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Developing a novel flavoured low alcohol beer using New Zealand honeydew honey and yacon concentrate

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ABSTRACT

Demand for low alcohol beer (LAB) is growing around the world as consumer attitudes towards alcohol consumption shift, with health consciousness and sober driving listed among the possible drivers. In New Zealand, LAB is defined as having no >1.15 % alcohol by volume (ABV). In industry, physical or biological processes are used to produce no- and low- alcohol beer; however, these methods are typically inaccessible to home brewers, and literature on accessible methods is non-existent. New Zealand honeydew honey (NZHDH) and New Zealand yacon concentrate (NZYC) have recently been chemically profiled in the literature. NZHDH and NZYC contain fermentable sugars, while NZYC also contains a very high percentage of fructooligosaccharides (FOS). This paper aims to incorporate these food materials into LABs using a method accessible to home brewers. The resultant LABs had an alcohol content between 0.61 and 0.86 % ABV, well under the 1.15 % threshold. Results from sensory analysis showed that all beer samples were somewhat acceptable to consumers; however, penalty analysis determined that hedonic scores could be improved by making the beers more sweet, bitter, and hoppy. Correspondence analysis revealed that the beers containing NZYC were more 'foamy' and 'brown' than all other beer samples, properties most likely related to the protein content and colour of NZYC respectively. Beers containing NZYC were also found to contain FOS and/or inulins, which are non-digestible sugars and therefore hypocaloric. This study provides an accessible method to produce LAB and shows how NZYC may be incorporated into LABs to yield a functional food.

1. Introduction

According to Food Standards Australia New Zealand (2023), a beer may be labelled as 'low alcohol' in New Zealand if it contains no >1.15 % alcohol by volume (ABV). Demand for low alcohol beer (LAB) and non-alcoholic beer (NAB, typically defined as <0.50 % ABV in literature) is growing around the world as consumer attitudes towards alcohol consumption shift. Steady declines in adolescent alcohol use have been observed between 2002 and 2014 in most high-income European nations as well as Australia, New Zealand, Canada, Japan, and the United States (Vashishtha et al., 2020). In the European Union approximately 1.38 billion litres of NAB was produced in 2019, representing 3.8 % of beer volume and 4.1 % of beer value; although overall market share was low, the growth of NAB volume and value between 2013 and 2019 was greater than that for regular beer (Kokole, Jané Llopis & Anderson, 2022). Possible drivers for growth in consumer interest in NAB and LAB include health consciousness, and social responsibility (for instance, sober driving) (Kokole et al., 2022).

Industrial processes for LAB and NAB production can be classified as physical (thermal treatments or membrane separation) or biological. Physical processes are carried out after full fermentation; thermal treatments use heat to completely or partially remove alcohol while membrane separation processes selectively remove alcohol across semi-permeable membranes (Salanță et al., 2020). Membrane separation processes have some advantages over thermal treatments (mild operating temperatures, low energy consumption, little or no need for enhancing agents, and reduced operating costs); however, both membrane separation and thermal treatments require specialised equipment not found in standard breweries (Salanță et al., 2020). Biological processes are carried out during fermentation, and many do not require the use of specialised equipment; however, modifications to the fermentation process can result in undesirable changes to the sensorial

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characteristics of the beer (Salanță et al., 2020). Using a higher quantity of non-fermentable sugar will lead to an unpleasant sweetness and wort-like flavour, arrested or limited fermentation can lead to deficiencies in aromatic compounds, and cold-contact brewing generates aldehydes which contribute to off-flavours (Salanță et al., 2020). Although industrial methods to produce LAB and NAB are well-described in the literature, research on the production of LAB or NAB in a home-brew context is non-existent. Most industrial processes are inaccessible to home-brewers due to the need for specialised equipment or inputs only available to industry (e.g. specialised yeasts). The most accessible method to home brewers – thermal distillation – is disregarded in the literature, as the removal of alcohol under higher temperatures has been found to have significant adverse effects on beer taste (Brányik, Silva, Baszczyňski, Lehnert & Almeida e Silva, 2012).

Honeydew honey (HDH) is produced from nectar exuded from insects such as aphids and scale insects; most commercial New Zealand HDH (NZHDH) is produced from nectar exuded by *U. assimile* which feed on trees in the Nelson beech forest (Chessum, Chen, Hamid & Kam, 2022). HDH typically has a stronger flavour and darker colour than blossom honey, and has distinct amino acid, polyphenol, and sugar profiles (Pita-Calvo & Váquez, 2017; Seraglio et al., 2019; Vasić et al., 2019). Despite its high antioxidant activity and comparable chemical profile to other varieties of HDH (Chessum et al., 2022), NZHDH remains an underutilised resource due to the dominance of Mānuka honey.

Yacon concentrate (YC) is a sweet syrup derived from juice extracted from the roots of the yacon plant (*Smallanthus sonchifolius*), and has physical and sensorial characteristics similar to honey or sugar cane syrup. However, unlike other syrups, YC is hypocaloric owing to its high concentration of fructooligosaccharides (FOS) and inulin, which are non-digestible oligosaccharide fructans that cannot be utilised by *Saccharomyces cerevisiae* (Genta et al., 2009; Wang & Li, 2013; Yan, Welch, Rush, Xiang & Wang, 2019). The bioactive profile and total FOS content of yacon roots, and therefore YC, varies between cultivars. New Zealand YC (NZYC) is produced from the roots of yacon cultivar 'New Zealand', which was developed as recently as the 1980s (Chessum, Chen, Kam & Yan, 2023). According to Chessum et al. (Chessum et al., 2023), the FOS content of NZYC varies widely, ranging from 17.625 ± 0.325 to 52.276 ± 0.808 g/100 g.

Functional foods are commonly defined as those which contain, in addition to nutrients, other components that may be beneficial to health (Kumar, Sripada & Poornachandra, 2018; Temple, 2022). Yacon has previously been described in the literature as a functional food due to its biologically active components, particularly FOS, inulin, and phenolic compounds (Yan, Permal, Quach, Chessum & Kam, 2022). NZYC has recently been incorporated into functional beverages containing collagen, blackcurrant, or vitamin C (Yan, Chessum, Nand, Terzaghi & Kam, 2023).

This work aims to fill a gap in the literature by developing an innovative way to utilise NZHDH (an underutilised resource) and NZYC (a recent introduction to the market) in a functional LAB beverage, utilising a brewing technique (thermal distillation) readily available to home-brewers. Their high concentrations of fermentable sugars make NZHDH and NZYC excellent alternatives to priming sugars during secondary fermentation. However, as stated, thermal distillation is typically disregarded in the literature due to its significant adverse effects on beer taste. It is hypothesised that utilising NZHDH and/or NZYC during secondary fermentation will introduce acceptable flavours which mask the adverse effects of thermal distillation, either through their pre-existing volatile profiles or by the generation of new volatiles via yeast metabolism, while keeping alcohol content below 1.15 % ABV.

2. Materials and methods

2.1. Materials

All reagents used in this study are ≥99 % in purity unless otherwise stated. NZHDH (three different production batches over the course of one year) was sourced from Streamland Honey Group Ltd, Rotorua, New Zealand. Three different production batches of NZYC were sourced from Yacon New Zealand Ltd., Auckland, New Zealand. Ultrapure water (UPW) was produced using a Purite Select Fusion water deionisation unit (L300760, Purite, Oxford, England).

Butan-1-ol (>95 %) (AJA107–2.5GL) and hydrochloric acid (36 %) (AJA1367–2.5 L) were sourced from AJAX FineChem, New South Wales, Australia. Methyl-2-methyl butyrate (8219AL) was sourced from AK Scientific, San Francisco, USA. 6-aminoquimolyl-N-hydroxysuccinimidyl carbamate (BIB6284) was sourced from Apollo Scientific, Stockport, England. Sodium chloride (BDH9286–500 G) was sourced from BDH Chemicals, Poole, England. Disodium phosphate (47,201) was sourced from ECP Ltd, Auckland, New Zealand. Acetonitrile (A998–212), chloroform (C607–4), ethanol (10,428,671), formic acid (AC270480010), and methanol (A452SK-4) were sourced from Fisher Scientific, Loughborough, England. Rice hulls, Gladfield wheat malt (PLU-107), Mangrove Jack's Carbonation Drops, Gladfield ale malt (PLU-100), rolled wheat flakes, Safale US-05 Dry Ale Yeast, and Whirlfloc were sourced from Brewshop, Hamilton, New Zealand. Pacific Jade hops, Mangrove Jack's amber crown top beer bottles, and Mangrove Jack's crown seals were sourced from Hauraki Home Brew, Auckland, New Zealand. Sodium citrate dihydrate (1–3646) was sourced from J.T. Baker Chemical Co., Phillipsburg, USA. Mannitol (M150/18/89) was sourced from May and Baker Ltd., Dagenham, England. Fructose (14,278) was sourced from Panreac Química SLU, Barcelona, Spain. Sodium tetraborate dihydrate (borax) (N1017515–500) was sourced from Pure Science, Wellington, New Zealand. 1-kestose (72,555–100MG), Amino acid standard (A9906), C7 – C30 Saturated Alkanes (49,451-U), dry acetonitrile (271,004–1 L), L-alanine-2,3,3,3-d4 (485,845–1 G), and nystose (56,218–100MG) were sourced from Sigma-Aldrich, Burlington, USA.

2.2. Materials and methods

2.2.1. Brewing of low alcohol beer

2.2.1.1. Brewing. The LABs were brewed according to the following procedure. 2 kg of wheat malt, 2.56 kg ale malt, 0.55 kg flaked wheat, and 0.5 kg of rice hulls were milled using a three-roller grain mill with a 1 mm gap. The milled grains were then mashed with 20 kg of water at 75 °C for 1 hour in a Grainfather mash tun model no 10,191 (Grainfather, Auckland, New Zealand); the temperature was then increased to 80 °C and held for 10 min to mash out. The spent grains were then sparged with 8 kg of water at 72 °C. The temperature of wort was raised to 100 °C and boiled for 1 hour. A Magrove Jack's hop spider (Grainfather, Auckland, New Zealand) was placed in the mash tun and three grams of Pacific jade hops were added at the beginning of the boil; a further 7 gs was added along with one Whirlfloc tablet within the last 10 min of the boil. After the boil phase was completed, the hop spider was removed. The wort was then pumped through a Grainfather counterflow chiller (Grainfather, Auckland, New Zealand) to cool to 40 °C, and was then whirlpooled to oxygenate.

2.2.1.2. Primary fermentation. The cooled wort was then transferred to a Grainfather conical fermenter model no 10,162 (Grainfather, Auckland, NZ) and pitched with 1 packet of Safale US-05 Dry Ale Yeast. The conical fermenter was then closed and sealed with an airlock. The beer was fermented at 18 °C for 7–10 days, or until bubbling was no longer observed through the airlock.

2.2.1.3. Distillation and secondary fermentation. Once fermentation was complete, the yeast slurry was removed from the conical fermenter and set aside in a refrigerator set to 5 °C. The beer was transferred back into the mash tun and a T500 distillation column (Still Spirits, Wellington,

New Zealand) was attached (refer to Fig. 1, supplementary material). The beer was distilled at 100 °C until ethanol was no longer observed dripping from the condensing tube. 750 mL glass bottles were prepared according to Table 1, and were then filled with distilled beer. The addition of NZYC, NZHDH, and/or sucrose at this stage was both for flavour and for secondary fermentation - to re-carbonate the beer while keeping alcohol content below 1.15 % ABV. Sucrose was added in the form of carbonation drops; one carbonation drop is equivalent to 3.33 gs of sucrose. After beer samples were prepared, bottles were capped and conditioned in a dark cupboard at room temperature for three weeks. After conditioning, beer samples were stored in a refrigerator set to 5 °C.

Table 1
Formulation of low alcohol beers.

Sample	Beer volume (mL)	Yeast slurry (mL)	Sucrose (g)	NZHDH (g)	NZYC (g)
LoHDH	750	1	3.33	4.7	0
HiHDH	750	1	0	9.4	0
LoYC	750	1	3.33	0	4.7
HiYC	750	1	0	0	9.4
Control	750	1	6.66	0	0
HDHYC	750	1	0	4.7	4.7

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

2.2.2. Determination of ethanol content

2.2.2.1. Standard preparation. A working standard of ethanol was prepared at 1 % concentration by adding 500 µL of ethanol to 25 mL of UPW in a 50 mL volumetric flask and making up to the mark with UPW. A 5 % butan-1-ol solution (internal standard) was prepared by adding 2.5 mL of butan-1-ol to 25 mL of methanol in a 50 mL volumetric flask and making up to the mark with UPW. Calibration standards of ethanol were prepared at concentrations from 1 % to 0.03125 % ABV in UPW by serial dilution.

