

ORIGINAL  
RESEARCH

# Enhancing the sweetening power of lactose by enzymatic modification in the reformulation of dairy products

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*Lactose solutions of up to 50% (w/v) were incubated with lactases and glucose isomerases for subsequent implementation in dairy product samples to enhance sweetness. A degree of hydrolysis of >90% and of isomerisation of 50% were attainable. The sensory sweetening power of lactose in solutions of up to 50% (w/v) can be enhanced 2–3 times. Based on sensory experiments, application of this bi-enzymatic system in yoghurt and pudding samples allowed for a 10–20% (w/w) reduction in the total sugar content, whilst retaining equal sweetness. The growth of yoghurt starter cultures was not affected, yet furosine formation more than doubled in high heated, enzyme-modified milk.*

**Keywords** Dairy technology, Dairy microbiology, Lactose, Physicochemical properties, Sensory analysis, Yoghurt.

## INTRODUCTION

The worldwide increase in the frequency of non-communicable diseases, such as cardiovascular diseases or type-2 diabetes, presents healthcare systems with tremendous problems. In its 'Global strategy on diet, physical activity and health', the World Health Organization (World Health Organization 2004) associates main risk factors of these diseases with unbalanced diets and a lack of physical exercise. Following this strategy, the European Commission published a White Paper on 'A strategy for Europe on nutrition, overweight and obesity-related health issues' (European Commission 2007) to guide actions of its member states at local and national levels. In 2018, the German Federal Ministry of Food and Agriculture released a national strategy on food reformulation and innovation, in which a holistic approach to reducing the sugar, salt or saturated fatty acid content in prepacked foods without substantially altering the original

product characteristics is presented (Federal Ministry of Food and Agriculture 2018).

Random sampling of sweetened dairy products and flavoured yoghurts available in German supermarkets showed that these products contained up to 22 g of sugar per 100 g (Bagus *et al.* 2016). Hence, milk products such as pudding and sweetened yoghurt are major contributors to the daily sugar intake of consumers and present a suitable target for food reformulation applications. In principal, the sugar content of dairy products may either be reduced by adding smaller quantities of sugar, or by reducing the original lactose content of the milk using cross-flow membrane processes or chromatographic techniques, the latter of which are based on the separation of ionic (e.g. proteins and salts) and nonionic (e.g. lactose) substances in skim milk (Harju 2004). Lactose hydrolysis is also a suitable procedure to achieve a lower overall sugar content of dairy products without substantial deviation of the flavour characteristics, as the

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relative sweetness of glucose is higher than that of lactose, allowing a reduction in added sugar whilst sweetening the end product (Gänzle *et al.* 2008; McCain *et al.* 2018).

A further increase in the sweetness of milk can be achieved if the glucose resulting from lactose hydrolysis is partly converted to fructose by the enzyme glucose isomerase. In dairy products in which lactose was hydrolysed, a degree of glucose isomerisation of between 25–52% could be achieved, whilst the relative sweetness of the resulting syrups was 80–100% that of a corresponding sucrose solution (Poutanen *et al.* 1978; Chiu and Kosikowski 1986; Abril and Stull 1989). Temiz *et al.* (2004) hydrolysed lactose in various aqueous, two-phase systems, with subsequent glucose isomerisation in a glass-column system. The final product contained an approximate fructose to glucose ratio of 60:40. Lorenzen *et al.* (2013) used ultra-filtration (UF)-permeates of skim milk, sweet whey, acid whey and lactose solutions for incubation with lactase and glucose isomerase. Lactose hydrolysis was 96–99%, whilst glucose isomerisation was between 47 and 53%. On a scale of 0–5, with 0 being ‘not present’ and 5 indicating ‘very strong’, the intensity of sweetness increased from 1 to 3, as determined by a trained sensory panel (Lorenzen *et al.* 2013). Preliminary studies on the application of the bi-enzymatic system in yoghurt manufacture have also been conducted. Due to the inhibiting effect of calcium on glucose isomerase, only 36% isomerisation of the glucose moiety was observed (Lorenzen *et al.* 2013). To our knowledge, further studies using the bi-enzymatic system in the manufacture of dairy products have to date not been performed. However, Torres and Batista-Viera (2017) reported on the application of a tri-enzymatic system of immobilised enzymes consisting of  $\beta$ -galactosidase, D-xylose isomerase and L-arabinose isomerase in a model system.

Reducing the lactose content, or replacing it by other types of sugar or non-nutritive sweeteners, may have a significant impact not only on the overall sensory properties, but also on preservation or the fermentability of dairy products (Buttriss 2013). The microorganisms used as starter cultures in fermented milk products such as yoghurt depend on the composition of available sugars for lactic fermentation. To date, it has not been investigated whether commercial yoghurt starter cultures can ferment bi-enzymatically sweetened milk in the same way as unprocessed bovine milk.

The present study used a novel approach in which solutions containing up to 50% (*w/v*) lactose were incubated with different soluble lactases and immobilised glucose isomerases using a bi-enzymatic system and subsequently implemented in dairy sample manufacture. Demineralised UF-permeates of skim milk were enzymatically modified and combined with UF-retentates of skim milk and cream for the manufacture of pudding and yoghurt samples. Sensory evaluation of both modified sugar syrups, as well as pudding and yoghurt produced with enzymatically sweetened milk, was performed. For such enhanced yoghurt samples, the microbiological

quality and growth of starter cultures were also monitored during production and storage at 4 °C.

## MATERIALS AND METHODS

### Origin of materials

Raw bovine milk, obtained from the experimental farm (Schädtbek, Germany) of the Max Rubner-Institut in Kiel, Germany, was warmed to  $45 \pm 1$  °C and skimmed. Lactose monohydrate was provided by Molkerei Meggle GmbH & Co. KG (Wasserburg, Germany).  $\beta$ -galactosidases NOLA™ Fit 5500 (a wide spectrum *Bifidobacterium bifidum*  $\beta$ -galactosidase produced by submerged fermentation using *Bacillus licheniformis*; activity 5500 bifido lactase units/g) and Ha-Lactase 2100 (a neutral lactase produced by submerged fermentation using *Kluyveromyces lactis*; activity 2100 neutral lactase units/g) were kindly provided by Chr. Hansen (Nienburg, Germany). The immobilised glucose isomerase Gensweet® IGI-HF (activity 251–300 GIGIC U/g) and the soluble glucose isomerase Gensweet® SGI (activity 3000 GIU/g) were donated by DuPont (Leiden, the Netherlands). Immobilised glucose isomerase Sweetzyme IT® (activity 380–420 IGIU/g) was a gift from Novozymes® (Bagsvaerd, Denmark). Mg-lactate·H<sub>2</sub>O was supplied by Dr. Paul Lohmann (Emmerthal, Germany). Glucose–fructose syrup (C\*TruSweet™ 01743) was kindly provided by Cargill (Hamburg, Germany). Premixes for the production of chocolate pudding (type: 1933 E; Schoko-Creme ‘Minus L’) and vanilla pudding (type: 2778 G; Vanille-Creme ‘Minus L’) were delivered by Condetta GmbH & Co. KG (Halle/Westfalen, Germany).

