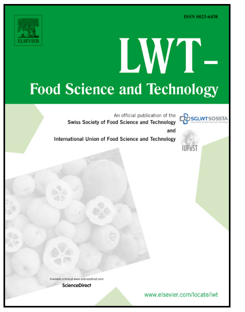
Accepted Manuscript



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

Nazir Kizzie-Hayford, Doris Jaros, Susann Zahn, Harald Rohm

PII: S0023-6438(16)30478-9

DOI: 10.1016/j.lwt.2016.07.067

Reference: YFSTL 5641

To appear in: *LWT - Food Science and Technology*

Received Date: 9 March 2016

Revised Date: 14 June 2016

Accepted Date: 29 July 2016

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

Nazir Kizzie-Hayford, Doris Jaros, Susann Zahn, Harald Rohm

Chair of Food Engineering, Technische Universität Dresden, 01069 Dresden, Germany

Correspondence:

Nazir Kizzie-Hayford

Chair of Food Engineering

Technische Universität Dresden

01069 Dresden

Germany

Fax: +49 351 463 37761

Mail: nazir.kizzie-hayford@tu-dresden.de

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.

Keywords: Tiger nut milk, fermentation, phase separation, proteins, sensory.

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 et al., 2006). Thus, adding proteins and polysaccharides to TNM might be beneficial for improving the physical 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 *L. 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)

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

where pH_0 is initial pH, pH_∞ 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, & 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 x 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 (2010).

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, & 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 fermented TNM was measured using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria). As mixing of fermented samples prior to measurement resulted in lumpy products, an ultra turrax mixer was used to homogenize the samples (11,000 rpm, 40 s). After 24 h at 6 °C, samples were transferred into the rheometer's cylinder geometry (inner diameter, 24.66 mm; outer diameter, 26.66 mm; height, 40 mm) and equilibrated to 20 ° C for 5 min before applying a shear rate sweep from 0.01/s to 100/s. For each fermentation experiment, syneresis and viscosity measurements were carried out in triplicate.

When possible, gel firmness of fermented TNM was determined by penetration. 20 mL aliquots were fermented in screw-top glass vessels (inner diameter, 38 mm). After keeping the samples at 6 °C for 24 h, the gels were penetrated by a cylindrical plunger (diameter, 15 mm; height, 10 mm) mounted on an RSA 3 solids analyzer (TA Instruments GmbH, Alzenau, Germany) at 0.5 mm/s. The initial slope of the force/distance curves was taken as an indicator for gel firmness (Jaros, Pätzold, Schwarzenbolz, & Rohm, 2006). Measurements were carried out in quadruplicate.

2.4. Sensory analysis

Flash profiling of fermented TNM was conducted using the procedure of Delarue & Sieffermann (2004) as modified by Thamke, Dürrschmid, & Rohm, (2009). A 13 member panel (4 male, 9 female; average age, 31 yrs.) was recruited for the study.

Samples subjected to analysis were the fermented plain TNM, and the four fermented products with caseinate or whey protein. To assess the discriminatory quality of the test, a duplicate of sample 1WPX was included. Samples were encoded with random 3-digit codes and simultaneously served in counterbalanced order in 20 mL transparent cups. The experiments were performed in a standard sensory laboratory. Raw data on the attributes and the corresponding intensities were analyzed by principal component and generalized procrustes analyses using the Senstools.Net software (OP&P Product Research BV, Utrecht, Netherlands). Results are based on duplicate experiments.

2.5. Statistics

Results of analytical experiments were evaluated using one-way analysis of variance. Tukey HSD or Games-Howell post hoc analysis was further used to compare the mean values when necessary. All significant values refer to $P < 0.05$. SPSS software package version 16.0 was used for performing the analysis (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Acidification and gel formation during fermentation

Fig. 1 shows that acidification during fermentation of plain or enriched TNM follows profiles that are similar to milk fermentation (Soukoulis et al., 2007). The addition of tiger nut protein reduced pH of the plain TNM (6.23) by approx. 0.25 units, probably because tiger nut proteins contain more acidic amino acids than basic amino acids (Aremo, Bamidele, Agere, Ibrahim, & Aremu, 2015). Conversely, sodium caseinate addition increased initial pH to 6.40. The Gompertz lag time parameter λ, was, on average, 1.6 h for plain TNM and systems enriched with dairy proteins but significantly higher (λ = 3.2 h) when tiger nut protein was used as enrichment medium. A similar effect of an initially reduced pH on lactic acid bacteria lag time in yogurt fermentation was observed by, e.g., Öztürk & Öner (1999). Maximum pH decay rate was 0.43/h – 0.45/h for plain TNM and TNM enriched with whey protein isolate, which is lower than μ ~ 0.65/h – 0.70/h reported for cow milk yogurt (De Brabandere & De Baerdemaeker, 1999). The addition of tiger nut protein significantly reduced μ to 0.25/h, whereas TNM with sodium caseinate came close to dairy systems (μ ~ 0.55/h). The reduced acidification rate can be partly attributed to the type of fermentable sugars. Apart from fructose traces, approx. 6 g/100 g sucrose serves as carbohydrate source (Table 1), and *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* showed a delayed growth on these sugars (Amoroso, Manca de Nadra, & Oliver, 1989). The results suggest that the protein source is relevant for the time to reach a particular pH in tiger nut milk fermentation. After approx. 15 h incubation, a pH of 4.4, which is comparable to that of dairy yogurt, was achieved for all systems.

