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Enrichment of tiger nut milk with microbial transglutaminase cross-linked protein improves the physico-chemical properties of the fermented system

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ABSTRACT

Milk proteins cross-linked with microbial transglutaminase were investigated for their potential to improve the microbiological and physico-chemical properties of fermented tiger nut milk. Fermented systems with cross-linked proteins did not affect *S. thermophilus* viable counts but decreased that of *L. delbrueckii* ssp. *bulgaricus* compared to the untreated protein systems. Systems with cross-linked proteins showed shorter microbial lag time and a higher pH reduction rates during fermentation. During storage of the fermented product, viable counts of *L. delbrueckii* ssp. *bulgaricus* decreased faster than that of *S. thermophilus*, and systems with cross-linked proteins revealed a lower decrease in *L. delbrueckii* ssp. *bulgaricus* cell counts compared to untreated proteins during 15 d. Products from cross-linked sodium caseinate or whey protein showed 16.4 fold and 3.6 fold increase in viscosity, and approx. 30% and 36% decrease in syneresis compared to their untreated counterparts, respectively. The addition of proteins to tiger nut milk improved the lightness of the fermented product and minimized lightness decrease during storage, and casein cross-linking further improved lightness. The enrichment of tiger nut milk with cross-linked protein has therefore a large potential for improving the physical characteristics of fermented tiger nut milk.

1. Introduction

Lactic acid fermentation of tiger nut (*Cyperus esculentus* L.) aqueous extracts, also denoted as tiger nut milk (TNM), is known to give lactose-free, sweet-sour products that might serve as important source of food nutrients (Akoma, Elekwa, Afodunrinbi, & Onyeukwu, 2000; Wakil, Ayenuro, & Oyinola, 2014). However, lactic acid fermentation of plain tiger nut milk leads to products with low viscosity and high susceptibility to phase separation (Kizzie-Hayford, Jaros, Zahn, & Rohm, 2016), which adversely affects consumer acceptance of the product (Akoma et al. 2000). Our recent report revealed that the enrichment of TNM with milk proteins and subsequent lactic acid fermentation resulted in yoghurt-like products with acceptable textural and sensory properties (Kizzie-Hayford et al., 2016). Tiger nut milk itself shows a protein content as low as < 1 g/100 g, which does not allow the fermented product to build up a sufficient texture (Kizzie-Hayford et al., 2016). Thus, addition of milk proteins is necessary for enhancing texture and sensory properties of the fermented product, and may help to improve the protein supply of consumers.

For marketing purposes, additional knowledge on the storage properties of fermented TNM is essential to monitor and predict product quality. Depending on product composition, changes in the physico-chemical attributes of the fermented system might occur during storage because of microbial imbalances, post acidification and syneresis (MacBean, 2009). Exemplarily, syneresis might have profound effects on the storage quality of fermented systems as even plain TNM exhibits a limited colloidal stability (Kizzie-Hayford, Jaros, Schneider, & Rohm, 2015). This contributes to appearance and texture defects of the fermented system, and impacts consumer acceptance (Walstra, Geurts, & Wouters, 2006). Microbial imbalances and post acidification contribute to textural defects, promote wheying-off and might cause excessive sourness of yoghurt (Yildiz, 2010).

For stirred yoghurt, an increase in viscosity and a reduction of syneresis during storage were observed after pre-treatment of the base cow milk with microbial transglutaminase (mTGase, EC 2.3.2.13; Jaros, Heidig, & Rohm, 2007). This enzyme is mainly

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produced by *Streptomyces mobaraensis*, and commercially available for the food industry. It cross-links proteins through the formation of isopeptide bonds between protein-bound lysine and glutamine residues, which improves the texture of acid protein gels made thereof (Jaros, Partschefeld, Henle, & Rohm, 2006; Rohm, Ullrich, Schmidt, Löbner, & Jaros, 2014). Pre-treatment of cow milk with mTGase was also reported to prolong fermentation time, increase gel strength and reduce post acidification in set-style yoghurt (Lorenzen, Neve, Mautner, & Schlimme, 2002; Ozer, Avni Kirmaci, Oztekin, Hayaloglu, & Atamer, 2007). In contrast, Romeih, Abdel-Hamid, and Awad (2014) showed that mTGase had no effect on the acidification rate of buffalo skim-milk. Instead, the simultaneous addition of mTGase and butter milk powder to buffalo skim-milk resulted in shorter fermentation time. Effects of mTGase treatment on microbial acidification might therefore depend on the type of fermentation substrate.

Currently, there is no evidence in the literature regarding the effect of mTGase cross-linked proteins on the microbiological properties of fermented tiger nut milk. Exploring the potential of mTGase cross-linked proteins for improving the physico-chemical properties of fermented TNM is novel, as it might lead to products with enhanced texture and improved storage properties (Kizzie-Hayford et al., 2016). Therefore, the present study investigates the effects of mTGase-modified proteins added to tiger nut milk on the microbiological and physico-chemical properties during fermentation and storage.