The commercial yoghurt cultures YoFlex® Premium 4.0 and ABT-100 were obtained from Chr. Hansen. The lactic acid bacteria (LAB) contained in the YoFlex® Premium 4.0 starter culture, as indicated by the manufacturer, were *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The ABT-100 starter culture contained *S. thermophilus* together with *L. acidophilus* and *Bif. animalis*. All cultures were received as frozen pellets and stored at –80 °C before inoculation into milk.

All chemicals used were of analytical grade. Sodium hydroxide (50% *w/w*) and sodium acetate (anhydrous), as well as glucose, galactose, fructose and lactose, which were used as external standards, were supplied by Sigma-Aldrich (Steinheim, Germany). Potassium hexacyanoferrate (II) {K<sub>4</sub>[Fe(CN)<sub>6</sub>]·3H<sub>2</sub>O} and zinc sulphate [ZnSO<sub>4</sub>·7H<sub>2</sub>O] used for Carrez solutions I and II were purchased from VWR (Darmstadt, Germany).

### Lactose hydrolysis and glucose isomerisation

Aqueous lactose solutions (300–500 g/L) were obtained by dissolving lactose in water and heating the mixture to  $71 \pm 2$  °C whilst stirring. Prior to lactose hydrolysis, these lactose solutions were cooled to the optimal enzyme temperatures of 45 or 50 °C, at which the lactose solutions

remained stable. Lactose hydrolysis was then performed with the  $\beta$ -galactosidases, according to the manufacturer's specifications for up to 7 h and applying the parameters shown in Table 1. Hydrolysed lactose solutions were heated to  $71 \pm 2$  °C for 3 min to inactivate both the  $\beta$ -galactosidase and the possible contaminating microorganisms, and stored at 4–6 °C until required for glucose isomerisation.

About 25 g of the immobilised glucose isomerases were filled into an XK 26/20 chromatography column (diameter: 26 mm, height: 200 mm, filled volume: 95 cm<sup>3</sup>; GE Healthcare GmbH, Solingen, Germany) and equilibrated according to the manufacturer's specifications. For glucose isomerisation, the parameters shown in Table 2 were applied. Hydrolysed lactose solutions containing 36 ppm Mg-lactate·H<sub>2</sub>O were pumped through the column for up to 6 h in a closed circuit with varying flow velocities to adjust enzyme-substrate contact time and hence the degree of isomerisation. Finally, the modified sugar solutions were heated for 3 min at  $71 \pm 2$  °C to inactivate possible contaminating microorganisms and stored at 4–6 °C until further use.

Calculation of lactose hydrolysis and glucose isomerisation was performed as follows:

$$\text{Degree of hydrolysis (DH, \%)} = \frac{\text{hydrolysed lactose}}{\text{initial lactose}} \times 100$$

$$\text{Degree of isomerisation (DI, \%)} = \frac{\text{fructose}}{\text{glucose} + \text{fructose}} \times 100$$

#### Determination of carbohydrates and calcium

UV-test kits were used according to the respective manufacturer's protocols for the determination of lactose/D-galactose

(Boehringer Mannheim/R-BioPharm, Darmstadt, Germany) and D-glucose, D-fructose and calcium (Thermo Fisher Scientific GmbH, Dreieich, Germany). Analysis was performed using the automatic photometer system Konelab 20i (Thermo Fisher Scientific GmbH). For each method, a 4-level calibration with commercially available standard solutions was carried out. Three independent experiments were performed and the standard deviation calculated. The coefficients of correlation in these studies were >0.95.

#### Preparation of milk for pudding and yoghurt production

Due to the inhibiting effect of calcium on glucose isomerase, demineralised UF-permeates of skimmed bovine milk were used for isomerisation during pudding and yoghurt production. For the preparation of permeates, pasteurised skimmed milk was warmed to  $50 \pm 2$  °C and ultra-filtered (NMWCO 5000 Dalton; Type HF 1.0-43-PM5-PB; Koch Membrane Systems, Wilmington, MA, USA). Residual lactose in the UF-retentates was hydrolysed using the NOLA™ Fit 5500  $\beta$ -galactosidase according to the manufacturer's specifications and applying the parameters in Table 1. After pasteurisation ( $71 \pm 2$  °C, 2 min), UF-retentates were stored at 4–6 °C until further use. The UF-permeates were demineralised using the cation exchanger Purolite® S930 Plus (Purolite Deutschland GmbH, Ratingen, Germany) according to the manufacturer's guidelines. To improve the performance of the glucose isomerase, 36 ppm Mg (from Mg-lactate·H<sub>2</sub>O) was added. Lactose hydrolysis and glucose isomerisation in the UF-permeates were conducted using the NOLA™ Fit 5500  $\beta$ -galactosidase and the soluble Gensweet® SGI glucose isomerase according to the manufacturer's protocols.

#### Pudding production and analysis of physical properties

Pudding production was performed as outlined in Figure 1. The mixture of UF-retentate, UF-permeate, cream and pudding premix was supplemented with sucrose and heated to 90 °C for 3 min under permanent stirring in a closed system. Pudding was cooled to 50 °C and filled into lockable cups in portions of 30 g.

Colour determination of pudding samples was performed using the colorimeter PCE-CSM3 (PCE Deutschland, Meschede, Germany). After calibration (black/white), 0.1 mL of the samples was measured and the results were specified in  $L^*a^*b^*$  units. Colour differences were calculated as  $dE^*$ . The gel firmness was analysed using a texture analyser (TA.XTplusConnect; Stable Micro Systems, Godalming, UK) using the following parameters: stamp P/0.5R; diameter 12 mm; test speed 0.5 mm/s; path length 10 mm; and trigger force 0.5 g. Gel firmness was calculated as the trigger force divided by the area of the stamp (113 mm<sup>2</sup>). All measurements were performed in quadruplicate, and the standard deviation was calculated as an error indicator. The coefficients of variation in these studies were between 0.17 and 3.48%.

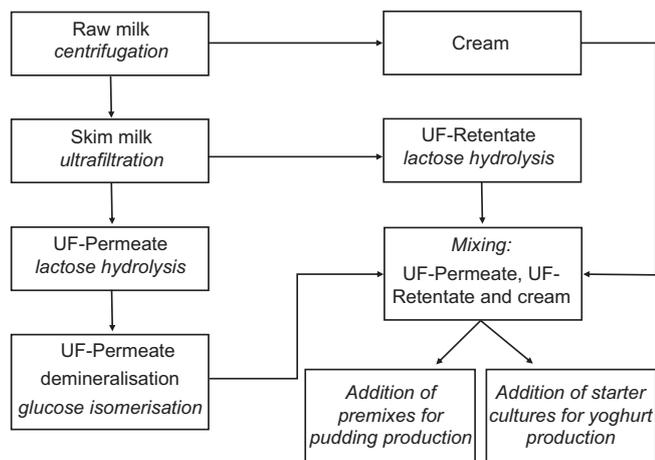
**Table 1** Parameters used for lactose hydrolysis.