Whereas the systems with additional tiger nut protein remained liquid during fermentation, it was possible to monitor gel formation by small amplitude oscillatory shear when dairy proteins were used for enrichment (Fig. 2). For both proteins, the higher concentration resulted in a pH 0.2 – 0.3 units higher at gelation onset (the point when a storage modulus (G') of 1 Pa was achieved; Jacob et al., 2011) and, as a consequence of almost identical acidification profiles, in a shorter time until gelation onset. pH at gelation onset of TNM enriched with dairy proteins was 0.04 – 0.52 units lower than the range reported for cow milk (Lee & Lucey, 2006). It is also evident from Fig. 2 that the amount of added protein significantly affected gel stiffness, and that stiffer gels were obtained when sodium caseinate was used for enrichment. A storage modulus of approx. 300 Pa is comparable to that of set yogurt from skimmed cow milk with 12 g/100 g dry matter (Jaros, Rohm, Haque, Bonaparte, & Kneifel, 2002). Whey proteins form gels mainly through disulphide interactions whilst casein forms gels through hydrophobic, van der Waals, hydrogen bonding and electrostatic interactions (Alting, Hamer, de Kruif, & Visschers, 2000; Dalgleish, 1997). Differences in rearrangement mechanisms during gelation might account for the variations in gel stiffness in supplemented fermented TNM. However, stiffness of acid gels from TNM enriched with dairy proteins was lower than that of casein or whey protein gels at comparable concentration (Alting et al., 2004; Lucey, van Vliet, Grolle, Geurts, & Walstra, 1997). Reasons for this might include effects of substrate composition, the rate of acidification and fermentation temperature (Nguyen et al., 2014; Lucey et al., 1997). The broad variation of gel stiffness achieved by varying type and content of dairy proteins may be advantageous for tailoring product texture.

3.2. Microbiological, chemical and physical properties of fermented tiger nut milk

The substrate had only a minor influence on lactic acid bacteria development. In all samples, viable counts of *S. thermophilus* ranged from 2.8 – 5.6 x 10^8 cfu/g and were higher than that of *L. delbrueckii* ssp. *bulgaricus* (2.4 – 5.0 x 10^6 cfu/g). These viable counts were higher than reported by Wakil et al. (2014) who, however, worked with isolates from wild TNM fermentations as starter cultures. Compared to the plain fermented TNM and TNM enriched with tiger nut protein, viable counts for both *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were higher when sodium caseinate or whey proteins were present in the fermentation substrate.

Related to dry matter, the base tiger nuts contained 5.32 ± 0.11 g/100 g protein, 20.72 ± 0.56 g/100 g fat, 1.85 ± 0.01 g/100 g ash, 20.49 ± 0.21 g/100 g total fiber, and 51.62 ± 0.42 g/100 g carbohydrates. Protein, ash and carbohydrate content were slightly higher than that from our previous report (Kizzie-Hayford et. al., 2015b), probably because of the different harvest period. Total solids content of plain TNM used as fermentation substrate was 10.40 g/100 g, which comprised of 1.02 ± 0.01 g/100 g protein, 2.23 ± 0.02 g/100 g fat, 0.28 ± 0.01 g/100 g ash, and 6.87 ± 0.05 g/100 g total carbohydrate. Table 1 shows that the main fermentable carbohydrate is sucrose. The difference between total carbohydrate and sugar content originates from starch and fiber. Generally, the sugar content in tiger nuts depends on the cultivar and the ripening stage (Coşkuner, Ercan, Karababa, & Nazlican, 2002). Compared to plain TNM, the fermented products showed higher fructose contents, presumably because of microbial sucrose hydrolysis and the further utilization by yogurt microorganisms, which prefer glucose to fructose for growth (Amoroso et al., 1989). The content of lactic acid was higher than what was reported by Akoma et al. (2000). Although pH at the end of fermentation was similar, there were significant differences in the lactic acid content of the fermented TNM, with the highest values in the substrates with the highest protein content. It is evident that the buffering capacity especially of the milk proteins is responsible for these differences. Increased protein content in fermented TNM might enhance the production of microbial by-products such as fatty acids and amino acids as flavor-active compounds (Sadler & Murphy, 2010).