2.2.2.2. Sample preparation. 980 µL of standard, beer sample, or UPW (blank) were added to 1.5 mL short thread amber glass vials (P/N THC1109250, Thermo Fisher Scientific, Waltham, USA) and spiked with 20 µL of internal standard. Vials were capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA). Samples were then analysed using the following instrumentation and parameters.

2.2.2.3. Standard and sample analysis. Ethanol content of the LABs was determined by gas chromatography coupled with a flame ionisation detector (GC-FID). An Agilent 7890A GC-FID (Agilent, Santa Clara, USA) equipped with a J&W DB-FATWAX Ultra Inert column (30 m × 250 µm × 0.25 µm) (Agilent, Santa Clara, USA) was used for separation. The oven temperature program was set at 50 °C for 3 min, followed by an increase of 80 °C/min to 220 °C, then held for 6 min with a constant column flow of 1.505 mL/min of hydrogen gas. The injector temperature was set at 250 °C and the split ratio was 20:1. The detector temperature was set at 280 °C, with a hydrogen flow rate of 30 mL/min, an airflow rate of 400 mL/min, and a makeup flow rate of 3.495 mL/min. The injection volume was 0.5 µL.

2.2.2.4. Standard curve. A standard curve for ethanol was generated with concentration ranging from 0.03125 % to 1 % ABV. The curve equation was $Y = 1233.13064X - 16.99745$, where Y represents peak area and X represents%ABV. The R^2 value was 0.99826.

2.2.3. Qualitative determination of fructooligosaccharides and inulins by acid hydrolysis and determination of fructose

2.2.3.1. Reagent and standard preparation. A citric acid buffer was prepared by mixing 30.7 mL of 0.2 M citric acid with 19.3 mL of 0.2 M disodium phosphate and making it up to 100 mL with UPW. An internal standard of mannitol was prepared in UPW at a concentration of 50,000 ppm, and a stock solution of fructose was prepared in UPW at a concentration of 1500 ppm. Fructose standards were then prepared from the stock solution by dilution in UPW at concentrations ranging from 100 to 1500 ppm.

2.2.3.2. Sample preparation. LAB samples (30 mL) were transferred to 50 mL centrifuge tubes (339,652, Thermo Fisher Scientific, Waltham, USA) and degassed in an Elmasonic S 10 H ultrasonic bath (Elma, Singen, Germany) using the degas function. Degassed LAB samples were then adjusted to pH 4.0 with citric acid buffer. pH was measured using a Eutech pH 700 meter (Thermo Fisher Scientific, Waltham, USA). Eight

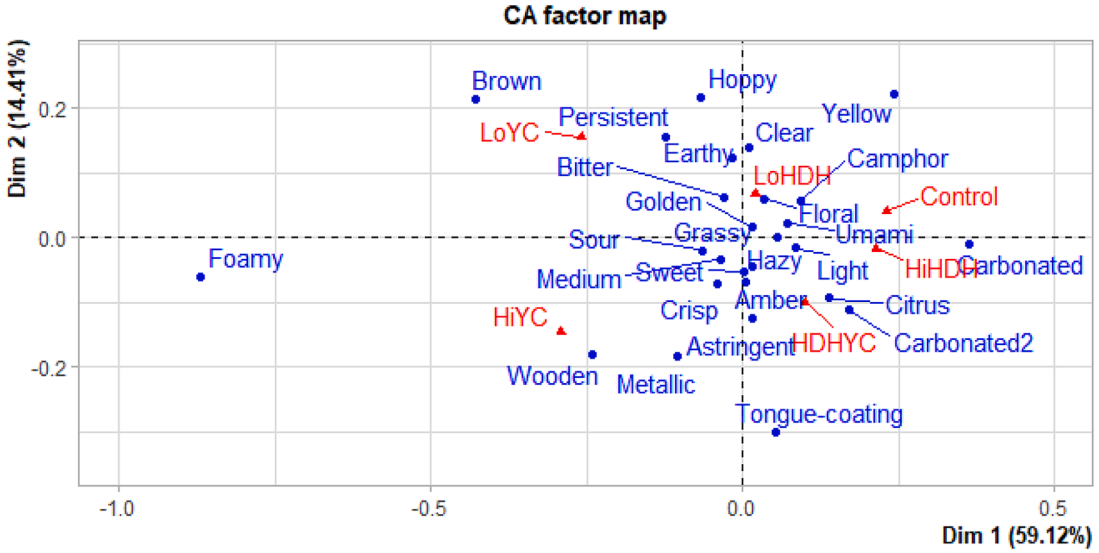

Fig. 1. Correspondence analysis of the Check-All-That-Apply terms and the cluster distribution of low-alcohol beer samples.
Note: 'Carbonated' = appearance term; 'Carbonated2' = mouthfeel term. 'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

mL of LAB samples were then transferred into 10 mL amber vials (THC18 09 1310, Thermo Fisher Scientific, Waltham, USA), capped (THC18 03 1578, Thermo Fisher Scientific, Waltham, USA), and heated in a 120 °C oven for 90 min. After 90 min, glass vials were placed on ice to stop the hydrolysis and cooled to room temperature. Three millilitres of hydrolysed LAB samples were then transferred to 15 mL falcon tubes (339,650, Thermo Fisher Scientific, Waltham, USA), washed with 3 mL of chloroform, and centrifuged at 2500 relative centrifugal force (RCF) for 5 min. The aqueous layer was used for subsequent analysis.

One mL of standard, sample, or blank (UPW) were transferred to 1.5 mL short thread amber glass vials (P/N THC1109250, Thermo Fisher Scientific, Waltham, USA) and spiked with 20 μL of mannitol internal standard. Vials were capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA). Fructose was then determined by high-performance liquid chromatography (HPLC) with an evaporating light scattering detector (ELSD) using the following instrumentation and parameters.

2.2.3.3. Standard and sample analysis. HPLC/ELSD analysis was carried out using a Shimadzu LC-10AT liquid chromatogram (Shimadzu, Auckland, New Zealand) coupled with an Agilent 385-ELSD (Agilent, Santa Clara, USA). Samples were injected with a Shimadzu SIL-10A auto-injector (Shimadzu, Auckland, New Zealand). The column was a Luna Omega 3 μm Sugar 100 Å 250 × 4.6 mm (P/N 00G-4775-E0, Phenomenex. Torrens, USA) with a SecurityGuard Cartridge (P/N AJ0–4495, Phenomenex, Torrens, USA).

The HPLC parameters were as follows: the mobile phase was acetonitrile:UPW (80:20, v/v), isocratic elution, pump flow rate of 0.5 mL/minute, analysis time of 75 min, injection volume of 10 μL, sample loop volume of 50 μL, temperature of 25 °C. The ELSD parameters were as follows: evaporator temperature was 80 °C, nebuliser temperature was 50 °C, and the inert gas flow rate (N_2) was 1.20 standard liters per minute (SLM).

2.2.3.4. Standard curve. A standard curve for fructose was generated with concentration ranging from 100 to 500 ppm. The curve equation was $Y = 0.000315996X + 64.0396$, where Y represented concentration and X represented peak area. The r^2 value was 0.9938321.

2.2.4. Determination of volatile compounds

Volatile compounds present in the beer samples, NZHDH, and NZYC were extracted by solid-phase microextraction (SPME), analysed by gas chromatography – mass spectrometry (GCMS–), and measured in terms of internal standard response ratio (ISRR).

2.2.4.1. Sample preparation. To prepare the NZHDH and NZYC, 1.0 g was dissolved in 5 mL UPW; LAB samples were used undiluted. 2 mL of sample or UPW (blank) were added to a 10 mL amber vial (THC18 09 1310, Thermo Fisher Scientific, Waltham, USA) along with 0.5 g sodium chloride. Internal standard (1 ppm) was prepared by dissolving 1 μL of methyl-2-methyl butyrate into 100 mL of UPW. 10 μL of internal standard was added to each vial; samples were then capped (THC18 03 1578, Thermo Fisher Scientific, Waltham, USA) and vortexed for 30 s using a Benchmark Benchmixer (BV1000, Benchmark Scientific, Sayreville, USA).

2.2.4.2. Sample analysis. The headspace vials were incubated at 60 °C for 15 min. During incubation, samples were agitated in cycles of 60 s on and 10 s off, with an agitator speed of 1300 rpm. The SPME fiber used for this study was Supelco 50 mm/30 μm DVB/CAR/PDMS, Stableflex, 24GA Fiber Assembly (P/N 57,329-U, Sigma-Aldrich, Burlington, USA). The extraction period was 25 min, and the desorption time was 5 min. SPME volatile analysis was performed on an Agilent 7890 GC system (Agilent, Santa Clara, USA) coupled with an Agilent 5977B MSD (Agilent, Santa Clara, USA), equipped with a Gerstel MultiPurpose Sampler (Gerstel, Linthicum, USA). The column used was an J&W DB-FATWAX Ulta Inert column (0.25 μm film thickness, 0.25 mm internal diameter, 30 m length) (Agilent, Santa Clara, USA). The carrier gas was helium with a constant flow rate of 1.1 mL/min. The injection was splitless, with the inlet temperature for the injection port set at 250 °C. For the chromatographic conditions, the temperature was set at 40 °C, held for 2 min, increased to 240 °C at a rate of 8 °C/min, and held for 3 min. The total run time of mass spectrometry (MS) was 30 min. The MS conditions were set at a source temperature of 250 °C and a quad temperature of 150 °C, with the electron ionization energy set at 70 eV. The total MS total ion chromatogram scanned masses ranged from 29 to 450 *m/z*.

2.2.4.3. Compound identification. To aid in the identification of unknown compounds, C7 – C30 saturated alkane reference material was run using the same GC–MS method used for sample analysis. The retention times for alkanes from heptane to triacontane were uploaded to the MassHunter Unknowns software program (Agilent, Santa Clara, USA) to augment the NIST14 library compound identification with retention index (RI) values.

The spectra and semipolar column RI values and spectra for compounds from the existing NIST database were compared to the experimentally obtained spectra and RI values. If (a) spectra were similar and (b) RI values were within 20 points, this was identified as a positive hit. If a positive hit was not identified, then the RI values of spectrally similar compounds in the NIST database were manually compared to the experimentally obtained RI value to obtain a hit. Once a hit was obtained, the compound was added to the MassHunter library. The completed library was then run against the entire sample set to obtain relative concentrations, normalised to the response of the internal standard (methyl-2-methyl butyrate). As such the results are semi-quantitative. GC–MS quantification was not performed, and absolute concentration was not obtained.

2.2.5. Determination of colour

2.2.5.1. Sample preparation. 10 mL of LAB samples were placed into beakers and degassed by sonication in ice water for 10 min using an Elmasonic S 10 H ultrasonic bath (Elma, Singen, Germany). Degassed LAB samples were then transferred to 15 mL centrifuge tubes (339,650, Thermo Fisher Scientific, Waltham, USA) and centrifuged at 5000 rpm for 15 min.

2.2.5.2. Sample analysis. Samples were transferred to plastic cuvettes. Absorbance was measured at 430 nm in triplicates against a blank (UPW) using an Ultrospec 7000 UV–Vis spectrophotometer (11,608,853, Thermo Fisher Scientific, Waltham, USA). Colour (in EBC units) was obtained by multiplying absorbance at 430 nm by 25.

2.2.6. Determination of amino acids

2.2.6.1. Reagent and standard preparation. AccQTag reagent was prepared by warming and sonicating 2.8 mg 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in 1 mL dry acetonitrile to dissolve using a Quantrex 90H sonicator (L&R Ultrasonics, Kearny, USA). Sodium citrate buffer was prepared by dissolving 5.88 g sodium citrate dihydrate in 100 mL UPW. Borate buffer was prepared by warming and sonicating 7.63 g of sodium tetraborate decahydrate in 90 mL UPW and 10 mL acetonitrile, and adjusting pH to 8.8 using 0.1 M hydrochloric acid. pH was measured using a Eutech pH 700 meter (Thermo Fisher Scientific, Waltham, USA). A neutralising solution was prepared by mixing 10 mL formic acid with 90 mL UPW. Internal standard spiked methanol (ISSM) was prepared by dissolving 1 mg d4-alamine in 100 mL methanol.

