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Dietary fiber as nutritional and sustainable malt substitute for reducing glycemic load in non-alcoholic beer

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14 **Abstract**

15 Non-alcoholic beer (NAB) is mostly produced with energy-intensive dealcoholization, and its digestible
16 carbohydrates contribute to glycemic load, limiting suitability for people with impaired blood glucose regulation.
17 Reducing digestible carbohydrates lowers glycemic load but can compromise NAB palatability. Here, we propose
18 a strategy to produce palatable NAB with reduced digestible carbohydrates and environmental impact by replacing
19 dealcoholization with maltose-negative yeasts and malt with resistant dextrins. The approach combines inhibited
20 β -amylase activity, higher liquor-to-grist ratios, and elimination of base malt to reduce maltose, maltotriose, and
21 residual extract, while resistant dextrins restore body and reduce bitterness. The resulting reduction in glycemic
22 load is governed by malt substitution with resistant dextrins, while replacing dealcoholization with maltose-
23 negative yeast delivers the largest reduction in global warming potential and resistant dextrins substantially reduce
24 land use. Together, these findings demonstrate a scalable approach to producing NAB that balances sensory
25 quality, nutritional properties, and environmental sustainability.

Introduction

In most high income countries, beer consumption has declined in the past two decades due to the desire for healthier living ^{71,72}. Non-alcoholic beer (NAB) is increasingly considered a healthier alternative to alcoholic beverages and its consumption in high income countries is growing rapidly ⁷³. Despite the health benefits of eliminating alcohol, NAB presents a high glycemic load (GL) and an elevated environmental impact due to the energy-intensive dealcoholization process. Overcoming the limitations of current production technologies requires alternative routes to dealcoholization and strategies to replace digestible carbohydrates (DCs) with low GL alternatives that preserve palatability.

The energy intensive dealcoholization step can be eliminated using maltose-negative yeast to restrict alcohol formation to below 0.5vol%¹ (Fig. 1). Maltose-negative yeasts do not ferment maltose but fructose and glucose which account for less than 15% of fermentable carbohydrates in typical beer wort ². The residual high amount of sweet maltose in the finished beer increases palatability (S. Fig. 5) by contrasting bitterness and improving body but as well increasing GL due to its high glycemic index (GI) ^{3,4}. Reducing maltose without compromising on sensory quality is therefore crucial to improve the nutritional quality of NAB made with maltose-negative yeasts ⁵.

Previous studies have attempted to reduce maltose by mashing above the optimal β -amylase and α -amylase temperature to limit its formation ⁶, or by enzymatically converting maltose into glucose such that it is metabolized by maltose-negative yeast ⁷. Neither approach or combination can fully eliminate maltose, as it is already present in the grain, and converting it entirely into glucose would produce excessive alcohol ^{6,7}. Increasing liquor-to-grist ratio (LTGR) to lower maltose levels is the easiest method, however the resulting decrease in residual extract (RE) yields a thin bodied and overly bitter final product. Balancing nutritional quality, palatability and environmental impact of NAB remains therefore unresolved.

In the present study, we use maltose-negative yeasts combined with the replacement of malt with resistant dextrins (RDs), a form of dietary fiber (DF), to produce more sustainable and nutritionally improved NAB compared to the industry standard. The effect of RDs on NAB bitterness and body was assessed by producing low-maltose, low-residual-extract worts and supplementing them with different types of RD. The tribological and rheological properties of the selected RDs were chosen to substitute DCs and were correlated with their bitterness reducing and body enhancing effects in a NAB matrix using a trained sensory panel. The glycemic response, measured by the interstitial fluid glucose levels, of a single dietary fiber-enriched NAB (f-NAB) formulation compared to a commercial reference NAB (c-NAB) was assessed with an intervention study including 22 participants. Furthermore, a life-cycle assessment (LCA) was

conducted to quantify the different environmental impacts between the f-NAB and c-NAB.

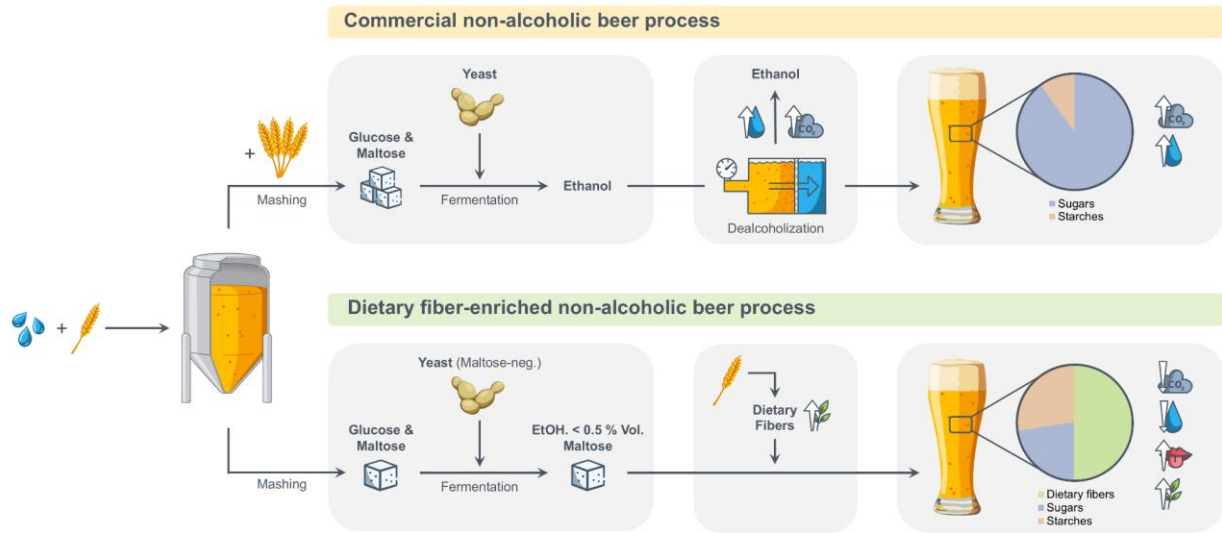


Fig. 1: Non-alcoholic beer (NAB) production using commercial dealcoholization (top) versus dealcoholization-free and dietary fiber integrated production (bottom). Top: Production of a commercial non-alcoholic beer (c-NAB). Alcoholic beer is produced and the ethanol is subsequently removed by dealcoholization and ethanol recovery (nanofiltration with subsequent vacuum distillation). **Bottom:** Proposed production concept for fiber-enriched non-alcoholic beer (f-NAB). Mashing conditions and grist composition are adjusted to modify the wort carbohydrate profile, particularly by reducing maltose formation. Dietary fibers are integrated to compensate for reduced extract and body while lowering the digestible carbohydrate load. Ethanol formation is limited during fermentation by using maltose-negative yeast, thereby avoiding subsequent dealcoholization.

Results and discussion

NAB production without dealcoholization

The use of maltose-negative yeasts with adapted LTGR and mashing protocols allow the production of NAB without dealcoholization (Fig. 1). Maintaining ethanol <0.5 vol% requires restricting total fermentable carbohydrates, predominantly glucose, to below 7.7 g/L. Glucose is mainly formed during mashing since the endogenous amylases active during typical mashing conditions form maltose, maltotriose, maltooligosaccharides (MOS, DP 3-10), and dextrins (DP 3-100) but no glucose in significant amounts⁸. Maltose is a glucose dimer with a relative sweetness of 30-50% compared to sucrose (glucose at 50-75% sucrose sweetness)⁹ and is hypothesized to be the primary culprit for the high GI of NAB. Therefore, minimizing maltose formation during mashing is hypothesized to be essential for a low-GL formulation. To arrest maltose formation, mashing temperatures above 70 °C are utilized to thermally inhibit β -amylase⁸.

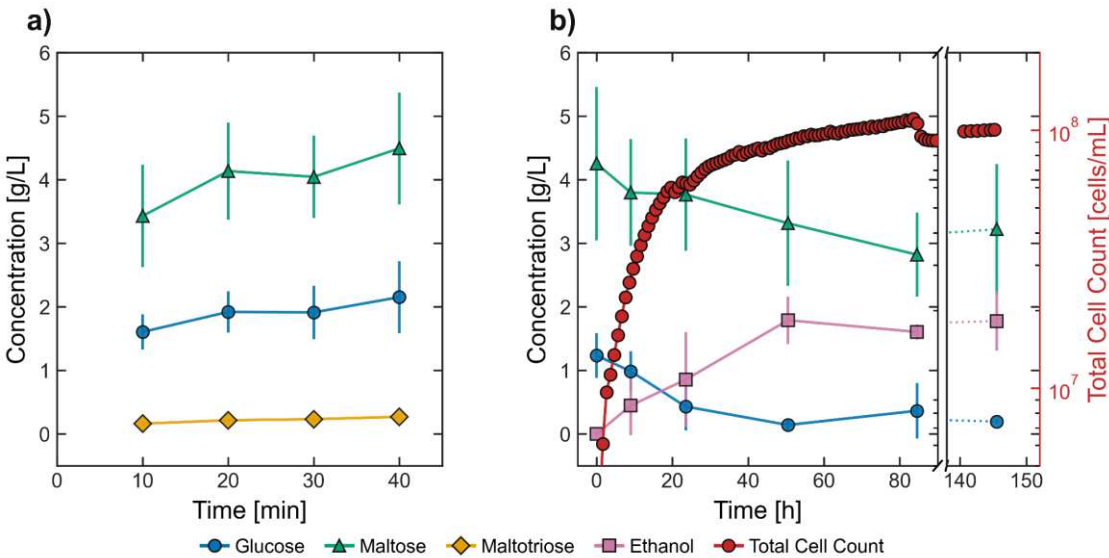


Fig. 2: Temporal evolution of key process dynamics during mashing and fermentation of NAB. **a)** Glucose, maltose and maltotriose, as a function of mashing time for a specific liquor-to-grist ratio (LTGR) and grist composition (GC). Data are shown for n = 3 biological replicates. **b)** Wort carbohydrate concentrations, including glucose and maltose, and ethanol concentration (left y-axis) and total cell count (right y-axis) as a function of fermentation time. Fermentation was conducted with a maltose-negative *S. cerevisiae* strain at 20 °C before cooling to 4 °C at t = 84.5 h. NIR refers to ethanol measurement by near-infrared spectroscopy. Data are shown for n = 3 biological replicates, except for viable cell density, where n = 1 biological replicate. All error bars correspond to the standard deviation of the average from triplicate measurements.