During fermentation, TNM separated into a transparent lower liquid part and an opaque upper part (Fig. 3). Phase separation in TNM systems might result from differences in solute properties such as particle size, molecular shape and charge (de Jong, Klok, & van de Velde, 2009; Kizzie-Hayford et al., 2015a). Thin protein membranes that coat fat droplets enhance stability of emulsions by lowering interfacial energy and surface tension; during fermentation, phase separation might increase in low protein emulsions because of the acidification, which distorts the protein film and facilitates oil droplet contact, and which causes flocculation (Kinsella, Damodoran, & German, 1985). Addition of tiger nut protein to TNM before fermentation probably reduced surface tension by increasing the thickness of the interfacial protein membrane, thereby, enhancing the stability of the emulsion (Sun & Gunasekaran, 2009). Protein denaturation by thermal treatment was not appropriate to inhibit phase separation. Inclusion of CnX and WPX, however, ensured product stability during fermentation. Firstly, whey proteins and sodium caseinate exhibit emulsifying properties (Amine, Dreher, Helgason, & Tadros, 2014) and probably improved the emulsion stability of the plain TNM. Secondly, the milk proteins with their gel forming capacity allowed structures which entrap the liquid phase in a three-dimensional network, arresting further phase separation (de Jong et al., 2009).

Fig. 4 shows that, in all systems, fermented TNM exhibited shear thinning behavior. At shear rate of 1.0/s, mixtures containing TNP showed a lower viscosity than plain fermented TNM, probably caused by surface tension reduction through coating of TNM lipid droplets with proteins. The increase in viscosity observed in 3CnX or 3WPX might originate from the protein gels or soluble protein aggregates from the thermal denaturation of whey proteins. A similar increase in yogurt viscosity on addition of sodium caseinate or whey protein concentrate was observed by Akalın, Unal, Dinkci, & Hayaloglu (2012). The viscosity difference at higher shear rates was pronounced, showing that WPX systems had a higher shear resistance and viscosity than CnX systems. Although homogenization, which was necessary to dissolve lumps, may have attenuated the effects of intact gels on fermented TNM viscosity, the mixing procedure improved their texture. The results depict that enriching TNM with dairy proteins before fermentation results in products of more viscous characteristics.

Table 2 shows that the firmness of gels from TNM enriched with dairy proteins was higher when enrichment increased, possibly because of a denser protein-protein network that was generated during gelation. Additionally, gel firmness was higher when sodium caseinate rather than whey protein was used for enrichment. This is comparable with observations from regular yogurt fortified with either sodium caseinate or whey proteins (Modler, Larmond, Lin, Froehlich, & Emmons, 1983; Rohm & Schmid, 1993). Evidently, susceptibility to forced syneresis, which also reflects the physical quality of protein gels (Rohm & Kovac, 1994), was more reduced the more protein was added to TNM. Again, enrichment with sodium caseinate resulted in a more pronounced effect. The inverse relationship between gel firmness and syneresis is similar to that in dairy yogurt as long as it is generated by differences in the protein content (Jaros et al., 2002) but differs from that when yogurt made by different starter cultures is compared (Rohm & Kovac, 1994).

3.3. Sensory properties of fermented tiger nut milk

Principal component analysis of the consensus matrix showed that dimension 1 and dimension 2 accounted for a fraction of 83.59% of the total variance, and dimension 3 for an additional 11.14%. In Fig. 5, the sample-related coordinates of the two identical samples that were presented to the panelists grouped together, which is a strong indicator for the discriminative reliability of the assessment (Diaz-Maroto, Vinas, & Cabezudo, 2003). Emerging descriptors out of the 34 different descriptors used for fermented TNM were *sweet*, *watery*, *brown*, *almond*, *phase separation*, *woody* and *nutty*. Those for 1WPX were *fruity*, *sweet* and *oil droplets on surface* whilst 3WPX were *fruity*, *banana*, *viscous*, *earthy*, *sour*, *adstringent*, *creamy*, *lightness* and *homogenous*. The most frequent descriptors for 1CnX were *musty*, *foamy*, *oil droplets* *on surface* and *nutty*, and for 3CnX were *graininess*, *viscous*, *balanced*, *sour*, *foamy* and *mushroom*. The results show that each product generated key sensory attributes that can be exploited when producing fermented tiger nut milk.

4. Conclusions

Enrichment of TNM with tiger nut proteins or dairy proteins with xanthan gum improved the properties of fermented TNM. Particularly, enrichment with dairy proteins caused a slight increase in the viable counts and increased the acidification rate of fermented TNM. All proteins significantly increased lactic acid content of the fermented system. Fermented TNM showed semi-solid, yogurt-like properties on enrichment of plain TNM with dairy proteins. Inclusion of all the types of proteins significantly reduced phase separation in the fermented product. Fermented systems fortified with sodium caseinate showed higher gel stiffness and lower whey drainage than gels from whey proteins. However, the latter showed stirred products of higher viscosity. Important sensory descriptors for fermented TNM were *sweet*, *watery*, *brown*, *almond*, *phase separation*, *woody* and *nutty*. Inclusion of dairy proteins showed unique sensory attributes that may be relevant for tailoring fermented TNM products. Further studies on the effects of the additives on shelf-life of fermented TNM might be relevant.