2. Materials and methods

2.1. Materials

Tiger nuts were supplied by farmers at Twifo Praso in the Central Region of Ghana, and were prepared by cleaning and drying, and subsequently stored as described previously (Kizzie-Hayford et al., 2015). Sodium caseinate was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), xanthan gum from Cargill France SAS (Saint-Germain-en-Laye, France) and whey protein isolate (<97 g/100 g protein) was supplied by Sports Supplements Ltd. (Colchester, UK). Microbial transglutaminase Activa MP from *Streptomyces mobaraensis* was supplied by Ajinomoto Foods Deutschland GmbH, Hamburg, Germany. The activity of the enzyme preparation, which was measured by using the Folk and Cole (1966) method, was 90 units per g.

2.2. Preparation of substrates

Tiger nut milk (TNM) was prepared by wet-milling of soaked and washed tiger nuts using a cutting mill and filter pressing of the mush (Kizzie-Hayford et al., 2016). Concentrated TNM (~30 g/100 g total solids), that was obtained after mush separation and evaporation in an R-124 rotational evaporator coupled to a B-172 vacuum controller (BÜCHI Labortechnik AG, Flawil, Switzerland) at 70 °C, was diluted to 10 g/100 g total solids and used as the reference fermentation substrate.

Dispersions of sodium caseinate (8 g/100 g), whey protein isolate (8 g/100 g) and xanthan gum (1 g/100 g) was separately prepared by dispensing the necessary amount in aqua demin. and mixing with a magnetic stirrer at 25 °C for at least 2 h. When applicable, protein solutions were heated for protein denaturation in a water bath at 80 °C for 10 min, cooled to room temperature and divided into two parts. One part was treated with mTGase according to Jaros et al. (2014a,2014b): after thermal equilibration of the protein solution and addition of 3 U mTGase per g milk protein, incubation was carried out in a water bath at 40 °C for 2 h. Subsequently, the mixture was heated to 80 °C for 10 min for enzyme inactivation, and immediately cooled in ice water. To prevent effects due to this heat treatment, the protein solution without enzyme treatment was also subjected to all heating and cooling steps. Subsequently, substrates for the fermentation of protein-enriched TNM systems were prepared by mixing TNM with xanthan gum to result in 10.0 g tiger nut solids, 0.1 g xanthan and 3.0 g sodium caseinate or 3.0 g whey protein isolate without mTGase treatment (CnX, WPX) or with mTGase treatment (CnXe, WPXe) per 100 g substrate.

2.3. Fermentation of tiger nut milk substrates

After enrichment, TNM was pasteurized at 70 °C for 15 min in 500 mL plastic jars under continuous agitation, cooled and inoculated with 0.01 g/100 g FVV-211 yogurt starter, a mixed culture of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* (DSM Food Specialties, Delft, Netherlands), and fermented by placing samples in a water bath at 38 °C for 16.5 h. During fermentation, pH was continuously monitored using an InoLab 730 pH meter (WTW GmbH, Weilheim, Germany), and lag time λ (h) and maximum rate of pH reduction μ (1/h) were estimated from pH/time plots using the Gompertz model as described previously (Kizzie-Hayford et al., 2016). After acidification, semi-solid TNM gels were homogenized at 11,000 rpm for 20 s using a T25 ultra turrax (IKA GmbH & CO. KG, Staufen, Germany) to ensure smooth texture products. Samples were filled into 120 mL sterile plastic jars and firmly sealed with lids for 24 h. Subsequently, TNM products were analyzed after 0, 5, 10 and 15 d of storage at 6 °C. Fermentation of TNM products was performed in triplicate.

2.4. Analysis of protein cross-linking and of the fermented tiger nut milk products

2.4.1. Size exclusion chromatography of enzymatically cross-linked proteins

To assess the extent of mTGase cross-linking, protein analysis was performed by size exclusion chromatography (AZURA Assistant ASM 2.1L, Knauer Wissenschaftliche Gerate GmbH, Berlin, Germany) with a UVD 2.1S detector at 280 nm (Knauer Wissenschaftliche Gerate GmbH, Berlin, Germany). The elution buffer, composed of 1 g/L CHAPS, 6 mol/L Urea, 0.1 mol/L NaCl, and 0.1 mol/L Na_2HPO_4, was adjusted to pH 6.8. For dissociating protein aggregates and reducing disulphide bonds, protein solutions were diluted with elution buffer and treated with dithiothreitol of a concentration of 0.15 g/L. Samples were separated and detected by 0.5 mL/min isocratic elution using a Superdex 200 increase 10/30 column (GE Healthcare, Uppsala, Sweden) at ambient temperature. Chromatographic data was acquired using the ClarityChrom v.3.07 software (Knauer Wissenschaftliche Gerate Gmbh) and corresponding peak areas (A) were analyzed for the fractions of monomers, dimers and polymers. Degree of polymerisation (DP, %) was calculated according to Bönisch, Lauber, and Kulozik (2004) by DP = 100 Σ (Area[dimers + trimers + polymers])/Σ (Area [monomers + dimers + trimers + polymers]).

2.4.2. Viable counts

Viable counts of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in the fermented products were determined by pour plating of the samples diluted in peptone water using MRS or M-17 media, respectively (IDF, 2003). Determinations were done in triplicate.

2.4.3. pH and titratable acidity

pH of the fermented products was measured at 20 ± 1 °C. Titratable acidity was determined according to a previously described procedure (Kizzie-Hayford et al., 2016). The average titre