Parameters	NOLA™ Fit 5500	Ha-Lactase 2100
pH Value	6.0	6.5
Temperature	50 °C	45 °C
Substrate concentration	300, 400, 500 (g/L)	300, 400, 500 (g/L)
Enzyme/substrate ratio	10 or 80 BLU/g lactose	10 or 80 NLU/g lactose

BLU, bifido lactase units; NLU, neutral lactase units.

**Table 2** Parameters applied for glucose isomerisation.

Parameters	Gensweet® IGI-HF	Sweetzyme IT®
pH value	7.8	7.5
Temperature	62 °C	57 °C
Substrate concentration	300, 400, 500 (g/L)	300, 400, 500 (g/L)
Flow velocity	1.49–9.10 (mL/min)	1.59–9.38 (mL/min)



**Figure 1** Schematic diagram of pudding and yoghurt production with modified sugar syrups. Adapted and modified from Lorenzen *et al.* (2013).

#### *Yoghurt production and analysis of physical and chemical properties*

Yoghurt production was performed as outlined in Figure 1. The mixture of UF-retentate, UF-permeate and cream (with the addition of glucose–fructose syrup for production of a standard yoghurt with similar sweetness to enhanced yoghurt for sensory tests) was heated to 90 °C for 3 min. Yoghurt was manufactured in 4 L pitchers. Starter cultures were thawed in a water bath at 25 °C for 20 min. After homogenisation at 60 °C and 200 bar, and cooling to 45 °C, the milk was inoculated either with YoFlex<sup>®</sup> Premium 4.0 or ABT-100 starter cultures according to the manufacturer's instructions for laboratory production with DVS<sup>®</sup> ripening cultures (Chr. Hansen). After inoculation and stirring of the pre-warmed milk, yoghurt was poured into 150-mL yoghurt cups. The cups were sealed, and the yoghurt milk was left to ferment at 43 °C. The pH during fermentation was monitored in one of the yoghurt samples using a laboratory pH meter (inoLab pH Level 2; WTW GmbH & Co. KG, Weilheim, Germany). Once the pH reached 4.5, the yoghurt samples were cooled to 4 °C and post-acidification was monitored at this temperature for 3 weeks. Yoghurt was manufactured in three independent experiments for both cultures. The dry matter and fat content of standard and sweetness-enhanced milk and yoghurt samples were determined according to German standard method C35.5 for dry matter and C15.3.2 for fat content (VDLUFA 2003).

Viscosity measurements were carried out using an MCR 302 rheometer (Anton Paar Germany GmbH, Ostfildern, Germany), equipped with a cone-plate measuring system (CP50-1-SN 29358, diameter 50 mm, cone angle 1°, truncation 0.103 mm). After equilibration of the samples (0.7 mL) at 20 °C for 2 min, a frequency sweep started at 1/s and was raised to 1000/s in 16 steps at 20 °C, with a 30 s length for each step. Each sample was measured three times.

Colour determination of yoghurt samples was performed using the colorimeter PCE-CSM3 (PCE Deutschland). After calibration (black/white), 0.1 mL of the samples was measured and the results were specified in  $L^*a^*b^*$  units. Colour differences were calculated as  $dE^*$ .

#### *Microbiological analysis of yoghurt*

Microbiological samples were taken immediately after inoculation of the pre-warmed milk to determine inoculation levels of starter LAB, as well as just prior to cooling when the yoghurt reached a pH of 4.5 and after five and 21 days of storage and post-acidification at 4 °C, to determine starter LAB counts.

For microbiological testing, 10 g of yoghurt was placed in a BagFilter<sup>®</sup> 400 P lab blender bag with a <250-µm lateral filter (Interscience for Microbiology, Saint Nom, France), to which 90 mL of pre-warmed sodium citrate solution (2% w/v) was added. The bag was placed in a BagMixer<sup>®</sup> (Interscience for Microbiology) laboratory blender for 2 min at maximum speed. Of the supernatant, 1 mL was diluted in a tenfold dilution series. To determine the lactic acid bacterial count (cfu/g; colony forming units per gram), 100 µL volumes of appropriate dilutions were spread-plated onto M17 agar (Terzaghi and Sandine 1975) to enumerate *S. thermophilus*. For counts of *L. delbrueckii* subsp. *bulgaricus* contained in YoFlex<sup>®</sup> Premium 4.0, MRS agar (VWR International GmbH) was used. For counts of *L. acidophilus* in the ABT-100 starter culture, Bile-MRS (MRS agar + 0.15% w/v bile) was used (Vinderola and Reinheimer 1999). For counts of *Bif. animalis* contained in the ABT-100 starter culture, LP-MRS (MRS agar + 0.2% w/v lithium chloride + 0.3% w/v sodium propionate) was used (Vinderola and Reinheimer 1999).

Samples taken after 21 days of storage at 4 °C were also tested for possible contamination with either enterobacteria (using VRBD medium; Merck, Darmstadt, Germany), enterococci (using KAA medium; Merck), yeast or moulds (using YGC agar; VWR International GmbH), and pseudomonads (using CFC agar as described by Merck (2010) with the addition of 1% w/v Delvolid<sup>®</sup> Instant [DSM Food Specialties, Delft, the Netherlands] and 10% v/v glycerine [Carl Roth, Karlsruhe, Germany]). The selective agar plates were incubated aerobically at 43 °C for 48 h (M17), 37 °C for 48 h (MRS, Bile-MRS, KAA), 25 °C for 48 h (YCG, CFCD) or 30 °C for 24 h (VRBD). LP-MRS media plates were incubated in anaerobic jars with Oxoid<sup>™</sup> AnaeroGen<sup>™</sup> sachets (Thermo Fisher Scientific GmbH) at 37 °C for 48 h.