Coupling an elevated LTGR with the complete exclusion of base malts (LTGR2-GC2) arrests maltose

accumulation during isothermal mashing at 74 °C (Fig. 2a). Glucose, maltose, and maltotriose concentrations showed no apparent increase, remaining within the range of experimental variation during 40 min isothermal mashing at 74 °C for LTGR2- GC2. The absent glucose increase above the experimental variation confirms that LTGR and GC dictate glucose concentration. The absent maltose increase above the experimental variation confirms the effective β -amylase thermal inactivation⁸. The wort density increased from 10 to 40 min mashing exceeding the experimental variation (S.Fig. 1), suggesting a continuous solubilization of MOS and dextrans from the malt into the wort without a measurable breakdown into maltose and maltotriose. The comparison of LTGR1-GC1 with LTGR2-GC2 shows that adding Pilsner malt disproportionately increases glucose, maltose, and maltotriose relative to its grist weight (S.Tab. 1). Expectedly, gently kilned base malts yield more low-molecular-weight sugars than hotter-kilned caramel malts and unmalted wheat flakes⁸.

Fermentation with a maltose-negative *Saccharomyces (S.) cerevisiae* strain achieved rapid glucose depletion without above experimental variation maltose consumption, effectively restricting ethanol accumulation (Fig. 2b). Glucose was consumed within the first 50 h, whereas dissolved oxygen (DO) was depleted in the first hour (S.Fig. 3). Other studies revealed that for various *non-Saccharomyces* strains cultivated under similar conditions glucose concentrations of 4.4 g/L were not fully metabolized after 144 h¹⁰. The relatively fast glucose depletion reported in this study is most likely due to the low initial concentration and the used *S. Cerevisiae* strain that was produced via hybridization with a maltose-negative wild type according to the technical data sheet¹¹. Viable cell counts remained stable upon glucose depletion and maltose concentration remained constant at ~3-4 g/L confirming the maltose-negativity of the strain. The reduction of maltose within the experimental variation is in line with previous studies¹⁰. NIR-measured ethanol production inversely mirrored glucose depletion, with yields remaining consistent across NIR, GC-FID, and theoretical stoichiometry (S.Fig. 2a). The rapid initial DO depletion reflects immediate oxygen uptake for cell recovery, which quickly establishes an anaerobic environment. The stable maltose concentration confirms the strain's maltose-negativity, while the strict synchronization of glucose depletion, ethanol accumulation, and growth cessation demonstrates a dominantly anaerobic ethanolic metabolism. For LTGR1-GC1, an initial DO of 4-5 ppm (S.Fig. 3) diverted a fraction of glucose toward biomass synthesis rather than ethanol. Because this early carbon diversion shifts the 7.7 g/L threshold based on the yeast's respiration-fermentative balance, both stoichiometric glucose consumption and apparent attenuation overestimate final ethanol yields under these higher DO conditions (S.Fig. 2b).

Physical and sensory properties of resistant dextrins

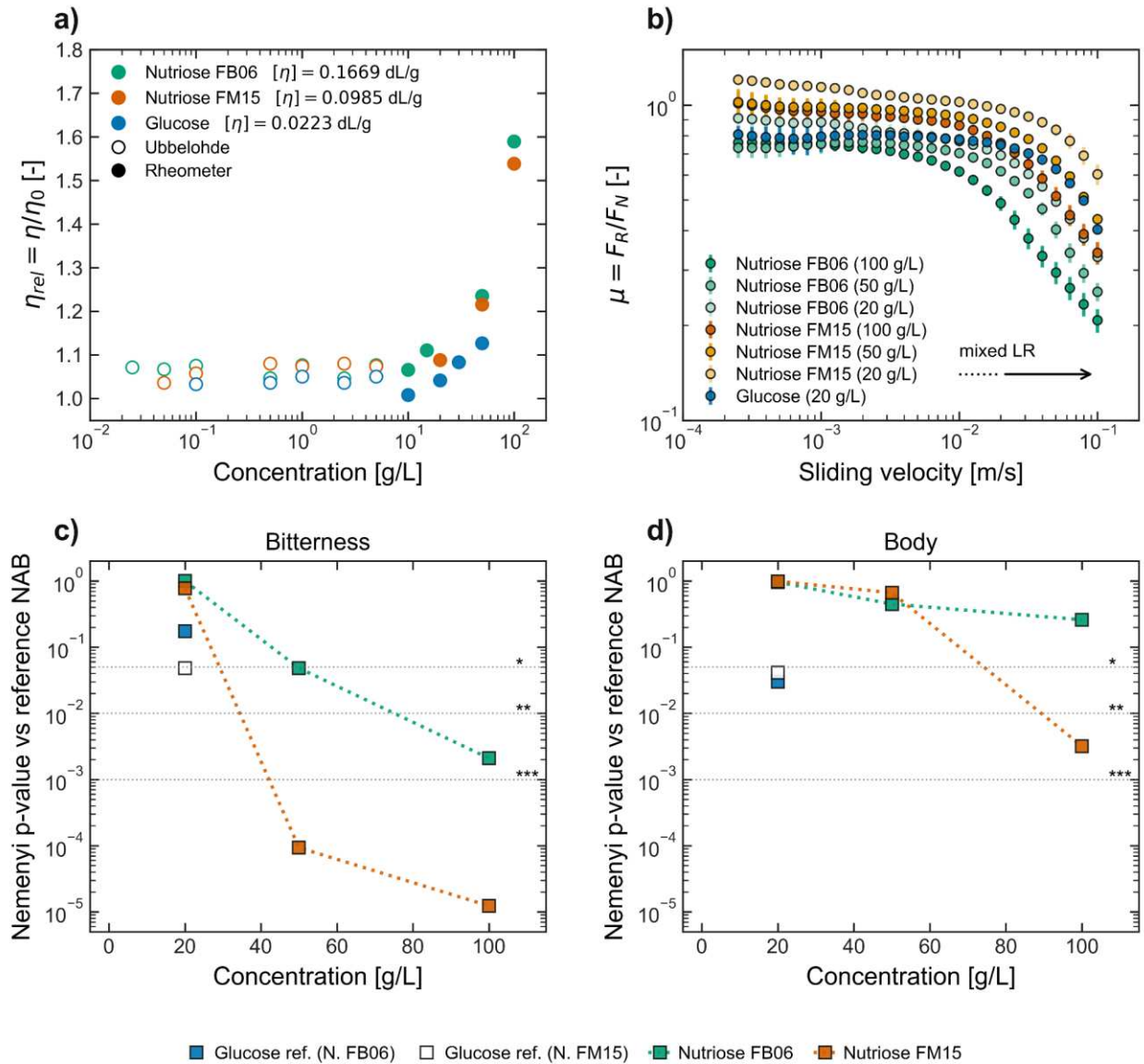


Fig. 3: Rheological, tribological, and sensory properties of glucose and resistant dextrins. **a)** Relative viscosity as a function of concentration for glucose, Nutriose FM15 and Nutriose FB06 dissolved in water (n = 3 replicates, open symbols, Ubbelohde capillary viscometer) and a model NAB matrix (n = 2 replicates, closed symbols, rotational rheometer concentric-cylinder). Intrinsic viscosity values, obtained from Kraemer plots (S.Fig. 4) are indicated. **b)** Friction coefficient as a function of sliding velocity (Stribeck curve) for glucose (20 g/L), Nutriose FM15 (20, 50, and 100 g/L) and Nutriose FB06 (20, 50, and 100 g/L) in a model NAB matrix (n = 5 replicates). Error bars correspond to the standard deviation of the five replicates. **(c, d)** Nemenyi post hoc p-values for the sensory attributes **c)** bitterness and **d)** body as a function of glucose, Nutriose FB06, and Nutriose FM15 concentration. Values represent comparisons between each treatment and the reference NAB (LTGR1-GC1). Colored dashed lines guide the eye; black dotted lines

indicate significance thresholds (*p < 0.05, p < 0.01, ***p < 0.001).

Reducing maltose with β -amylase inhibition at 74°C, a high LTGR, and the absence of base malts in the grist reduced the RE, yielding a thin-bodied and excessively bitter NAB. RE in beer has been reported to reduce bitterness; however, the high molecular heterogeneity did not allow to disentangle the individual contributions of viscosity, lubrication and sweetness to the reduction^{25,26}. A recent evaluation of commercial NABs revealed that maltose is a good predictor for overall liking (S.Fig. 5)¹². To test GL-neutral alternatives to RE and maltose, Nutriose FB06 and Nutriose FM15, two RDs classified as resistant starch, a type of DF, were chosen based on their high solubility and low viscosifying effect. In this section, the rheological and tribological properties of two selected RDs are correlated with their sensory capacity to enhance the body and mask bitterness in the final NAB. We benchmark the RDs to glucose, a well-defined compound representing DCs. The molecular properties of Nutriose FB06 and FM15 are displayed in Table 1:

Table 1: Chemical and physical properties of used resistant dextrins compared to glucose.