Acknowledgement

Nazir Kizzie-Hayford is supported via a joint scholarship by the Government of Ghana, Ministry of Education (GOG-MOE) and the German Academic Exchange Services (DAAD), Ref. No. 91548121. The authors thank Eva-Maria Kneschke for technical assistance with HPLC measurements.

5. References

Akalın, A. S., Unal, G., Dinkci, N., & Hayaloglu, A. A. (2012). Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *Journal of Dairy Science, 95*, 3617–3628.

Akoma, O., Elekwa, U. O., Afodunrinbi, A. T., & Onyeukwu, G. C. (2000). Yogurt from Coconut and Tigernuts. *Journal of Food Technology in Africa*, *5*, 132-134.

Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydrate Polymers*, *97*, 253–261.

Alting, A. C., Hamer, R. J., de Kruif, C. G., & Visschers, R. W. (2000). Formation of disulfide bonds in acid-induced gels of preheated whey protein isolate. *Journal of Agricultural and Food Chemistry*, *48*, 5001–5007.

Alting, A. C., Weijers, M., de Hoog, E. H. A., van de Pijpekamp, A. M., Cohen Stuart, M. A., Hamer, R. J., et al. (2004). Acid-induced cold gelation of globular proteins: effects of protein aggregate characteristics and disulfide bonding on rheological properties. *Journal of Agricultural and Food Chemistry*, *52*, 623–631.

Amine, C., Dreher, J., Helgason, T., & Tadros, T. (2014). Investigation of emulsifying properties and emulsion stability of plant and milk proteins using interfacial tension and interfacial elasticity. *Food Hydrocolloids*, *39*, 180–186.

Amoroso, M. J., Manca de Nadra, M. C., & Oliver, G. (1989). The growth and sugar utilization by *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus salivarius ssp. thermophilus* isolated from market yogurt. *Le Lait*, *69*, 519–528.

Aremo, M. ., Bamidele, T. ., Agere, H., Ibrahim, H., & Aremu, S. O. (2015). Proximate composition and amino acid profile of raw and cooked black variety of tiger nut (*Cyperus esculentus l.*) grown in Northeast Nigeria. *Journal of Biology, Agriculture and Healthcare, 5*, 213–221.

Belewu, M. A., Bamidele, R. A., & Belewu, K. . (2010). Cyper-coconut yoghurt: preparation, compositional and organoleptic qualities. *African Journal of Food Science and Technology*, *1*, 010–012.

Coşkuner, Y., Ercan, R., Karababa, E., & Nazlıcan, A. N. (2002). Physical and chemical properties of chufa (*Cyperus esculentus L*) tubers grown in the Çukurova region of Turkey. *Journal of the Science of Food and Agriculture*, *82*, 625–631.

Dalgleish, D. G. (1997). Structure-function relationships of caseins. In S. Damodaran & A. Paraf (Ed.), *Food proteins and their applications* (pp. 225–255). New York: Marcel Dekker.

De Brabandere, A. G., & De Baerdemaeker, J. G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering*, *41*, 221–227.

de Jong, S., Klok, H. J., & van de Velde, F. (2009). The mechanism behind microstructure formation in mixed whey protein–polysaccharide cold-set gels. *Food Hydrocolloids*, *23*, 755–764.

Delarue, J., & Sieffermann, J.-M. (2004). Sensory mapping using Flash profile. Comparison with a conventional descriptive method for the evaluation of the flavour of fruit dairy products. *Food Quality and Preference*, *15*, 383–392.

Diaz-Maroto, M. C., Vinas, M. A. G., & Cabezudo, M. D. (2003). Evaluation of the effect of drying on aroma of parsley by free choice profiling. *European Food Research and Technology*, 216, 227–232.

IDF (2003). Yogurt – Enumeration of characteristic microorganisms: Colony count technique at 37 degrees C. Standard 117. International Dairy Federation, Brussels.

IDF (2008). Skimmed milk, whey and buttermilk – Determination of fat content: Gravimetric method (reference method). Standard 22. International Dairy Federation, Brussels.

Jacob, M., Nöbel, S., Jaros, D., & Rohm, H. (2011). Physical properties of acid milk gels: Acidification rate significantly interacts with cross-linking and heat treatment of milk. *Food Hydrocolloids*, *25*, 928–934.

Jaros, D., Heidig, C., & Rohm, H. (2007). Enzymatic modification through microbial transglutaminase enhances the viscosity of stirred yogurt. *Journal of Texture Studies*, *38*, 179–198.

