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Development of strategies for the successful production of yogurt-like products from tiger nut (*Cyperus esculentus* L) milk

Dissertation

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Nazir Kizzie-Hayford

DEVELOPMENT OF STRATEGIES FOR THE SUCCESSFUL PRODUCTION OF YOGURT-LIKE PRODUCTS FROM TIGER NUT MILK

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Tiger nuts (*Cyperus esculentus* L) are recognized as a high potential, alternative source of food nutrients. However, there is limited scientific literature on the technological possibilities for developing value-added foods, such as fermented products from tiger nut milk. Therefore, strategies for producing and improving the properties of fermented tiger nut milk were investigated for generating lactose-free, nutritious yogurt-like products with acceptable sensory properties and a prolonged shelf life quality.

A wet-milling procedure was standardized for extracting tiger nut milk from tiger nuts, and the effects of the extraction process on nutrient distribution, colour properties and colloidal stability of the milk were analyzed. Next, tiger nut milk was enriched with proteins and/or hydrocolloids and the impact of the additives on the physical properties of the milk were determined. Enriched tiger nut milk was fermented by using classical yogurt cultures and the obtained products were analyzed for the microbiological, physico-chemical and sensory characteristics. Additionally, effects of enriching tiger nut milk with microbial transglutaminase cross-linked proteins on the microbiological and physico-chemical properties were evaluated.

Higher wet-milling intensity improved the nutrient composition, colloidal stability and colour of the milk. Enrichment of tiger nut milk with milk proteins and xanthan gum enhanced the viscosity and stability, and after fermentation, led to homogenous gel-like products with superior microbiological, physico-chemical and different sensory properties compared to the fermented plain tiger nut milk. Microbial transglutaminase cross-linked proteins improved the physical characteristics of the fermented product, especially during storage. This product would be relevant in many developing countries with high prevalence of lactose intolerance, limited access to nutritious food but show a high distribution of tiger nut vegetation.

ENTWICKLUNGSSTRATEGIEN FÜR DIE ERFOLGREICHE HERSTELLUNG VON JOGURTÄHNLICHEN PRODUKTEN AUS ERDMANDELMILCH

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Erdmandeln (*Cyperus esculentus* L) haben ein hohes Potential als alternative Quelle Lebensmittelinhaltsstoffen. Allerdings gibt es nur in begrenztem Ausmaß Literatur über technologische Möglichkeiten zur Entwicklung von Mehrwert-Lebensmitteln wie fermentierter Erdmandelmilch. Daher wurden Strategien zur Herstellung und Verbesserung der Eigenschaften von fermentierter Erdmandelmilch zur Erzeugung laktosefreier joghurtähnlicher Produkte mit akzeptablen sensorischen Eigenschaften untersucht.

Für die Extraktion der Erdmandelmilch wurde ein Nassmahlverfahren standardisiert und der Einfluss des Verfahrens auf die Nährstoffverteilung, die Farbeigenschaften und die kolloidale Stabilität der Milch analysiert. Als nächstes wurde Erdmandelmilch mit Proteinen und/oder Hydrokolloiden angereichert, und der Einfluss der Additive auf die physikalischen Eigenschaften des Extrakts bestimmt. Angereicherte Erdmandelmilch wurde mit klassischen Joghurtkulturen fermentiert, und die mikrobiologischen, physikalisch-chemischen und sensorischen Eigenschaften der Produkte wurden untersucht. Zusätzlich wurden Effekte der Anreicherung von Erdmandelmilch mit enzymatisch vernetzten Proteinen auf die mikrobiologischen und physikalisch-chemischen Eigenschaften bewertet. Eine höhere Nassmahlintensität verbesserte die Nährstoffzusammensetzung, die kolloidale Stabilität und die Farbe der Milch. Die Anreicherung erhöhte die Viskosität und Stabilität und führte nach der Fermentation zu homogenen gelartigen Produkten mit verbesserten mikrobiologischen, physikalisch-chemischen und sensorischen Eigenschaften im Vergleich zur fermentierten Erdmandelmilch. Mikrobielle Transglutaminase-vernetzte Proteine verbesserten die physikalischen Eigenschaften des fermentierten Produkts, insbesondere während der Lagerung. Dieses Produkt wäre in vielen Entwicklungsländern mit hoher Prävalenz von Laktoseintoleranz und begrenztem Zugang zu nahrhaften Lebensmitteln als Alternative von Interesse.