	Nutritiose FB06 (FB06)	Nutritiose FM15 (FM15)	Glucose
DE in g/100g	5 (TDS)	10-15 (TDS)	100
DP	12-25 ¹³	7-12 ^{14,15} (from Mw)	1
Mw (g/mol)	4-6*10 ³ (weight) ¹³	1.2-2*10 ³ (number) ¹⁴	180
Predicted $[\eta]$ (DP) (dL/g) ¹⁵	0.062-0.072 dL/g	0.048-0.062 dL/g	
Residual sugars in g/100g	<0.5 (TDS)	5-10 (TDS)	100
Dietary fiber content in g/100g	82-88 (TDS)	90-95 (TDS)	0

Despite comparable relative viscosity compared to Nutriose FM15 at equivalent concentrations, Nutriose FB06 demonstrates superior boundary and mixed-regime lubrication (Fig. 3a, b). The relative viscosity as a function of concentration is depicted for glucose, Nutriose FB06, and FM15 (Fig. 3a). Viscosity increases more effectively for Nutriose FB06 and FM15 compared to glucose. Nutriose FB06 exhibits a higher intrinsic viscosity (0.1669 dL/g) than FM15 (0.0985 dL/g) (S.Fig. 4). However, their bulk viscosities align closely at the evaluated sensory concentrations (20–100 g/L). The difference between Nutriose FB06 and FM15 becomes more apparent in the Stribeck curves, where the friction coefficient is a function of sliding velocity (Fig. 3b). We observe the transition from boundary to mixed lubrication regime (LR) at approximately 10⁻² m/s from the analysis of the friction coefficient as a function of the Hersey number (S.Fig. 6)¹⁶. Nutriose

FB06 demonstrates lower friction coefficients across both boundary and mixed lubrication regimes relative to Nutriose FM15. Nutriose FM15 exhibits higher friction, structurally mirroring the mixed-regime lubrication profile of glucose more closely than Nutriose FB06. Interestingly, 20 g/L Nutriose FB06 shows very similar lubrication to 100 g/L Nutriose FM15 in the mixed regime. While the relative difference in intrinsic viscosity between Nutriose FB06 and FM15 scales as predicted by their degree of polymerization (DP), their absolute values deviate from linear maltodextrin models¹⁵. Both are higher compared to the model predictions, where the value for Nutriose FM15 aligns more closely with the model predictions than FB06, a discrepancy potentially attributable to the degree of branching (DB). The extensive branching and resulting reduced average chain length (CLn) should theoretically yield lower intrinsic viscosities than their linear maltodextrin counterparts. The anomaly between DP and physical properties is also evident in the lubrication behavior of Nutriose FB06 compared to FM15. Low molecular weight maltodextrins were found to lubricate more effectively in the boundary and mixed lubrication regime and with increasing concentration¹⁷. Other studies report that rigid-rod polymers show higher friction coefficients than random coil polymers¹⁸. Based on the manufacturing process and the concentration dependent viscosity, Nutriose FM15 is likely to exhibit longer CLn and a lower DB than Nutriose FB06. This is supported by the dependency of CLn and DB on DP during their manufacture. At a DP>18, a sudden increase of DB and reduction of CLn is reported¹⁹. This structural divergence explains their similar viscosities yet contrasting lubrication behaviors: the extensive branching of Nutriose FB06 yields a hydrodynamic radius comparable to Nutriose FM15, while maintaining a random coil rather than a rigid-rod conformation.

While bitterness suppression is reliably governed by bulk viscosity and lubrication, body perception is primarily driven by sweetness, with physical viscosity and lubrication serving only as secondary, coupled factors that fail to enhance body in the absence of sweet-taste cues (Fig. 3c, d). Both RDs significantly reduce perceived bitterness at concentrations of 50 g/L and above compared to the NAB reference (Fig. 3c). Conversely, body perception differentiates the two fibers (Fig. 3d). Nutriose FB06 yields no significant body enhancement across all tested concentrations, whereas Nutriose FM15 at 100 g/L significantly increases perceived body to a similar extent as a 20 g/L glucose addition. The investigated glucose, Nutriose FB06 and FM15 concentrations help us relate the change in bitterness and body to different mechanisms. At 20 g/L, viscosities are very similar across the three compounds and the samples differ only in their sweetness and lubrication behavior. Since the molecular structure of Nutriose FB06 and FM15 is very close to DP6-20 maltodextrins, sweetness effects are expected to be low based on previous work with RDs^{20,21,22}. Nutriose FB06 increases whereas FM15 reduces lubrication compared to glucose, yet bitterness reduction and body enhancement were only significant for glucose suggesting that the sweetness of glucose suppressed bitterness and enhanced body perception. These results are consistent with previous works reporting a 30% bitterness reduction for 20 g/L glucose in a 10 IBU beer matrix^{23,24} and showing that DP1-DP3

carbohydrates correlate significantly with beer fullness whereas >DP10 dextrans do not⁷⁴. At concentrations of 50 and 100 g/L, the viscosity of Nutriose FB06 and FM15 is increased compared to glucose and bitterness reduction becomes significant. Previous studies estimated that the viscous contribution to bitterness reduction in a maltodextrin-sucrose solution is between 20% and 30%, trying to distinguish physical effects from molecular interactions²⁷. However, Nutriose FM15 reduces bitterness with greater statistical significance than FB06 while their viscosities are nearly identical. This can be attributed to either the difference in lubrication behavior or in residual sugars (Tab. 1). Nutriose FM15 has 5-10 g/100g residual sugars compared to the 0.5 g/100g of Nutriose FB06 and shows reduced lubrication compared to Nutriose FB06. Furthermore, at 50 g/L, the Stribeck curve of Nutriose FM15 is very similar to 20 g/L glucose. The effect of Nutriose FB06 and FM15 on body perception is similarly insignificant up to 50 g/L, despite the increased viscosity compared to 20 g/L glucose and the similar lubrication behavior of 50 g/L Nutriose FM15 to 20 g/L glucose. These results tentatively point towards bitterness suppression being predictably governed by physical viscosity and oral lubrication, whereas body perception remains largely insensitive to matched lubrication or moderate viscosity increases in the absence of gustatory sweetness. At 100 g/L, Nutriose FM15 and FB06 both reduced bitterness with greater statistical significance and diverged in their statistical significance of increasing body. The increased body perception for FM15 at such high concentration is most likely a combination of residual sugars, high viscosity and lubrication behavior. While bitterness reduction is driven by sweetness and viscosity, body perception is, based on our results, primarily driven by sweetness, with physical viscosity and lubrication likely contributing as secondary, coupled factors that are difficult to separate at higher concentrations. Similar findings were reported for maltodextrins with varying molecular weight where viscosity was a poor predictor for palate fullness²⁸.

Taken together, these findings suggest that incorporating 50–100 g/L of either DF type can effectively reduce bitterness and, in the case of 100 g/L Nutriose FM15, enhance body perception to a degree comparable to 20 g/L glucose. To assess the impact of the investigated DFs on the nutritional quality of NAB, an intervention study with a commercial NAB (c-NAB) and fiber-enriched NAB (f-NAB) was done.

Reducing Glycemic Load in Non-Alcoholic Beer

Evaluating the nutritional quality of NAB requires an accurate assessment of its postprandial impact via the GL. The GL is calculated based on the DC content and respective GI of a given food and serving size^{3,4}:

$$GL(g) = \frac{GI \cdot DC(g)}{100}$$

A food with high DC content and low GI (i.e. yellow peas) can have the same GL as a food with little DC exhibiting high GI (i.e. glucose rich drink). We hypothesize that the introduction of Nutriose FB06 lowers

the GL via a reduction of the GI as shown previously for other DFs ²⁹. Nutriose FB06 was chosen for the intervention study as it contains little to no residual sugars potentially affecting the blood glucose response. The LTGR2-GC2 system was produced at 500 L scale and 50 g/L Nutriose FB06 dissolved into the wort to create the f-NAB. A c-NAB with a similar digestible carbohydrate content and RE composition was identified. Both the f-NAB and c-NAB were sent to an external laboratory for analysis of DC composition and glucose, maltose and maltotriose was measured additionally in-house.

Table 2: Total digestible carbohydrate content, glucose, maltose, and maltotriose as well as dietary fiber content and composition of samples used for intervention study. * denotes residual extract with FB06 contribution, d.l. = detection limit, n/a = not applicable

Sample	Total DC (g/100g)	Glucose (g/100g)	Maltose (g/100g)	Maltotriose (g/100g)	Total DF (g/100g)	HMWDF, DP>10 (g/100g)	LMWDF, DP3-9 (g/100g)	RE (g/ml)
c-NAB	2.6	0.11	0.51 - 0.58	0.11	n/a	n/a	n/a	1.015
f-NAB	2.7	<0.1	0.16 - 0.19	< d.l.	3.7	1.4	2.4	1.011/1.025*
Glucose ref	2.5	2.5	0	n/a	n/a	n/a	n/a	n/a

The sample composition of the f-NAB and c-NAB compared to the glucose reference reveals that while both c-NAB and f-NAB have similar total digestible carbohydrate contents, the c-NAB has higher glucose and maltose concentrations (Tab. 2). The lower maltose concentration of the f-NAB compared to the data shown in Fig. 2 and S.Tab. 1 are due to the change in malt lot and the production scale. Total DF content of the f-NAB was determined to be 3.7 g/100g of which 1.4 g/100 g are DP>10, high-molecular weight (HMW) DF and 2.4 g/100 g are DP 3-9 low-molecular weight (LMW) DF. The RE of the c-NAB was 1.015 g/ml and of the f-NAB 1.011 g/ml prior to the Nutriose FB06 addition and 1.025 g/ml after addition. The low recovery rate of the added Nutriose FB06 as DF is related to the partial digestibility of the product. Based on the NAB RE value of 1.011 g/ml, a theoretical digestible carbohydrate concentration of 1.8-2 g/100g is expected without Nutriose FB06 addition. Consequently 0.7-0.9 g/100g of digestible carbohydrates were contributed by the addition of 50 g/L Nutriose FB06 (14-18 g/100g), which is in accordance with the specified 12-18 g/100g non-DF moieties which are most likely DCs ³⁰. However, the determined DF content of 3.7 g/100g is slightly below the specified 82-88 g/100g. The residual difference is likely due to the inaccuracy of the volume determination in the fermentation tank and applied method to determine total DF content. For the design of the intervention study the important metric is the total DC content which was similar between c-NAB and f-NAB resulting in similar ingested sample volume and hence gastric emptying.