Standards were prepared ranging from 200 μM to 0.781 μM by diluting amino acid standard A9906 in UPW. 50 μL of ISSM was added to 50 μL of each standard.

2.2.6.2. Sample preparation. Samples were prepared by mixing 1 mL of LAB or UPW (blank) with 5 mL of 6 M HCl in a 20 mL glass vial (320018R-2375, JG Finnergan, Vineland, USA). The mixtures were purged under nitrogen gas, capped (THC18 03 1578, Thermo Fisher Scientific, USA) and digested overnight in a drying oven (MOV-112, Sanyo, Osaka, Japan) at 110 °C. The digested samples were filtered using 9.0 cm Whatman 40 filter paper (144,090, W&R Balston Ltd., Kent, England), and 100 μL of each sample or blank was transferred into a microcentrifuge tube. Hydrochloric acid was dried off at 100 °C in a drying oven (MOV-112, Sanyo, Osaka, Japan), and the dried samples and blank were resuspended in 500 μL of 200 mM sodium citrate buffer. 40 μL of resuspended sample or blank was added to 40 μL of ISSM in microcentrifuge tubes, which were then centrifuged at 4466 xG for 5 min. The supernatant was used for AccQTag deriviatisation.

2.2.6.3. AccQTag derivatisation. 10 μL of sample, standard, or blank was added to 70 μL of borate buffer in a microcentrifuge tube, followed by 10 μL of AccQTag reagent. The tubes were vortexed immediately, capped, and incubated at 55 °C in an Agilent 7890A GC-FID oven (Agilent, Santa Clara, USA) for 15 min. Neutralising solution (400 μL) was then added to each tube. Solutions were transferred to 0.7 mL short thread micro-vials (P/N 11 19 1706, Thermo Fisher Scientific, Waltham, USA) and capped with 9 mm short thread screw caps (P/N 09 15 0838, Thermo Fisher Scientific, Waltham, USA).

2.2.6.4. Standard and sample analysis. Liquid chromatography-mass spectrometry (LC-MS) analyses were conducted using an Agilent 1260 Infinity Quaternary LC System (Agilent, Santa Clara, USA). The LC-MS system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), 1260 infinity diode array detector (DAD) (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionisation (ESI) source (model number: G1948B). The column used was a Kinetex Evo C18 (2.1 × 150 mm, 1.7 μm) (Phenomenex, Torrens, USA).

The MS ionisation source conditions were as follows: capillary voltage of 4 kV, drying gas temperature of 300 °C, drying gas flow rate of 10 L/min, and nebuliser pressure of 30 psi. The positive ion mode was performed with multiple reaction monitoring (MRM) for quantitative analysis. The LC-MS conditions were as follows: Mobile phase A was 0.1 % formic acid in UPW. Mobile phase B was 0.1 % formic acid in acetonitrile. The flow rate was 0.225 mL/min, and the column temperature was 25 °C.

The gradient program was as follows: The initial percentage of mobile phase B (B%) was 5 %, and held for 3 min. B% was then raised at a rate of 1 % per minute to 10 %, and was held for 2 min. B% was then raised at a rate of 1.2 % per minute to 17 %, then raised at a rate of 42 % per minute to 80 % and held for 0.5 min. B% was then lowered at a rate of 50 % per minute to 5 % and the run was complete.

2.2.6.5. Standard curve. The analyte concentration ranges, curve equations, and fits are presented in Table 2.

Table 2
Standard curve information for amino acid analysis.

Analyte	Concentration range (μM)	Equation	r^2 value
L-Proline	0.78 - 200	$Y = 0.011366X$	0.9995
L-Glutamic acid	0.78 - 200	$Y = 0.003799X + 0.003717$	0.9994
L-Histidine	0.78 - 200	$Y = 1.4528*10^{-4}X$	0.9901
Glycine	0.78 - 100	$Y = 0.006435X + 0.004427$	0.9991
L-Aspartic acid	0.78 - 100	$Y = 0.004764X + 0.003803$	0.9981
L-Alanine	0.78 - 200	$Y = 0.003604X + 0.001898$	0.9998
L-Serine	0.78 - 100	$Y = 0.005368X$	0.9976
L-Leucine	0.78 - 100	$Y = 0.022675X + 0.003198$	0.9989
L-Valine	0.78 - 50	$Y = 0.008518X$	0.9993
L-Arginine	0.78 - 100	$Y = 1.3617*10^{-4}X$	0.9957
L-Threonine	0.78 - 50	$Y = 0.006420X$	0.9983
L-Phenylalanine	0.78 - 50	$Y = 0.013880X - 0.005159$	0.9987
L-Lysine	0.78 - 50	$Y = 0.005488X$	0.9989
L-Tyrosine	0.78 - 50	$Y = 0.010623X - 0.003470$	0.9989
L-Isoleucine	0.78 - 50	$Y = 0.018771X - 0.004326$	0.9994
Glutamine	0.78 - 50	$Y = 8.2846*10^{-4}X$	0.9928
γ-Amino-n-butyric acid	0.78 - 100	$Y = 0.008709X$	0.9997
L-Cystine	0.78 - 50	$Y = 0.005466X$	0.9949
L-Methionine	0.78 - 50	$Y = 0.009534X$	0.9986
Asparagine	0.78 - 100	$Y = 2.800*10^{-4}X - 3.9303*10^{-4}$	0.9964
Ethanolamine	0.78 - 200	$Y = 0.006561X$	0.9992
Hydroxy-L-proline	0.78 - 100	$Y = 0.06742X$	0.9991
L-Ornithine	0.78 - 50	$Y = 0.005230X$	0.9997
β-Alanine	0.78 - 100	$Y = 0.005159X$	0.9997

Y = Relative response, X = Concentration (μM)

2.2.7. Sensory evaluation

2.2.7.1. Study protocol. The sensory evaluation was presented in a questionnaire format including unstructured line scales, just-about-right (JAR) scales, check-all-that-apply (CATA) questions, and sociodemographic questions. An information sheet about the experiment, a consent form, and a quick-response (QR) code linking to the questionnaire (on Qualtrics) were presented to the subject upon arrival at the Food Science Laboratory (Auckland University of Technology). Subjects were instructed to answer the questions according to the order presented on the questionnaire. For each LAB, a randomised three-digit code was presented at the top of each section of the questionnaire, and subjects were required to answer the questions specifically to the LAB with the corresponding randomised three-digit code. The subjects first evaluated acceptance using unstructured line scales, then the appropriateness of the level of a specific attribute using JAR scales, and finally CATA questions to understand consumer perception of the LABs. In between LABs, subjects were instructed to eat some unsalted crackers and drink some water to cleanse the palate. After all samples were tasted, subjects answered the sociodemographic questions.

2.2.7.2. Subjects. Subjects were recruited from the Auckland area through posters and word-of-mouth. Subjects were screened for the following criteria: (1) the subject was at least 20 years of age (due to conditions of ethics approval), (2) the subject had no food allergies (such as wheat, yacon, honey, barley, hops, and alcohol), (3) the subject was in good health and not pregnant or trying to conceive a child, (4) the subject was not operating heavy machinery or driving within two hours of completing the sensory test, and (5) the subject was a regular (at least once a month) consumer of beer. Fifty-four subjects attended the sensory evaluation; however, one was removed due to the subject withdrawing from the sensory evaluation. 58.5 % of subjects were male, the largest age group was 20–29 (41.5 %), and most subjects drank beer on a fortnightly-to-monthly basis (71.7 %). A summary of the subject's sociodemographic details is presented in Table 3. This sensory evaluation was reviewed and approved by the AUT Ethics Committee with an

Table 3
Sociodemographic information of subjects with percentages including age, gender, and frequency of beer consumption.

Characteristic		Sample *n* (%)
Age	20-29	22 (41.5%)
	30-39	16 (30.2%)
	40+	15 (28.3%)
Gender	Male	31 (58.5%)
	Female	22 (41.5%)
Beer consumption	At least once a week	15 (28.3%)
	Fortnightly-to-monthly	38 (71.7%)

approval number of 23/265.

2.2.7.3. Sample presentation and sensory evaluation procedure. The sensory evaluation took place in the Food Laboratory at the AUT city campus with the temperature set at 22 °C. White fluorescent light was used in the Food Laboratory. A researcher was available to explain the experiment and how to answer the questionnaire to the subjects. Subjects were instructed to consume the LABs one at a time following the order of the randomised three-digit codes provided on the questionnaire and answered the survey questions accordingly. Samples were poured into a 30-mL transparent portion cup on a subject-by-subject basis to prevent loss of carbonation. LABs were stored in a refrigerator set at 5 °C before the sensory evaluation.

2.2.7.4. Scales and questionnaire. The sensory evaluation consisted of (1) unstructured line scales labelled from "dislike extremely" to "like extremely" for determining the overall liking, as well as liking of appearance, aroma, flavour, and mouthfeel, of the LABs, (2) JAR scales labelled from "Much too little" to "Much too much" for determining whether hoppiness, bitterness, or sweetness could be reformulated or adjusted to increase product overall liking, and (3) CATA questions to determine which sensory terms were relevant or perceived by the subjects.

Terms used in the CATA questionnaire were formulated by a semi structured focus group. Ten subjects were recruited from Auckland University of Technology; all were regular beer drinkers, and eight had previous experience with sensory evaluation. Based on the results from the focus group, eight appearance terms (hazy, clear, carbonated, foamy, golden, amber, yellow, brown), four taste terms (bitter, sour, sweet, umami), eight flavour terms (earthy, floral, grassy, crisp, hoppy, metallic, wooden, citrus), and six mouthfeel terms (light, medium, persistent, carbonated, astringent, tongue-coating) that best described the LABs were selected. A list of terms and their corresponding definition were provided to subjects when evaluating samples in terms of the CATA sensory attributes. Subjects were asked to select all the terms that they considered best to describe each LAB evaluated.

2.2.8. Statistical analysis

Statistical analysis was carried out using R Studio version 2023.09.0. R packages used in this paper include 'agricolae' for Tukey's honestly significant difference (HSD) test; 'factoextra', 'FactoMineR', and 'gplots' for correspondence analysis; 'rcompanion', 'DecTools', and 'coin' for Cochran's *Q* test; and 'FactoMineR' and 'SensoMineR' for penalty analysis.

For sensory evaluation, results from unstructured line scales were analysed by one-way and two-way analysis of variance (ANOVA) using R studio. Statistical significance was defined to exist at $p < 0.05$ for both one-way and two-way ANOVA. Post hoc analysis was carried out using Tukey's HSD test with a 95 % family-wise confidence interval. Results from CATA questions were analysed using Cochran's *Q* test, with statistical significance set at <0.05. When statistical significance was reached for CATA terms, McNemar's test was used for post hoc testing. In addition, correspondence analysis was conducted to visualise the CATA terms. For JAR analysis, the mean drop in overall liking was calculated from unstructured line scales (0 to 100), JAR results were further processed by categorising attributes into "not enough" (e.g., 1 and 2), "just-about-right" (e.g., 3), and "too much" (e.g., 4 and 5) categories from the 5-point scale used in the sensory survey. JAR questions were analysed using SensoMineR, which produced the penalty score and degree of significance.

Ethanol, fructose, colour, and amino acid measurements were done in triplicates from three separate production batches; results were reported as mean values ± standard deviation as calculated in Excel and analysed by the Kruskal-Wallis test using R Studio. Post hoc analysis was carried out in R studio using Dunn's test of multiple comparisons with the Holm method. Volatile compound measurements were done in triplicates; results were reported as mean values ± standard deviation as calculated in Excel and analysed by the Kruskal-Wallis test using R Studio.

3. Results and discussion

3.1. Ethanol content in beer samples

The ethanol contents of the LABs, as determined by GC-FID, are presented in Table 4. All results are lower than the 1.15 % ABV threshold for a beer to be labelled as 'low alcohol' in New Zealand. The amount of NZYC and NZHDH to be added to the LABs was determined by the amount of fermentable sugars present in these samples as described in the literature (Chessum et al., 2022; Chessum et al., 2023).

