

The Effect of Lactic Acid Bacteria on Congener Composition and Sensory Characteristics of Scotch Malt Whisky

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ABSTRACT

Lactic acid bacteria (LAB) comprise a major part of the natural microflora of Scotch malt whisky fermentations, due to their tolerance of heat and elevated ethanol concentrations. In this study, their effects on the organoleptic properties of the spirit were investigated. Samples from late (>70 h) fermentations were obtained from whisky distilleries throughout Scotland. Bacteria of varying colony morphologies were isolated, purified, and characterised initially using random amplification of polymorphic DNA – polymerase chain reaction (RAPD-PCR). Isolates with differing RAPD patterns were retained and their ability to produce 10-hydroxystearic acid (10-HSA) from oleic acid was determined qualitatively using high performance thin layer chromatography. 10-HSA is the primary precursor of γ -dodecalactone, which is an important flavour compound in malt whisky responsible for the desirable “sweet and fatty” characteristic of the spirit. Thirty-nine isolates had strong or weak bioconversion activity while 89 isolates displayed negligible or no activity. Forty-two strains, largely from the former category were identified using partial 16S rRNA gene sequences. *Lactobacillus paracasei* was the predominant organism but *L. brevis* and *L. plantarum* were also identified. These 42 strains were assessed for their bioconversion capacity in a semi-quantitative manner using gas chromatography – mass spectrometry (GC-MS) and five isolates, comprising *L. brevis*, two strains of *L. paracasei*, and two strains of *L. plantarum* were selected for further study. These isolates were used in laboratory-scale, simulated whisky fermentations with *Saccharomyces cerevisiae*. Fermentation liquor (wash) was distilled to produce new-make spirit, which was analysed organoleptically by quantitative descriptive analysis. Spirit from fermentations inoculated with *L. brevis* had an enhanced “sweet” character, probably due to the higher γ -lactone levels detected in this whisky, as well as increased “sulfury” and “meaty” notes, most likely due to yeast autolysis. *L. paracasei* enhanced the “green/grassy” notes of new-make spirit, while also adding a “sour” aroma probably resulting from the elevated levels of lactic acid detected in the wash. Like *L. paracasei*, *L. plantarum* increased the “green/grassy” notes of new-make spirit. Further fermentations were carried out in which *L. brevis*, one strain of *L. paracasei*, and one strain of *L. plantarum* were inoculated into fermentations with yeast comprising 90% *S. cerevisiae* and 10% *Torulaspora delbrueckii*, which had been

isolated previously from Scotch whisky fermentations and shown to enhance the concentration of γ -lactones in new-make spirit. Co-fermentation of *L. brevis* with *S. cerevisiae* and *T. delbrueckii* resulted in a spirit with increased “green/grassy”, “sweet”, and “oily” notes, with decreases in “sulfury” and “meaty” observed when the wild yeast was not present. Spirit derived from co-fermentations of *L. paracasei* and *T. delbrueckii* exhibited increased “soapy”, “sour”, and “sulfury” notes. Co-fermentation of *L. plantarum* and *T. delbrueckii* caused increases in “green/grassy”, “soapy”, “sweet”, “sour”, and “sulfury” notes. Increased concentrations of γ -lactones were detected in new-make spirit distilled from fermentations inoculated with *L. brevis*, presumably contributing to the enhanced sweet character of this spirit. This effect was further amplified by the inclusion of *T. delbrueckii* in the laboratory-scale fermentations.

For Christine, Neil, and Christopher

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ABBREVIATIONS

6-PG/PK	6-phosphogluconate/phosphoketolase
10-HPA	10-hydroxypalmitic acid
10-HSA	10-hydroxystearic acid
10-KSA	10-ketostearic acid
ABV	alcohol by volume
ATP	adenosine triphosphate
Å	Angstrom(s)
BHI	brain-heart infusion
BLAST	basic local alignment search tool
C	carbon
conc.	concentration
D	aspartic acid
Da	Dalton
DHAP	dihydroxyacetonephosphate
DNTP(s)	deoxyribonucleoside 5'-triphosphate
E	glutamic acid
<i>E.</i>	<i>Escherichia</i>
EDTA	ethylenediaminetetraacetic acid
f	forward
Fe	iron
GAP	glyceraldehyde-3-phosphate
GC	gas chromatography
G+C	guanine and cytosine content
h	hour(s)
HPAE	high performance anion exchange
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
ICBD	International Centre for Brewing and Distilling
K	lysine
K_m	Michaelis-Menten kinetics
<i>L.</i>	<i>Lactobacillus</i>

LAB	lactic acid bacteria
LOX	lipoxygenase
min	minute(s)
mol%	molar percentage
MS	mass spectrometry
NAD ⁺	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
O.D.	optical density
o/n	overnight
<i>P.</i>	<i>Pseudomonas</i>
PCA	principal component analysis
PCR	polymerase chain reaction
PEP-PTS	phosphoenolpyruvate dependent-phosphotransferase system
pK _a	acid dissociation constant
PMF	proton motive force
ppb	parts per billion
ppm	parts per million
r	reverse
RAPD	randomly amplified polymorphic DNA
rRNA	ribosomal ribonucleic acid
<i>S.</i>	<i>Saccharomyces</i>
SD	standard deviation
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SG	specific gravity (g l ⁻¹)
<i>Sp.</i>	<i>Sporobolomyces</i>
spp.	species
SWRI	Scotch Whisky Research Institute
<i>T.</i>	<i>Torulaspota</i>
Taq/taq	<i>Thermus aquaticus</i>
vol.	volume
(v/v)	volume:volume ratio
(w/v)	weight:volume ratio
x g	times gravity

PRESENTATIONS

“The Effects of Lactic Acid Bacteria on the Sensory Characteristics of New-Make Scotch Whisky.”

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