Both NABs showed a high GI exceeding pure glucose, indicating that RDs lower glycemic load strictly via iso-sensory malt replacement, as their additive inclusion does not attenuate glycemic response (Fig. 4). The averaged interstitial glucose levels for the $n = 22$ observations for each sample are within each other's 95% confidence intervals ($CI = 1.96 * s/\sqrt{n}$) (Fig. 4a). The average increase in interstitial glucose is identical for the three sample conditions until $t = 30$ min. The glucose reference and c-NAB peak at $t = 35$ min, whereas the f-NAB peaks at $t = 40$ min. Average peak height is in the order f-NAB > c-NAB > glucose followed by a similar glucose depletion rate and a stable glucose level at $t = 85$ min. To better understand the difference of the samples, the incremental area under the curve (iAUC) for the three samples was calculated. No statistically significant difference was detected in a Type III F-test ($F(2, 42) = 1.81, p = 0.176$) between the iAUC (average $\pm s$) of the glucose (51.4 ± 21.6 mmol $\times$ min /L), the c-NAB (58.0 ± 21.8 mmol $\times$ min /L), and the f-NAB (59.2 ± 14.7 mmol $\times$ min /L) as shown in S.Fig. 7. Tukey-adjusted pairwise comparisons showed no statistically significant differences between the glucose reference and f-NAB ($p = 0.194$), the glucose reference and c-NAB ($p = 0.301$), or f-NAB and c-NAB ($p = 0.962$). The non-significance could be attributed to the high inter-individual iAUC variability ranging from 9.6 to 116.0 mmol $\times$ min /L among all 66 observations. The low DC load (12.5 g) and the lack of replicate testing for each individual, potentially compounded by measuring interstitial rather than capillary blood glucose, likely exacerbated this variance and reduced the statistical power to detect differences³¹. The within subject effects not captured by average values were analysed in a spaghetti plot shown in S.Fig. 8. Although the average iAUC values were not significantly different, the high inter-individual variability appears largely random and can be considered non-directional. Therefore, we assume that the average values are accurate but not precise. To calculate the GL and enhance interstudy comparability, the GI of the f-NAB and c-NAB were calculated for each individual by:

$$GI = \frac{iAUC_{sample}}{iAUC_{glucose}}$$

In accordance with Wolever et al.⁶⁹, two statistical outliers exceeding $\bar{x} \pm 2s$ were excluded. One additional trial was excluded due to an uncharacteristic physiological non-response curve (iAUC 9.6 mmol $\times$ min /L see S.Fig 9, P20), yielding a final cohort of $n = 19$. The resulting average GI values ($\pm s$) were 120.1 ± 39.2 (CV = 32.7%) for c-NAB and 119.6 ± 42.0 (CV = 35.1%) for f-NAB.

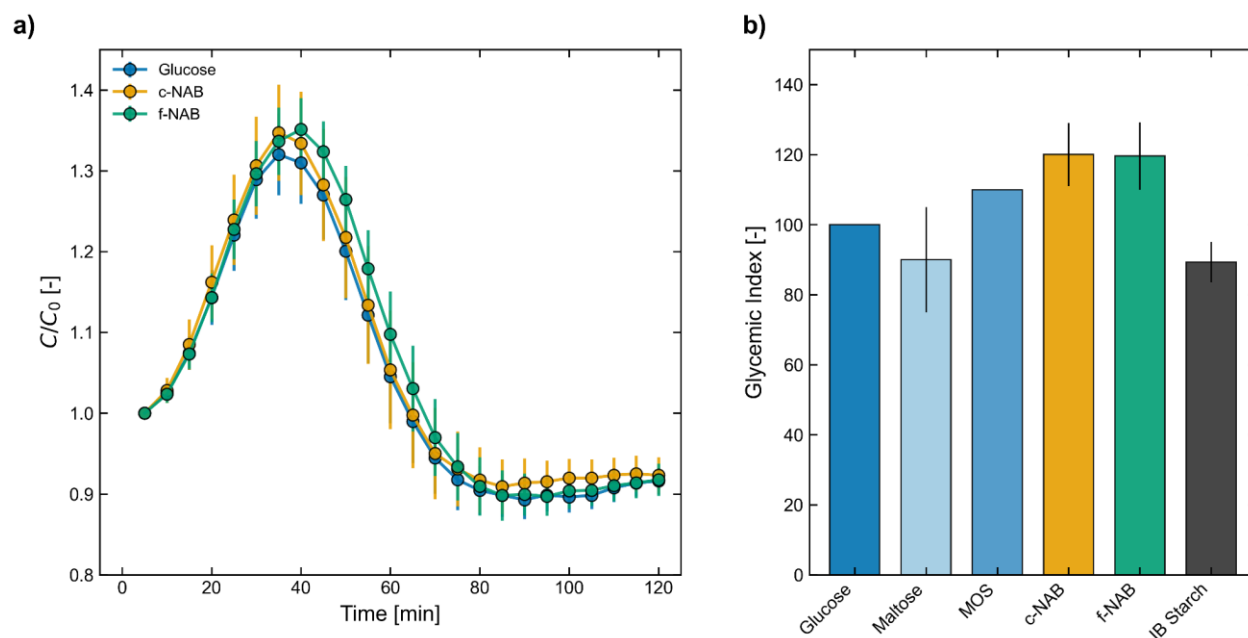


Fig. 4: Impact on interstitial glucose levels from f-NAB and c-NAB compared to glucose. a) Normalized interstitial glucose response based on $C_0 = C(t = t_0)$ over 120 minutes after glucose, f-NAB, and c-NAB consumption with 12.5 g of digestible carbohydrates. Values are presented as average $\pm$ 95% CI ($1.96 \cdot s/\sqrt{n}$) assuming normal distribution and independence of 22 participants. **b)** The glycemic index of glucose, maltose, maltooligosaccharides (MOS, DP3-10), c-NAB, f-NAB, and isolated barley starch. Glycemic index for f-NAB and c-NAB are reported as average $\pm$ 95% CI ($1.96 \cdot s/\sqrt{n}$) based on an outlier corrected cohort of 19 participants. Maltose values are indicated as average, minimum and maximum values reported in literature^{3,4} and the estimate of MOS and IB starch are from a selected publications^{32,70} and their reported uncertainties⁷⁰.

The observed standard deviations s are consistent with GI methodology data⁶⁹, where absolute within-laboratory s span 14 to 48 GI units and corresponding inter-individual CVs of 37-44% are reported. Because reference glucose was evaluated on a single occasion rather than in duplicate or triplicate, normal day-to-day intra-individual variation CV_{intra} of $\sim 20 - 25\%$ was directly propagated into the individual quotient values. However, because this error propagation is symmetric and non-directional, the population mean remains unbiased. Furthermore, analyzing $n = 19$ participants substantially exceeds the standard requirement of $n = 10$ reflected in the resulting standard error of the mean ($s/\sqrt{n}$) = SEM) of 9.0 (f-NAB) and 9.6 (c-NAB) GI units. Given the relatively low SEM, we conclude that the true population mean GI for both f-NAB and c-NAB is > 100 , exceeding pure glucose (100) and maltose (75-105), and potentially estimated values for glucose oligomers such as maltotriose and maltotetraose (110)^{3,4,32} (Fig. 4b).

The surprisingly high estimated average GI for c-NAB and f-NAB can be in part explained by previous findings. Similar blood glucose responses were made between glucose and α -amylolysates in rodents³³. Linear (α -1,4 glycosidic bond) MOS present in the α -amylolysates were identified as the main contributors to the elevated glycemic response. Other studies found a complete conversion of linear MOS of DP 2-5 into glucose within 20 minutes by C-terminal maltase-glucoamylase which are located along the length of the small intestine from duodenum to ileum^{34,35}. Branched α -limit dextrins (α -1,4 and α -1,6, DP>4) show a

reduced glycemic response compared to linear MOS even though the mucosal bound α -glucosidases hydrolyze more than 60% within 20 minutes.^{33,34} The low effect of RDs on the glycemic response observed in f-NAB is in accordance with previous studies where significant attenuation of the glycemic response for sucrose, rice, and wheat³⁶ but not for glucose and maltodextrin was found³⁷. For linear MOS with higher DP, similar GI to maltotriose is expected based on findings with alpha-amylolysates in rodents³³. In alcoholic beers, linear MOS (DP 2-7) can make up to 35% of total digestible carbohydrates amounting to concentrations of 1 g/100g, while larger and more branched dextrans (DP>10) constitute up to 38% of total carbohydrates^{38,39}. The exact DP and DB of the RE is not well described. Based on the glycemic responses of both the f-NAB and c-NAB, we estimate that their molecular structure permits a similar digestion as the alpha-limit dextrans produced by the enzymatic hydrolysis with pancreatic amylases described in previous studies^{33,34,40}.

These findings indicate that eliminating maltose does not lower the GI of NAB, pointing instead to a potential role for rapidly digested MOS and intermediate dextrans in maintaining this elevated glycemic response. Furthermore, the addition of RD failed to attenuate this high glycemic response. Consequently, RD governs the GL primarily through malt substitution while preserving essential sensory attributes of DCs such as bitterness reduction and body improvement. Previous studies reduced the GL in NAB with isomaltulose and RDs and reported improved insulin sensitivity after dietary interventions⁴¹. The GL reduction of substituting malt with RD can either be expressed at equal RE or at iso-sensory impact. Quantifying GL reduction at equal RE requires to spike NABs with RDs followed by dilution to equal RE and their assessment on overall liking. Quantifying GL reduction based on iso-sensory impact can be done exemplarily for Nutriose FB06 compared to glucose for the sensory attribute bitterness:

$$\begin{aligned} \frac{\Delta GL (g)}{100} &= \frac{GL_{fNAB} - GL_{Glucose}}{100} = GI_{fNAB} DC_{FB06} - GI_{Glucose} DC_{Glucose} \\ &= GI_{fNAB} C_{fNAB} V x_{DC} - GI_{Glucose} DC_{Glucose} \end{aligned}$$

where C_{fNAB} is the iso-sensory attribute concentration, V the volume or mass of the consumption unit, x_{DC} the volume or mass fraction of digestible carbohydrates in the substitute. The DCs present from other sources than glucose or Nutriose FB06 are assumed to be equal in both NABs. Calculating $\Delta GL (g)$ for 330 g NAB where 20 g/L glucose is substituted with 50 g/L FB06 with $x_{DC} = 0.16$ and $GI_{FB06} = 120.1$ yields 3.2 g - 6.6 g = - 3.4 g. Hence, substituting DC with RD on an iso-sensory impact basis lowers the GL of the overall NAB. However, the impact of the malt substitution with RDs on the environmental sustainability of the NAB remains unclear.