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Symbols and Abbreviations

Symbol/Abbreviation	Interpretation	Units
n_i	Number of particles	µm
τ	Shear stress	Pa
λ	Microbial lag time	s
ω	Angular frequency	rad/s
ΔH	Transition enthalpy	J/g
η_i	Flow index	-
η_o	Viscosity	Pa.s
τ_o	Yield stress	Pa
$\dot{\gamma}$	Shear rate	1/s
µ	Max rate of pH reduction	1/s
1TNP	Tiger nut milk mixed with 1 g/100 g tiger nut protein	-
2TNP	Tiger nut mixed with 2 g/100 g tiger nut protein	-
a*	Red-green intensity	-
Al	Albumin	-
b*	Yellow-blue intensity	-
C*	Chroma	-
Ci	Creaming index	-
CMC	Carboxymethyl cellulose	-
CnG	Tiger nut mixed with sodium caseinate and guar gum	-
CnX	Tiger nut milk mixed with sodium caseinate and xanthan gum	-
CnXe	Tiger nut milk mixed with microbial transglutaminase cross-linked sodium caseinate and xanthan gum	-
D_{10}	Lower decile of volumetric particle distribution	µm
$d_{4,3}$	Volume-weighed particle distribution	µm
D_{50}	Median value of volumetric particle distribution	µm
D_{90}	Upper decile of volumetric particle distribution	µm
d_i	Particle diameter	m
DM	Dry matter	-
DP	Degree of polymerization	%

Symbol/Abbreviation	Interpretation	Units
g	Acceleration due to gravity	m/s^2
γ	Interfacial tension	J/m^2
G_e	Elastic interaction free energy	J
Glo	Globulin	-
Glu	Glutamin	-
G_{mix}	Free energy of interaction	J
GPA	Generalized procrustees analyses	-
GRAS	Generally recognized as safe	-
G_s	Steric interaction free energy	J
h_{ab}	hue	-
H_c	Height of creamy layer	mm
H_e	Height of emulsion layer	mm
H_o	Height of oil layer	mm
Hs	Height of serum layer	mm
I_f	Final intensity of transmitted light	-
I_o	Initial intensity of transmitted light	-
K	Consistency of TNM	-
L*	Lightness	-
M_{DPR}	Mass of dry pressing residue	g/100 g
MRS	Medium according to De Man, Rogosa and Sharp Agar	-
M_{SDTN}	Mass of soaked dried tiger nut	g/100 g
M_{STN}	Mass of soaked tiger nuts	g
mTGase	Microbial transglutaminase	-
M_{TNM}	Mass of tiger nut milk	g
M_{WPR}	Mass of wet pressing residue	g/ 100g
NC_{SDTN}	Nutrient content of soaked and dried tiger nuts	g/100 g
NC_{TNM}	Nutrient content of tiger nut milk	g/100 g
NC_{WPR}	Nutrient content of wet pressing residue	g/100 g
pH_∞	Final pH	-
pH_0	Initial pH	-

