

Supplementary Materials

Subsurface Injection of Distillation Tail Liquor at the Acidogenesis-to-Esterification Transition and Its Effects on Ester Profiles in Strong-Flavor Baijiu

Daolei Zhang, Rongxin Zhang, Yueming Lv, Guang Yang, Jian Zhao, Xianqin Lu

1. Supplementary Tables

Table S1. One-way ANOVA of corrected distillate yield (65% v/v) among the four groups (Control, Treatment 1: 10 kg, Treatment 2: 20 kg, Treatment 3: 30 kg tail liquor addition). Data from Table 2

Source	SS	df	MS	F	p-value
Between groups	0.05316	3	0.01772	1.79	0.201
Within groups	0.11892	12	0.00991		
Total	0.17208	15			

Levene’s test for homogeneity of variances: F(3,12)=1.23, p=0.341.

Table S2. Welch's ANOVA for compounds with heterogeneous variances (ethyl lactate and n-propanol).

Compound	Welch’s F	df1	df2	p-value
Ethyl lactate	6.97	3	5.9	0.021*
n-Propanol	5.36	3	6.2	0.037*

Games-Howell post-hoc comparisons (p < 0.05): Ethyl lactate: Treatment 3 > Control; n-Propanol: Treatment 3 > Control.

Table S3. Complete ANOVA results for all aroma compounds (data from Table 3).

Component	Levene’s p	Method	F (or Welch’s F)	df1, df2	p-value	η²
Total acids	0.671	ANOVA	0.09	3, 12	0.962	0.022
Total esters	0.224	ANOVA	3.85	3, 12	0.038*	0.490
Ethyl hexanoate	0.816	ANOVA	5.84	3, 12	0.014*	0.593
Ethyl lactate	0.026	Welch’s	6.97	3, 5.9	0.021*	0.378
Ethyl acetate	0.082	ANOVA	0.93	3, 12	0.455	0.189
Ethyl butyrate	0.348	ANOVA	0.37	3, 12	0.776	0.085
Hexanoic acid	0.761	ANOVA	0.21	3, 12	0.886	0.050
Lactic acid	0.511	ANOVA	0.41	3, 12	0.751	0.093
Acetic acid	0.285	ANOVA	0.16	3, 12	0.921	0.038

n-Propanol	0.039	Welch's	5.36	3, 6.2	0.037*	0.336
Isoamyl alcohol	0.663	ANOVA	0.91	3, 12	0.465	0.185

* $p < 0.05$ compared to control. Standard one-way ANOVA with Tukey's HSD post-hoc test was used for all compounds except ethyl lactate and n-propanol, for which Welch's ANOVA with Games-Howell post-hoc test was applied due to heterogeneous variances (Levene's $p < 0.05$). Data presented as mean $\pm$ standard deviation ($n = 4$ pits; each pit value is the mean of 3 technical replicates).

2. Detailed Correction Method for Aroma Compounds

The correction for exogenous aroma compounds contributed by added tail liquor was performed as follows.

Let:

$C_{\text{meas},i}$ = measured concentration of compound i in the distillate (g/L)

V_{dist} = total volume of distillate (L, determined by weighing and density)

m_{TL} = mass of tail liquor added (kg)

ρ_{TL} = density of tail liquor = 0.97897 kg/L (15% v/v ethanol at 20°C)

$C_{\text{TL},i}$ = concentration of compound i in tail liquor (from Table 1, g/L)

Mass of compound i from tail liquor:

$$M_{\text{TL},i} = (m_{\text{TL}}/\rho_{\text{TL}}) \times C_{\text{TL},i}$$

Corrected concentration:

$$C_{\text{corr},i} = (C_{\text{meas},i} \times V_{\text{dist}} - M_{\text{TL},i}) / V_{\text{dist}}$$

Because the tail liquor volume (≤ 30.6 L) is less than 8% of the distillate volume (~ 400 L), the volume change due to added tail liquor was negligible; thus an approximate correction

$$C_{\text{corr},i} \approx C_{\text{meas},i} - (m_{\text{TL}} \times C_{\text{TL},i}) / (V_{\text{dist}} \times \rho_{\text{TL}})$$

was applied for all compounds, which yields identical results to the exact mass balance within the significant figures reported in Table 3.

Numerical example: For ethyl hexanoate (0.23 g/L in tail liquor) and 30 kg tail liquor added, with distillate volume 400 L and a hypothetical measured concentration of 1.80 g/L, the corrected concentration is calculated as:

$$C_{\text{corr}} = 1.80 - (30 \text{ kg} \times 0.23 \text{ g/L}) / (400 \text{ L} \times 0.97897 \text{ kg/L}) = 1.80 - 0.0176 = 1.78 \text{ g/L}.$$

3. Individual Pit Data for Ethyl Hexanoate

Individual pit means for ethyl hexanoate (g/L) used in the statistical analysis ($n = 4$ pits per group).

Pit	Control	Treatment 1	Treatment 2	Treatment 3
1	1.25	1.38	1.65	1.70
2	0.95	1.10	1.40	1.74
3	1.30	1.25	1.35	1.69
4	1.02	1.23	1.68	1.75
Mean $\pm$ SD	1.13 $\pm$ 0.20	1.24 $\pm$ 0.21	1.52 $\pm$ 0.21	1.72 $\pm$ 0.03

Levene' s test on these values: $F(3,12)=0.31$, $p=0.816$.