which were under detection limit were stated as <LOD.

Standard parameters	
pH [N.S.]	-1
Colour	11
Sensitive proteins	19
Soluble proteins [N.S.]	6
TBI	-5
Haze parameters	
Cold haze 90°	130
Cold haze 25° [N.S.]	10
Permanent haze 90°	-44
Permanent haze 25°	-65
Polyphenols	
Total polyphenol content	-13
Proanthocyanidins [N.S.]	-17
Flavanoids	-10
TRAP	-7
Oxidative stability	
ESR lag time [N.S.]	2
ESR rate of radical formation [N.S.]	-2
Sulphites	< LOD
Thiols	< LOD

Bitter acids	
trans-iso-Cohumulone	-47
cis-iso-Cohumulone [N.S.]	-3
trans-iso-Humulone	-49
cis-iso-Humulone [N.S.]	-6
trans-iso-Adhumulone	-31
cis-iso-Adhumulone	-7
Total iso-α-acids	-16
Volatile aldehydes	
2-Methylpropanal	281
2-Methylbutanal	78
3-Methylbutanal	23
Methional	61
Phenylacetaldehyde	29
Furfural	25606
Hexanal	178
trans-2-Nonenal	< LOD
Cysteinylated aldehydes	
All cysteinylated markers	< LOD
Sensory	
Staleness Intensity	99