Symbol/Abbreviation	Interpretation	Units
pI	Isoelectric point	-
PR	Pressing residue	-
Pro	Prolamin	-
R	Residual protein	-
r	Droplet radius	µm
R_m	Material recovery	-
RM_{DPR}	Relative mass of dry pressing residue	g/100 g
RM_{WPR}	Relative mass of wet pressing residue	g/100 g
Si	Serum index	-
TA	Titratable acidity	g/100 g
T_d	Denaturation temperature	°C
TNM.1	Tiger nut milk from 1 min milling	-
TNM.2	Tiger nut milk from 2 min milling	-
TNM.3	Tiger nut milk from 3 min milling plus ultra turrax dispersion	-
TNP	Tiger nut protein	-
T_o	Onset melting temperature	°C
V_r	Reduced Stokes sedimentation or creaming velocity	m/s
V_s	Stokes sedimentation or creaming velocity	m/s
WPX	Tiger nut mixed with whey protein isolate and xanthan gum	-
WPXe	Tiger nut mixed with microbial transglutaminase cross-linked whey protein and xanthan gum	-
W_{SDTN}	Wet content of pre-soaked, dried tiger nuts	g/g
W_{WPR}	Wet content of wet pressing residue	g/100 g
Y_{TNM}	Yield of tiger nut milk	g/100 g
S^{conf}	Entropy of dispersion	J/K
ρ	Density	g/cm^3
ϕ	Hydrodynamic volume	nm^3

1. Introduction and aim

Tiger nuts are sweet nut-like vegetable root tubers of the perennial grass-like cyperaceous plant called *Cyperus esculentus* L (Coskuner et al., 2002). The plant thrives in the tropical and Mediterranean regions, and is commonly known in Nigeria, Ghana, Togo, Ivory Coast and in Spain and Egypt where the root tubers are mainly used as a source of food nutrients (Omode et al., 1995; Pascual et al., 2000). Tiger nuts are rich in carbohydrate, lipids, fibre, some proteins, minerals, ascorbic acids and α-tocopherols (Ekeanyanwu and Ononogbu, 2010). To exploit their nutritional potential, tiger nuts have recently been experimented for enriching the fibre content in gluten-free bread and biscuits (Aguilar et al., 2015; Zahra and Ahmed, 2014). The process for producing milk-like aqueous extracts from tiger nuts, called tiger nut milk, (TNM) or composites with other vegetable milk extracts for beverage was explored by few authors (Belewu, 2007; Cortés et al., 2004; Sanful, 2009a; Ukwuru and Ogbodo, 2011). Lactic acid fermentation of TNM is of particular interest because of the prospects to generate lactose-free, yogurt-like products of improved microbial stability and extended shelf life with acceptable sensory properties. Such fermented systems might be promising as a valuable alternative source of food nutrients, especially in many developing countries where the population shows high prevalence of lactose intolerance and has limited access to nutritious food (Vesa et al., 2000).

For the production of TNM, tiger nuts are pre-soaked in water to soften the fibrous tissues, washed, wet-milled and pressed to separate the mush from the tiger nut milk (Djomdi et al., 2007; Ejoh et al., 2006). Although the composition of tiger nuts is extensively documented in the literature (Aremo et al., 2015; Bado et al., 2015; Codina-Torrella et al., 2015; Ekeanyanwu and Ononogbu, 2010; Karababa et al., 2001; Oladele et al., 2009), little is known about the nutrient transfer from tiger nuts to the TNM after the extraction procedure. This is partly because of limited literature on a standard protocol for TNM extraction, so that the properties of TNM such as total yield on dry matter, nutrient composition, colloidal stability and colour as affected by factors such as milling

are not well documented in the literature.