Control 1 had significantly lower ethanol content than HiHDH 1, HiHDH 3, and LoHDH 3. No other significant differences were identified between the LAB samples when correcting for multiple comparisons. This suggests that the levels of fermentable sugars in the NZHDH used for this work may be higher than that identified in the literature. These results also demonstrate that it may be possible to add higher levels of NZHDH and/or NZYC to LABs without exceeding the 1.15 % ABV threshold; however, this could lead to over-carbonation of the beer.

3.2. Fructooligosaccharides and inulins in the beer samples

A preliminary trial was carried out to quantitatively determine FOS and inulins using the method described by Chessum et al. (2023). However, the HPLC spectra (refer to Fig. 2, supplementary material) had a high number of peaks and it could not be determined which peaks corresponded to FOS, inulins, and other carbohydrates. In the method described by Chessum et al. (2023), two FOS standards (1-kestose and nystose) are used, and subsequent peaks are assigned to higher-order FOS and inulins based on the commonly accepted assumption that the retention time of structurally similar carbohydrates increases as the degree of polymerisation increases. This method of peak designation could not be used in the present study for the aforementioned reasons.

According to Navarro, Vela and Navarro (2012), around 90 % of the carbohydrates that are present in beer post-fermentation are dextrins, while around 10 % are polysaccharides that originate from the cell walls of the grains. It is assumed that these dextrins and other polysaccharides were also present in the LABs. In order to break down these dextrins and polysaccharides to their constituent sugar units to yield a cleaner HPLC chromatogram, acid hydrolysis was carried out. However, acid hydrolysis also breaks down FOS and inulins to their constituent sugar units (refer to Fig. 3, supplementary material). As dextrins and these other polysaccharides are comprised of glucose units, while FOS and inulins are comprised of fructose units with a terminal glucose unit, it was hypothesised that if FOS and/or inulins were present in the LABs, the LABs would be characterised by the presence of fructose. The results

Table 4
Ethanol content (ABV%) of low alcohol beers (n=3).

Sample	ABV% Batch 1	ABV% Batch 2	ABV% Batch 3
Control	0.61 ± 0.02^{a}	0.65 ± 0.01^{ab}	0.65 ± 0.04^{ab}
HDHYC	0.67 ± 0.01^{ab}	0.69 ± 0.03^{ab}	0.71 ± 0.07^{ab}
LoHDH	0.72 ± 0.0^{ab}	0.77 ± 0.02^{ab}	0.84 ± 0.05^{b}
HiHDH	0.85 ± 0.02^{b}	0.84 ± 0.02^{ab}	0.86 ± 0.03^{b}
LoYC	0.66 ± 0.02^{ab}	0.72 ± 0.00^{ab}	0.70 ± 0.00^{ab}
HiYC	0.68 ± 0.04^{ab}	0.69 ± 0.02^{ab}	0.69 ± 0.02^{ab}

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.
'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

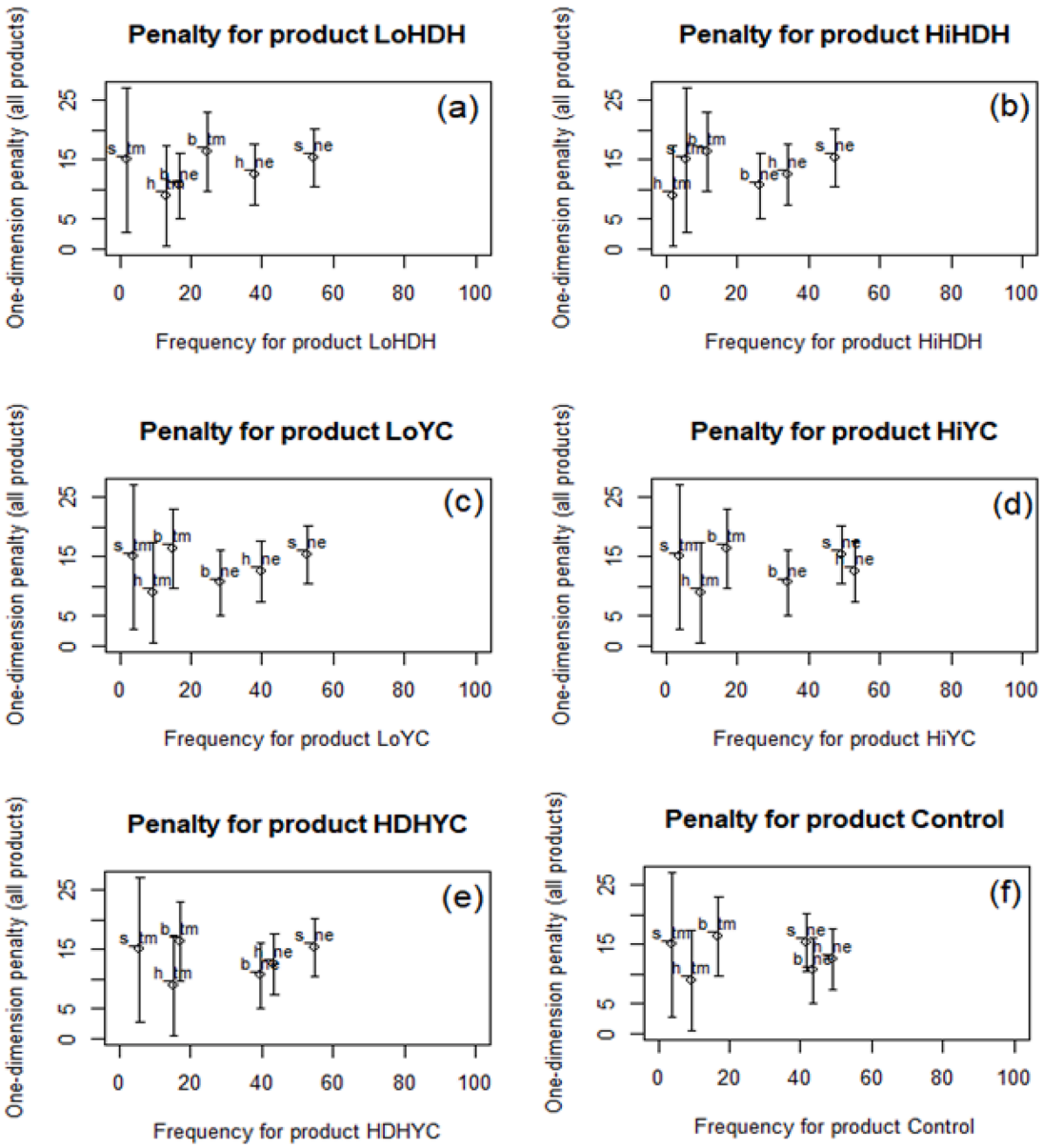

Fig. 2. Penalty analyses of low-alcohol beer samples. (a) LoHDH, (b) HiHDH, (c) LoYC, (d) HiYC, (e) = HDHYC, (f) = Control. 'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.
Note: Sensory attribute is denoted to the left of the underscore: *b* = bitterness, *h* = hoppiness, *s* = sweetness. Intensity of the attribute is denoted to the right of the underscore: ne = not enough, tm = too much.

Table 5
Results from fructose analysis (n=3).

Sample	mg/L fructose (batch 1)	mg/L fructose (batch 2)	mg/L fructose (batch 3)
LoYC	193.35 ± 5.57^{ab}	195.33 ± 15.98^{ab}	196.48 ± 18.99^{ab}
HiYC	262.73 ± 23.63^{b}	243.72 ± 19.08^{b}	206.98 ± 40.54^{ab}
HDHYC	186.94 ± 20.05^{ab}	146.12 ± 30.49^{ab}	122.36 ± 7.60^{a}
LoHDH	n.d.	n.d.	n.d.
HiHDH	n.d.	n.d.	n.d.
Control	n.d.	n.d.	n.d.

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.
'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

from the determination of fructose are presented in Table 5.

These results support the hypothesis that FOS and/or inulin were present in the LABs, as those samples that contained NZYC did have fructose present following acid hydrolysis, while those samples that did not contain NZYC contained no fructose. Samples HiYC 1 and HiYC2 contained significantly more fructose than sample HDHYC 3 after hydrolysis, which suggests that these samples may also have contained significantly more FOS and/or inulin than HDHYC 3.

These results also support the claim that *Saccharomyces cerevisiae* is unable to metabolise FOS and inulin. Hence, FOS is a viable option to be incorporated into beer as a hypocaloric sweetener and thereby yield a functional beverage. Studies show that daily FOS intake between 4 and 15 gs is sufficient to reduce constipation (Sabater-Molina, Larqué, Torrella & Zamora, 2009), while various studies on the effect of daily FOS intake on satiety and appetite reduction were inconclusive using doses from 8 to 21 gs (Hess, Birkett, Thomas & Slavin, 2011). In the study

carried out by Hess et al. (2011), daily doses of 10 or 16 gs of FOS resulted in dose-dependent increases in breath hydrogen response, indicating fermentation of FOS in the gut which has been associated with digestive benefits and improved gut health.

3.3. Volatile compounds

With respect to volatiles, the most significant compounds in beer fall into the following categories: aldehydes, higher alcohols, esters, vicinal diketones, sulfur compounds, and hop-derived compounds (Blanco, Andrés-Iglesias & Montero, 2016; Piornos, Koussissi, Balagiannis, Brouwer & Parker, 2023; Riu-Aumatell, Miró, Serra-Cayuela, Buxaderas & López-Tamames, 2014). Aldehydes are formed mainly from sugars and amino acids via the Maillard reaction and Strecker degradation during mashing and boiling stages, and are additionally formed by yeast metabolism during fermentation (Piornos et al., 2023; Riu-Aumatell et al., 2014). During normal fermentation, aldehydes are subsequently converted to higher alcohols via the anabolic pathway of yeast metabolism, and to esters by an enzyme-catalysed reaction between acetyl-CoA and higher alcohols (Blanco et al., 2016; Piornos et al., 2023). There is a relationship between the concentrations of higher alcohols and esters; in lagers, the ideal ratio of higher alcohols to esters falls between 4:1 and 4.7:1 (Blanco et al., 2016). Vicinal diketones are a byproduct of the synthesis of some amino acids, sulfur compounds form as a result of a variety of reactions including the light-induced degradation of isoα-acids from hops, while the essential oil of hops may contain over 1000 compounds which can also be converted to other volatiles by yeast metabolism (Blanco et al., 2016; Piornos et al., 2023; Riu-Aumatell et al., 2014).

As thermal distillation was carried out in this work to produce LAB samples, a significant proportion of volatile compounds present after primary fermentation would have been lost. For many LABs and NABs, the final volatile profile is only determined when flavours are added at the end of production (Piornos et al., 2023), so it was theorised that the addition of NZHDH and/or NZYC would play a significant role in the aroma and flavour of the final beers. For instance, yeast metabolism of NZHDH and NZYC during secondary fermentation could lead to the formation of aldehydes, and subsequently higher alcohols and/or esters. Additionally, volatile compounds present in NZHDH and NZYC may have contributed to the final aroma and flavour.

SPME analysis was carried out for the beer samples, as well as for neat NZHDH and NZYC. For the beer samples, principal component analysis was carried out, along with hierarchical cluster analysis and k-means clustering. However, there was no clear separation of beer samples along the principal components, nor did any clear trends emerge by either method of cluster analysis. This may be due to the relatively low concentration of NZHDH and NZYC in the beers; the volatile compounds common to beer would be binding competitively with the NZHDH and NZYC volatiles to the SPME fibre, thus making it harder to distinguish NZHDH beers from NZYC beers.

