LWT - Food Science and Technology 74 (2016) 319–324

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

# Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk

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ARTICLE INFO

*Article history:*
Received 9 March 2016
Received in revised form
14 June 2016
Accepted 29 July 2016
Available online 30 July 2016

*Keywords:*
Tiger nut milk
Fermentation
Phase separation
Proteins
Sensory

ABSTRACT

This study aims to explore and improve the quality of fermented tiger nut milk by investigating effects of enrichment with tiger nut proteins or dairy proteins mixed with xanthan gum on the microbiological, physicochemical and sensory properties. Enrichment with tiger nut protein decreased pH of the base system, increased microbial lag time and reduced acidification rate. Dairy proteins marginally increased the viable counts of starter culture and significantly increased acidification rate. Tiger nut milk enriched with tiger nut protein remained liquid after fermentation, whilst dairy protein enrichment caused formation of semi-solid, yogurt-like gels. Fermented gel systems containing sodium caseinate showed higher gel stiffness and lower whey drainage than gels with whey protein enrichment. However, fortification with whey proteins resulted in stirred products of higher viscosity. Frequent sensory descriptors for fermented tiger nut milk were *sweet*, *watery*, *brown*, *almond*, *phase separation*, *woody* and *nutty*. Fortification with dairy proteins resulted in sensory attributes that may be pivotal for improving the properties of fermented tiger nut milk.



## 1. Introduction

Tiger nut (*Cyperus esculentus* L.) is a sweet almond-like tuber that has already been recognised as a high potential, alternative source of food nutrients (Sánchez-Zapata, Fernández-López, & Angel Pérez-Alvarez, 2012). Particular interest in a fermented extract of tiger nuts, usually addressed as tiger nut milk (TNM), has emerged because of its sensory, nutritional and probiotic prospects, but also because fermentation might be appropriate to generate microbially stable products with extended shelf life. For such applications, tiger nuts are pre-soaked to soften the fibrous tissues, washed, wet-milled, pressed to obtain the milk-like extract, pasteurised and fermented with lactic acid bacteria into a sweet-sour drink (Akoma, Elekwa, Afodunrinbi, & Onyeukwu, 2000; Wakil, Ayenuro, & Oyinola, 2014).

Fermented TNM might be of at least local relevance in some countries considering that worldwide about 75% of adults experience a decreased lactase activity, and lactose intolerance prevalence rate is ~5% in Northern Europe, ~70% in Sicily and ~90% in Asian and African countries (Vesa, Marteau, & Korpela, 2000). To exploit the potential of TNM for producing fermented drinks, scientific reports were mainly dedicated to investigating its physicochemical composition, and its sensory and microbiological characteristics (Akoma et al., 2000; Belewu, Bamidele, & Belewu, 2010; Sanful, 2009; Wakil et al., 2014). Presently, as regards the physical characteristics of fermented TNM, literature information is scarce although they show a direct impact on consumer acceptability (Walstra, Geurts, & Wouters, 2006). Exemplarily, phase separation might be a factor that accounts for low sensory scores in appearance and textural attributes of fermented TNM (Akoma et al., 2000; Sanful, 2009; Wakil et al., 2014).

We have previously observed that TNM has only a limited colloidal stability which might cause phase separation in fermented systems (Kizzie-Hayford, Jaros, Schneider, & Rohm, 2015a). Therefore, we investigated the effect of adding sodium caseinate or soy protein isolate together with polysaccharides (carboxymethyl cellulose, xanthan gum or guar gum) on the stability of plain TNM. We found that, after adding 0.1 g/100 g xanthan gum with 1 or 3 g/100 g sodium caseinate to tiger nut milk, phase separation was considerably reduced (unpublished). It is well-known that physical and rheological properties of dairy yogurt can be improved by base milk enrichment with dairy proteins (Jaros & Rohm, 2003; Walstra, Wouters, & Geurts, 2006). Thus, adding proteins and polysaccharides to TNM might be beneficial for improving the physical

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http://dx.doi.org/10.1016/j.lwt.2016.07.067
0023-6438/

properties of the fermented TNM, but also contribute to protein based nutritional energy. Our previous report suggests that the globular tiger nut protein might show emulsifying effects, which could additionally contribute to the reduction of phase separation in TNM (Kizzie-Hayford, Jaros, Schneider, & Rohm, 2015b).

Therefore, the aim of this study is to explore effects of enriching TNM with tiger nut protein, xanthan gum and whey protein or sodium caseinate on the viability of microbial cultures, on acidification dynamics, and on physicochemical characteristics and sensory properties.

## 2. Materials and methods

### 2.1. Materials

A batch of tiger nuts that was provided by farmers of Twifo Praso in the Central Region of Ghana was prepared for the experiments by cleaning and drying (Kizzie-Hayford et al., 2015a). Whey protein isolate (<97 g/100 g protein) was obtained from Sports Supplements Ltd. (Colchester, UK), sodium caseinate from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and xanthan gum from Cargill France SAS (Saint-Germain-en-Laye, France). All reagents were of analytical grade.

