Sönke Kienitz, Christoph Neugrodda, Thomas Becker

Impact of reverse-osmosis process parameters on the physicochemical, aroma and sensory profile of non-alcoholic lager beer

Reverse-osmosis (RO) dealcoholization of lager beer was investigated in two separate one-factor series. The concentration degree (CD) at the start of diafiltration (SD) was set to 60, 50, 40 or 30 % of the original volume (OV) at 288 K. Process temperature was varied from 283 to 293 K at a constant CD of 50 % OV. All trials were performed at a retentate flow of 163 L h^{-1} and a permeate-flow setting of 25 L h^{-1}. Reducing CD from 60 to 30 % OV decreased diafiltration-water consumption from 191.2 to 127.3 L and total process time from 542 to 432 min. One-way ANOVA confirmed significant effects on both responses ($p < 0.001$). Increasing temperature from 283 to 293 K reduced total process time from 567 to 400 min ($p < 0.001$), while diafiltration-water consumption decreased from 168.2 to 157.9 L ($p = 0.019$). After blending to a common real-extract target, lower CD values resulted in higher viscosity, turbidity and colour. Lower process temperature resulted in higher concentrations of acetate esters, ethyl esters, terpene alcohols and higher alcohols and higher floral, citrus-like, hoppy-spicy and fruity sensory intensities. CD therefore affected water demand and the physicochemical composition of the final non-alcoholic beer, whereas temperature strongly affected process duration, pressure requirement and volatile retention.

Descriptors: Non-alcoholic beer, reverse osmosis, dealcoholization, process parameters, aroma compounds, odour activity value

1 Introduction

The brewing industry is facing declining volumes of conventional beer in several established markets while non-alcoholic beer (NAB) continues to grow. Global and European market reports describe a markedly more positive development for alcohol-free beer than for conventional beer [1, 2, 3]. In Germany, production of alcohol-free beer almost doubled between 2014 and 2024 while total alcoholic beer production declined over the same period [4]. Product quality is therefore increasingly relevant because consumer acceptance depends strongly on taste and on the perceived similarity of NAB to conventional beer [5].

Non-alcoholic beer can be produced by biological restriction of ethanol formation or by physical removal of ethanol from fully fermented beer [6, 7, 8]. Physical dealcoholization offers the advantage that fermentation can proceed conventionally before ethanol is removed, but this benefit is accompanied by the risk of losing volatile aroma compounds and altering non-volatile matrix constituents [9, 10, 11]. Commercial and experimental surveys have shown that physically dealcoholised beers often exhibit lower aroma intensity and reduced body compared with their alcoholic counterparts, although the extent depends on the process, beer matrix and any subsequent blending step [11, 12, 13].

Reverse osmosis (RO) is an established membrane-based route for beer dealcoholization and is typically operated at low temperature to limit thermal reactions [14, 15, 16]. Batch RO dealcoholization comprises an initial concentration phase followed by constant-volume diafiltration. During diafiltration, deaerated water replaces permeate while ethanol is removed [46, 47]. The required process time and diafiltration-water volume depend on the concentration degree (CD) at the start of diafiltration (SD) because the retentate volume determines the liquid inventory during diafiltration. Lower CD values correspond to a smaller retentate volume and higher concentrations of retained solutes. The resulting increase in viscosity and osmotic pressure can increase the transmembrane pressure (TMP) required at a given permeate-flow setpoint [47, 48].

Temperature and hydraulic operating conditions affect both process performance and product composition. Increasing temperature changes water and solute mobility in polyamide RO membranes [21, 22]. In beer dealcoholization, higher temperature increased permeate flux and reduced solute rejection. Pressure and feed flow also changed alcohol and ester rejection [23, 24]. Studies

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with model aroma solutions likewise found effects of pressure, feed flow, temperature and feed composition on aroma rejection [23, 24, 25, 26]. In low- and no-alcohol wine, RO pressure altered volatile retention and sensory properties [27].

Beer-matrix composition also affects RO transport. Polyphenols, polysaccharides and proteins can contribute to membrane deposits and alter hydraulic resistance [28, 29]. Arabinoxylan and β-glucan are relevant for viscosity and filtration behaviour, while high-molecular-weight arabinoxylan can contribute to the mouthfeel of NAB [30, 31, 32]. Changes that reduce process time can therefore occur together with changes in the retention of volatile and non-volatile beer constituents.

Previous beer studies found compound-specific retention of higher alcohols, esters and hop-derived compounds during RO [9, 10]. For neutral organic solutes, permeation through thin-film composite polyamide membranes depends on partitioning into the active layer and diffusion through the polymer. Molecular dimensions, functional groups and solute-membrane interactions affect these transport steps [33, 34, 35, 36]. Process parameters can therefore influence aroma retention by changing bulk concentration, membrane-surface concentration, TMP and the transport of water and solutes through the active layer.

The objective of this study was to determine how concentration degree (CD) at the start of diafiltration (SD) and process temperature influence reverse-osmosis dealcoholization of lager beer. Process performance was quantified by total dealcoholization time, transmembrane pressure (TMP) at the end of diafiltration and diafiltration-water consumption. The main focus was the physicochemical, volatile and sensory composition of the final blended non-alcoholic beer (NAB) after adjustment to a common real-extract target. Aroma changes were evaluated as percentage concentration differences of chemically related compound groups, compound-specific odour activity values (OAVs) and descriptive sensory attributes.

2 Materials and methods

2.1 Dealcoholization process

Lager beer was dealcoholised using an AromaPlus® RO pilot plant (GEA Group Aktiengesellschaft, Düsseldorf, Germany) equipped with one AromaPlus® 3838 spiral-wound thin-film composite polyamide on polysulfone membrane. The active membrane area was 7.0 m^2 and the specified average NaCl rejection was 95 %. Reverse osmosis was selected because it enables ethanol removal at low process temperature and has previously been applied to beer with substantial retention of selected aroma compounds [10, 15].