Lactic acid fermentation of TNM into various types of sweet-sour beverages has already been attempted (Akoma et al., 2000; Belewu et al., 2010; Wakil et al., 2014). These reports focused on the physico-chemical composition, and on the sensory and microbiological characteristics of fermented TNM. Currently, there is limited scientific literature on the physical characteristics of fermented TNM although they have a direct impact on consumer acceptability (Walstra et al., 2006). For instance, phase separation in fermented TNM might be a factor that accounts for the low sensory scores in appearance and textural attributes (Akoma et al., 2000; Sanful, 2009b; Wakil et al., 2014). Development of strategies to impede phase separation is fundamental for improving the textural properties of fermented TNM. A well-known fact is that the physical and rheological properties of dairy yogurt can be improved by base milk enrichment with milk proteins (Jaros and Rohm, 2003; Walstra et al., 2006). It is however not evident whether enrichment of fermented TNM with tiger nut proteins affects the colloidal stability and/or leads to textural modification of the fermented system. Developing a process for tiger nut protein isolation is important for investigating its potential as a colloidal stabilising compound for TNM. Alternatively, by enriching TNM with stabilising additives from various well-characterised protein sources such as soy bean protein, sodium caseinate or hydrocolloids including guar gum, carboxymethyl cellulose, xanthan gum or mixtures thereof, stabilised systems can be prepared by enhancing the textural properties through the formation a gel network, or by increasing the viscosity to trap or sterically hinder phase separation (Bouyer et al., 2013; Dickinson, 2011; Kinsella, 1979; Tadros, 2009).

It is also known that, when milk is treated with microbial transglutaminase (mTGase, EC 2.3.2.13) and then fermented using lactic acid bacteria, it leads to yogurt products of improved viscosity, less syneresis and reduction in post acidification during storage (Jaros et al., 2007; Lorenzen et al., 2002). This enzyme cross-links proteins and improves the texture of acid protein gels made thereof. Addition of mTGase cross-linked proteins to TNM might lead to

fermented products with enhanced texture and improved storage properties. However, there is no evidence in the literature regarding the effect of mTGase cross-linked proteins on the microbiological or physico-chemical properties of fermented tiger nut milk.

The aim of this study is to develop strategies for the successful production of yogurt-like products from TNM. The first part of the study proposes a standard procedure for the preparation of TNM and determines nutrient transfer from nut to milk during extraction. The effects of the tiger nut milling programme on the physical characteristics of the obtained milk is also analysed. Secondly, experiments to improve the dispersion stability of TNM are carried out by using various types of proteins and hydrocolloids as stabilising additives to achieve a more suitable substrate for generating fermented systems with acceptable sensory properties. Thirdly, tiger nut proteins are isolated and characterised for enhancing the physical and nutritional properties of fermented tiger nut milk. The effects of enriching TNM with proteins and/or hydrocolloids on the microbiological, physico-chemical and sensory properties of the lactic acid fermented products are reported. Lastly, cross-linking of proteins using mTGase and subsequent enrichment in TNM is investigated for improving the physico-chemical and microbiological qualities of the fermented product during storage.

2. Literature review

2.1 Tiger nut, origin, nutritional value and food use

Tiger nuts (*Cyperus esculentus L*) also called earth almonds, rush nut, yellow nut sedge, edible galingale or northern nut grass are oblong shaped, yellow, brown or black bumpy-skinned with an encircling leaf scar (**Fig. 2.1**) and ivory internal nut-like flesh (Small, 2014). Tiger nuts vary in size with an average diameter of ~ 6 mm and Length of ~ 12 mm. They are hard and wrinkled when dry with an average weight and raw density of 0.257 g and 1.187 g/mL, respectively (Coskuner et al., 2002). Furthermore, they show a size and chemical composition-related hydration capacity with an average of 0.11 g/g tuber (Coskuner et al., 2002). Purportedly, tiger nuts are native to ancient Egypt where they were roasted and eaten as sweet nuts. The tubers thrive in the warmer regions of the northern hemisphere (Europe, Northern America, Asia, Africa, and South America) and are now cultivated or gathered in the wild for food in some parts of Europe (for example, Spain) and West Africa (Ghana, Togo, Nigeria among others) (Small, 2014). The plant has spread to other parts of the world including South-western France, Switzerland, Austria, Germany and the Netherlands because of its nutritional prospects but more importantly, because it is an invasive plant (Follak et al., 2015; Small, 2014).