Jaros, D., Pätzold, J., Schwarzenbolz, U., & Rohm, H. (2006). Small and large deformation rheology of acid gels from transglutaminase treated milks. *Food Biophysics*, *1*, 124–132.

Jaros, D., & Rohm, H. (2003). Controlling the texture of fermented dairy products: the case of yoghurt. In G. Smit (Ed.), *Dairy processing: Improving quality* (pp. 155–184). Cambridge: Woodhead publishing.

Jaros., D., Rohm, H., Haque, A., Bonaparte, C. & Kneifel, W. (2002). Influence of the starter culture on the relationship between dry matter content and physical properties of set-style yogurt. Milchwissenschaft – Milk Science International 57, 325–328.

Kinsella, J.E., Damodoran, S.,& German, B. (1985); Physicochemical and functional properties of oil seed proteins with emphasis on soy proteins. In A.M. Altschul,

& H.L. Wilcke (Ed.), New protein foods (pp. 108-172) Washington DC: Academic Press Inc.

Kizzie-Hayford, N., Jaros, D., Schneider, Y., & Rohm, H. (2015a). Characteristics of tiger nut milk: effects of milling. *International Journal of Food Science & Technology*, *50*, 381–388.

Kizzie-Hayford, N., Jaros, D., Schneider, Y., & Rohm, H. (2015b). Physico-chemical properties of globular tiger nut proteins. *European Food Research and Technology*, *241*, 835–841.

Lee, W.-J., & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science*, *89*, 2374–2385.

Lucey, J. A., van Vliet, T., Grolle, K., Geurts, T., & Walstra, P. (1997). Properties of acid casein gels made by acidification with glucono-δ-lactone. 1. Rheological properties. *International Dairy Journal*, *7*, 381–388.

Modler, H.W., Larmond, M.E., Lin, C.S., Froehlich, D. & Emmons, D.B., (1983). Physical and sensory properties of yogurt stabilized with milk proteins. *Journal of Dairy Science*. 66, 422–429.

Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2014). The effect of fermentation temperature on the microstructure, physicochemical and rheological properties of probiotic buffalo yoghurt. *Food and Bioprocess Technology*, *7*, 2538–2548.

Öztürk, B. A., & Öner, M. D. (1999). Production and evaluation of yogurt with concentrated grape juice. *Journal of Food Science*, *64*, 530–532.

Rohm, H., & Kovac, A. (1994). Effects of starter cultures on linear viscoelastic and physical properties of yogurt gels. *Journal of Texture Studies*, *25*, 311–329.

Rohm, H. & Schmid, W. (1993). Influence of dry matter fortification on flow properties of yogurt. 1. Evaluation of flow curves. Milchwissenschaft – Milk Science International 48, 556-560.

Sadler, G. D., & Murphy, P. A. (2014). pH and titrable acidity. In Nielsen, S. S. (Ed.), *Food Analysis* (pp. 219–238). New York: Springer.

Sánchez-Zapata, E., Fernández-López, J., & Angel Pérez-Alvarez, J. (2012). Tiger nut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties, and food applications. *Comprehensive Reviews in Food Science and Food Safety*, *11*, 366–377.

Sanful, R. E. (2009). The use of tiger nut (*Cyperus esculentus*), cow milk and their composite as substrates for yogurt production. *Pakistan Journal of Nutrition*, *8*, 755-758.

Soukoulis, C., Panagiotidis, P., Koureli, R., & Tzia, C. (2007). Industrial Yogurt Manufacture: monitoring of fermentation process and improvement of final product quality. *Journal of Dairy Science*, *90*, 2641–2654.

Sun, C., & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids*, *23*, 165–174.

Thamke, I., Dürrschmid, K., & Rohm, H. (2009). Sensory description of dark chocolates by consumers. *LWT - Food Science and Technology*, *42*, 534–539.

Vesa, T. H., Marteau, P., & Korpela, R. (2000). Lactose intolerance. *Journal of the American College of Nutrition*, *19*, 165S–175S.

Wakil, S. M., Ayenuro, O. T., & Oyinola, K. A. (2014). Microbiological and nutritional assessment of starter-developed fermented tigernut milk. *Food and Nutrition Sciences*, *50*, 495.

Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). *Dairy science and technology*. Boca Raton, FL: CRC press.

Table 1

Effect of enrichment on viable counts and composition of fermented tiger nut milk.