#### *Determination of sugar profiles and measurement of furosine content*

Sugar profiles were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Frenzel *et al.* 2015). Analysis was

carried out on a Dionex ICS-3000 chromatographic system (Thermo Fisher Scientific GmbH) consisting of an SP gradient pump, an SP autosampler and a detector compartment using an ED 40 electrochemical detector with a gold working electrode and an Ag/AgCl reference electrode (Bruggink *et al.* 2005). Data acquisition and processing were performed with the Chromeleon™ 6.8 chromatography data system software (Thermo Fisher Scientific GmbH). Before injection, 0.25–1 g of samples was clarified using 5 mL of Carrez solutions I and II (Carrez I = 3.60% *w/v* potassium hexacyanoferrate (II)  $\{K_4[Fe(CN)_6] \cdot 3H_2O\}$ ; Carrez II = 7.20% *w/v* zinc sulphate  $[ZnSO_4 \cdot 7H_2O]$ ) and 10 mL of 0.1 M NaOH. The samples were filled to a volume of 100 mL, filtered and diluted 1–200 with water. Samples or standard solutions were injected at a volume of 20  $\mu$ L, and separations were performed with a flow rate of 0.2 mL/min at 25 °C on a pellicular anion-exchange resin-based analytical column CarboPac PA-100 (2 mm  $\times$  250 mm) with a Dionex™ CarboPac™ PA-100 guard column (2 mm  $\times$  50 mm; Thermo Fisher Scientific GmbH). Elution was carried out with a gradient of NaOH and sodium acetate, beginning with a stationary elution with 15.5 mM NaOH and 3 mM sodium acetate for 15 min, followed by stepwise increases over 35 min to reach a final concentration of 100 mM NaOH and 250 mM sodium acetate. After each run, the column was washed for 10 min with 100 mM NaOH and 250 mM sodium acetate and re-equilibrated for 15 min with starting conditions. Analysis of the samples was performed in duplicate. The carbohydrates glucose, galactose, fructose and lactose eluted as separate peaks, allowing their quantitative determination by using standard calibration curves.

Measurement of furosine content was performed according to the ISO 18329:2004 [IDF 193:2004] ion-pair reverse-phase high-performance liquid chromatography method (International Organization for Standardization 2004).

#### Assessment of sensory properties

Sensory evaluation of the modified sugar solutions as well as of pudding and yoghurt samples was carried out according to DIN 10967 (quantitative descriptive analysis). A panel of 30 assessors with previous experience in the descriptive sensory analysis of dairy products was appointed for the sensory evaluation. To evaluate the modified sugar solutions and pudding samples, taste descriptions were applied. Enzyme-treated lactose solutions were diluted to 5% (*w/w*) of the total sugar content. Pudding samples were evaluated after one and 14 days of storage. Yoghurt samples were evaluated after five and 21 days of storage at 4–6 °C. Samples (enzyme-treated lactose solutions: 20–30 mL at an evaluation temperature of 20–22 °C; pudding: 30 g at 10–12 °C; yoghurt: 30 g at 6–8 °C) were given to the assessors in a sensory laboratory. One session per day with a

maximum of six samples was conducted. The intensity of the taste descriptors was examined and rated according to a structured scale from 0 (not present) to 5 (very strong).

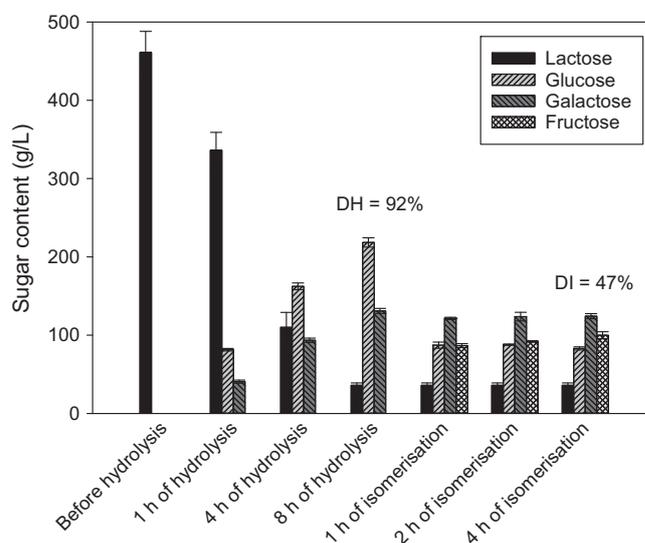
#### Statistical analyses

Three independent repetitions of each production experiment were performed for all pudding and yoghurt variations. Determination of sugar content and measurements of physical properties were done in technical triplicates, as were all bacterial counts. For statistical evaluation, the arithmetic mean, standard deviation, confidence intervals (student distribution,  $\alpha$ : 0.95,  $n$  = 15–25) and standard error of the mean were calculated. To confirm the independence of two trials or data sets, Student's *t*-test was applied. Data were analysed using Microsoft Excel (Microsoft Office Professional Plus 2019) and Sigma Plot version 13 (Systat Software GmbH, Erkrath, Germany).

## RESULTS AND DISCUSSION

### Incubation of lactose solutions with $\beta$ -galactosidases and glucose isomerases

Aqueous solutions with a concentration of up to 50% (*w/w*) lactose were processed using the bi-enzymatic system (Lorenzen *et al.* 2013). Figure 2 provides an exemplary overview of the sugar content of intermediary solutions during lactose hydrolysis and partial glucose isomerisation. The initial lactose concentration of  $461 \pm 27$  g/L was hydrolysed within 8 h to a final concentration of  $36 \pm 3$  g/L, obtaining a degree of hydrolysis (DH) of 92%. The lower



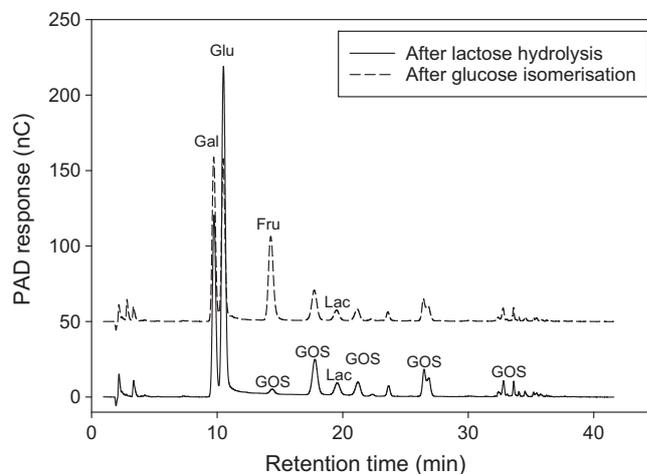
**Figure 2** Sugar content of solutions during lactose hydrolysis and subsequent partial glucose isomerisation with indication of the final degree of hydrolysis (DH) and degree of isomerisation (DI). The  $\beta$ -galactosidase was inactivated after hydrolysis, and the level of residual lactose is shown during isomerisation. Depiction: arithmetic mean ( $n$  = 3)  $\pm$  SD.

galactose content in relation to the glucose concentration after 8 h of hydrolysis was due to the presence of galactooligosaccharides (GOS) that were formed by the transgalactosylation reaction in concentrated lactose solutions, as was also described previously by Gänzle *et al.* (2008). The subsequent partial isomerisation of the glucose moiety was largely completed after 1 h. A final degree of isomerisation (DI) of 47% was achieved after 4 h of incubation.