A total of 49 volatile compounds were detected in the neat NZHDH and/or NZYC samples by SPME-GC–MS, including 9 alcohols, 6 aldehydes, 2 carboxylic acids, 8 esters, 11 ketones, 5 terpenoids, and 8 others. These compounds are detailed in Table 6. Of the 49 identified compounds, 44 were identified in the NZHDH samples. A previous study by Revell, Morris and Manley-Harris (2014) identified 23 volatile compounds in NZHDH of the southern beech (*Nothofagus* spp.) variety; 11 of these compounds were identified in the present study. These compounds include 3,7-dimethylocta-1,6-dien-3-ol (linalool), (5E)−3,7-dimethylocta-1,5,7-trien-3-ol (hotrienol), phenylmethanol (benzyl alcohol), 2-phenylethanol, benzaldehyde, phenylacetaldehyde (benzeneacetaldehyde), 1-phenylethan-1-one (acetophenone), 1-(2-methoxyphenyl)ethenone (*o*-methoxyacetophenone), 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (terpineol), cycloocta-1,3,5,7-tetraene, and 2-methyl-1-benzofuran. Revell et al. (2014) analysed a total of ten types of New Zealand monofloral kinds of honey and found that linalool was present

Table 6
Volatile profile of New Zealand honeydew honey (NZHDH) and New Zealand yacon concentrate (NZYC) (n = 3).

Volatile	ITSD response ratio (NZHDH)	ITSD response ratio (NZYC)	Aroma notes
Alcohol			
Hexan-1-ol	0.01 ± 0.00	0.01 ± 0.00	Herbaceous, grassy[4]
2-Ethylhexan-1-ol*	0.08 ± 0.00	0.11 ± 0.00	Mild, oily, sweet, floral-rose, fruity[6]
3,7-Dimethylocta-1,6-dien-3-ol*	1.31 ± 0.10	0.13 ± 0.01	Sweet, tender, fresh floral[2]
(5E)-3,7-Dimethylocta-1,5,7-trien-3-ol*	0.62 ± 0.05	N.D.	Floral[5]
Decan-1-ol*	0.01 ± 0.00	0.00 ± 0.00	Fatty, waxy[7]
Undecan-1-ol*	0.05 ± 0.00	0.02 ± 0.00	-
Phenylmethanol*	0.03 ± 0.00	0.00 ± 0.00	Green, pungent[8]
2-Phenylethanol*	0.66 ± 0.16	0.29 ± 0.17	Honey, sweet, yeast, floral, spicy, herbal, rose[1]
Dodecan-1-ol*	0.24 ± 0.01	0.07 ± 0.01	Waxy, soapy[7]
Aldehyde			
Benzaldehyde*	0.36 ± 0.03	0.06 ± 0.01	Almond, cherry, stone[3]
5-Methylfuran-2-carbaldehyde*	0.05 ± 0.00	0.31 ± 0.01	Almond, caramel[9]
(2S)-2-[(2S,5S)-5-Ethenyl-5-methyloxolan-2-yl] propanal*	0.06 ± 0.01	N.D.	Floral[10]
Phenylacetaldehyde*	0.32 ± 0.02	0.08 ± 0.01	Rose, floral, peach[2]
3,4-Dimethylbenzaldehyde*	0.06 ± 0.00	0.10 ± 0.02	Almond[11]
4-Methoxybenzaldehyde*	0.37 ± 0.06	N.D.	Hawthorn, aniseed[12]
Carboxylic acid			
Acetic acid	0.02 ± 0.00	0.03 ± 0.01	Sour, vinegar, pungent[1]
3-Methylbutanoic acid*	0.36 ± 0.03	N.D.	Cheesy[7]
Ester			
Ethyl acetate*	0.05 ± 0.01	0.01 ± 0.00	Fruity, solvent-like[3]
Methyl benzoate*	0.04 ± 0.00	0.05 ± 0.01	Fruity, herbal, floral[13]
Ethyl decanoate*	0.00 ± 0.00	N.D.	Floral[14]
Methyl-2-hydroxybenzoate*	0.03 ± 0.00	N.D.	Wintergreen, mint[15]
Ethyl-2-phenylacetate*	0.13 ± 0.01	0.01 ± 0.00	Floral, honey[7]
2-Phenylethyl acetate	0.20 ± 0.09	0.10 ± 0.05	Rose, floral, fruity, sweet, honey[1]
2-Phenylethyl butanoate*	0.00 ± 0.00	0.00 ± 0.00	Floral, fruity[7]
Bis(2-methylpropyl) benzene-1,2-dicarboxylate*	0.04 ± 0.00	0.02 ± 0.01	-
Ketone			
3-Methylbutan-2-one*	0.02 ± 0.00	0.06 ± 0.00	-
4-Methylpent-3-en-2-one*	0.07 ± 0.01	N.D.	-
1-(Furan-2-yl)ethan-1-one*	0.06 ± 0.00	0.85 ± 0.03	Coffee, caramel, sweet[16]
1-(Furan-2-yl)propan-1-one*	0.02 ± 0.00	0.07 ± 0.00	Roasty, nutty[13]
3,5,5-Trimethylcyclohex-2-en-1-one*	0.06 ± 0.01	0.01 ± 0.00	Rooibos-woody[17]
1-Phenylethan-1-one*	0.04 ± 0.00	0.05 ± 0.01	Orange blossom, jasmine[18]
2,6,6-Trimethylcyclohex-2-ene-1,4-dione*	0.57 ± 0.05	N.D.	Seaweed[17]
1-(1H-pyrrol-2-yl)ethenone*	0.00 ± 0.00	0.07 ± 0.00	Nutty[17]
1-(2-Methoxyphenyl) ethanone*	2.15 ± 0.18	N.D.	-
1-(Furan-2-yl)-2-hydroxyethanone*	N.D.	0.02 ± 0.00	Sweet[19]
1-(2-Hydroxy-5-methylphenyl)ethanone*	0.01 ± 0.00	N.D.	Floral, herbaceous[20]

(*continued on next page*)

Table 6 (*continued*)

Volatile	ITSD response ratio (NZHDH)	ITSD response ratio (NZYC)	Aroma notes
Terpenoid			
(1R,2R,4S)-1,3,3-Trimethyl-2-norbornanol*	N.D.	0.04 ± 0.00	Camphor[21]
4-Methyl-1-(propan-2-yl) cyclohex-3-en-1-ol*	N.D.	0.07 ± 0.00	Terpene-like, musty[21]
2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol*	0.16 ± 0.01	0.52 ± 0.01	Pine, terpene, lilac, mint, floral, citrus, orange[15]
(2E)-3,7-Dimethylocta-2,6-dien-1-ol*	0.01 ± 0.00	0.02 ± 0.00	Floral, geranium[12]
(6E)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol*	N.D.	0.00 ± 0.00	Herby, woody[22]
Other			
Cycloocta-1,3,5,7-tetraene*	0.01 ± 0.00	N.D.	-
2-[(2S,5R)-5-ethenyl-5-methyloxolan-2-yl] propan-2-ol*	0.16 ± 0.02	0.01 ± 0.00	Floral, earthy[23]
1,2,3,4-tetramethylbenzene*	N.D.	0.10 ± 0.01	-
2-methyl-1-benzofuran*	0.02 ± 0.00	N.D.	Burnt phenolic[24]
(Furan-2-yl)methanol	0.05 ± 0.00	0.05 ± 0.00	Burnt sugar, fermented, creamy, caramel[1]
2-methoxyphenol*	0.01 ± 0.00	N.D	Smokey[25]
5-pentyloxolan-2-one	0.01 ± 0.00	0.01 ± 0.00	Coconut[7]
2,4,5-trimethylphenol*	0.02 ± 0.00	N.D.	-

[1] Alves et al. (2020)
[2] Ji (2021)
[3] Ferreira and Guido (2018)
[4] Olaniran, Hiralal, Mokoena, and Pillay (2017)
[5] Pino and Fajardo (2011)
[6] Api et al. (2016)
[7] Herkenhoff, Brödel, and Frohme (2024)
[8] Zhao et al. (2014)
[9] Lee, Jo, and Kim (2010)
[10] Schneider, Dötterl, and Seifert (2013)
[11] Zhao, Fan, and Xu (2021)
[12] Evans et al. (1999)
[13] Bettenhausen et al. (2018)
[14] Bettenhausen et al. (2020)
[15] Rajendran, Silcock, and Bremer (2023)
[16] Dusart et al. (2022)
[17] du Preez et al. (2020)
[18] Soucy (2014)
[19] Kameoka (1986)
[20] Wang et al. (2024)
[21] Schieberle and Grosch (1988)
[22] Satora and Pater (2023)
[23] Monacci et al. (2024)
[24] Food and Agricultural Organization of the United States (2024)
[25] Sterckx, Missiaen, Saison, and Delvaux (2011).
* Significant differences existed between samples at the .05 probability level

in significantly greater concentration in NZHDH than all other varieties, although it was also found in some manuka, pohutukawa, clover, kamahi, thyme, southern rata, and tawari honey samples. Therefore linalool, a floral and spicy terpene alcohol, may be considered to be a poor marker for southern beech HDH (Howe, 2020; Revell et al., 2014).

Another study by Brown (2013) analysed the headspace of honeydew (nectar exuded by other insects which are collected by bees and processed into HDH) and black sooty mould found on black beech trees (*Nothofagus solandri*) in Arthur's Pass National Park on the South Island of New Zealand. Eleven compounds were identified, seven of which were identified in NZHDH in the present study. These compounds include phenylmethanol, 2-phenylethanol, benzaldehyde, phenylacetaldehyde, methyl-2-hydroxybenzoate (methyl salicylate), ethyl 2-phenylacetate, and 2-phenylethyl acetate.

Of the remaining 30 compounds identified in NZHDH in the present study, a further 16 have been identified in other varieties of HDH in the literature (Castro-Vázquez, Díaz-Maroto & Pérez-Coello, 2006; Duru, Taş, Çayan, Küçükaydın & Tel-Çayan, 2021; Janoskova, Vyviurska & Špánik, 2014; Jerković & Marijanović, 2010; Karabagias, 2022, 2014; Lušić, Koprivnjak, Ćurić, Sabatini & Conte, 2007; von Eyken Bonafonte, 2019; Yang, Battesti, Costa, Dupuy & Paolini, 2018; Yildiz et al., 2022), while to the best of the author's knowledge 14 have not been previously identified in literature. Those compounds not previously identified include undecan-1-ol, dodecan-1-ol, 5-methylfuran-2-carbaldehyde, 3,4-dimethylbenzaldehyde, 4-methoxybenzaldehyde, methyl benzoate, 2-phenylethyl butanoate, 1-(furan-2-yl)propan-1-one, 2,6,6-trimethylcyclohex-2-ene-1,4-dione, 1-(1H-pyrrol-2-yl)ethenone, 2-[(2S,5R)−5-ethenyl-5-methyloxolan-2-yl]propan-2-ol, 2-methoxyphenol, 5-pentyloxolan-2-one, and 2,4,5-trimethylphenol. Common aroma descriptors associated with these compounds include floral (with hawthorn and rose specified), fruity, and earthy (see Table 6). Interestingly, 2,6,6-trimethylcyclohex-2-ene-1,4-dione (also known as 4-oxoisophorone) has been associated with the aroma of seaweed (du Preez, de Beer, Moelich, Muller & Joubert, 2020).

Of the 49 volatile compounds detected, 35 were identified in NZYC, of which 9 had previously been identified in yacon-related materials in the literature. Benzaldehyde and acetic acid have been identified in the roots of fresh Colombian yacon (Cuervo, Benitez & Castellanos, 2018). 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol, (2E)−3,7-dimethylocta-2,6-dien-1-ol, acetic acid, ethyl acetate, methyl benzoate, 2-phenylethanol, 3,7-dimethylocta-1,6-dien-3-ol, and benzaldehyde have been identified in a mixed juice containing yacon, litchi, and longan (Chen et al., 2019). Phenylmethanol has been identified in essential oil extracted from the leaves of the yacon plant (Li, Liu, Lan, Zheng & Rong, 2009). Literature concerning the volatile compounds present in yacon or YC is limited with the remaining 26 compounds found in the current study not previously identified. Aroma descriptors for these compounds include sweet, floral, almond, caramel, coffee, pine, and citrus, among others (see Table 6). However, bis(2-methylpropyl) benzene-1,2-dicarboxylate, also known as diisobutyl phthalate, is a plastic-related contaminant that has previously been identified in honey and so likely originates from the packaging material rather than being botanical in nature (von Eyken Bonafonte, 2019).

It is important to note that the method used to identify volatile compounds in the present study was semi-quantitative. Results are expressed in terms of internal standard response ratio, which cannot be used to determine concentration and can only be used to compare the relative concentration of each compound between samples that have been treated equally. Therefore, no inferences can be made on whether the concentration of these compounds is sufficient to be detected by humans. They also cannot be used to compare the concentration of different compounds within the same sample.