System[a]	Viable counts (x10^6 cfu/g)[b]		Sugars (g/100 g)		Lactic acid (g/100 g)
	Streptococci	Lactobacilli	Sucrose	Fructose	
TNM	-	-	$6.01^{a} \pm 0.02$	$0.07^{a} \pm 0.01$	-
Fermented TNM	$340.0^{a} \pm 34.0$	$2.40^{a} \pm 0.47$	$5.58^{bd} \pm 0.10$	$0.14^{b} \pm 0.01$	$0.54^{a} \pm 0.00$
1TNP	$280.0^{a} \pm 52.0$	$2.50^{a} \pm 0.36$	$5.92^{b} \pm 0.02$	$0.18^{c} \pm 0.02$	$0.69^{b} \pm 0.00$
2TNP	$358.0^{a} \pm 26.0$	$2.95^{a} \pm 0.19$	$5.70^{c} \pm 0.06$	$0.15^{b} \pm 0.01$	$0.73^{c} \pm 0.01$
1CnX	$380.0^{a} \pm 49.0$	$3.25^{b} \pm 0.21$	$5.49^{d} \pm 0.01$	$0.13^{b} \pm 0.01$	$0.76^{d} \pm 0.00$
3CnX	$553.0^{c} \pm 36.0$	$5.08^{d} \pm 0.44$	$5.31^{e} \pm 0.06$	$0.13^{b} \pm 0.00$	$1.08^{f} \pm 0.00$
1WPX	$460.0^{b} \pm 47.0$	$3.78^{c} \pm 0.31$	$5.28^{e} \pm 0.63$	$0.14^{b} \pm 0.02$	$0.70^{b} \pm 0.01$
3WPX	$498.0^{bc} \pm 41.0$	$4.88^{cd} \pm 0.82$	$4.87^{f} \pm 0.65$	$0.13^{b} \pm 0.02$	$0.88^{e} \pm 0.01$

[a] TNM, tiger nut milk; 1TNP (2TNP), TNM enriched with 1 (2) g/100 g tiger nut protein, 1CnX (3CnX), TNM enriched with 1 (3) g/100 g sodium caseinate and 0.1 g/100 g xanthan; 1WPX (3WPX), TNM enriched with 1 (3) g/100 g whey protein isolate and 0.1 g/100 g xanthan.
[b] Results are arithmetic mean ± standard deviation from (n=3) determinations. Values in the same column marked by a different superscript differ significantly at $P < 0.05$.

Table 2.

Effect of enrichment with dairy proteins on gel properties of fermented tiger nut milk.

Fermented system[a]	Gel firmness (N/mm)[b]	Whey drainage (%)[b]
1CnX	0.021 ± 0.004[a]	14.1 ± 0.97[a]
3CnX	0.115 ± 0.02[b]	1.9 ± 0.70[b]
1WPX	0.013 ± 0.002[c]	31.8 ±1.45[c]
3WPX	0.038 ± 0.008[d]	15.2 ± 1.24[a]

[a] 1CnX (3CnX), TNM enriched with 1 (3) g/100 g sodium caseinate and 0.1 g/100 g xanthan; 1WPX (3WPX), TNM enriched with 1 (3) g/100 g whey protein isolate and 0.1 g/100 g xanthan.
[b] Results are arithmetic mean ± standard deviation from (n=4; gel firmness) or (n=3; whey drainage) determinations. Values in the same column marked by a different superscript differ significantly at $P < 0.05$.

Figure captions

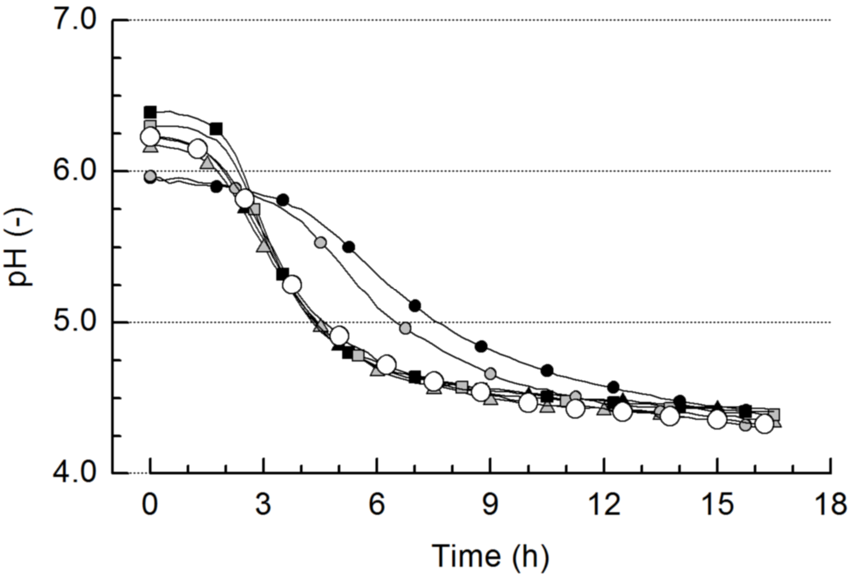
Fig. 1. Acidification profiles during fermentation of plain (TNM) or enriched tiger nut milk. ○, plain tiger nut milk; ○, ●, TNM enriched with 1 (2) g/100 g tiger nut protein; □, ■, TNM enriched with 1 (3) g/100 g sodium caseinate and 0.1 g/100 g xanthan; △, ▲, TNM enriched with 1 (3) g/100 g whey protein isolate and 0.1 g/100 g xanthan. pH measurement was continuous, only selected data points are displayed.