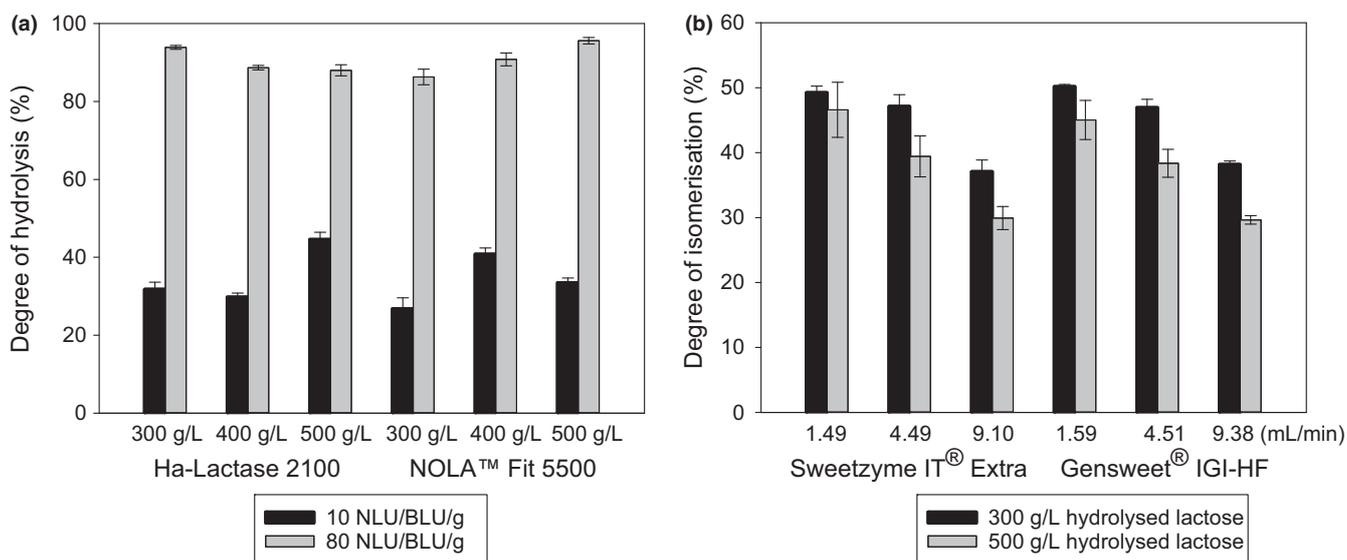
Two highly purified  $\beta$ -galactosidases, Ha-Lactase 2100 and NOLA™ Fit 5500, were used for lactose hydrolysis, applying the parameters outlined in Table 1. DH-values of 27–45% were obtained with a low enzyme to substrate ratio of 10 lactase units/g lactose and initial lactose concentrations between 300 and 500 g/L (Figure 3a). Applying a high enzyme to substrate ratio of 80 lactase units/g lactose resulted in DH-values between 86 and 96%. In Figure 3(b), the degree of isomerisation achieved with two different glucose isomerases in relation to the substrate concentration and the flow velocity of the hydrolysed lactose solution through the column reactor are shown (for further parameters see Table 2). As demonstrated in Figure 3(b), the degree of isomerisation decreased with increasing substrate concentration and flow velocity. At a hydrolysed lactose concentration of 500 g/L and a flow velocity of 1.49 or 1.59 mL/min, DI percentages of 49.3% (Sweetzyme IT® Extra) and 50.2% (Gensweet® IGI-HF) could be calculated, respectively.

The sugar composition of hydrolysed lactose and isomerised glucose solutions measured by HPAEC-PAD are shown in Figure 4. Due to the high degree of lactose hydrolysis, the main sugar components are the

monosaccharides galactose and glucose. Only low amounts of residual lactose could be determined following hydrolysis with both  $\beta$ -galactosidases (results of the neutral  $\beta$ -galactosidase, Ha-Lactase 2100, not shown). Furthermore, the GOS generated by the transgalactosylation activity of the  $\beta$ -galactosidases during hydrolysis were previously shown to be mainly di-, tri- and tetrasaccharides, but the exact composition depended on the  $\beta$ -galactosidase used (Frenzel *et al.* 2015). The neutral  $\beta$ -galactosidase Ha-Lactase 2100 preferentially produces  $\beta$ 1-6 linkages between saccharides



**Figure 4** High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) profile of lactose solutions (500 g/L) hydrolysed with NOLA™ Fit 5500 followed by partial glucose isomerisation with Gensweet® IGI-HF (Gal, galactose; Glu, glucose; Lac, lactose; Fru, fructose; GOS, galactooligosaccharides).

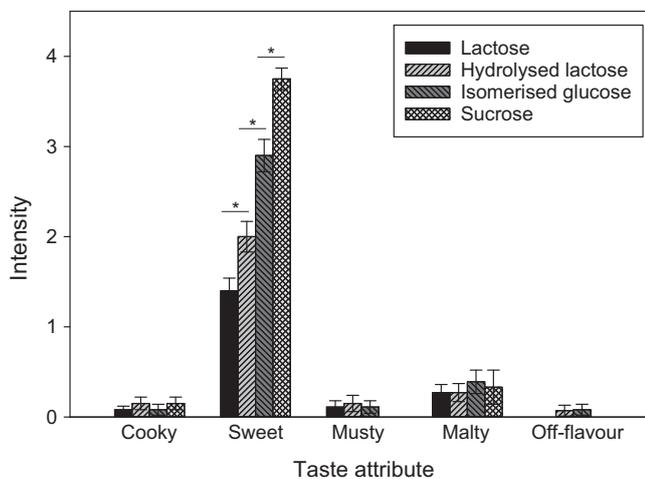


**Figure 3** (a) Degree of lactose hydrolysis (%) with different lactases and in relation to substrate concentrations, and (b) degree of isomerisation (%) with different isomerases in relation to substrate concentration and flow velocity (mL/min) of the hydrolysed lactose solutions. Depiction: arithmetic mean ( $n = 3$ )  $\pm$  SD.

with 6'-galactosyllactose as the main reaction product, whilst the *Bif. bifidum*  $\beta$ -galactosidase NOLA™ Fit 5500 produces considerable amounts of 3'- and 4'-galactosyllactose in addition to the di- and tetrasaccharides (Depeint *et al.* 2008). Comparison of the sugar composition before and after glucose isomerisation showed a significant decrease in glucose and the generation of fructose, but no influence of the glucose isomerase on GOS formation or the residual lactose content during the enzymatic reaction. These results showed that enhancing the sweetening power of lactose through its hydrolysis and subsequent glucose isomerisation can also deliver an additional benefit through the production of prebiotic GOS. In particular, GOS generated by the *Bif. bifidum*  $\beta$ -galactosidase NOLA™ Fit 5500 have

the potential to function as prebiotics for LAB and bifidobacteria in human and animal nutrition (Vulevic *et al.* 2018). However, further investigations of the stability of GOS during the manufacture of dairy products, especially during heat treatment and fermentation, should be done.