Life-cycle assessment

We conducted an LCA to compare industry-scale environmental impacts of f-NAB production from maltose-negative yeasts with commercial c-NAB production. The c-NAB was modelled based on a conventional brewing process with subsequent ethanol removal via nanofiltration and ethanol purification and recovery via distillation. To separate the effects of dealcoholization and RD addition, we additionally modelled a scenario where RD is replaced by an equivalent RE-dose of malt (m-NAB). f-NAB LTGR1 and 2 represent the formulations used for sensory study and glycemic response, respectively.

Producing f-NAB using maltose-negative yeast and RDs lowers global warming potential (GWP), while also reducing land- and water use compared to c-NAB (Fig. 5). Because alcohol removal is energy intensive, c-NAB showed the highest GWP among the three production methods with 0.35 kgCO₂-eq per kg beer (Fig. 5a). In comparison, f-NAB LTGR1, f-NAB LTGR2 and m-NAB had a GWP of 0.31, 0.29, and 0.28 kgCO₂-eq per kg beer, respectively. Although the higher malt content in m-NAB resulted in higher contributions from malting and barley cultivation, the overall GWP impacts were higher for both f-NABs due to the production of RD (Fig. 5a). Thereby the main driver of the GWP was the heat demand to spray dry the RD. Thus, the GWP of f-NABs could be further reduced by forming the RD *in situ* during the mash making the spray drying obsolete. Land use was dominated by barley and wheat (f-NAB LTGR2) cultivation (Fig. 5b). Consequently, the land use of the three NAB types was distributed proportionally to their malt and wheat flake dosage with 0.32, 0.25, 0.2, and 0.15 m² * year for m-NAB, c-NAB, f-NAB LTGR1, and f-NAB LTGR2, respectively. RD, which was used to replace malt in both f-NABs, is commonly obtained from wheat starch slurry as a co-product of the separation of wheat starch and gluten⁴². Therefore, only a small share (3.4%) of the land used for wheat cultivation was allocated to wheat starch slurry based on the economic values of the co-products, which led to a lower land use of RD-based f-NABs compared to malt-based m-NAB overall. The largest share of water was used by the brewing stage followed by the cultivation of barley (Fig. 5c). Additionally, the energy-intensive nanofiltration and distillation steps consumed significant amounts of water, mainly due to the high share of hydropower in the Swiss electricity mix. Consequently, we found the highest water use for c-NAB with 2.5 liters per kg beer. Similar to land use, RDs had a lower water use compared to barley. Thus, we find a lower total water use for both f-NABs (1.9 - 2.1 liters / kg) compared to m-NAB (2.3 liters / kg). Considering the estimated global consumption of 8 billion liters of non-alcoholic beer in 2025⁴³, our findings indicate that a widespread use of maltose-negative yeast could lead to relevant sustainability gains compared to retroactive alcohol removal by nanofiltration and distillation, especially for dietary fiber-rich products (Fig. 5). At the same time, it should be noted that the removed ethanol from c-NAB can be reused, e.g., in the production of other alcoholic beverages where it potentially replaces food-grade ethanol from primary production⁴⁴. Depending on the prevented environmental impacts from the substituted ethanol production system, we find that c-NAB could eventually perform better than m-NAB

and f-NABs (S.Fig. 10). However, whether the removed ethanol is actually reused in practice and for which purpose depends on the business model of each brewery. Finally, the utility consumption for beer brewing can strongly vary among different producers, beer types, and production methods^{45–47}. Hence, the use of maltose-negative yeast to produce non-alcoholic beer does not supersede the introduction of efficient cooling and heating systems, water-saving measures, renewable energy sources, recycling concepts for biowaste, and sustainable packaging^{47,48}.

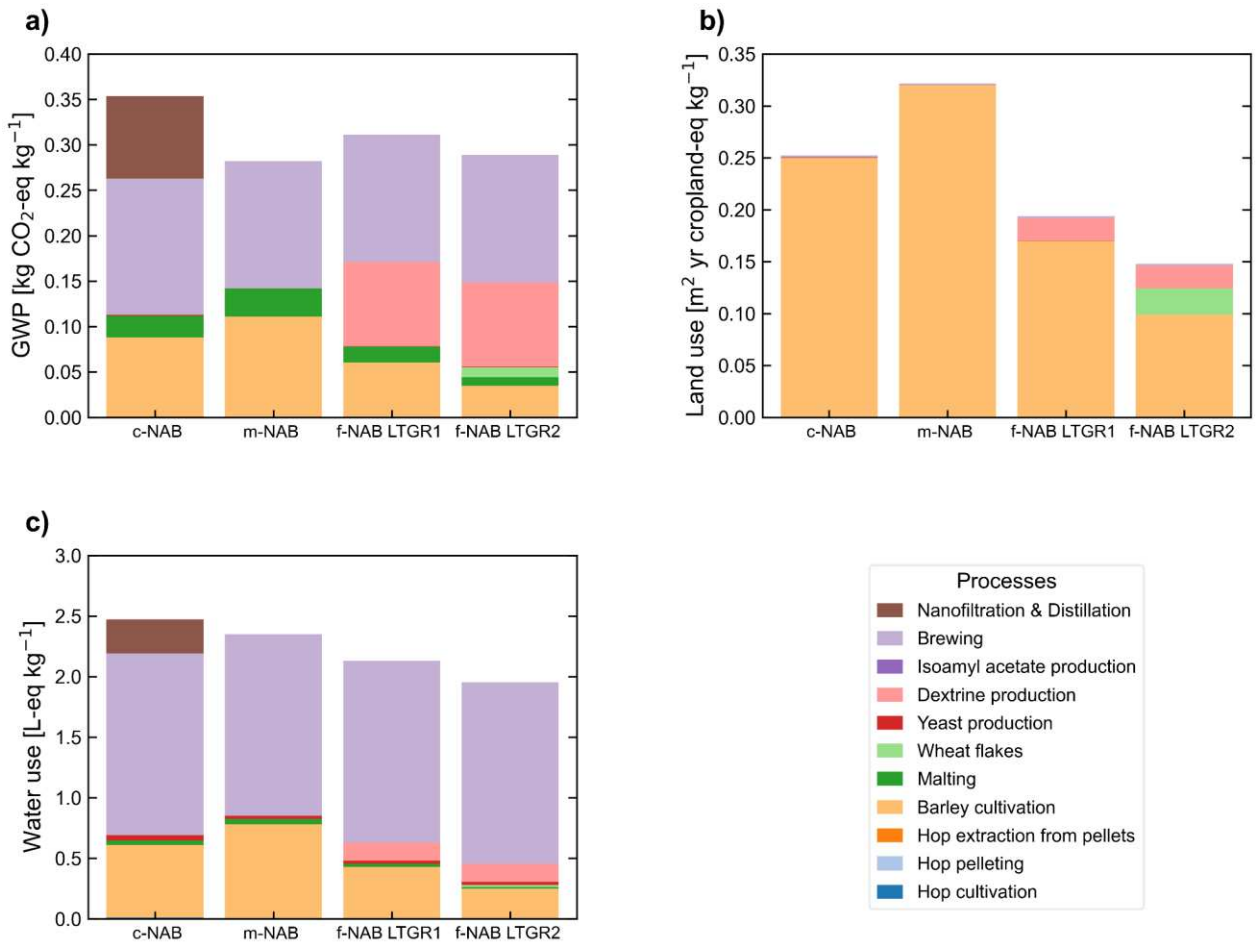


Fig. 5: Sustainability analysis comparing production methods for NAB. Environmental impacts for **a)** global warming, **b)** land use, and **c)** water use of 1 kg NAB with high malt content (m-NAB), fiber-rich NAB as used for sensory testing (f-NAB LTGR1), fiber-rich NAB as used for measuring glycemic load (f-NAB LTGR2) (all three produced with maltose-negative yeast to inhibit ethanol formation), and NAB produced by conventional brewing with subsequent alcohol removal by nanofiltration and ethanol recovery by distillation (c-NAB). The underlying data can be found together with the results for other impact categories in S.Tab 2-5.

Conclusion

We developed a novel brewing approach that avoids energy-intensive dealcoholization and replaces malt with resistant dextrins to resolve the trade-off between sensory palatability, nutritional quality, and environmental sustainability in NAB. We find that lowering maltose concentrations fails to reduce the glycemic index and therefore the glycemic load of NAB, pointing instead to a potential role for rapidly digested maltooligosaccharides and dextrins in maintaining the elevated glycemic response. Resistant dextrins do not attenuate glycemic response, however, they can lower the overall glycemic load by displacing malt on an iso-sensory impact basis. Combining maltose-negative yeast and resistant dextrins lowers climate, land, and water footprints, providing a scalable strategy to lower environmental impact of NAB production. To advance more nutritious and sustainable NAB production, this study establishes that raw materials, mashing- and fermentation conditions for NAB production without dealcoholization should be directed towards minimal digestible carbohydrate rather than low maltose and maltotriose content. The contribution of maltose to palatability should be leveraged, while maltooligosaccharides and dextrins should instead be replaced with resistant dextrins to reduce overall digestible carbohydrates. The use of resistant dextrins delivers iso-sensory formulations with 3.7 g/100g dietary fibers where the consumption of a typical 330 ml unit would result in a dietary fiber intake of approximately 12.2 g and a 3.4 g reduction in glycemic load. Consuming 12.2 g of dietary fiber is more than 40% of the recommended daily intake in Switzerland ⁴⁹ and was shown to promote gut microbiota able to produce short-chain fatty acids for a similar type of dietary fiber ⁵⁰. Future work should be directed towards the *in-situ* formation of resistant dextrins in the mash and the study of their molecular structure and impact on organoleptic properties. The adoption of NAB production according to our results holds great promise to reduce the GWP impact and improve the nutritional quality of NAB while giving breweries the possibility to transition their business model from alcoholic fermentation to nutritionally valuable and palatable beverages with reduced glycemic load.

Materials & methods

NAB production without dealcoholization

Materials and recipes

Table 3: Recipes for LTGR1-GC1 and LTGR2-GC2 wort production.