Dealcoholization trials were performed as biological duplicates. Two separate one-factor parameter series were investigated. Concentration degree (CD) at the start of diafiltration (SD) was expressed as the remaining retentate volume relative to the original volume (OV) and was set to 60, 50, 40 or 30 % OV at 288 K. These settings correspond to theoretical volumetric concentration ratios of 1.67, 2.00, 2.50 and 3.33, respectively. Process temperature was varied between 283 and 293 K at a constant CD of 50 % OV. For both series, the retentate flow was set to 163 L h^{-1} and the permeate-flow setpoint was 25 L h^{-1} (see table 1). The 50 % OV and 288 K condition occurred in both series, but each series was treated as a separate experimental block.

Table 1 **Set process parameters for the reverse-osmosis dealcoholization trials using the AromaPlus® pilot plant. CD denotes concentration degree at the start of diafiltration (SD) and is expressed as percentage of the original volume (OV). Retentate flow and permeate-flow setting were kept constant in both process-parameter series**

Trial series	CD at SD [% OV]	Temperature [K]	Retentate flow [L h^{-1}]	Permeate flow [L h^{-1}]
Concentration degree	60, 50, 40, 30	288	163	25
Temperature	50	293, 288, 283	163	25

During the concentration phase, permeate removal was continued to the respective target volume. Diafiltration water was deaerated to < 0.1 ppm O_2 using a DGS 10 membrane-deaeration unit (CENTEC GmbH, Maintal, Germany) with CO_2 as stripping gas. Constant-volume diafiltration was continued until the retentate ethanol concentration was < 0.5 % vol. alc. Ethanol was monitored using a Cobrix 5600 Multibev inline sensor (Anton Paar, Graz, Austria). Total process time, diafiltration-water consumption and TMP at the end of diafiltration (RED) were documented. The final retentates were blended to 4.0 % real extract with deaerated brewing water. The produced NAB were carbonated to 5.5 g L^{-1} CO_2, bottled, pasteurised at 336 K to 30 pasteurisation units and stored at 277 K.

After each experiment, the plant was cleaned with P3-ultrasil 132 for alkaline precleaning for 30 min at 313 K, P3-ultrasil 62A for enzymatic cleaning for 45 min at 313 K and Ultrasil 78 for acidic cleaning for 30 min at 313 K (Ecolab GmbH, Reinach, Switzerland). The system was disinfected with P3-oxonia active for 15 min at 293 K and conditioned with P3-ultrasil 73. Before each trial, membrane performance was checked with brewing water at 288 K. Post-cleaning water-flux values ranged from 95 to 105 % of the initial reference value. The same membrane element was used for all trials to avoid element-to-element variability.

2.2 Physicochemical and aroma-compound analyses

Alcohol, density and extract were determined according to MEBAK B-590.10.181. Viscosity was analysed according to MEBAK B-590.05.281, pH according to MEBAK B-590.00.040, 90 ° turbidity according to MEBAK B-430.01.732, colour according to MEBAK B-400.03.115, β-glucan (BG) according to MEBAK B-400.26.112 and iso-α-acids according to MEBAK B-500.02.131. Arabinoxylan (AX) was determined using a modified Douglas method [37, 38]. Mother beer (MB), RED retentates and the blended NAB were analysed. Each analytical determination was performed in triplicate for each biological process replicate.

Sample preparation and headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME GC-MS) analysis followed the validated method described previously [9]. Samples

Table 2 **Mean values ± standard deviation of physicochemical properties, transmembrane pressure (TMP) at the end of diafiltration (RED), total dealcoholization time and diafiltration-water volume (VDia) for the mother beer (MB) and end-of-diafiltration retentates. Process trials were performed as biological duplicates and analytical determinations were performed in triplicate. OV denotes original volume**

Sample	Original extract	Real extract	Alcohol	pH	ρ	η	90° turbidity	Colour	BG	AX	Total polyphenols	FAN	Iso-α-acid	TMP EDF	Total time	V_{Dia}
	[°P]	[m.-%]	[vol.-%]	[-]	[g cm^{-3}]	[mPa s]	[EBC]	[EBC]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[bar]	[min]	[L]
MB	11.55 ± 0.220	2.59 ± 0.026	4.92 ± 0.019	4.55 ± 0.020	1.009 ± 0.0001	1.54 ± 0.019	0.68 ± 0.02	9.0 ± 0.12	153 ± 10.3	420 ± 15.7	147 ± 15.8	108 ± 2.1	12.3 ± 0.15	-	-	-
60 % OV	7.28 ± 0.100	6.30 ± 0.050	0.48 ± 0.020	4.71 ± 0.091	1.024 ± 0.0011	1.48 ± 0.160	2.5 ± 0.06	11.4 ± 0.16	204 ± 7.5	538 ± 13.8	201 ± 9.7	152 ± 2.4	18.5 ± 0.54	6.9 ± 0.11	542 ± 5.8	191.2 ± 2.15
50 % OV	8.22 ± 0.155	7.56 ± 0.152	0.47 ± 0.010	4.64 ± 0.050	1.030 ± 0.0015	1.56 ± 0.187	3.8 ± 0.08	13.3 ± 0.18	234 ± 8.8	580 ± 21.7	218 ± 12.6	153 ± 5.1	19.9 ± 0.41	7.2 ± 0.05	498 ± 10.2	162.7 ± 1.87
40 % OV	9.33 ± 0.145	8.69 ± 0.105	0.48 ± 0.020	4.61 ± 0.055	1.046 ± 0.0100	2.19 ± 0.205	5.6 ± 0.05	20.5 ± 0.22	241 ± 7.4	592 ± 20.5	230 ± 10.1	169 ± 5.5	21.2 ± 0.17	7.6 ± 0.13	458 ± 8.7	138.3 ± 1.90
30 % OV	9.81 ± 0.155	9.15 ± 0.099	0.46 ± 0.020	4.59 ± 0.032	1.050 ± 0.0020	2.55 ± 0.231	7.1 ± 0.10	26.5 ± 0.30	251 ± 8.5	610 ± 22.1	243 ± 11.5	180 ± 5.8	21.8 ± 0.25	8.2 ± 0.14	432 ± 6.3	127.3 ± 2.80
293 K	8.13 ± 0.142	7.48 ± 0.160	0.49 ± 0.050	4.59 ± 0.091	1.029 ± 0.0014	1.52 ± 0.170	3.5 ± 0.08	13.0 ± 0.18	229 ± 8.5	465 ± 20.9	214 ± 12.0	151 ± 4.9	16.8 ± 0.49	5.2 ± 0.13	400 ± 4.7	157.9 ± 2.1
288 K	8.22 ± 0.155	7.56 ± 0.152	0.47 ± 0.010	4.64 ± 0.050	1.030 ± 0.0015	1.77 ± 0.187	3.8 ± 0.08	13.3 ± 0.18	234 ± 8.8	470 ± 21.7	218 ± 12.6	153 ± 5.1	19.9 ± 0.41	7.2 ± 0.05	498 ± 10.2	162.7 ± 1.87
283 K	8.23 ± 0.100	7.57 ± 0.105	0.47 ± 0.014	4.68 ± 0.055	1.031 ± 0.0016	2.05 ± 0.199	4.1 ± 0.08	13.6 ± 0.20	239 ± 9.1	478 ± 22.1	222 ± 12.0	156 ± 5.2	24.2 ± 0.17	8.0 ± 0.12	567 ± 7.2	168.2 ± 0.59