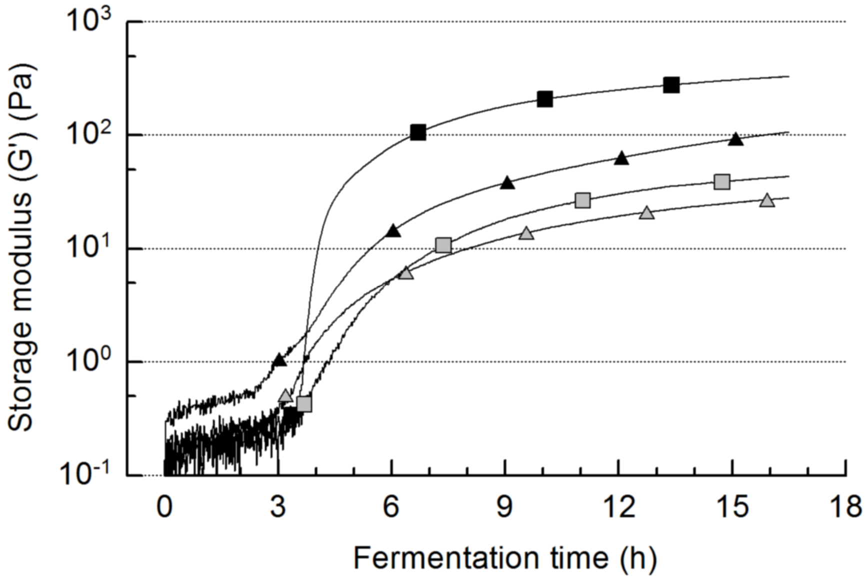
Fig. 2. Development of gel stiffness during acidification of enriched tiger nut milk. □, ■, TNM enriched with 1 (3) g/100 g sodium caseinate and 0.1 g/100 g xanthan; △, ▲, TNM enriched with 1 (3) g/100 g whey protein isolate and 0.1 g/100 g xanthan. Stiffness measurement was continuous, only selected data points are displayed. Each curve represents the arithmetic mean of triplicate measurements.

Fig. 3. Phase separation, expressed as percentage transparent lower layer, of fermented tiger nut milk (TNM) with different composition. 1TNP and 2TNP, tiger nut milk was enriched with 1 (2) g/100 g tiger nut protein. Black bars, tiger nut protein was heated to 85 °C for 10 min prior to fermentation. Samples "Milk protein" contained 1 g/100 g sodium caseinate or whey protein isolate.

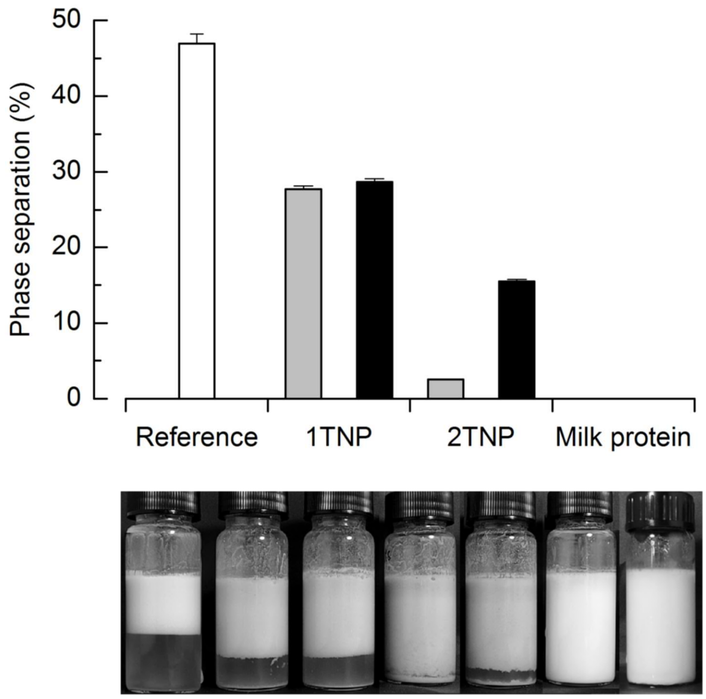
Fig. 4. Apparent viscosity of fermented tiger nut milk (TNM) with different composition. ○, plain tiger nut milk ; ●, TNM enriched with 2 g/100 g tiger nut protein ; ■, TNM enriched with 3 g/100 g sodium caseinate and 0.1 g/100 g xanthan; ▲, TNM enriched with 3 g/100 g whey protein isolate and 0.1 g/100 g xanthan. Each curve represents the arithmetic mean of triplicate measurements.