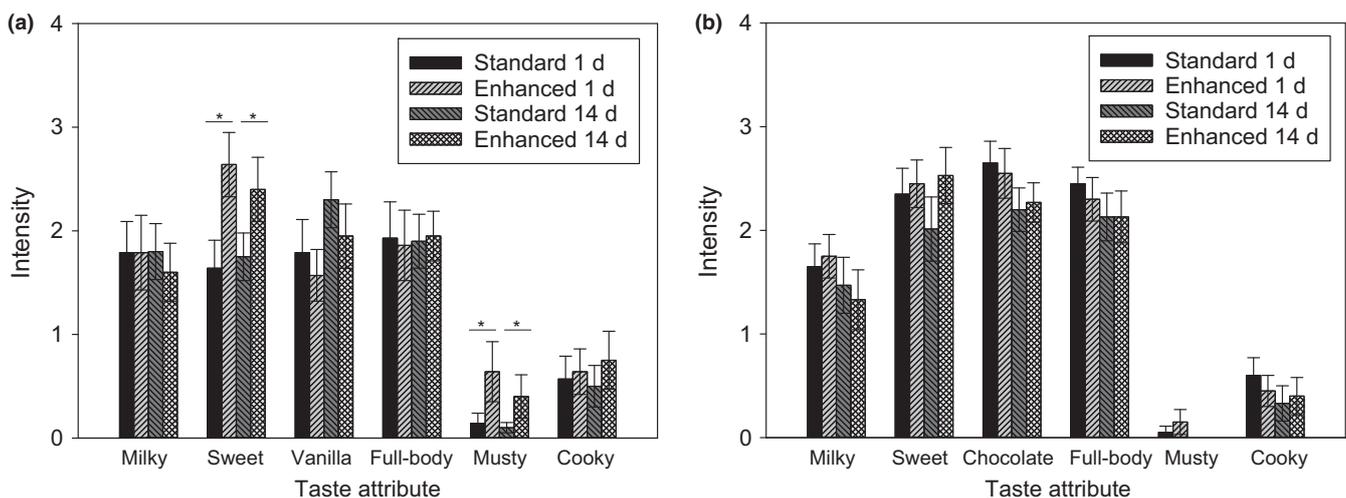
Selected taste attributes of solutions (5% w/w) of lactose, hydrolysed lactose, isomerised glucose and sucrose, determined by sensory studies, are shown in Figure 5. It is evident from these results that the sweetening power of lactose doubled when the bi-enzymatic system was applied. When the sweetness of sucrose was set to 100%, the enzyme-modified sugar solution amounted on average to 80% sweetness, thus twice that of lactose. The odour characteristics tested for were negligible (results not shown). All sugar solutions exhibited faintly recognisable flavours such as cooky, musty and malty. A very slight off-flavour was detected in the hydrolysed lactose and partly isomerised glucose solutions.



**Figure 5** Selected taste attributes of solutions of lactose, hydrolysed lactose and hydrolysed lactose with partly isomerised glucose in relation to sucrose. Depiction: arithmetic mean ( $n = 13$ )  $\pm$  SD. Unpaired  $t$ -test, \* $P < 0.05$ .

### Sensory and physical properties of pudding samples

Pudding samples were manufactured as described earlier and outlined in the schematic diagram in Figure 1. Due to the inhibiting effect of calcium on glucose isomerase, demineralised UF-permeates of skimmed milk were used during production. About 99% (w/v) of calcium was removed by cation exchange before application of the bi-enzymatic system. Standard and modified pudding samples were adjusted to roughly the same sensory sweetness by varying the amount of sucrose added. In Figure 6, the results of selected taste attributes of vanilla and chocolate pudding produced with standard and sweetness-enhanced milk are shown. The intensity of most of the attributes tested for in standard and sweetness-enhanced pudding samples did not differ significantly between samples. The sweetness of pudding samples made with sweetness-enhanced milk was slightly higher



**Figure 6** Selected taste attributes of (a) vanilla and (b) chocolate pudding from pilot plant production using whole milk (standard) or lactose modified milk (enhanced) in relation to the storage time. Depiction: arithmetic mean ( $n = 18$ )  $\pm$  SD. Unpaired  $t$ -test, \* $P < 0.05$ .

than that of standard samples, especially in vanilla pudding. In addition, vanilla pudding produced with enzyme-modified milk showed higher intensities of an undesired musty flavour than the standard samples (Figure 6a). It is surprising that there was no difference between the intensity of cooky flavour in standard and sweetness-enhanced pudding samples because the furosine content in high heated, enzyme-modified milk amounted to roughly 204 mg/100 g protein, which is more than twice that of standard milk (about 105 mg/100 g protein). This indicates an increasing *Maillard* reaction in the sweetness-enhanced milk due to the different sugar composition.

The sugar content and composition of standard and sweetness-enhanced vanilla and chocolate pudding samples are outlined in Table 3. These results suggest that the total sugar content of pudding samples (% w/w) may only be reduced by 9% (vanilla) to 22% (chocolate) whilst retaining equal sensory intensities of sweetness. The possibility of

reducing sugar in desert products thus seems lower than expected based on the preliminary studies using lactose as a raw material.

Gel firmness measurements of the pudding samples stored at 4–6 °C were performed 14 days after manufacture. Samples of standard and sweetness-enhanced vanilla and chocolate pudding stored for 14 days showed no significant differences in gel firmness (Table 4). The colour was measured as  $L^*a^*b$  colour index and calculated as the colour difference between the standard and the sweetness-enhanced samples. The resulting colour difference in the pudding samples was <1, indicating that any differences in colour were not detectable by the human eye (Table 4).

### Sensory properties of yoghurt samples and analysis of starter culture composition

Yoghurt samples were manufactured as described earlier and outlined in the schematic diagram in Figure 1. Two different starter cultures, a traditional (YoFlex<sup>®</sup> Premium 4.0) and a milder-tasting culture (ABT-100), were applied. Adjustment of standard and modified yoghurt samples to roughly the same sweetness for sensory tests was achieved by adding glucose–fructose syrup to the standard milk. Selected taste attributes of traditional and mild-tasting yoghurt samples produced with standard and sweetness-enhanced milk are shown in Figure 7. Yoghurt prepared using the traditional starter culture YoFlex<sup>®</sup> Premium 4.0 with enzyme-modified milk was perceived to be sweeter than the standard sample (Figure 7a). Musty flavour characteristics were not detected in standard or sweetness-enhanced yoghurt samples when the traditional starter culture was applied. Mild-tasting yoghurt samples (ABT-100 starter culture) manufactured with enzyme-modified milk showed a higher sweetness than those produced with standard milk (Figure 7b). Musty flavour was detected using the mild starter culture, especially in the yoghurt samples produced with enzyme-modified milk.

The sugar content and composition of standard and sweetness-enhanced yoghurt samples are outlined in Table 5. It is

**Table 3** Sugar content and composition of standard and sweetness-enhanced vanilla and chocolate pudding after 14 days of storage ( $n = 3 \pm \text{SD}$ ).