were spiked with an internal-standard solution containing ethyl 2-methylpentanoate as standard 1 and menthol as standard 2. Volatile aroma compounds were extracted using a divinylbenzene/carbon wide range/polydimethylsiloxane (DVB/C-WR/PDMS) SPME Arrow at 313 K for 30 min and subsequently analysed by GC-MS. Matrix-matched calibration is important for beer headspace methods because ethanol and non-volatile matrix constituents affect partitioning and fibre response [39, 40].

2.3 Odour activity values and aroma-group evaluation

Odour activity values were calculated for each quantified aroma compound by dividing the measured concentration by its literature odour threshold. The thresholds were taken from the compound-specific references compiled and validated in the previously published HS-SPME GC-MS method [9]. Where a threshold range was available, the lower value of that range was used consistently with that method to provide a sensitive screen for potential odour activity. This approach follows the established use of concentration-to-threshold ratios in beer aroma research (eq. 2-1) [41, 42].

$$OAV_i = \frac{c_i}{OT_i} \qquad 2\text{-}1$$

where c_i is the measured concentration of aroma compound i and OT_i is the corresponding odour threshold in the same concentration unit. An OAV ≥ 1 was interpreted as an indication that the compound can potentially contribute directly to aroma. OAV was not interpreted as a direct sensory-intensity scale because mixture effects, suppression and synergistic interactions can modify perception [42, 43, 44].

For a class-level assessment of process-dependent changes in volatile composition, the quantified compounds were assigned to acetate esters, ethyl esters, terpene alcohols, higher alcohols and sulphur compounds. The compound grouping followed the analytical classification used in the validated HS-SPME GC-MS method [9]. The percentage concentration difference of each compound group relative to the MB was calculated as follows (eq. 2-2):

$$\Delta C_{group}[\%] = \left[\left(\frac{\Sigma C_{sample}}{\Sigma C_{MB}}\right) - 1\right] \cdot 100 \qquad 2\text{-}2$$

where positive values indicate a higher summed concentration than in the MB and negative values indicate a lower summed concentration. The sulphur group comprised dimethyl sulphide (DMS) and dimethyl trisulphide (DMTS). Because their odour thresholds differ

Table 3 **Relative differences in total dealcoholization time, diafiltration-water consumption and end-of-diafiltration transmembrane pressure within each process-parameter series. The central condition in each series was used as the series-specific reference**

Series	Setting	Δ total time [%]	Δ diafiltration water [%]	Δ TMP EDF [%]
Concentration degree	60 % OV	8,8	17,5	–4,2
Concentration degree	50 % OV	0	0	0
Concentration degree	40 % OV	-8	–15	5,6
Concentration degree	30 % OV	–13,3	–21,8	13,9
Temperature	293 K	–19,7	–3	–27,8
Temperature	288 K	0	0	0
Temperature	283 K	13,9	3,4	11,1

Table 4 **Theoretical volumetric concentration ratio and measured concentration ratio of selected non-volatile constituents relative to the theoretical ratio at the end of diafiltration. The ratio was calculated as shown and is used as a descriptive concentration benchmark rather than as a density-corrected mass balance.** $\left(\frac{c_{RET}}{c_{MB}}\right)/\left(\frac{1}{f_{OV}}\right) \cdot 100$

CD at SD [% OV]	Theoretical ratio	Expected increase [%]	BG / theoretical [%]	AX/theoretical [%]	Polyphenol/ theoretical [%]	FAN/ theoretical [%]	Iso-α-acid/ theoretical [%]
60 % OV	1,67	66,7	80	76,9	82	84,4	90,2
50 % OV	2	100	76,5	69	74,1	70,8	80,9
40 % OV	2,5	150	63	56,4	62,6	62,6	68,9
30 % OV	3,33	233,3	49,2	43,6	49,6	50	53,2

Table 5 **Mean values ± standard deviation of selected physicochemical parameters in the blended non-alcoholic beers after adjustment to approximately 4.0 % real extract. Values represent biological duplicates. BG denotes β-glucan and AX denotes arabinoxylan**