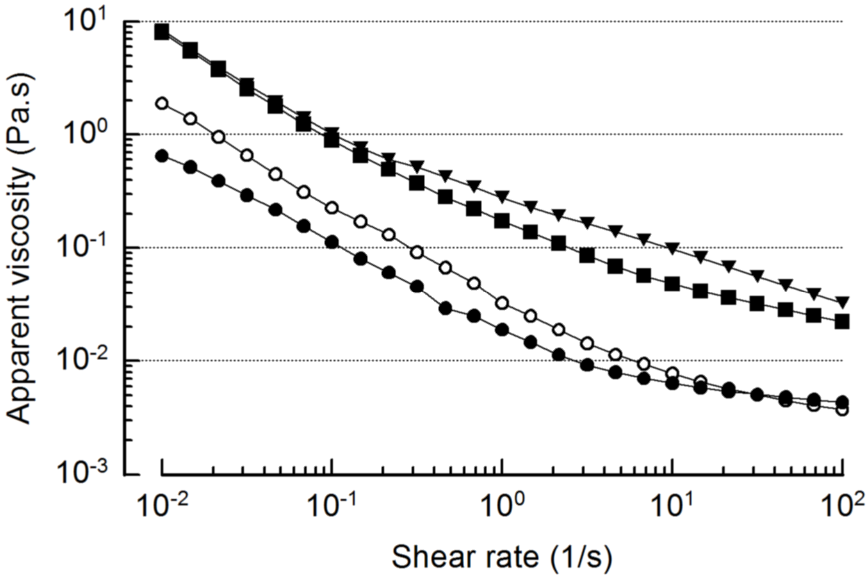
Fig. 5. GPA group average plots for descriptors of fermented tiger nut milk (TNM) with different compositions. In the consensus space are CnX, enrichment with sodium caseinate and 0.1 g/100 g xanthan; WPX, enrichment with whey protein isolate and 0.1 g/100 g xanthan. Numbers in the code refer to addition of sodium caseinate or whey protein isolate (g/100 g). Plots are based on duplicate experiments.


7.0
6.0
5.0
4.0
pH (-)
0
3
6
9
12
15
18
Time (h)

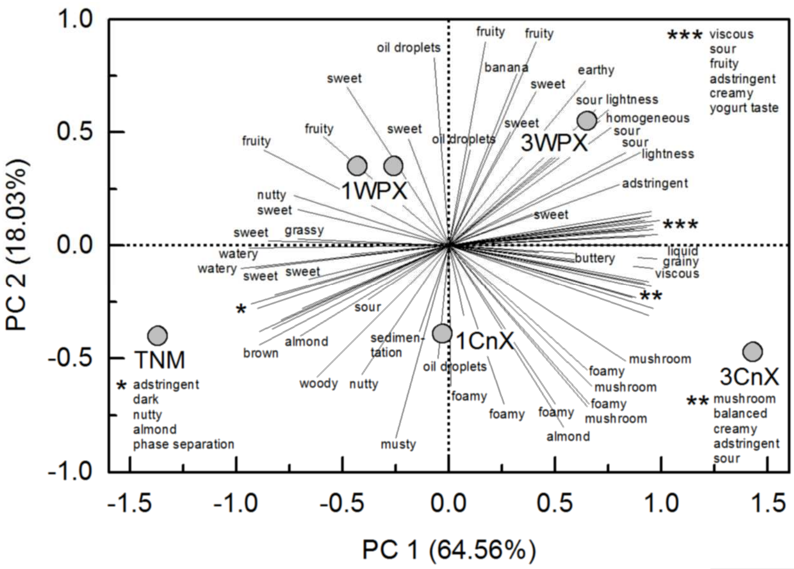


Storage modulus (G') (Pa)
10^3
10^2
10^1
10^0
10^-1
0
3
6
9
12
15
18
Fermentation time (h)


Phase separation (%)
50
40
30
20
10
0
Reference
1TNP
2TNP
Milk protein



Apparent viscosity (Pa.s)
10^1
10^0
10^-1
10^-2
10^-3
10^-2
10^-1
10^0
10^1
10^2
Shear rate (1/s)


PC 2 (18.03%)
PC 1 (64.56%)
1.0
0.5
0.0
-0.5
-1.0
-1.5
1.5
1WPX
3WPX
1CnX
3CnX
TNM
*** viscous
sour
fruity
adstringent
creamy
yogurt taste
** mushroom
balanced
creamy
adstringent
sour
* adstringent
dark
nutty
almond
phase separation
fruity
oil droplets
sweet
banana
earthy
sour lightness
homogeneous
lightness
adstringent
grassy
watery
buttery
liquid
grainy
viscous
brown
almond
sour
sedimen-
tation
woody
nutty
musty
foamy
mushroom

Highlights:

- Tiger nut milk shows pronounced phase separation during fermentation
- Protein enrichment of tiger nut milk inhibits phase separation in fermented systems
- Protein enrichment improves textural properties of the fermented system
- Protein enrichment leads to fermented systems with different sensory properties