Material	Supplier	LTGR1-GC1	LTGR2-GC2
Liquor-to-grist ratio (l/kg)	-	7.75	13.65
Premium Pilsner malt (kg/hl liquor)	Weyermann	5.56	-
Carapils malt (kg/hl liquor)	Weyermann	5.56	5.56
Wheat malt pale (kg/hl liquor)	Weyermann	1.78	-
Wheat flakes (kg/hl liquor)	Brau- und Rauchshop (Densbüren, CH)	-	1.78
Total malt (kg/hl liquor)	-	12.9	7.34
Total malt (kg/hl final beer)	-	11.6	6.6
Calcium sulphate (g/hl)	Brau- und Rauchshop (Densbüren, CH)	50	50
Calcium chloride (g/hl)	Brau- und Rauchshop (Densbüren, CH)	100	100
Sodium Chloride (g/hl)	Schweizer Salinen AG (Pratteln, CH)	15	15
Lactic acid (80%, ml/hl)	Brau- und Rauchshop (Densbüren, CH)	205	220
Hops extract (6%, ml/hl)	Brouwland (Beverlo, BE)	85.5	85.5
LoNa yeast (g/hl)	Lallemand (Montreal, CA)	250	210
Nutriose FB06 (kg/hl)	Roquette (Lestrem, FR)	25.0	-

CO2 carbonation (kg/hl)

Linde Gas Schweiz
AG (Dagmersellen,
CH)

3.5

3.5

399

400 **LTGR1-GC1 NAB production**

401 Approximately 450 l of tap water (average hardness, 15.7 °fH; supplied by the city of Zürich) was heated to
402 75-76 °C and adjusted with 50 g CaSO₄, 100 g CaCl₂ and 15 g NaCl. At approximately 75 °C, 25 kg Pilsner
403 malt, 25 kg Carapils malt and 8 kg wheat malt were added. The mash was held at 74 °C for 40 min, with
404 samples collected at 10-min intervals, before heating to 78 °C. The mash was transferred to a lauter tun and
405 recirculated through the grain bed for approximately 1 h until clarification, then returned to the brew kettle.
406 Mash volumes were 330-340 l. The grain bed was sparged with 180 - 185 l of water at 78 °C. Wort pH was
407 adjusted to 4.5 with lactic acid from an initial pH of approximately 5.7; total additions were 205-220 ml. At
408 boiling temperature, the wort was boiled for 5 min. 85.5 ml hop extract was added to obtain a target
409 bitterness of 10 IBU. Once the wort was at 22 °C, pitching with LoNa yeast was done directly without
410 rehydration. Fermentation was performed at 20 °C, with samples collected at defined time points (10-11 h,
411 20-24 h, 44 h, 59 h and 89-90 h) to monitor specific gravity . Samples that were not analyzed immediately
412 were frozen directly after collection. After specific gravity stabilization, the beer was cold-crashed at 4 °C.

413 **LTGR2-GC2 NAB production**

414 The wort for LTGR2 batches was made in a B80 Pro Brewing System (Brewtools, Grimstad, Norway).
415 Approximately 60 l of tap water (average hardness, 15.7 °fH; supplied by the City of Zurich) was heated to
416 75 °C and adjusted with 6.67 g CaSO₄, 13.33 g CaCl₂ and 2 g NaCl. At approximately 75 °C, 3.33 kg Carapils
417 malt and 1 kg wheat flakes were added. The mash was held at 74 °C for 40 min, with samples collected at
418 10-min intervals, before heating to 78 °C. The mash was then recirculated through the grain bed for
419 approximately 1 h until clarification. The grain bed was sparged with water at 78 °C to achieve an
420 endvolume of 69 l. Wort pH was adjusted to 4.5 with lactic acid from an initial pH of approximately 5.7;
421 total additions were 9 - 12 ml. At boiling temperature, the wort was boiled for 5 min. 11.4 ml hop extract
422 was added to obtain a target bitterness of 10 IBU. Once the wort was at 20 °C, pitching with LoNa yeast was
423 done directly without rehydration. Fermentation was performed at 20 °C, with samples collected at defined
424 time points (9 h, 23.5 h, 50.5 h, 84.5 h and 145.5 h) to monitor specific gravity. Samples that were not
425 analyzed immediately were frozen directly after collection. After specific gravity stabilization, the beer
426 was cold-crashed at 4 °C.

Physicochemical and microbiological characterization

Dissolved oxygen

Dissolved Oxygen was measured using a VisiFerm DO Arc 120 H2 optical DO sensor (Hamilton Bonaduz AG, Bonaduz, Switzerland). The probe was directly integrated into the fermenter during fermentation and data were recorded every 12 min. Data acquisition was performed using ArcAir software version 3.11.1 connected to the probe.

Sugar - HPLC-RID

For sample preparation, 2 mL of each sample was centrifuged at 14,000 rpm and 10 °C for 5 min using a centrifuge (Sigma 3-16KL, Sigma Laborzentrifugen GmbH, Germany). The supernatant was subsequently filtered through a 0.45 µm syringe filter (Chromafil Xtra PTFE-45/13, Macherey-Nagel GmbH & Co. KG). For HPLC analysis, 1 mL of the filtered sample was transferred into an HPLC vial and capped.

Sugar analysis was performed by high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity II system equipped with a quaternary pump (G7111B), autosampler (G7129A), column compartment (G7116A), diode array detector (G7117C), and refractive index detector (RID; G7162A). Chromatographic separation was carried out at 40 °C under isocratic conditions using 5 mM H₂SO₄ as the mobile phase at a flow rate of 0.60 mL min⁻¹. The total run time was 20 min, followed by a 1 min post-run period. Samples were injected in standard injection mode with an injection volume of 10 µL.

Detection was performed using the RID operated at 40 °C, with positive signal polarity and automatic zero adjustment before each analysis. UV detection was not used for data acquisition.

The HPLC method parameters were as follows: mobile phase, 5 mM H₂SO₄; flow rate, 0.60 mL min⁻¹; column temperature, 40 °C; injection volume, 10 µL; run time, 20 min; post-run time, 1 min; and RID temperature, 40 °C.

Calibration curves for glucose, maltose, fructose and maltotriose can be found in S.Fig.11.

Density

Density was measured using either a DMA 5002 density meter or a DMA 4501 density meter (Anton Paar GmbH, Graz, Austria). Before measurement, samples were degassed and centrifuged at 4500 rcf and 20 °C for 15 min. Measurements were performed using a sample volume of 2 mL. The instruments were calibrated with air and deionized water.

Ethanol - Density

Ethanol concentration (C_{EtOH}) based on the decrease in density was calculated according to the following equation:

$$C_{EtOH} = (D_0 - D_t) * 131.25 * 7.893$$

where D_0 is the initial density of the fermentation medium and D_t is the density of the sample at the respective fermentation time point. The density difference was converted to ethanol concentration using the constants 131.25 and 7.893.

Ethanol - NIR

Near-infrared spectroscopy (NIR) was used to determine ethanol content using an Alcolyzer 7001 (Anton Paar GmbH, Graz Austria). Before measurement, samples were degassed and centrifuged at 4500 rcf and 20 °C for 15 min. A minimum sample volume of 30 ml was used. The instrument was calibrated using water and a 10% EtOH solution.

Ethanol - GC-FID

Ethanol analysis was performed by headspace gas chromatography with flame ionization detection (HS-GC-FID) using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and a Combi PAL autosampler. For sample preparation, approximately 1 mL of sample was centrifuged at 14,000 rpm and 10 °C for 5 min using a Sigma 3-16KL centrifuge. The supernatant was subsequently filtered through a 0.45 µm syringe filter (Chromafil Xtra PTFE-45/13, Macherey-Nagel GmbH & Co. KG). 200 µL of the supernatant was transferred into a 22 mL headspace vial together with 500 µL of ionic solution and 200 µL of 1-propanol internal standard. The vial was sealed and vortexed prior to analysis.

Chromatographic separation was carried out using an MN OPTIMA-5-MS capillary column (30 m × 250 µm × 0.25 µm). Samples were injected in split mode (50:1) with an inlet temperature of 250 °C. Nitrogen was used as the carrier gas under constant-flow conditions. The oven temperature was held at 40 °C for 3 min and increased to 220 °C at 30 °C min⁻¹, resulting in a total run time of 9 min.

Headspace sampling was performed with an injection volume of 1 mL. Samples were incubated at 40 °C for 15 min with agitation at 500 rpm prior to injection, and the syringe temperature was maintained at 50 °C. Detection was performed using the FID operated at 300 °C with hydrogen, air, and nitrogen makeup gas flow rates of 40, 400, and 30 mL min⁻¹, respectively.

Ethanol concentrations were determined using an internal-standard calibration with 1-propanol. Calibration standards were prepared from an ethanol stock solution, and concentrations were calculated from a linear calibration curve based on the ethanol-to-internal-standard peak area ratio. Calibration curves for glucose, maltose, fructose and maltotriose can be found in S.Fig.12.

Ethanol - Stoichiometry

Ethanol concentration (C_{EtOH}) based on the sugar consumption was calculated according to the following equation:

$$C_{EtOH} = (C_{Glu} - C_{Glu,t}) * 0.511$$

where C_0 is the initial sugar concentration and $C_{Glu,t}$ is the residual sugar concentration at the respective fermentation time point. The factor 0.511 corresponds to the theoretical yield coefficient for the conversion of glucose to ethanol based on the stoichiometry of alcoholic fermentation.

Permittivity Measurements

Permittivity was measured with an Incyte sensor (Hamilton Bonaduz AG, Bonaduz, Switzerland). The probe was directly integrated into the fermenter during fermentation and measurements were recorded every 12 min throughout the fermentation. The measurement frequency range was set to 300 - 1000 kHz using the medium moving average setting. Data acquisition was performed with ArcAir software (version 3.11.1) connected to the probe.

Calibration of Total Cell Count

Samples were collected at several time points during fermentation to determine the total cell count. Total cell count was determined by haemocytometer counting to establish a calibration curve (S.Fig.13).