NAB	Real extract	Alcohol	Viscosity	90° turbidity	Colour	BG	AX	Iso-α-acid
	[m.-%]	[vol.-%]	[mPa s]	[EBC]	[EBC]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]
60 % OV	3.97 ± 0.16	0.302 ± 0.022	1.304 ± 0.110	1.58 ± 0.08	7.18 ± 0.32	128.6 ± 8.8	339.1 ± 19.4	11.66 ± 0.71
50 % OV	4.03 ± 0.13	0.251 ± 0.008	1.299 ± 0.103	2.02 ± 0.06	7.09 ± 0.18	124.8 ± 6.2	309.2 ± 15.1	10.61 ± 0.34
40 % OV	3.96 ± 0.12	0.219 ± 0.013	1.544 ± 0.103	2.56 ± 0.08	9.36 ± 0.28	110.0 ± 5.4	270.2 ± 14.3	9.67 ± 0.25
30 % OV	4.07 ± 0.13	0.204 ± 0.013	1.690 ± 0.118	3.15 ± 0.12	11.77 ± 0.40	111.5 ± 6.3	271.1 ± 15.8	9.69 ± 0.33
293 K	3.95 ± 0.17	0.259 ± 0.031	1.276 ± 0.096	1.85 ± 0.08	6.87 ± 0.25	121.0 ± 7.1	245.7 ± 16.3	8.87 ± 0.45
288 K	4.02 ± 0.16	0.250 ± 0.010	1.411 ± 0.107	2.02 ± 0.08	7.07 ± 0.23	124.5 ± 7.0	249.9 ± 16.2	10.59 ± 0.42
283 K	4.04 ± 0.21	0.251 ± 0.016	1.562 ± 0.127	2.19 ± 0.12	7.25 ± 0.37	127.5 ± 9.5	254.9 ± 21.1	12.90 ± 0.57

Table 6 **One-way ANOVA and Tukey HSD results for total dealcoholization time and diafiltration-water consumption as affected by concentration degree (CD) at the start of diafiltration (SD) and process temperature. Degrees of freedom (df) are reported as model df, error df. F denotes the ANOVA F statistic. Eta squared (η^2) is the effect-size measure and represents the proportion of total variance associated with the investigated process parameter in the present dataset. Different superscript letters within a process-parameter series indicate significant pairwise differences according to Tukey's HSD test at $p < 0.05$**

	t_{tot}					V_{dia}			
Process parameter	df	F	p	η^2	Tukey HSD	F	p	η^2	Tukey HSD
CD	3, 4	72,57	< 0.001	0,982	60^a, 50^b, 40^c, 30^c	334,49	< 0.001	0,996	60^a, 50^b, 40^c, 30^d
T	2, 3	238,08	< 0.001	0,994	293^a, 288^b, 283^c	19,44	0,019	0,928	293^a, 288^{ab}, 283^b

by orders of magnitude, the summed sulphur concentration was interpreted together with the compound-specific OAV rather than as a direct indicator of sulphury aroma [9, 45].

2.4 Sensory analysis

Mother beer and NAB samples were stored at 277 K under light-protected conditions and were evaluated within three weeks after bottling. A trained panel (n = 12) assessed the samples using a rate-all-that-apply (RATA) procedure with intensity ratings from 0, not perceptible, to 5, very high intensity. The procedure followed DIN 10964 and the tasting rooms complied with DIN EN ISO 8589. The process-parameter sensory comparison reported in this study was performed exclusively with the final blended NABs. End-of-diafiltration retentates were not included in the sensory evaluation.

2.5 Statistical analysis

The dealcoholization experiments were conducted as biological duplicates. Each physicochemical analytical determination was performed in triplicate. Technical triplicates were averaged within each biological process replicate before inferential analysis to avoid pseudoreplication. The effects of CD at SD and process temperature on total dealcoholization time and diafiltration-water consumption were evaluated separately by one-way analysis of variance (ANOVA) using OriginPro 2021b (OriginLab Corporation, Northampton, MA, USA). When the omnibus ANOVA was significant, Tukey's honestly significant difference (HSD) test was applied for pairwise comparisons at $\alpha = 0.05$. Degrees of freedom (df) are reported as model df and error df. Effect size was expressed as eta squared (η^2), calculated as the factor sum of squares divided by the total sum of squares. Because each process level comprised only two biological replicates,