Compounds which have a much greater internal standard response ratio in NZHDH than in NZYC include 3,7-dimethylocta-1,6-dien-3-ol, (5E)−3,7-dimethylocta-1,5,7-trien-3-ol, 2-phenylethanol, dodecan-1-ol, benzaldehyde, phenylacetaldehyde, 4-methoxybenzaldehyde, 3-methylbutanoic acid, ethyl-2-phenylacetate, 2,6,6-Trimethylcyclohex-2-ene-1,4-dione, 1-(2-Methoxyphenyl)ethenone, and 2-[(2S,5R)−5-ethenyl-5-methyloxolan-2-yl]propan-2-ol. Aroma notes for these compounds are detailed in Table 6; common terms include sweet, floral, honey, rose, and earthy. Compounds which have a much greater internal standard response ratio in NZYC than in NZHDH include 5-methylfuran-2-carbaldehyde, 1-(furan-2-yl)ethan-1-one, 2-(4-methylcyclohex-3-en-1-yl) propan-2-ol, and 1,2,3,4-tetramethylbenzene. Aroma notes for these compounds are detailed in Table 6; descriptors include almond, caramel, coffee, pine, and floral. Of these terms, floral and earthy were identified by the semi structured focus group. Hence, if the concentration of

compounds associated with 'floral' and 'earthy' were above the detection limit in the final LABs, then differences may have been identified in sensory analysis.

3.4. Colour in beer samples

The results from the colour analysis are displayed in Table 7. Low EBC colour corresponds to a lighter or paler beer; high EBC colour corresponds to a darker beer. As the production method for the different LAB samples was identical up to the addition of NZHDH, NZYC, and/or carbonation drops, it was theorised that any differences in the colour of the LAB samples would be the result of these additions. The colour of honey is determined by both its botanical origin and its mineral composition; the L* (or lightness) score for honeydew honeys has been correlated to the concentration of arsenic, cadmium, iron, sulfur, lead, and calcium (Jara-Palacios et al., 2019; Pita-Calvo & Váquez, 2017). Additionally, some researchers have found correlation between honey colour and antioxidant activity (Bergamo et al., 2019), suggesting that phenolic compounds may play a role in determination of colour. It is assumed that the mineral content and phenolic profile of yacon concentrate is also determinative of colour.

Although there was noticeable difference in colour of NZYC and NZHDH, with NZYC being significantly darker than NZHDH, relatively few significant differences between LAB samples were identified. HiHDH3 had significantly lower EBC colour (i.e. was significanly lighter in colour) than HiYC1 and HiYC2, while control 1 had significantly lower EBC colour than HiYC1. Although significant differences were not observed across all LAB samples, the observed significant differences are consistent with the respective colours of NZHDH and NZYC. It is possible that, at higher concentrations of NZHDH and NZYC, more significantly different colours may be achieved between LAB samples.

3.5. Amino acids in beer samples

Twenty-four amino acids were quantified in the present study and are detailed in Table 8. Although significance ($p < 0.05$) was identified by the Kruskal Wallis test for many of the amino acids, post-hoc analysis using Dunn's test for multiple comparisons with the Holm method only identified one significant difference across all amino acids; LoHDH 1 had significantly lower L-arginine content (68.43 ± 2.80 mg/L) than HiYC 1 (113.09 ± 2.30 mg/L) ($p = 0.0429$).

Amino acids present in the LAB would have mainly originated from the grains used for brewing. While most of the amino acids are metabolised during fermentation, as will be later discussed, around 30 % of nitrogen compounds derived from barley are present in the final beer (Fontana & Buiatti, 2009). In the literature, amino acids such as alanine, arginine, cysteine, gamma aminobutyric acid, leucine, proline, threonine, and valine have been reported at concentrations in excess of 100 mg/L, with proline typically the most abundant (Fontana & Buiatti, 2009). In the present study, proline and L-glutamic acid were by far the most abundant amino acids at concentrations of around 700 mg/L. L-histidine, glycine, L-aspartic acid, and L-serine were all reported at concentrations in excess of 100 mg/L. While these concentrations are higher than those identified in the literature, they have all been previously identified at concentrations of 50 mg/L or higher in various beers (Fontana & Buiatti, 2009).

Essential and non-essential amino acids themselves typically have negligible taste, although L-alanine and L-tryptophan are associated with sweet and bitter taste respectively (Ferreira & Guido, 2018). However, when metabolised they significantly influence the development of the aroma and taste profile (Hazelwood, Daran, van Maris, Pronk & Dickinson, 2008; Wong et al., 2023). Yeasts such as *Saccharomyces cerevisiae* metabolise amino acids into volatile compounds such as aldehydes, fusel alcohols, and fusel acids via the Ehrlich pathway, and these volatile compounds do have a significant impact on the flavour of LAB (Wong et al., 2023). Similarly, *Saccharomyces cerevisiae* can metabolise non-essential amino acids such as glycine, alanine, and serine to pyruvate, which is then broken down into esters via the anabolic pathway (Wong et al., 2023). Amino acids also participate in the Maillard reaction; some products of the Maillard reaction such as furfural and 5-hydroxymethylfurfural are correlated with bitter and stale flavours and aromas (Ferreira & Guido, 2018). Strecker degradation, a reaction between amino acids and α-dicarbonyls formed in the Maillard reaction, forms Strecker aldehydes, which also contribute to flavour and aroma (Ferreira & Guido, 2018).

It was hypothesised that the different amino acid profiles of NZHDH and NZYC would result in different flavour profiles in the LABs, as different volatile compounds would be formed by the metabolic action of *S. cerevisiae*, as well as the Maillard reaction and Strecker degradation. However, SPME analysis of the LABs suggested otherwise, with principal component and cluster analyses yielding no significant results as previously discussed. This may be because the concentration of NZYC and NZHDH added to the LABs was too low to cause significant difference.

This is supported by the lack of significant difference in the amino acid profile as shown in Table 8. As stated earlier, around 30 % of nitrogen compounds remain in beer after fermentation. Assuming the composition of the remaining 30 % is representative of the initial composition, it would theoretically be possible to identify any significant differences arising from the addition of NZYC and NZHDH, if significant differences did exist.

3.6. Sensory evaluation

One-way ANOVA showed no significant differences ($p > 0.05$) between any of the LABs in terms of appearance, aroma, flavour, or mouthfeel. Significant differences were identified in terms of overall liking ($p = 0.0491$). However, Tukey's HSD test showed no significant differences between any of the paired means. Two-way ANOVA showed that the interactions between the LABs and panelist age, gender, or frequency of beer consumption were not significant ($p > 0.05$) for overall liking, appearance, aroma, flavour, or mouthfeel. As stated, relatively few significant differences were identified between LAB samples in terms of colour analysis, and this was reflected in the liking of the appearance of the LABs with no significant differences identified. In terms of aroma and flavour, the lack of significant differences between the LABs is supported by the results from SPME analysis, where the volatile profiles of the different LABs were similar or identical. The mean scores for overall liking, appearance, aroma, flavour, and mouthfeel are detailed in Table 9. All scores had relatively broad standard deviations with mean values that tended to fall between 50 and 70 on unstructured line scales, indicating that samples were not strongly liked but tended to be more liked than disliked. With respect to overall liking, the highest score was for the control (64.11 ± 18.28), while the lowest was for LoHDH (54.60 ± 26.83).

Seven CATA sensory attributes were statistically significant between LABs using Cochran's *Q* test. Four of these terms were appearance terms

Table 7
Results from European Brewery Convention (EBC) colour analysis (n=3).

Sample	EBC (batch 1)	EBC (batch 2)	EBC (batch 3)
Control	13.76 ± 0.03^{ab}	14.10 ± 0.02^{abc}	14.20 ± 0.02^{abc}
HDHYC	17.81 ± 0.03^{abc}	17.26 ± 0.03^{abc}	16.83 ± 0.04^{abc}
LoHDH	14.76 ± 0.03^{abc}	15.38 ± 0.10^{abc}	14.60 ± 0.18^{abc}
HiHDH	15.18 ± 0.04^{abc}	14.77 ± 0.01^{abc}	13.55 ± 0.09^{a}
LoYC	17.34 ± 0.05^{abc}	17.04 ± 0.04^{abc}	16.18 ± 0.01^{abc}
HiYC	20.23 ± 0.00^{c}	17.95 ± 0.02^{bc}	16.70 ± 0.10^{abc}

Significant differences existed between samples at the .05 probability level. Values with different superscript letters are significantly different.
'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Table 8
Amino acid profile of low alcohol beer (LAB) as determined by liquid chromatography – mass spectrometry (n = 3).