Physical and sensory evaluation of NAB

Physical Evaluation

Ubbelohde Capillary Viscometry

Solutions were prepared by dissolving Nutriose FB06, Nutriose FM15, or glucose in water at concentrations ranging from 0.05 to 5 g/dL. Flow times were measured in triplicate using a LAUDA Ubbelohde capillary viscometer (Type I, capillary constant $K = 0.01 \text{ mm}^2 \text{ s}^{-2}$; inner capillary diameter 0.63 mm). The viscometer

was operated in a temperature-controlled water bath maintained at 20 °C. Measurements were performed using a sample volume of 10 mL. Relative viscosity was calculated using the following equation with water as the blank and t_0 as the flow time of water and t the flow time of the sample.:

$$\eta_{rel} = \frac{t}{t_0}$$

Sample Preparation for Rotational Rheometry and Tribology

A commercial NAB with no added sugars (Feldschlösschen Alkoholfrei, Lager, 50 cl can) was chosen as reference beer for the viscosity and tribology measurements. Prior to all measurements, the beer was degassed to minimize potential interference from nucleating gas bubbles. The beer can was opened up to 24 h before the degassing procedure. For degassing, approximately 200 mL of beer was transferred to a 400 mL beaker and allowed to stand until most of the foam had dissipated. The beaker was subsequently subjected to vacuum treatment at 0.75 bar using a vacuum pump for at least 10 min. When freshly opened beer was used, the degassing procedure was either extended or repeated to ensure adequate removal of dissolved carbon dioxide. Solutions for viscosity and tribology measurements were prepared by dissolving Nutriose FB06, Nutriose FM15, or glucose in water at the desired concentrations.

Rotational Rheometry

To measure the viscosity a MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a Couette system (CC 27) measuring geometry was used. The prepared solutions were transferred into the measuring geometry and equilibrated at 35 °C to simulate oral conditions. To ensure that the desired temperature is reached the measurement did not start before the temperature was in a range of 35 ± 0.2 °C over a surveillance time of 1 minute. A logarithmic $\dot{\gamma}$ -ramp from 1 to 1000 s⁻¹ was performed with 41 measurement points and a steady state setting with a maximum of 30 s. Following the same interval but with a $\dot{\gamma}$ -ramp from 1000 to 1 s⁻¹.

Tribology

A MCR 702 rheometer (Anton Paar GmbH, Graz, Austria) with a T-BTP measuring cell and a ball-on-three-pins tribology attachment (BC 12.7) was used to conduct the measurements. The used pins as well as the ball consisted of Poly dimethylsiloxane (PDMS). The applied settings are based on the test sequence of ⁵¹

for different alcoholic containing beers and NABs. The step of dry-running before the sample measurement was waived. Approximately 1 mL of sample was deposited onto the sample holder using a pipette. The temperature remained constant at 20 °C and the normal force at 3 N. First, the ball was held on 3 N for an interval of 3 minutes with three points. Second, 5 intervals with a logarithmically increasing speed from 10^{-8} to 0.1 m/s with 71 data points was performed. Between the intervals a resting period of 40 s consisting of 8 points and a decreasing speed ramp (0.1- 10^{-8} m/s) was employed.

Sensory Evaluation

Preparation of NAB samples for sensory evaluation

For the sensory evaluation, ten samples were prepared using the LTGR1-GC1 NAB formulation. The required amount of glucose or dietary fibre was weighed into a 5 L measuring cup and mixed with NAB. NAB was added to obtain a final volume of 1 L for each sample. The mixtures were transferred into 1 l flip-top glass bottles (Bormioli Rocco, Migros Genossenschaftsbund). Hop extract was added at 0.25 ml per bottle to obtain a bitterness of 25 IBU. The bottles were closed and mixed thoroughly. Samples were not carbonated.

Pasteurisation

Kegs and 1 L bottles were pasteurised by heating to an average core temperature of 65 °C with a holding time of at least 3 min. For pasteurisation of the 1 L flip-top bottles, approximately 5 L of water was heated to 75 °C in an Instant Pot Duo Evo Plus 80 (Instant Pot, Ottawa, Canada). The bottles were placed in the hot water bath for approximately 35 to 40 min. A control bottle was used to monitor the sample temperature. The initial sample temperature was approximately 19 °C. The sample temperature reached 65 °C after approximately 26 min, and the total pasteurisation time was 37 min, ensuring a holding time of at least 3 min at 65 °C.

Participants

The sensory study was conducted in the sensory laboratory of the Bern University of Applied Sciences (BFH-HAFL) with trained expert assessors. Ethical approval was obtained from the Ethics Advisory Board of BFH prior to the study, and all panelists provided informed consent before participation. All assessors are routinely screened for their bitterness, sensitivity and discrimination ability as part of BFH-HAFL's internal quality assurance procedures.

Sample size estimations were based on ISO 8587:2006 which recommends a minimum of 12 to 15 assessors for descriptive ranking tasks to ensure adequate test sensitivity and reliability. Each ranking series was

conducted in duplicate by 8 assessors for the attribute *body* and 9 assessors for the attribute *bitterness* resulting in 16 or 18 responses per ranking. In total, twelve different trained assessors participated in the sensory study. Four assessors completed all four ranking sessions, while the remaining assessors varied between sessions depending on availability.

Samples used for ranking

Each ranking series included five samples: a reference NAB according to LTGR1-GC1 without added glucose or RD, one sample with 20 g/L glucose added to NAB, and three samples with either Nutriose FB06 or Nutriose FM15 added at concentrations of 20, 50, and 100 g/L. Approximately 30 mL of each sample was served in opaque dark brown cups to mask slight visual differences introduced by fibre addition. All samples were non-carbonated and served at room temperature. Unsalted crackers and still water (25 °C) were provided for palate cleansing.

Procedure

Ranking tests were performed in accordance with ISO 8587:2006 to evaluate the perceived intensity of two pre-defined sensory attributes: the basic taste *bitterness* and the mouthfeel descriptor *body*. To calibrate the panel on the attribute *body*, a short training session was conducted prior to the ranking series where *body* was measured. During this session, assessors were provided with a definition of the attribute (“the capacity to fill the mouth” and “opposite of watery”) ⁵² and tasted three alcohol-free beers that demonstrated the concept: Feldschlösschen Alkoholfrei Lager (low body), Feldschlösschen Alkoholfrei Weizenfrisch (high body), and Leffe Blonde 0% Alcohol Free (high body), the latter selected based on literature reports of its relatively high mouthfeel intensity within the non-alcoholic beer category ⁵³. Samples were coded with randomized three-digit numbers and presented simultaneously to assessors. The sample arrangement followed a Latin square randomization design. Assessors performed the rankings individually in sensory booths using a computer. 10-minute breaks were scheduled between sessions.

Data Analysis

Data collection was carried out using the sensory software EyeQuestion (Version 6), and Statistical analysis was performed in R (version 4.4.3, see supplementary information for sensory R script). For each dataset (*bitterness/body* × FB06/FM15), the Friedman test evaluated the omnibus null hypothesis *H0*: the distributions of ranks are identical across products within blocks (i.e., no product effect), against the alternative *H1*: at least one product differs. Blocks were defined as assessor×replicate sessions (each ranking session treated as a complete block). All tests were two-sided with $\alpha = 0.05$. Upon a significant Friedman result, Nemenyi all-pairs comparisons were used to identify differing pairs while controlling family-wise

error via the test's critical-difference framework; significance is denoted as $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***. Estimates of effect sizes were quantified by calculating Kendall's W and are displayed in S.Fig.14.

Glycemic load and index of NAB

Intervention Study

Participants

The study was conducted with 22 healthy normal weight adults (body mass index between 18.5-29.9 kg/m²), aged 20-62 years (9 males and 13 females). Participants were recruited through a study advertisement in the BFH employee and student newsletter and via indirect personal contacts in the Bern area, with enrollment conducted through an online registration form linked to the advertisement on the BFH website. Written informed consent was obtained following eligibility assessment and before enrolment in the study. Excluded were participants with acute, untreated or unstable metabolic or hormonal diseases (including diabetes), diseases of the gastrointestinal tract, having known allergies or intolerances to ingredients of beer, practicing intensive competitive sports (>10 hours per week), following special metabolically relevant diets (e.g. low-carb or intermittent fasting), suffering from sleep disorders or lacking possibility of regular sleep rhythm (e.g. due to shift work), are pregnant or breastfeeding, having active smoking status, problematic consumption or abuse of alcohol, drugs or medication, lacking of German or English skills and lacking ability to give consent. Of 39 individuals screened for eligibility, 23 were included in the study. One participant withdrew because of repeated technical failure of the sensor.

Sample Preparation

The three different samples (c-NAB, f-NAB, Ref Glucose) were filled according to Tab. 4 into 50 cl unlabelled aluminum cans to yield 12.5 g of digestible carbohydrates per can. The c-NAB and f-NAB were carbonated and poured from their original container into the unlabelled aluminum cans. The glucose reference was not carbonated. The sample liquids were dosed with a precision of ± 2 g into the cans. The can headspace was purged with N₂/CO₂ gas (Biogon C30 ,Linde Gas Schweiz AG) before the lid was sealed. Cans were pasteurized.

Table 4: Carbohydrate concentration of samples used for the dietary intervention study

Sample	Total Carbohydrate (g/100g)	Of which sugars (g/100g)	Net weight (g)
Reference NAB	2.6	0.5	468
Fiber NAB	2.7	0	444

Sample	Total Carbohydrate (g/100g)	Of which sugars (g/100g)	Net weight (g)
Glucose ref	2.5	2.5	500

Randomization

To prevent position, order, and carryover effects, sample presentation orders were randomized individually for each participant ($n = 22$) using a Fisher–Yates shuffling algorithm implemented in Python (v.3.13.9, random.shuffle). Each panelist evaluated the three treatments, identified by unique three-digit blinding codes (188, 720, and 896), in a balanced, fully randomized permutation across three sequential serving positions.

Double-Blinding

An independent researcher uninvolved in the study procedures generated the randomized presentation orders and packed the unlabelled aluminum cans into individual participant containers in their defined testing sequence. To allow for replacement in case of spillage, cans were marked on the bottom with a three-digit code. Only this independent researcher had access to the code allocation for the glucose reference, f-NAB, and c-NAB. Unblinding of the sample allocation occurred only after the completion of all statistical analyses. For individual GI calculation of each participant, sample allocation was known.