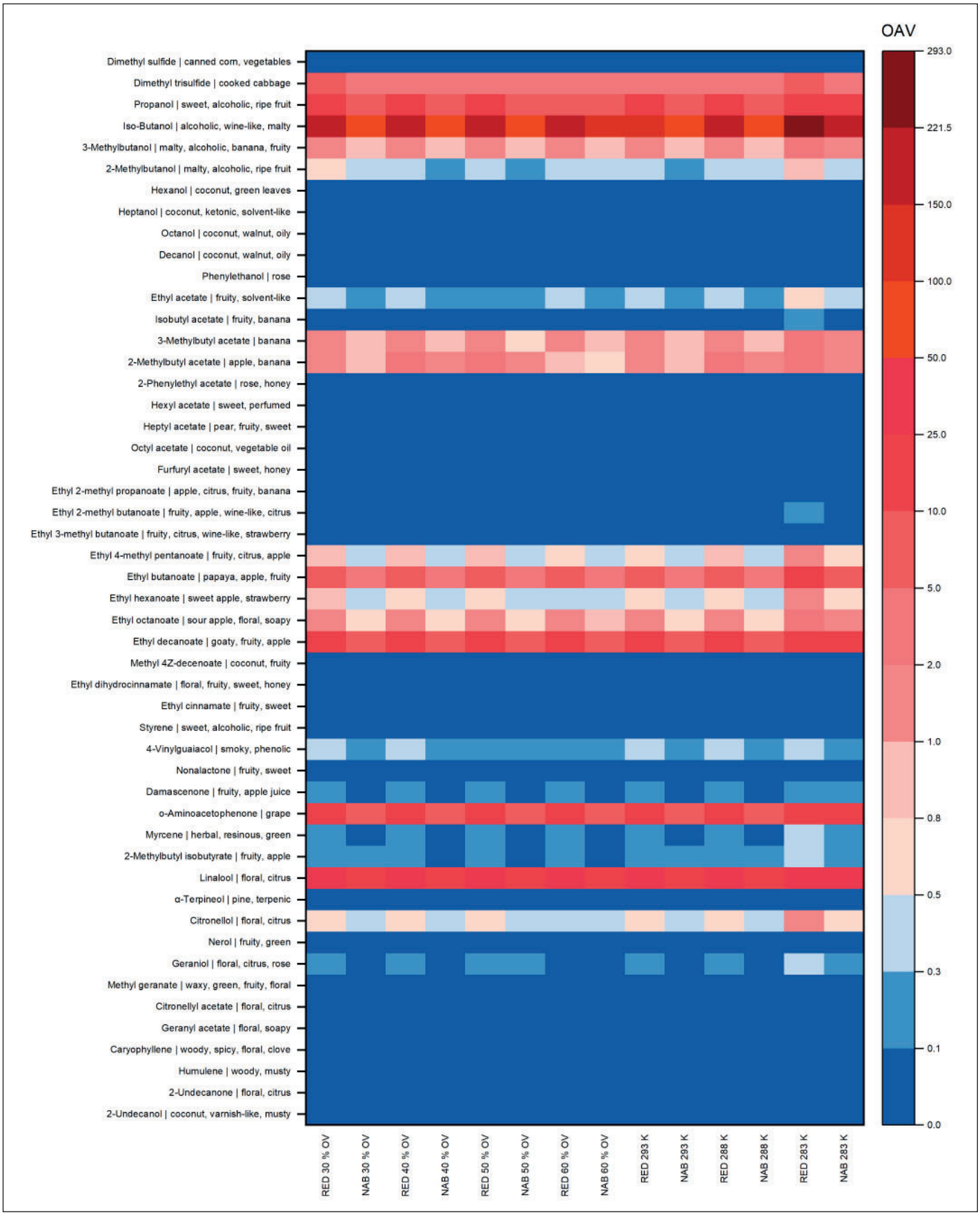

Fig. 1 **Odour activity value (OAV) heat map of selected aroma compounds in the end-of-diafiltration retentates (RED) and final blended non-alcoholic beers (NAB) produced at different concentration degrees (CD) at the start of diafiltration (SD) and process temperatures. OAV was calculated according to equation (2-1). The colour scale visualises compounds below and above an OAV of one within the same heat map**

Table 7 **Percentage concentration difference of summarised aroma groups in end-of-diafiltration retentates (RED) and final blended non-alcoholic beers (NAB) relative to the alcoholic mother beer (MB), measured by HS-SPME GC-MS. Values were calculated from summed concentrations according to equation (2-2). Positive values indicate concentrations above the MB and negative values indicate concentrations below the MB**

Process series	Setting	Sample	Acetate esters [%]	Ethyl esters [%]	Terpene alcohols [%]	Higher alcohols [%]	DMS + DMTS [%]
Concentration degree	60 % OV	EDF	20,0	28,1	102,4	226,0	–77,4
Concentration degree	60 % OV	NAB	–22,8	–17,6	30,1	109,6	–85,4
Concentration degree	50 % OV	EDF	29,4	62,3	129,3	202,7	–77,4
Concentration degree	50 % OV	NAB	–28,1	–9,8	27,4	68,2	–87,4
Concentration degree	40 % OV	EDF	62,1	75,9	158,5	258,5	–73,6
Concentration degree	40 % OV	NAB	–22,9	–16,4	22,9	70,4	–87,4
Concentration degree	30 % OV	EDF	94,9	89,4	186,6	314,3	–69,8
Concentration degree	30 % OV	NAB	–10,5	–13,0	31,5	90,2	–86,1
Temperature	293 K	EDF	55,9	51,6	129,9	231,4	–75,5
Temperature	293 K	NAB	–13,5	–15,9	27,5	83,9	–86,4
Temperature	288 K	EDF	62,1	75,9	158,5	258,5	–73,6
Temperature	288 K	NAB	–12,1	–4,6	40,2	94,4	–85,7
Temperature	283 K	EDF	163,7	186,1	321,3	483,2	–58,5
Temperature	283 K	NAB	43,9	56,1	129,9	218,2	–77,4

formal variance-homogeneity testing was not interpreted. ANOVA results were evaluated together with biological-replicate variation and effect size. Sensory intensities, aroma-group concentrations and OAVs were evaluated descriptively.

3 Results

3.1 Process performance and physicochemical composition

The two process parameters affected technological performance differently. Reducing CD at SD from 60 to 30 % OV reduced total dealcoholization time from 542 to 432 min and diafiltration-water consumption from 191.2 to 127.3 L, while RED TMP increased from 6.9 to 8.2 bar. At a constant CD of 50 % OV, increasing temperature from 283 to 293 K reduced total time from 567 to 400 min and TMP from 8.0 to 5.2 bar, whereas diafiltration-water consumption decreased from 168.2 to 157.9 L (see table 2).

Relative to the series-specific central condition, CD at SD affected diafiltration-water consumption more strongly, whereas temperature produced the larger relative change in TMP and total process time. Changing CD from 60 to 30 % OV reduced water consumption by 33.4 % and total process time by 20.3 %, while TMP increased by 18.8 %. Increasing temperature from 283 to 293 K reduced total process time by 29.5 %, TMP by 35.0 % and diafiltration-water consumption by 6.1 % (see table 3).

The RED composition was affected more extensively by CD at SD than by temperature. When CD was changed from 60 to 30 % OV, real extract, density, viscosity, turbidity, colour, BG, AX, total polyphenols, FAN and iso-α-acids all increased, with the largest proportional changes observed for turbidity and colour (see table 2). The measured increases in retained non-volatile constituents remained below the theoretical volumetric factors, particularly at a CD of 30 % OV (see table 4). In the temperature series at a constant CD of 50 % OV, changes in BG, AX, total polyphenols and FAN were small, whereas viscosity, turbidity and iso-α-acids increased as temperature decreased from 293 to 283 K.