Sugar (% w/v)	Vanilla pudding		Chocolate pudding	
	Standard <sup>a</sup>	Enhanced	Standard <sup>a</sup>	Enhanced
Lactose	5.2 ± 0.10	0.1 ± 0.01	5.1 ± 0.07	0.1 ± 0.01
Glucose	4.2 ± 0.07	5.3 ± 0.08	6.1 ± 0.08	5.9 ± 0.12
Galactose	0.1 ± 0.01	2.4 ± 0.05	0.1 ± 0.01	2.4 ± 0.09
Fructose	2.8 ± 0.03	3.4 ± 0.04	4.7 ± 0.05	4.1 ± 0.06
∑ Sugar <sup>b</sup>	12.3	11.2	16.0	12.5
∑ Sugar reduction compared with the standard product <sup>b</sup>	NA	8.9	NA	21.8

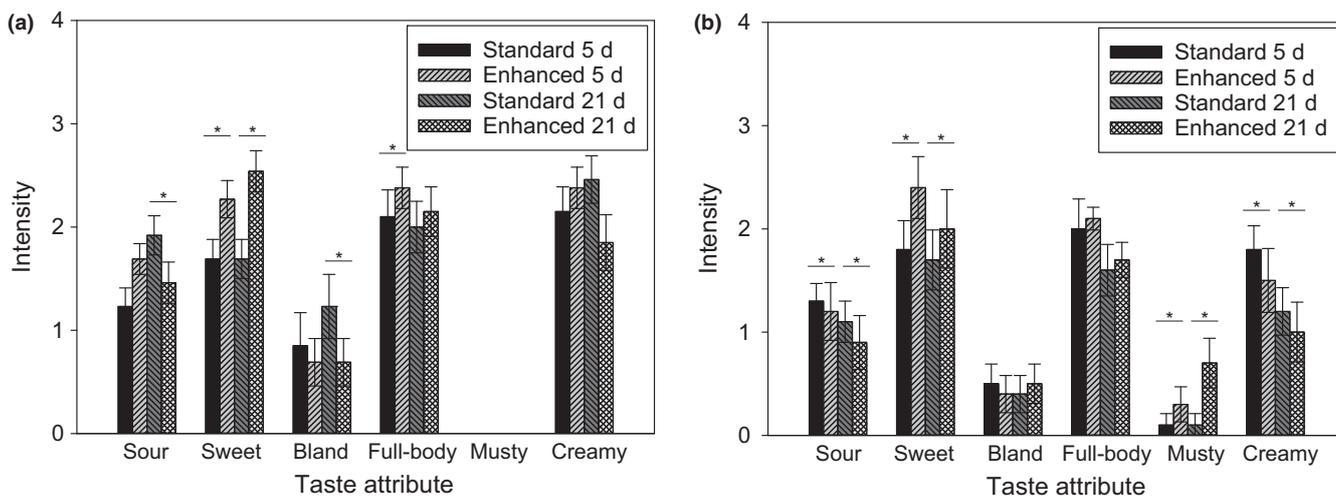
NA, not applicable.

<sup>a</sup>Adjusted to the same sweetness as enhanced samples.

<sup>b</sup>Calculated.

**Table 4** Gel firmness and colour analysis of vanilla and chocolate pudding produced with standard and sweetness-enhanced milk after 14 days of storage ( $n = 4 \pm \text{SD}$ ).

	Vanilla pudding		Chocolate pudding	
	Standard	Enhanced	Standard	Enhanced
Gel firmness	$3.498 \times 10^{-4} \pm 1.061 \times 10^{-5} \text{ N/mm}^2$	$3.503 \times 10^{-4} \pm 8.849 \times 10^{-6} \text{ N/mm}^2$	$1.39 \times 10^{-3} \pm 6.549 \times 10^{-5} \text{ N/mm}^2$	$1.186 \times 10^{-3} \pm 4.867 \times 10^{-5} \text{ N/mm}^2$
$L^* a^* b^*$ colour index	$L^* 66.65 \pm 0.0026$ $a^* 1.725 \pm 0.0086$ $b^* 19.32 \pm 0.0097$	$L^* 67.56 \pm 0.0041$ $a^* 1.640 \pm 0.014$ $b^* 19.37 \pm 0.012$	$L^* 40.11 \pm 0.0040$ $a^* 6.516 \pm 0.0078$ $b^* 8.042 \pm 0.014$	$L^* 39.57 \pm 0.0049$ $a^* 6.67 \pm 0.013$ $b^* 8.20 \pm 0.0046$
Colour $dE^*$	0.92		0.59	



**Figure 7** Selected taste attributes of standard and sweetening power enhanced yoghurt fermented with (a) YoFlex® Premium 4.0 culture and (b) ABT-100 culture after five and 21 days of storage (standard yoghurt with addition of 1.5% Glu-Fru syrup). Depiction: arithmetic mean ( $n = 15$ )  $\pm$  SD. Unpaired *t*-test, \* $P < 0.05$ .

evident that the total sugar content of yoghurt samples (% w/w) may only be reduced by 11% (traditional) to 15% (mild-tasting yoghurt) whilst retaining the same sweetness. Considering that traditional yoghurt made with enzyme-modified milk showed a significantly higher sweetness than the standard sample (Figure 7a), a 15% sugar reduction in yoghurt may thus be achievable.

Rheological measurements of the yoghurt samples, stored at 4–6 °C, were performed at 5 and 21 days after manufacture by

using a shear stress-controlled rheometer at three different shear rates. No significant differences between the rheological behaviour of the standard yoghurt samples with glucose–fructose syrup addition and the sweetness-enhanced yoghurt were noticeable with both starter cultures (YoFlex® Premium 4.0 and ABT-100; results not shown). The colour was analysed as  $L^*a^*b$  colour index and calculated as the colour difference between the standard and the sweetness-enhanced yoghurt samples. The resulting colour difference in the yoghurt samples was  $<1$ , which means that the differences in colour were not detectable by the human eye (results not shown). The pH, dry matter and fat content of standard and sweetness-enhanced

**Table 5** Sugar content and composition of standard and sweetness-enhanced yoghurt fermented with different starter cultures after 21 days of storage ( $n = 3 \pm$  SD).