Amino acid	mg/L LAB Control batch 1	mg/L LAB Control batch 2	mg/L LAB Control batch 3	mg/L LAB HDHYC batch 1	mg/L LAB HDHYC batch 2	mg/L LAB HDHYC batch 3	mg/L LAB LoHDH batch 1	mg/L LAB LoHDH batch 2	mg/L LAB LoHDH batch 3	mg/L LAB HiHDH batch 1	mg/L LAB HiHDH batch 2	mg/L LAB HiHDH batch 3	mg/L LAB LoYC batch 1	mg/L LAB LoYC batch 2	mg/L LAB LoYC batch 3	mg/L LAB HiYC batch 1	mg/L LAB HiYC batch 2	mg/L LAB HiYC batch 3
L-Proline*	721.84 ± 59.98	698.04 ± 25.11	668.55 ± 75.02	699.44 ± 57.42	710.93 ± 25.40	637.20 ± 16.49	673.58 ± 24.26	719.16 ± 3.85	706.45 ± 26.81	704.23 ± 54.63	765.86 ± 49.68	663.61 ± 34.13	657.27 ± 42.88	677.13 ± 38.36	634.59 ± 50.30	783.52 ± 35.39	718.06 ± 43.90	697.99 ± 24.77
L-Glutamic acid*	694.61 ± 59.81	721.38 ± 30.94	671.17 ± 75.09	708.29 ± 70.05	714.46 ± 28.54	622.80 ± 12.23	646.75 ± 13.21	733.60 ± 1.09	690.78 ± 33.82	688.93 ± 52.95	747.08 ± 47.64	633.18 ± 37.93	655.12 ± 41.88	707.36 ± 40.84	622.74 ± 65.35	783.28 ± 22.41	739.12 ± 49.56	707.61 ± 40.82
L-Histidine*	207.66 ± 17.54	196.97 ± 7.75	147.17 ± 71.17	189.60 ± 15.67	199.46 ± 14.13	173.72 ± 11.79	197.93 ± 26.51	221.67 ± 7.73	187.32 ± 21.33	187.29 ± 12.25	228.26 ± 7.90	184.18 ± 14.95	201.74 ± 18.17	178.43 ± 14.04	181.16 ± 16.38	224.61 ± 29.30	215.65 ± 27.44	169.02 ± 6.78
Glycine*	159.13 ± 13.51	165.94 ± 5.98	157.06 ± 24.82	154.17 ± 15.49	161.34 ± 2.12	139.98 ± 3.95	145.12 ± 4.00	162.85 ± 2.08	153.58 ± 4.91	150.64 ± 17.05	167.86 ± 8.71	145.27 ± 6.16	140.48 ± 9.56	150.89 ± 12.23	137.73 ± 12.53	167.15 ± 10.79	153.61 ± 8.98	147.56 ± 6.05
L-Aspartic acid	126.87 ± 10.24	131.93 ± 5.38	125.39 ± 14.73	125.08 ± 12.34	133.32 ± 5.79	117.27 ± 1.37	118.70 ± 2.10	134.15 ± 0.81	128.46 ± 4.92	124.76 ± 10.01	141.80 ± 10.39	120.20 ± 7.75	119.10 ± 8.30	126.34 ± 8.15	116.70 ± 9.73	137.12 ± 5.88	131.17 ± 8.36	125.91 ± 10.20
L-Alanine*	125.33 ± 10.60	132.68 ± 7.01	122.64 ± 13.87	118.88 ± 11.02	126.68 ± 3.65	109.95 ± 1.04	114.50 ± 2.22	134.56 ± 2.41	125.94 ± 3.74	122.73 ± 11.59	138.16 ± 8.83	115.79 ± 7.27	111.86 ± 7.35	119.92 ± 6.58	110.45 ± 10.52	131.38 ± 6.36	123.87 ± 10.40	118.92 ± 7.56
L-Serine**	122.10 ± 9.71	126.46 ± 9.33	111.52 ± 13.51	115.06 ± 9.87	116.93 ± 3.60	101.15 ± 3.64	110.19 ± 3.58	124.90 ± 1.30	116.08 ± 2.40	118.61 ± 16.04	133.76 ± 8.47	111.02 ± 6.27	104.62 ± 8.16	112.08 ± 7.29	103.75 ± 7.97	125.21 ± 5.75	109.25 ± 3.31	106.18 ± 5.70
L-Leucine**	117.83 ± 10.49	122.53 ± 2.84	112.92 ± 11.21	112.65 ± 9.79	116.84 ± 3.50	101.82 ± 3.07	111.57 ± 3.35	122.06 ± 1.77	115.70 ± 1.46	115.48 ± 8.93	134.51 ± 7.72	108.22 ± 5.34	105.40 ± 7.35	110.80 ± 6.20	101.09 ± 8.65	121.25 ± 5.61	109.66 ± 5.89	103.85 ± 3.76
L-Valine*	100.95 ± 7.75	104.74 ± 2.51	99.43 ± 11.75	95.77 ± 10.07	101.08 ± 3.98	88.33 ± 2.68	92.77 ± 3.43	103.52 ± 1.30	98.85 ± 4.30	97.41 ± 7.71	112.29 ± 7.19	92.17 ± 4.23	89.69 ± 6.33	97.26 ± 5.88	87.77 ± 7.03	106.15 ± 4.40	97.25 ± 7.28	92.39 ± 3.21
L-Arginine*	82.51 ± 6.27^{ab}	91.47 ± 8.02^{ab}	92.37 ± 9.22^{ab}	97.02 ± 10.85^{ab}	111.86 ± 5.59^{ab}	85.65 ± 0.56^{ab}	68.43 ± 2.80^{b}	96.78 ± 6.39^{ab}	82.68 ± 11.20^{ab}	82.54 ± 22.19^{ab}	107.92 ± 4.39^{ab}	83.95 ± 16.54^{ab}	89.29 ± 8.12^{ab}	97.95 ± 10.70^{ab}	77.60 ± 20.75^{ab}	113.09 ± 2.30^{a}	98.44 ± 20.02^{ab}	97.85 ± 8.14^{ab}
L-Threonine*	91.07 ± 6.93	94.99 ± 4.40	87.51 ± 9.27	87.69 ± 7.60	90.84 ± 4.29	80.23 ± 3.69	84.40 ± 2.16	94.34 ± 1.28	90.93 ± 0.97	88.24 ± 8.52	103.21 ± 5.81	85.09 ± 4.36	81.79 ± 5.26	86.06 ± 4.43	79.06 ± 8.12	97.22 ± 4.15	88.68 ± 6.02	87.33 ± 3.68
L-Phenylalanine*	87.25 ± 6.79	89.06 ± 3.67	84.64 ± 9.16	86.24 ± 6.33	87.05 ± 4.29	75.63 ± 1.68	82.30 ± 1.80	86.84 ± 2.88	82.22 ± 1.54	84.97 ± 8.40	93.26 ± 6.21	81.66 ± 4.24	79.35 ± 5.22	81.35 ± 6.25	73.60 ± 5.03	97.54 ± 7.17	84.61 ± 5.27	82.93 ± 3.28
L-Lysine*	89.14 ± 6.08	88.32 ± 5.95	80.84 ± 9.24	76.82 ± 5.82	87.08 ± 2.92	71.65 ± 2.03	81.34 ± 2.24	89.85 ± 1.18	84.23 ± 6.50	84.96 ± 5.37	92.37 ± 10.46	76.12 ± 2.65	76.41 ± 5.36	78.95 ± 5.09	72.84 ± 6.33	81.56 ± 3.98	77.78 ± 4.75	73.69 ± 7.97
L-Tyrosine*	80.91 ± 6.42	89.75 ± 5.50	81.84 ± 9.35	80.86 ± 6.19	88.62 ± 3.61	74.91 ± 1.36	76.31 ± 1.73	84.24 ± 3.93	82.52 ± 1.63	79.42 ± 7.37	93.73 ± 5.19	80.54 ± 2.35	74.65 ± 5.94	81.76 ± 6.87	75.20 ± 5.27	92.67 ± 8.27	85.64 ± 3.48	82.62 ± 5.30
L-Isoleucine**	76.35 ± 6.68	78.28 ± 4.20	72.57 ± 8.51	69.74 ± 4.98	73.37 ± 2.01	64.62 ± 0.13	68.87 ± 1.45	78.14 ± 0.84	73.32 ± 3.16	69.86 ± 6.82	83.11 ± 2.98	68.71 ± 3.47	66.74 ± 3.90	72.24 ± 5.78	64.41 ± 3.67	77.37 ± 3.25	70.26 ± 4.93	66.63 ± 2.23
Glutamine	72.16 ± 33.73	62.58 ± 6.33	64.70 ± 12.96	71.75 ± 9.02	82.69 ± 10.19	54.10 ± 6.65	53.67 ± 9.78	55.16 ± 1.95	52.33 ± 11.60	57.63 ± 27.89	60.61 ± 15.40	72.54 ± 6.36	43.82 ± 2.07	61.58 ± 9.97	48.31 ± 3.53	74.50 ± 9.66	52.90 ± 11.71	62.87 ± 7.54
γ-Amino-n-butyric acid***	19.00 ± 2.41	26.43 ± 2.38	25.43 ± 2.88	21.94 ± 1.89	26.08 ± 1.49	22.45 ± 0.80	19.34 ± 0.44	27.18 ± 0.63	26.82 ± 1.45	21.90 ± 1.65	27.49 ± 2.43	23.71 ± 1.55	20.87 ± 1.39	27.60 ± 2.78	23.25 ± 2.22	24.31 ± 1.20	28.33 ± 1.93	25.79 ± 1.70
L-Cystine*	20.69 ± 1.54	23.29 ± 7.49	18.79 ± 1.75	22.98 ± 4.45	22.57 ± 1.18	17.50 ± 1.28	23.26 ± 2.41	16.89 ± 1.38	22.65 ± 1.25	18.15 ± 1.92	20.32 ± 1.66	18.23 ± 1.74	27.41 ± 4.12	22.50 ± 1.17	20.24 ± 2.70	18.65 ± 7.10	22.72 ± 1.71	23.34 ± 1.25
L-Methionine*	18.75 ± 2.12	17.85 ± 0.58	18.49 ± 2.44	16.70 ± 1.22	17.02 ± 0.87	15.28 ± 0.87	16.65 ± 0.87	19.52 ± 0.50	19.05 ± 0.67	17.59 ± 1.35	19.67 ± 0.93	17.17 ± 1.10	16.01 ± 0.81	17.65 ± 2.42	16.58 ± 1.24	18.64 ± 0.97	16.80 ± 1.12	16.04 ± 0.57
Asparagine	13.69 ± 1.30	12.77 ± 0.38	12.74 ± 1.90	13.10 ± 2.13	10.74 ± 1.95	13.62 ± 0.90	12.99 ± 0.48	14.42 ± 1.75	11.84 ± 1.92	13.51 ± 1.58	11.83 ± 1.23	15.05 ± 3.64	12.36 ± 1.02	15.08 ± 1.53	12.38 ± 1.42	14.87 ± 2.46	13.68 ± 0.69	17.15 ± 3.73
Ethanolamine**	12.43 ± 0.97	12.33 ± 0.80	10.79 ± 0.68	11.58 ± 1.04	11.31 ± 0.47	10.13 ± 0.42	11.17 ± 0.65	12.16 ± 0.46	11.65 ± 0.61	11.99 ± 0.86	13.06 ± 1.03	10.91 ± 0.20	10.22 ± 0.57	11.62 ± 1.14	10.38 ± 0.66	12.47 ± 0.32	11.53 ± 0.93	10.95 ± 0.68
Hydroxy-L-proline*	6.82 ± 0.88	6.35 ± 0.94	6.43 ± 1.01	7.63 ± 1.09	7.59 ± 0.49	6.82 ± 0.18	7.47 ± 0.31	7.95 ± 0.37	6.77 ± 0.14	7.08 ± 1.00	8.19 ± 0.51	7.29 ± 1.10	7.47 ± 0.65	8.21 ± 0.89	7.01 ± 0.90	9.05 ± 0.83	8.58 ± 0.19	8.72 ± 0.37

(*continued on next page*)

Table 8 (*continued*)

Amino acid	mg/L LAB Control batch 1	mg/L LAB Control batch 2	mg/L LAB Control batch 3	mg/L LAB HDHYC batch 1	mg/L LAB HDHYC batch 2	mg/L LAB HDHYC batch 3	mg/L LAB LoHDH batch 1	mg/L LAB LoHDH batch 2	mg/L LAB LoHDH batch 3	mg/L LAB HiHDH batch 1	mg/L LAB HiHDH batch 2	mg/L LAB HiHDH batch 3	mg/L LAB LoYC batch 1	mg/L LAB LoYC batch 2	mg/L LAB LoYC batch 3	mg/L LAB HiYC batch 1	mg/L LAB HiYC batch 2	mg/L LAB HiYC batch 3
L-Ornithine	6.28 ± 1.63	7.25 ± 0.79	6.63 ± 0.77	6.08 ± 0.76	6.76 ± 0.18	6.18 ± 0.21	5.48 ± 0.41	7.56 ± 0.88	6.87 ± 0.62	7.19 ± 1.27	6.23 ± 1.67	6.38 ± 0.52	5.41 ± 0.66	6.42 ± 0.89	5.90 ± 1.81	6.24 ± 0.54	6.56 ± 0.47	6.86 ± 1.24
β-Alanine	3.68 ± 0.13	3.72 ± 0.21	3.37 ± 0.52	3.92 ± 0.86	3.37 ± 0.25	3.10 ± 0.24	3.32 ± 0.13	3.42 ± 0.26	3.90 ± 0.25	3.48 ± 0.20	3.30 ± 0.08	3.36 ± 0.36	3.42 ± 0.65	3.85 ± 0.59	3.11 ± 0.15	3.61 ± 0.19	3.37 ± 0.33	3.45 ± 0.50

* Significant differences existed between batches at the .05 probability level according to the Kruskal Wallis test. Values with different superscript letters are significantly different according to Dunn's test for multiple comparisons with Holm's method.

** Significant differences existed between batches at the .01 probability level according to the Kruskal Wallis test.

*** Significant differences existed between batches at the .001 probability level according to the Kruskal Wallis test.

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

Table 9
Results from unstructured line scale questions (n=53).

Sample	Overall liking	Appearance	Aroma	Flavour	Mouthfeel
LoHDH	54.60 ± 26.83	62.45 ± 21.06	48.58 ± 25.20	51.83 ± 28.75	59.34 ± 23.81
HiHDH	63.87 ± 21.90	70.09 ± 21.08	60.53 ± 24.29	59.55 ± 26.49	60.57 ± 26.25
LoYC	63.23 ± 21.01	69.36 ± 20.95	57.74 ± 22.72	60.32 ± 23.58	63.51 ± 23.80
HiYC	57.77 ± 20.77	65.83 ± 21.65	53.94 ± 20.14	53.19 ± 22.16	57.02 ± 22.02
Control	64.11 ± 18.28	67.28 ± 22.36	53.45 ± 22.98	60.09 ± 22.99	60.79 ± 21.57
HDHYC	54.49 ± 24.28	64.25 ± 21.70	52.92 ± 25.35	51.38 ± 28.73	56.91 ± 25.08

'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

- brown, carbonated (appearance), foamy, and yellow; two were mouthfeel terms – carbonated (mouthfeel), and tongue-coating; and one was a taste term – sour.