After blending to approximately 4.0 % real extract, process-dependent differences remained in the final NABs. Changing CD at SD from 60 to 30 % OV increased viscosity from 1.304 to 1.690 mPa s, turbidity from 1.58 to 3.15 EBC and colour from 7.18 to 11.77 EBC, while alcohol decreased from 0.302 to 0.204 % vol. alc. In contrast, decreasing temperature from 293 to 283 K increased viscosity from 1.276 to 1.562 mPa s and turbidity from 1.85 to 2.19 EBC, with only minor changes in BG and AX but an increase in iso-α-acids from 8.87 to 12.90 mg L^{-1} (see table 5).

The final NABs were adjusted to the same real-extract target, but differences between process conditions remained. The CD series changed both the concentration history and the amount of brewing water added during final adjustment. The temperature series was conducted at a constant CD. The two series were therefore analysed separately rather than as a factorial dataset.

One-way ANOVA showed significant effects of both CD at SD and process temperature on total dealcoholization time and diafiltration-water consumption. In the CD series, total process time differed significantly among the four settings ($F(3,4) = 72.57$, $p < 0.001$, $\eta^2 = 0.982$). Tukey's HSD separated 60, 50 and 40 % OV, while 40 and 30 % OV did not differ significantly ($p = 0.099$). Diafiltration-water

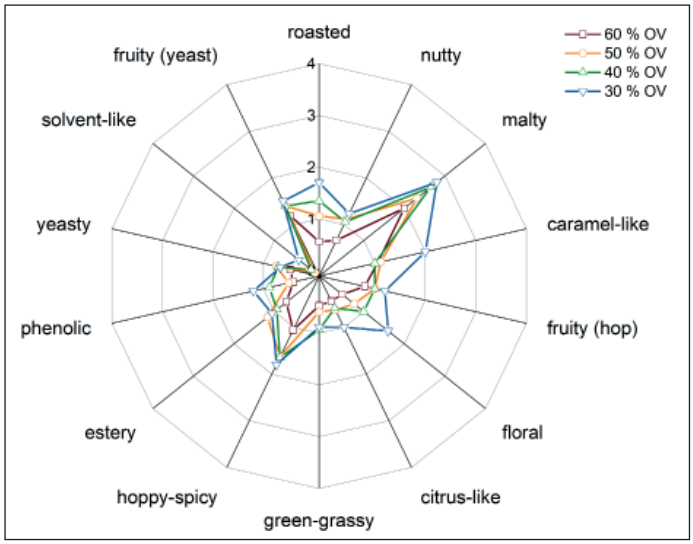

Fig. 2 **Mean sensory intensities of the final blended non-alcoholic beers produced at CD values at SD of 60, 50, 40 and 30 % of the original volume (OV). A trained panel evaluated the samples using a rate-all-that-apply test on a scale from 0, not perceptible, to 5, very high intensity. End-of-diafiltration retentates were not included in the sensory evaluation**

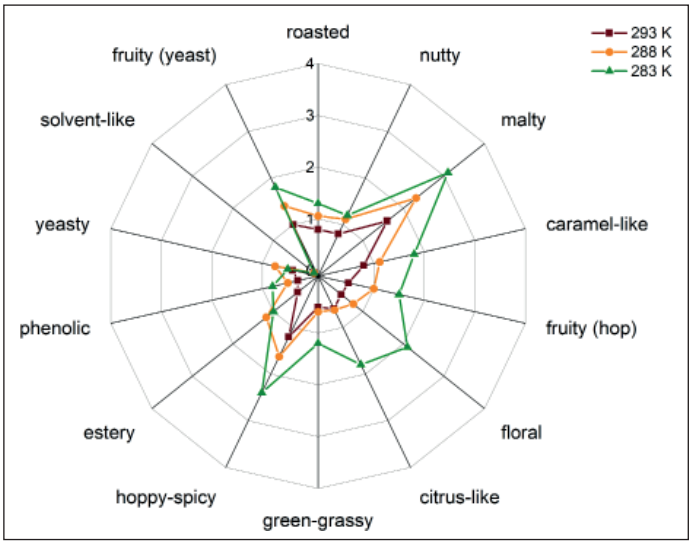

Fig. 3 **Mean sensory intensities of the final blended non-alcoholic beers produced at process temperatures of 293, 288 and 283 K. A trained panel evaluated the samples using a rate-all-that-apply test on a scale from 0, not perceptible, to 5, very high intensity. End-of-diafiltration retentates were not included in the sensory evaluation**

consumption was also affected by CD ($F(3,4) = 334.49$, $p < 0.001$, $\eta^2 = 0.996$), and all four CD settings differed significantly. Process temperature affected total process time ($F(2,3) = 238.08$, $p < 0.001$, $\eta^2 = 0.994$), with significant differences among 293, 288 and 283 K. Diafiltration-water consumption also differed among temperatures ($F(2,3) = 19.44$, $p = 0.019$, $\eta^2 = 0.928$), but Tukey's HSD identified a significant difference only between 283 and 293 K (see table 6).

3.2 Volatile composition and sensory profile

Changing CD at SD from 60 to 30 % OV caused smaller changes in the volatile composition of the final NAB than changing process temperature. In the CD series, acetate esters ranged from -28.1 to -10.5 %, ethyl esters from -17.6 to -9.8 %, terpene alcohols from +22.9 to +31.5 % and higher alcohols from +68.2 to +109.6 % relative to the MB. In the temperature series, the corresponding values changed from -13.5, -15.9, +27.5 and +83.9 % at 293 K to +43.9, +56.1, +129.9 and +218.2 % at 283 K (see table 7).