Procedure

This single-center, double-blind randomized cross-over clinical trial was conducted at the BFH in Bern between September 2025 and November 2025. The study followed a within-subject cross-over design with three study conditions. Study visits were scheduled individually prior to sending sensor packages containing the FreeStyle Libre 3 glucose sensor and written instructions for sensor application and test-day preparation checklist, with reminder and follow-up emails sent to ensure proper self-installation. Sensors were self-installed 2–4 days before the first test day to allow a minimum calibration period of 36 hours, with participants attaching the devices to the upper arm and connecting them to the Libre 3 smartphone application in accordance with the manufacturer’s instructions.

Each participant completed three experimental days within 7–21 days, with a minimum washout period of 48 hours between test days. All experiments were conducted in a meeting room at the BFH. Prior to each test day, participants were instructed to fast overnight and to refrain from caffeine, nicotine, chewing gum, after midnight, as well as from intensive physical activity 24 hours before a test day.

Test sessions were conducted in the morning between 07:46 (earliest start) and 11:16 (latest finish), with 1–

8 participants per test day. Each participant completed three identical test sessions in a randomized sequence. Each participant completed three identical test sessions in randomized sequence, consuming on each test day one of three test beverages (f-NAB, c-NAB, Glucose ref) with identical carbohydrate content, all provided in identical silver aluminium cans labelled with participant and study day identifiers. Randomization was performed by an independent project member using Python-generated random sequences, with participants assigned consecutively according to the order of enrolment. Each session included completion of a two-part questionnaire, beverage intake within 3 minutes, and a subsequent 120-minute measurement period. The two-part questionnaire assessed compliance with pre-test instructions (e.g., fasting), dietary habits, sleep quality, and perceived stress during the test period. On the first experimental day, participants also completed a brief questionnaire collecting demographic and anthropometric data. A detailed schedule is provided in the Appendix.

Data Acquisition and Analysis

Interstitial glucose measurements were obtained using FreeStyle Libre 3 sensors and the corresponding FreeStyle Libre 3 smartphone application (Version 3.6.5.11962, Abbott Diabetes Care Ltd., Witney, UK). Study staff accessed participants' glucose data via the web-based LibreView platform. Sensors recorded interstitial glucose concentrations in the upper arm at 5-minute intervals, yielding 24–25 measurements per participant and test day. After completion of data collection, all glucose data were exported as CSV files from LibreView. Additional data were collected digitally using REDCap (Research Electronic Data Capture).

Data analysis followed a predefined protocol for iAUC calculation for each participant using the trapezoidal rule according to FAO/WHO⁵⁴. Depending on the temporal alignment of sensor readings with beverage consumption, 24 or 25 measurements were available within the predefined observation period per participant and test day. To ensure a consistent number of observations across participants and conditions, the first 24 measurements from t_0 onwards were retained for analysis. The time point of the first available interstitial glucose measurement after completion of beverage consumption was defined as t_0 , and the corresponding glucose concentration as C_0 . C_0 was used as the baseline for iAUC calculation. Incremental areas above baseline were integrated over the predefined observation period, whereas areas below baseline were excluded. For intervals in which the glucose curve crossed the baseline, the intersection with the baseline was determined by linear interpolation and only the area above baseline was included. Data were processed using R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria; script provided in supplementary information). For each participant, a dataset was created containing glucose values from the relevant experimental time window (24 measurements per test day) and the corresponding blinded intervention code (188, 720, 896). Descriptive statistics (averages and standard deviations) were computed,

and data were visualized using boxplots and spaghetti plots. Differences in iAUC between the three experimental conditions (f-NAB, c-NAB and glucose reference) were analysed using a linear mixed-effects model. Experimental condition was included as a fixed effect, with the glucose solution as the reference category, and participant was included as a random intercept to account for repeated measurements within participants.

The overall effect of experimental condition was assessed using a Type III F-test with Satterthwaite approximation of the denominator degrees of freedom. Estimated marginal means (EMMs) were calculated for each experimental condition, followed by two-sided pairwise comparisons with Tukey adjustment for multiple comparisons. Statistical significance was defined as $\alpha = 0.05$. Effect estimates are reported with 95% confidence intervals. Linear mixed-effects models were fitted using the lme4 and lmerTest packages, and estimated marginal means and Tukey-adjusted pairwise comparisons were obtained using the emmeans package. Model assumptions were evaluated by visual inspection of the residuals. Histograms and Q-Q plots were used to assess the normality of residuals, and residual-versus-fitted plots were examined to evaluate homoscedasticity. Residuals were additionally inspected for potential outliers or influential observations; no indications of substantial violations of model assumptions or disproportionate influence of individual participants were observed. The significance level was set at $p < 0.05$. All statistical analyses were performed in R (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria).

The individual glycemic index was calculated based on individual iAUC values and calculated according to FAO/WHO⁵⁴ as follows:

$$GI = \frac{iAUC_{sample}}{iAUC_{glucose}}$$

The results for each participant and the statistical calculations were done with Excel 2607 and are provided in the supplementary information.

NAB Life Cycle Assessment

We conducted an LCA using the python-based framework Brightway 2.5⁵⁵ and the Recipe 2016 Midpoint (H) impact assessment method⁵⁶ to compare the environmental impacts of the two f-NAB formulations LTGR-GC1 and LTGR2-GC2 with two benchmarks based on the functional unit of 1 kg NAB. Like the f-NAB, the first benchmark was based on a brewing process with maltose-negative yeast to limit the alcohol production. To match the RE of the f-NAB, additional malt was simulated in the recipe (m-NAB). The second benchmark was modelled based on a conventional brewing process with subsequent ethanol removal via nanofiltration and ethanol recovery via distillation and the addition of isoamyl acetate to

maintain the flavor profile (c-NAB). Both benchmark approaches are presently applied in the commercial production of NABs. The system boundaries were cradle-to-factory gate. We included waste treatment of side-streams, where applicable, but excluded bottling and packaging, end-of-life treatment, and transport because we expected the differences between the three products to be minimal. Furthermore, we excluded capital goods due to limited data availability and their comparatively small influence on the impacts of beverage products⁵⁷. We modelled the foreground inventory for the context of Switzerland. It is accessible together with all calculations and data sources in the Supplementary File LCA. We based the utility consumption, i.e., hops, malt, water, electricity, and heat, of the conventional brewing process for the c-NAB on median values from existing literature on craft brewery- and industry-scale production^{48,58–63}. For the brewing process with maltose-negative yeast, we quantified the inputs of hops, malt, and yeast based on the recipe for f-NAB. To be able to compare the different production methods at industry-scale, we used the electricity and heat demand from conventional brewing to model the brewing with maltose-negative yeast, too. This seemed reasonable since the time and temperature profiles used to brew f-NAB aligned well with data for conventional brewing (Supplementary File LCA). The water used for brewing with maltose-negative yeast was directly measured. Cleaning water was added based on literature values. To quantify the amount of biowaste and waste water that can be expected from brewing with maltose-negative yeast, we applied the ratios between inputs and waste streams of biobased materials and water that were used in literature for the conventional process. Auxiliary inputs for brewing such as calcium sulphate, calcium chloride, sodium chloride, and lactic acid were not reported in scientific literature. Therefore, they were modelled for all three production methods based on measured data from the production of f-NAB to have a fair comparison. For malting⁶⁴, yeast production⁶⁵, nanofiltration and distillation⁴⁴, and isoamyl acetate production⁶⁶, we could directly use inventories from published LCAs or techno-economic models with minor modifications such as the geographic scope. In contrast, the production of hops, hop pelleting, hop extraction, and dextrin production from wheat starch slurry had to be modelled based on various data sources and thermodynamical calculations (Supplementary File LCA). We relied on the agrifootprint database v.6.3 (economic allocation)⁶⁷ to model the production of wheat starch slurry. Further, we used the ecoinvent database v. 3.11 (cut-off system)⁶⁸ for barley cultivation and all background processes such as waste treatment and electricity generation. After distillation, the removed ethanol from the c-NAB can be reused, e.g., as food-grade ethanol for the production of other alcoholic beverages⁴⁴. Therefore, we conducted a sensitivity analysis to understand how the environmental impacts would change if the obtained ethanol substituted conventional Swiss production covered by the ecoinvent database (Supplementary File LCA). They included ethanol production from sugar beet, grass, potatoes, wood, and whey.

LLMs

During the preparation of this work the author(s) used the large language model (LLM) *Gemini Pro* (Version 3.1) by Google to improve readability and language of existing formulations. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the publication. The author-verified Python 3.13.9 code used to generate data visualizations (Figures 2-5) was written with the assistance of *Gemini Pro* (Version 3.1) by Google and executed via Anaconda 2.7.0 in Jupyter Notebook 7.4.5.

Data availability

All source data of the manuscript and supplementary information as well as R-scripts used for evaluation of the source data and Python scripts used for visualization of the evaluated source data are available free of charge. For the dietary intervention study, anonymized data supporting the findings of this study are available from the corresponding author upon reasonable request. Individual-level participant data that could compromise participant privacy are not publicly available. Codes are made available on GitHub.

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Author Contribution Statement

Ivana Brun – Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Visualization, Project administration, Funding acquisition, Writing – original draft, Writing - review & editing

Lorena Neeser – Methodology, Validation, Investigation, Formal analysis, Data curation, Software, Visualization, Writing – original draft

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786 Kim Mishra – Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Validation,
787 Project administration, Supervision, Funding acquisition, Resources Writing – original draft, Writing -
788 review & editing

789 **Competing Interest Statement**

790 The authors declare no competing interests.

791 **Ethics declaration**

792 **Sensory:** Approved by the Ethics Advisory Board of the Bern University of Applied Sciences (Protocol
793 EAB2024_012). The board determined the study did not raise ethical concerns as no clinical data were
794 collected.

795 **Glycemic:** Approved by the Cantonal Ethics Committee Bern (Kantonale Ethikkommission für die
796 Forschung Bern, Project-ID: 2025-01272). The trial was registered at ClinicalTrials.gov (NCT07131904).
797 Participants provided written informed consent.

798

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