Sugar (%, w/v)	YoFlex® Premium 4.0 culture		ABT-100 culture	
	Standard <sup>a</sup>	Enhanced	Standard <sup>a</sup>	Enhanced
Lactose	4.2 $\pm$ 0.08	1.4 $\pm$ 0.03	4.5 $\pm$ 0.07	1.1 $\pm$ 0.06
Glucose	1.0 $\pm$ 0.02	1.8 $\pm$ 0.04	1.0 $\pm$ 0.03	1.4 $\pm$ 0.07
Galactose	1.2 $\pm$ 0.04	2.4 $\pm$ 0.05	1.4 $\pm$ 0.05	2.5 $\pm$ 0.09
Fructose	0.6 $\pm$ 0.02 <sup>b</sup>	0.6 $\pm$ 0.02	0.6 $\pm$ 0.03 <sup>b</sup>	1.4 $\pm$ 0.05
$\Sigma$ Sugar <sup>b</sup>	7.0	6.2	7.5	6.4
$\Sigma$ Sugar reduction compared with the standard product <sup>b</sup>	NA	11.4	NA	14.6

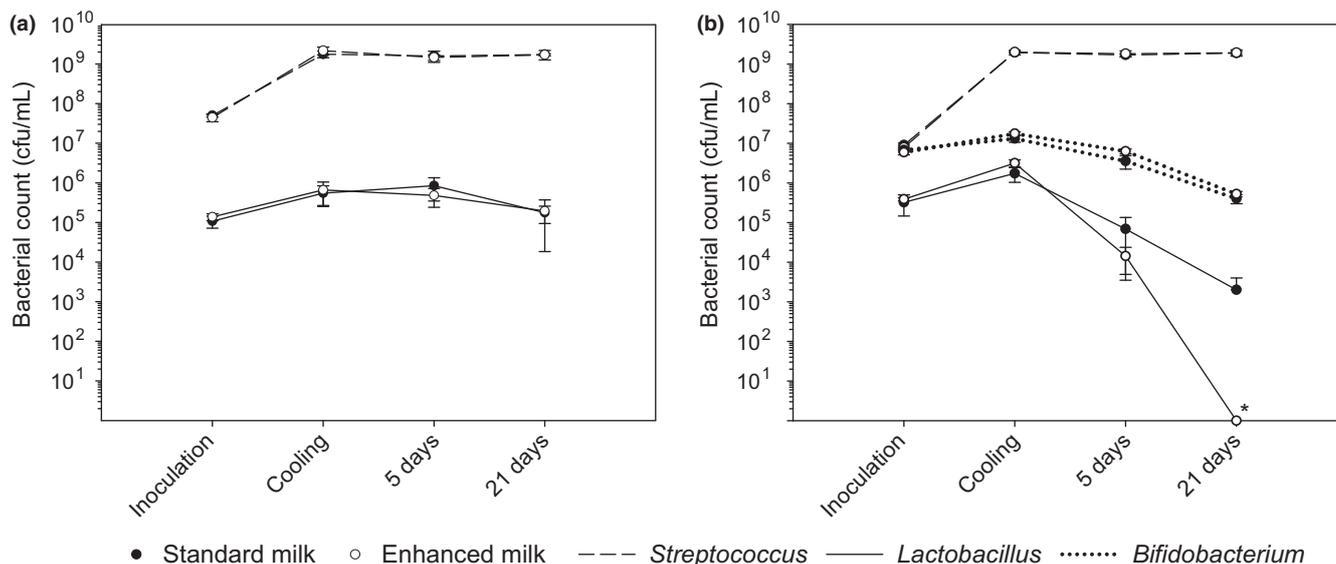
NA, not applicable.

<sup>a</sup>Adjusted to the same sweetness as enhanced samples using glucose–fructose syrup.

<sup>b</sup>Calculated.

**Table 6** pH, dry matter and fat content of standard and sweetness-enhanced milk prior to yoghurt production ( $n = 6 \pm$  SD), and fermented with different starter cultures after 5 days of storage ( $n = 3 \pm$  SD).

Sample	pH	Dry matter (%, w/v)	Fat (%, w/v)
Regular milk	6.67 $\pm$ 0.02	14.02 $\pm$ 0.03	3.55 $\pm$ 0.03
Enhanced milk	6.82 $\pm$ 0.07	14.85 $\pm$ 0.65	3.59 $\pm$ 0.02
Regular yoghurt at 5 days (YoFlex® Premium 4.0)	4.60 $\pm$ 0.01	13.87 $\pm$ 0.49	3.62 $\pm$ 0.05
Enhanced yoghurt at 5 days (YoFlex® Premium 4.0)	4.63 $\pm$ 0.02	14.31 $\pm$ 1.26	3.56 $\pm$ 0.04
Regular yoghurt at 5 days (ABT-100)	4.53 $\pm$ 0.02	14.03 $\pm$ 0.06	3.53 $\pm$ 0.05
Enhanced yoghurt at 5 days (ABT-100)	4.58 $\pm$ 0.04	14.69 $\pm$ 0.71	3.59 $\pm$ 0.04



**Figure 8** Lactic acid bacterial counts of *Streptococcus*, *Lactobacillus* and *Bifidobacterium* species during yoghurt production with standard and sweetness-enhanced milk using (a) YoFlex® Premium 4.0 and (b) ABT-100 cultures. Depiction: arithmetic mean ( $n = 3$ )  $\pm$  SE of the mean. \*Under the limit of detection set at  $1 \times 10^2$  cfu/mL.

milk prior to yoghurt production and after 5 days of storage are shown in Table 6. Sweetness-enhanced milk showed a slightly higher initial pH; however, acidification during fermentation and post-acidification during storage showed a similar progression in standard and sweetness-enhanced yoghurt samples. Dry matter content and fat content were comparable in both types of milk at all sampling time points.

Both in enzyme-modified milk and standard milk, the YoFlex® Premium 4.0 and ABT-100 starter cultures implemented in yoghurt production showed similar and expected growth patterns (Figure 8). This indicates a similar ability of the starter LAB to ferment enzyme-modified milk as compared with standard milk. Furthermore, tests for possible contamination with spoilage or opportunistically pathogenic bacteria showed that the microbiological quality of the sweetness-enhanced samples was not compromised in comparison with the standard samples (results not shown). The altered sugar combination can provide a sweeter tasting product without the need for added sugar and does not interfere with the manufacturing and fermentation processes of this product.

## CONCLUSIONS

The application of the bi-enzymatic system of lactose conversion for pudding and yoghurt production allowed a 10–20% ( $w/w$ ) reduction in the total sugar content of these samples as shown in laboratory-scale experiments. However, the possibility of reducing sugar in the dairy samples evaluated was lower than expected based on preliminary studies using aqueous lactose solutions as a raw material, with the exception of chocolate pudding samples. Even with

substrate concentrations of up to 50% ( $w/w$ ) lactose, DH-values of >90% and DI-values of 50% were attainable. Sensory studies showed that the sweetness power of lactose in solutions up to 50% ( $w/v$ ) can be enhanced 2–3 times. The intensity of most of the taste attributes tested in standard and sweetness-enhanced pudding and yoghurt samples was comparable. Production of yoghurt samples with sweetness-enhanced milk showed no influence of the altered sugar composition of the milk on the growth of and the acidification caused by two different starter cultures tested. The *Maillard* reaction was increased in bi-enzymatically modified milk as furosine formation more than doubled in high heated, enzyme-modified milk. Furthermore, prebiotic GOS generated during lactose hydrolysis were not affected by subsequent glucose isomerisation, thus delivering an additional benefit to the sweetness-enhanced milk. Further studies could focus on the application of starter cultures that utilise galactose as a carbohydrate source, as well as isomerisation of galactose to tagatose to further increase sweetness. Finally, the development of a glucose isomerase resistant to calcium might be of interest for industrial applications of the bi-enzymatic system of lactose conversion.

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