The OAV results showed the same difference between the two process parameters. In the CD series, linalool remained between 14.50 and 16.09 and ethyl butanoate between 3.98 and 4.59 in the final NABs. In the temperature series, linalool increased from 15.27 at 293 K to 27.09 at 283 K, ethyl butanoate from 4.16 to 7.82, ethyl octanoate from 0.69 to 1.22 and 3-methylbutyl acetate from 0.83 to 1.41 (see figure 1). DMS remained below threshold in all final NABs, whereas DMTS remained above threshold.

Sensory profiling also showed process-parameter-dependent differences. Changing CD at SD from 60 to 30 % OV was accompanied by higher mean intensities of roasted, malty, floral, hoppy-spicy and phenolic attributes, although the final-NAB ester and terpene groups did not show the same monotonic pattern (see figure 2). Decreasing temperature from 293 to 283 K increased floral intensity from 0.47 to 2.10, citrus-like from 0.60 to 1.80, hoppy-spicy from 1.20 to 2.40, fruity, hop-derived from 0.50 to 1.50 and fruity, yeast-derived from 1.00 to 1.80 (see figure 3).

Floral, citrus-like, hoppy-spicy and fruity attributes increased as temperature decreased, in the same direction as the GC-MS and OAV values. Solvent-like intensity remained zero despite OAVs above one for several higher alcohols. Roasted, nutty and caramel-like attributes were also higher at lower temperature. These latter sensory changes were not linked to a corresponding compound group in the targeted analysis.

The results show that CD at SD and process temperature affected different properties of the final NAB. CD had the larger effect on diafiltration-water consumption and on bulk physicochemical properties, whereas temperature produced the larger changes in volatile-group concentrations and the corresponding hop- and fermentation-associated sensory attributes.

4 Discussion

4.1 Comparative effects on process performance and physicochemical composition

CD significantly affected total process time and diafiltration-water consumption (see table 6). Eta squared was 0.982 for total process time and 0.996 for diafiltration-water consumption. Water consumption decreased at each lower CD. This follows from constant-volume diafiltration because less retentate is present at SD and less replacement water is required to reach the same ethanol target [46, 47]. TMP increased from 6.9 bar at 60 % OV to 8.2 bar at 30 % OV. The higher concentration of retained solutes increases osmotic pressure and thereby the pressure required for solvent transport [47, 48].

Between 40 and 30 % OV, diafiltration-water consumption decreased significantly by 11.0 L. Total process time decreased from 458 to 432 min, but this difference was not significant (Tukey HSD, p = 0.099). TMP increased from 7.6 to 8.2 bar. Under the investigated conditions, the lower CD therefore reduced water consumption further. The measured reduction in process time was not statistically significant.

The relative change in total process time was larger than the relative change in diafiltration-water consumption in the temperature series. Total process time differed significantly between all three temperatures, whereas diafiltration-water consumption differed significantly only between 283 and 293 K (see table 6). Increasing temperature from 283 to 293 K reduced total process time by 167 min and TMP from 8.0 to 5.2 bar. Diafiltration-water consumption decreased by 10.3 L. Increasing temperature lowers liquid viscosity and changes transport in the polyamide active layer [21, 22]. Catarino et al. reported higher permeate flux and lower rejection at higher temperature during beer RO [23, 24]. In the present study, the permeate-flow setpoint was 25 L h^{-1} for all temperature trials. The lower TMP at 293 K therefore represents a lower pressure requirement at the higher process temperature. The available process data do not separate the contribution of the concentration and diafiltration phases to the difference in total process time.

CD also changed the physicochemical properties of the final NAB. Reducing CD from 60 to 30 % OV increased viscosity, turbidity and colour after blending to approximately 4.0 % real extract. Final-NAB BG decreased from 128.6 to 111.5 mg L^{-1} and AX decreased from 339.1 to 271.1 mg L^{-1}. The higher viscosity therefore cannot be explained by higher BG or AX concentrations in the final product. The RED concentrations of BG, AX, polyphenols, FAN and iso-α-acids remained below the values calculated from the theoretical volume reduction. Solute rejection depends on molecular dimensions, functional chemistry and membrane interactions [33, 34, 35]. Macromolecular and colloidal material can also accumulate at membrane surfaces during beverage processing [28, 29, 49]. Higher turbidity and colour remained after adjustment to a similar final real extract. Protein-polyphenol association contributes to beer haze [50, 52]. BG and dextrins affect viscosity and filtration behaviour [30, 51]. AX also affects viscosity and mouthfeel depending on concentration and molecular characteristics [30, 31, 32].

4.2 Influence of process parameters on aroma and sensory properties of the final NAB

The aroma-group data (see table 7) show different responses to CD and temperature. In the CD series, reducing CD from 60 to 30 % OV increased the measured RED concentrations of acetate esters, ethyl esters, terpene alcohols and higher alcohols relative to the MB. At 30 % OV, the corresponding differences were +94.9, +89.4, +186.6 and +314.3 %. After final extract adjustment, the 30 % OV NAB showed -10.5 % acetate esters, -13.0 % ethyl esters, +31.5 % terpene alcohols and +90.2 % higher alcohols. The large differences measured in the RED retentate were therefore much smaller in the final NAB. The composition of the concentrated retentate does not directly describe the aroma composition after final blending.

Transport of a neutral aroma compound through a polyamide RO membrane is governed by partitioning into the active layer and diffusion through the polymer [33, 34, 35, 36]. Reducing CD increases the concentration of retained beer constituents and changes the concentration gradient, osmotic pressure and membrane-surface conditions during diafiltration. Pressure, feed flow, temperature and feed composition have all been reported to alter aroma rejection in beer and model beverage RO systems [23, 24, 25, 26]. Studies of neutral organic solutes and volatile molecules show that membrane sorption and molecular structure contribute to permeation in addition to molecular size [33, 34, 35, 36]. Functional chemistry also changes the rejection of low-molecular-weight neutral solutes [33, 34, 35].