32.1 % of panellists selected 'brown' for LoYC, which was significantly higher than for LoHDH (9.4 %), HiHDH (7.5 %), HiYC (13.2 %), and the control (3.8 %), and was not significantly different to HDHYC (18.9 %). This is somewhat in agreement with the results from colour analysis; although significant differences were not achieved across all samples, the EBU colour for LoYC samples was higher (i.e., darker) than LoHDH, HiHDH, and control samples, and was similar to HDHYC samples. However, HiYC samples tended to have similar or higher EBU colour than LoYC even though significantly more panellists selected 'brown for LoYC than HiYC. 9.4 % of panellists selected 'yellow' for HiYC, which was significantly less than LoHDH, HiHDH, and the control (all 28.3 %), while not significantly different to LoYC (20.8 %) or HDHYC (15.1 %). These results are in agreement with the overall trend observed in colour analysis, where LoHDH, HiHDH, and control samples had lower EBU colour (i.e., were lighter) than HiYC (although significance was not determined between all samples), while LoYC and HDHYC tended to have more similar EBU colour to HiYC.

64.2 % and 50.9 % of panellists selected 'foamy' as a descriptor for the appearance of HiYC and LoYC samples respectively which was significantly higher than all other samples, the highest of which was LoHDH (18.9 %). Conversely, only 26.4 % and 30.2 % of panellists selected 'carbonated' as a descriptor for the appearance of HiYC and LoYC samples respectively, significantly lower than all other samples except LoHDH (49.1 %), which was not significantly different to LoYC. Significantly more panellists selected 'sour' as a descriptor for the taste of HDHYC (69.8 %) than HiHDH (41.5 %), with no other significant differences identified.

Although statistical significance was determined by Cochran's *Q* test for carbonated (mouthfeel) and tongue-coating, McNemar's test identified no significance between the samples. The contingency table for check-all-that-apply terms is presented in Table 10.

Further analysis of CATA sensory attributes was carried out using correspondence analysis (CA) to visualise the association between sensory attributes and LAB, as shown in Fig. 1. The CA biplot map explains 73.53 % of the total variance (59.12 % in the first dimension and 14.41 % in the second dimension). The results displayed in Fig. 1 show that LoYC and HiYC are closely grouped in the negative scores of the first dimension and correlated with appearance terms of foamy and brown. According to Blasco, Viñas, and Villa (2011), foaming in beer occurs mainly as a result of the interactions between proteins and hop acids. Although barley proteins are the major contributors to beer foaming, with yeast proteins playing a secondary role (Blasco et al., 2011), it may be that the proteins present in NZYC contributed to the strong

Table 10
A contingency table of the fraction of consumers (n= 53) selecting the 20 terms from the Check-All-That-Apply questionnaire to describe low alcohol beer.

Attribute	Significance	LoHDH	HiHDH	LoYC	HiYC	Control	HDHYC
Appearance							
Amber	*0.887*	0.340	0.377	0.302	0.396	0.321	0.340
Brown	*<0.001***	0.094[a]	0.075[a]	0.321[b]	0.132[a]	0.038[a]	0.189[ab]
Carbonated	*<0.001***	0.491[bd]	0.642[ab]	0.302[cd]	0.264[c]	0.774[a]	0.660[ab]
Clear	*0.285*	0.283	0.226	0.321	0.321	0.358	0.208
Foamy	*<0.001***	0.189[a]	0.057[a]	0.509[b]	0.642[b]	0.057[a]	0.094[a]
Golden	*0.943*	0.453	0.528	0.509	0.472	0.472	0.472
Hazy	*0.697*	0.528	0.491	0.453	0.528	0.472	0.547
Yellow	*0.005***	0.283[a]	0.283[a]	0.208[ab]	0.094[b]	0.283[a]	0.151[ab]
Flavour							
Camphor	*0.407*	0.226	0.132	0.113	0.132	0.170	0.132
Citrus	*0.193*	0.245	0.358	0.245	0.283	0.340	0.396
Crisp	*0.584*	0.245	0.340	0.340	0.396	0.321	0.340
Earthy	*0.156*	0.358	0.170	0.283	0.226	0.283	0.245
Floral	*0.968*	0.226	0.226	0.264	0.208	0.264	0.245
Grassy	*0.969*	0.208	0.245	0.208	0.189	0.189	0.226
Hoppy	*0.078*	0.358	0.225	0.396	0.226	0.283	0.226
Metallic	*0.467*	0.151	0.113	0.358	0.208	0.113	0.208
Wooden	*0.281*	0.132	0.038	0.094	0.170	0.075	0.132
Mouthfeel							
Astringent	*0.846*	0.151	0.151	0.132	0.208	0.189	0.189
Carbonated	*0.019**	0.208[a]	0.321[a]	0.226[a]	0.302[a]	0.434[a]	0.396[a]
Light	*0.404*	0.358	0.528	0.434	0.415	0.491	0.472
Medium	*0.464*	0.434	0.302	0.358	0.396	0.321	0.434
Persistent	*0.582*	0.226	0.132	0.226	0.170	0.151	0.132
Tongue-coating	*0.024**	0.189[a]	0.113[a]	0.019[a]	0.151[a]	0.057[a]	0.151[a]
Taste							
Bitter	*0.409*	0.566	0.377	0.491	0.453	0.453	0.434
Sour	*0.006***	0.642[ab]	0.415[a]	0.604[ab]	0.604[ab]	0.491[ab]	0.698[b]
Sweet	*0.921*	0.208	0.283	0.245	0.283	0.245	0.245
Umami	*0.304*	0.170	0.264	0.170	0.189	0.189	0.113

Note: Letter within each row represents a statistical difference between each sample.
No asterisk represents statistical significance was not reached.
* Statistical significance was reached at $p < 0.05$
** Statistical significance was reached at $p < 0.01$
*** Statistical significance was reached at $p < 0.001$
'Lo' denotes a lower concentration of New Zealand honeydew honey (NZHDH) or New Zealand yacon concentrate (NZYC); 'Hi' denotes a higher concentration of NZHDH or NZYC. 'HDHYC' beer contains a lower concentration of both NZHDH and NZYC.

correlation of the 'foaming' variable with the LoYC and HiYC samples. The protein content of NZYC ranges from 4.744 ± 0.650 to 6.634 ± 0.158 g/100 g (Chessum et al., 2023), while the protein content of NZHDH – 0.47 ± 0.04 g/100 g - is approximately ten times lower (Chessum et al., 2022). NZYC is also darker in colour than NZHDH, which may explain why the 'brown' variable is strongly correlated with the LoYC and HiYC samples, while the 'yellow' variable is strongly correlated with the other samples. Control and HiHDH samples had high positive scores along dimension 1 and were correlated with attributes of yellow and carbonated. These results are somewhat supported by the results from colour analysis as previously discussed; furthermore, the results from the determination of ethanol content weakly support the correlation of HiHDH with the carbonated attribute. Although Dunn's test of multiple comparisons using Holm's method identified relatively few significant differences between samples, HiHDH LAB samples tended to have higher ethanol content and would therefore have the highest content of carbon dioxide.

The results from the second dimension are less significant, as they only explain 14.41 % of the total variance, and most of the descriptor terms lie relatively close to the origin. However, HiYC and HDHYC samples had relatively high negative scores and were correlated with flavour terms of wooden, metallic, and citrus, and mouthfeel terms astringent, carbonated, and tongue-coating. Of these flavour terms, citrus is associated with 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol, a compound with a higher internal standard response ratio in NZYC than in NZHDH.

Fig. 2 summarises the penalty analysis results for the LABs. Frequency, plotted on the x-axis, refers to the percentage of panelists who indicated either too much or too little of each attribute. Penalty, plotted on the y-axis, refers to the mean drop in overall liking as a result of that attribute having too little or too much intensity. Two attributes – not enough sweetness and not enough hoppiness – contributed significantly to the overall liking score for all six samples. Not enough sweetness had frequency ranging from 41.5 % in the control (Fig. 2**(f)** to 54.7 % in both the HDHYC (Fig. 2**e**) and LoHDH (Fig. 2**a**) samples, with a penalty of 15.3 ± 2.4. Not enough hoppiness had frequency ranging from 34.0 % in HiHDH (Fig. 2**b**) to 52.8 % in HiYC (Fig. 2**d**), with a penalty of 12.6 ± 2.6. Not enough bitterness also contributed significantly to almost all samples except LoHDH (Fig. 2**a**), where too much bitterness had a higher frequency (24.5 % compared to 17.0 %). Excluding LoHDH, the frequency for not enough bitterness ranged from 26.4 % in HiHDH (Fig. 2**b**) to 43.4 % in the control (Fig. 2**f**), with a penalty of 10.6 ± 2.7. Too much sweetness, too much hoppiness, and too much bitterness (except for LoHDH) had low frequency and thus did not contribute significantly to the overall liking of the LABs.

Reformulation could be considered to improve the overall liking scores for the LABs. Not enough bitterness had highest frequency for HiYC, HDHYC, and Control LABs, so it would be of most interest to increase the bitterness of these LABs. In order to increase bitterness, more hops could be added at the start of the boil phase to increase the concentration of isoalpha acids, while more hops could also be added towards the end of the boil phase to increase the concentration of volatile hop compounds and so increase hoppiness. Not enough hoppiness had frequency of 34.0 % or greater for all LABs, so it would be of interest to increase the hoppiness of all LABs. Not enough sweetness had frequency of 41.5 % or greater for all LABs, so it would be of interest to increase the sweetness of all LABs. To increase the sweetness of the beer, non-fermentable sugars such as lactose or artificial sweeteners could be

added to not generate additional alcohol or carbon dioxide. Starchier grain could also be used for brewing to increase the concentration of dextrin in the beer; however, changing the mash ingredients would change the flavour profile of the final beer depending on which grains were selected (Cadenas, Caballero, Nimubona & Blanco, 2021). FOS could also be added to the beer. In addition to increasing the sweetness of the beer, this would raise FOS content closer to the required dose to classify the LABs as functional beverages. Although increasing the bitterness of the beer may have an adverse effect on overall liking due to the high penalty of too much bitterness, it is important to note that sweetness does have a masking effect on bitterness and that the frequency of not enough bitterness tended to be approximately double that of too much bitterness, except in the LoHDH sample.

4. Conclusions

- LABs were successfully produced with respect to ethanol content, which ranged from 0.61 ± 0.02 % ABV (Control 1) to 0.86 ± 0.03 % (HiHDH 3). This also suggests that higher levels of NZHDH and/or NZYC may be added without exceeding the 1.15 % ABV threshold.
- After acid hydrolysis, fructose (indicating the presence of FOS) was detected in NZYC-containing samples but was not detected in other samples. This supports the incorporation of NZYC into LABs to develop a functional beverage, particularly with respect to gut health.
- Volatile analysis of NZHDH identified compounds associated with 'floral' and 'earthy' flavours (terms identified in LAB samples by the semi-structured focus group) at much higher levels than NZYC, while analysis of NZYC identified compounds associated with 'floral' at much higher levels than NZHDH. Although secondary fermentation results in chemical changes to volatile compounds, incorporating higher concentrations of NZHDH and NZYC may result in more significantly different flavours between LAB samples.
- Scores for overall liking, appearance, aroma, mouthfeel, and flavour on unstructured line scales were not significantly different between LAB samples, nor were interactions between LAB samples and panellist age, gender, or frequency of beer consumption. At their existing concentration, the additions of NZHDH and NZYC did not successfully differentiate flavoured LABs from the unflavoured controls, although samples were somewhat liked by panellists.
- CATA analysis showed that 'brown' was selected significantly more to describe the appearance of LoYC than all other samples except HDHYC, while 'yellow' was selected significantly less to describe the appearance of HiYC than LoHDH, HiHDH, and the control. 'Foamy' was selected significantly more, while 'carbonated' was selected significantly less, to describe the appearance of LoYC and HiYC samples. This suggests the addition of NZHDH and NZYC did have some effect on the appearance of LAB samples. Foaming properties may be related to the higher protein content of NZYC than NZHDH.
- Penalty analysis showed that all samples were not sweet enough and not hoppy enough, while all samples except LoHDH were not bitter enough. This result, along with results from unstructured line scales and CATA analysis, support reformulation of LAB beverages to increase liking and to more significantly differentiate samples from one another.

CRediT authorship contribution statement

Keegan Chessum: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Nazimah Hamid:** Writing – review & editing, Supervision, Methodology. **Barry Wong:** Methodology. **Tony Chen:** Methodology. **Mary Yan:** Resources. **Rothman Kam:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

The sensory evaluation in this study was reviewed and approved by the AUT Ethics Committee number 23/265. Written informed consent was obtained from all study participants.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2024.100544.

Data availability

No data was used for the research described in the article.

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