### 2.2. Preparation and fermentation of plain and enriched tiger nut milk

Tiger nut milk was prepared according to Kizzie-Hayford et al. (2015a) with modifications. After soaking in 1 g/100 mL citric acid for 24 h, tiger nuts were washed three times in aqua demin. and wet comminuted using a Kult pro mixer (WMF AG, Geislingen, Germany) for 3 min. TNM obtained after mush separation was concentrated in an R-124 rotational evaporator connected to a B-172 vacuum controller (BÜCHI Labortechnik AG, Flawil, Switzerland) at 70 °C for approx. 1 h. This procedure resulted in a TNM concentrate of approx. 30 g/100 g. Tiger nut protein (TNP) was isolated from TNM as described previously (Kizzie-Hayford et al., 2015b).

TNM concentrate diluted with aqua demin. to 10 g/100 g total solids served as reference fermentation substrate. For obtaining protein-enriched substrates, the concentrate was diluted with TNP dissolved in aqua demin. to ensure an additional 1 g/100 g (1TNP) or 2 g/100 g (2TNP) in the system. To investigate the impact of protein denaturation through heat treatment, TNP solutions were heated to 85 °C for 10 min in a water bath prior to mixing with plain TNM. In a second test set-up, xanthan and sodium caseinate, or xanthan and whey protein isolate were dispersed in aqua demin. and then mixed with TNM to obtain systems with 10 g tiger nut solids, 0.1 g xanthan and 1 or 3 g sodium caseinate (1CnX or 3 CnX) per 100 g substrate, or systems with 10 g tiger nut solids, 0.1 g xanthan and 1 or 3 g whey protein isolate (1WPX or 3 WPX) per 100 g.

Fermentation of plain and enriched TNM was carried out in glass bottles after heat treatment at 70 °C for 15 min under continuous agitation. After inoculation with 0.01 g/100 g FVV-211 yogurt starter, a mixed culture of *Lactobacillus delbrueckii* ssp. *bulgariucs* and *S. thermophilus* (DSM Food Specialities, Delft, Netherlands), the systems were acidified at 38 °C for 16.5 h. pH during fermentation was continuously monitored using an InoLab 730 pH meter (WTW GmbH, Weilheim, Germany). From the pH/time plots, lag time λ (h) and maximum pH reduction rate μ (1/h) were estimated using a graphical method based on the modified Gompertz equation for bacterial growth (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007)

$$pH = pH_0 + (pH_\infty - pH_0)exp\left\{ - exp\left[\mu e/(pH_\infty - pH_0)(\lambda - t) + 1\right]\right\} \quad (1)$$

where $pH_0$ is initial pH, $pH_\infty$ is final pH, μ is the maximum pH reduction rate, and λ is the lag time.

Gel formation during fermentation was investigated using an ARES RFS3 rheometer (TA Instruments GmbH, Eschborn, Germany) with a concentric cylinder geometry (inner diameter, 32 mm; outer diameter, 34 mm; height, 33.5 mm); temperature was maintained at 38 °C by a circulator. Approx. 11.2 mL inoculated TNM substrate was transferred into the cup. The inner cylinder was lowered into measuring position, the surface was covered with low viscosity silicone oil to prevent evaporation, and a time sweep was started by applying a strain of $\gamma = 0.003$ and an angular frequency of $\omega = 1$ rad/s (Jacob, Nöbel, Jaros, & Rohm, 2011). The dynamic moduli were recorded during acidification.

Fermentation with each substrate, and the corresponding acidification and gel formation measurements were carried out in triplicate. After fermentation, the samples were cooled to 6 °C and stored until analysis.

### 2.3. Analysis of the fermented tiger nut milk products

#### 2.3.1. Viable counts

Viable counts of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in fermented products were, after appropriate serial dilution, enumerated using MRS or M-17 media, respectively (IDF, 2003). Determinations were done in triplicate.

#### 2.3.2. Chemical analysis

TNM composition was analyzed as described previously (Kizzie-Hayford et al., 2015a). Total carbohydrate and fat content of TNM was determined according to Albalasmeh, Berhe, and Ghezzehei (2013) and IDF (2008), respectively.

Sugars were determined by HPLC combined with refractive index detection. After Carrez clarification and 0.45 μm filtration, separation was achieved by 0.5 mL/min isocratic elution using a 300 × 7.8 mm Rezex™ RPM-Monosaccharide Pb+2 (8%) monosaccharide analysis column (Phenomenex Ltd., Aschaffenburg, Germany). Temperature of the reference cell was maintained at 8 °C, and sucrose, glucose and fructose were detected by light scattering. Each determination was carried out in triplicate.

Titratable acidity (TA) was determined by diluting 10.0 g fermented TNM to 40 g using aqua demin. and subsequent titration with 0.1 mol/L NaOH against phenolphthalein. The volume of NaOH required to neutralise the analyte was recorded, and the lactic acid equivalent was calculated according to Sadler & Murphy (2014).

#### 2.3.3. Physical analysis

Phase separation as an indicator for stability under gravity was visually measured by relating the height of a clear lower phase to the total height of fermented TNM samples in small glass vessels. Separation time at incubation temperature (38 °C) was 16.5 h.

The susceptibility of fermented TNM to syneresis under accelerated gravity was determined as described by Jaros, Heidig, and Rohm (2007). 30.0 g TNM was incubated in pre-weighed sterile falcon tubes. After fermentation, samples were stored at 6 °C for 24 h, and then centrifuged at 600 g and 6 °C for 10 min. The separated liquid was removed using a Pasteur pipette, and is expressed in relation to the initial mass. Apparent viscosity of