The increase in TMP at lower CD adds a second transport-related change. Catarino et al. found that higher pressure increased alcohol rejection but decreased ester rejection during beer RO [23, 24]. A single proportional response of all aroma groups to CD is therefore not expected. Retentate flow was fixed at 163 L h^{-1} in the present experiments, so feed-flow differences were not an intentionally varied factor. Final blending further reduced the concentration differences generated during the RO step.

Temperature produced larger differences in the volatile composition of the final NAB than CD (see table 7). At 293 K, acetate esters and ethyl esters were 13.5 and 15.9 % below the MB, while terpene alcohols and higher alcohols were 27.5 and 83.9 % above the MB. At 283 K, the same groups were 43.9, 56.1, 129.9 and 218.2 % above the MB. Several compound-specific OAVs changed in the same direction.

Temperature affects diffusion in both the liquid phase and the polyamide active layer. Molecular-dynamics studies show that temperature changes molecular mobility and transport in polyamide RO membranes [21, 22]. During beer dealcoholization, Catarino et al. reported increasing permeate flux and decreasing solute rejection when temperature was increased from 278 to 293 K [23, 24]. Alvarez et al. also measured temperature-dependent aroma rejection in apple-juice model solutions [23, 24, 25, 26]. During RO concentration of chokeberry juice, cooling increased the retention of several volatile groups, including alcohols and terpenoids [20, 53]. The direction of the present temperature effect agrees with these studies.

Temperature and TMP were not independent in the present process. Lower temperature required a higher TMP at the same permeate-flow setpoint. Pressure can affect aroma classes differently. Catarino et al. reported different pressure responses for alcohols and esters in beer RO [23, 24]. Pressure-dependent losses of acetate and ethyl esters have also been reported during RO dealcoholization of wine [27]. The temperature series therefore represents the process condition obtained when temperature was changed at a fixed permeate-flow setpoint. The data do not separate the effect of temperature from the accompanying change in TMP on aroma transport.

The analytical and sensory results changed in the same direction for several temperature-dependent attributes. Linalool and other terpene alcohols contribute to floral and citrus-like hop character

[43]. Higher alcohols can contribute alcoholic, malty and fruity impressions [54, 55]. Acetate and ethyl esters contribute fruity and estery notes [56, 57]. At 283 K, higher linalool, ester and terpene-alcohol values occurred together with higher mean intensities of floral, citrus-like, hoppy-spicy and fruity attributes. In the CD series, several sensory attributes increased as CD decreased, but the grouped ester and terpene-alcohol concentrations in the final NAB did not follow the same monotonic pattern. The sensory changes in the CD series therefore cannot be assigned to the quantified volatile groups alone. Sensory perception depends on the combined beer matrix and aroma composition [42, 43, 44].

Not all sensory descriptors were explained by the quantified aroma compounds. Solvent-like intensity remained negligible despite OAVs above one for several higher alcohols. OAV indicates potential odour activity but does not represent sensory intensity in a multicomponent beer matrix [42, 43, 44]. Roasted, nutty and caramel-like ratings were higher at lower temperature, but the targeted analysis did not contain a compound group that could be linked directly to these attributes. No specific chemical cause is therefore assigned. Overall, temperature produced the clearest parallel changes in volatile composition and sensory profile. CD produced larger changes in water consumption and physicochemical composition than in the grouped volatile profile.

5 Conclusion/Summary

Concentration degree at SD and process temperature affected different properties of reverse-osmosis dealcoholization and of the final NAB. Reducing CD from 60 to 30 % OV significantly decreased diafiltration-water consumption and total process time. The change from 40 to 30 % OV reduced diafiltration-water consumption further, while the reduction in total process time was not significant. Lower CD also increased viscosity, turbidity and colour in the final NAB after adjustment to a common real-extract target. Increasing process temperature from 283 to 293 K significantly shortened total process time and reduced the required TMP. The change in diafiltration-water consumption was smaller. Processing at 283 K resulted in higher concentrations of several ester, terpene-alcohol and higher-alcohol groups than processing at 293 K, together with higher floral, citrus-like, hoppy-spicy and fruity mean sensory intensities. The literature on polyamide RO transport shows that temperature, pressure, concentration and compound-specific partitioning and diffusion can alter volatile rejection. In the present data, CD mainly affected water consumption and the physicochemical composition of the final NAB, while temperature had the larger effect on volatile retention and sensory profile.

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Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

CRediT authorship contribution statement

Sönke Kienitz: Conceptualization, Writing – original draft, Data curation, Formal analysis, Methodology, Visualization, Investigation; Christoph Neugrodda: Supervision, Writing – review and editing; Thomas Becker: Supervision, Writing – review and editing

Ethics statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Nomenclature and abbreviations

ANOVA = analysis of variance
AX = arabinoxylan
BG = β-glucan
CD = concentration degree
CO_2 = carbon dioxide
ci = concentration of aroma compound i
df = degrees of freedom
DIN = Deutsches Institut für Normung
DMS = dimethyl sulphide
DMTS = dimethyl trisulphide
DVB/C-WR/PDMS = divinylbenzene/carbon wide range/polydimethylsiloxane
EBC = European Brewery Convention
EN = European Standard
FAN = free amino nitrogen
GC-MS = gas chromatography-mass spectrometry
HSD = honestly significant difference
HS-SPME = headspace solid-phase microextraction
ISO = International Organization for Standardization
MB = mother beer
MEBAK = Mitteleuropäische Brautechnische Analysenkommission
NAB = non-alcoholic beer
NaCl = sodium chloride
O_2 = oxygen
OAV = odour activity value
OTi = odour threshold of aroma compound i
OV = original volume
RATA = rate-all-that-apply
RED = retentate end of diafiltration
RO = reverse osmosis
SD = start of diafiltration
SPME = solid-phase microextraction
T = process temperature
TMP = transmembrane pressure
ttot = total dealcoholization time
Vdia = diafiltration-water volume
α = significance level
η = dynamic viscosity
η^2 = eta squared, effect-size measure
ρ = density

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