In many parts of Africa, tiger nuts are eaten raw or roasted for snacks, wet-milled with or without the addition of cereals, cooked and served as sweetened gruels. In Nigeria, the root tubers are used for the preparation of "kunnu", a non-alcoholic beverage with low viscosity, sweet-acidic taste and milky appearance (Ade-Omowaye et al., 2008; Akoma et al., 2016; Belewu, 2007; Coskuner et al., 2002; Oladele and Aina, 2007). Tiger nuts are used in Spain for the commercial preparation of "horchata de chufa", which is a thin, sweetened aqueous tiger nut extract with a milky appearance (Martín-Esparza and González-Martínez, 2016; Sánchez-Zapata et al., 2012).

As regards the nutritional content, carbohydrates (starch, sugar, soluble and insoluble fibre) constitute the largest proportion of tiger nuts, followed by fats,

protein, minerals and vitamins (**Table 2.1**). The nutritional content of tiger nuts may vary depending on type, geographical location and the harvest period (Asante et al., 2014a; Codina-Torrella et al., 2015).

Figure 2.1: Tiger nuts (*Cyperus esculentus* L)

Tiger nuts have a high energy content (approximately 370 kcal/100 g dry matter), which mainly originates from the carbohydrates and fat (Bado et al., 2015; Ekeanyanwu and Ononogbu, 2010). Recent reports indicate that tiger nut oil shows comparable nutritional quality to olive oil, and might be economically competitive as an alternative source of food nutrients (Ezeh et al., 2014; Yoon, 2016). Because of the high fibre content, recent experiments explored tiger nut powder for the enrichment of bakery products to enhance the nutritional functionality of their product (Aguilar et al., 2015; Zahra and Ahmed, 2014). Although tiger nuts are low in protein, they are reported to contain considerable amounts of essential amino acids, especially arginine (Aremo et al., 2015; Temple et al., 1990). The more abundant minerals in tiger nuts include potassium, phosphorus, silicon, sulphur, zinc and magnesium, and the vitamins are ascorbic acid, α-tocopherol and β-carotene (Bado et al., 2015). Phytochemical analysis of tiger nuts showed trace amounts of alkaloids, resin, tannins, sterols, saponins and cynogenic glycosides (Ekeanyanwu et al., 2010). Currently, no hypersensitivity or allergy associated with raw or processed tiger

Table 2.1: Tiger nut composition (g/100 g tiger nut)

Nutrient compound	Bado et al. (2015) (dry matter)	Ekeanyanwu and Ononogbu (2010) (wet matter)
Moisture	-	11.40 ± 0.12
Protein	3.47 ± 0.71	8.07 ± 0.37
Fat	26.14 ± 0.71	24.30 ± 0.58
Saturated (g/100 g fat)	21.56 ± 0.71	-
Monounsaturated (g/100 g fat)	65.91 ± 1.75	-
Polyunsaturated (g/100 g fat)	12.53 ± 0.73	-
Carbohydrate	68.24 ± 1.28	30.00
Starch	30.54 ± 2.75	-
Sucrose	18.99 ± 0.56	-
Fructose	3.02 ± 0.37	-
Glucose	6.79 ± 1.34	-
Total fibre	-	24.00 ± 1.58
Minerals	1.81 ± 0.24	1.80 ± 0.10
Mineral elements (mg/100 g)		
K	608.3 ± 97.84	486.0 ± 59.90
P	229.6 ± 51.54	219.0 ± 10.00
Ca	32.27 ± 5.66	100.00 ± 2.65
Mg	100.5 ± 1.79	94.40 ± 1.28
Na	-	34.30 ± 1.53
Fe	11.44 ± 0.48	4.12 ± 0.10
Cu	0.71 ± 0.03	0.92 ± 0.05
Mn	1.55 ± 0.44	0.26 ± 0.01
Cl	167.0 ± 0.53	-
S	164.3 ± 18.23	-
Al	34.35 ± 2.55	-
Si	181.6 ± 50.35	-
Sr	0.36 ± 0.09	-
Cr	1.65 ± 0.05	-
Zn	2.34 ± 0.31	-

- not determined

nut consumption is known (Galindo Bonilla et al., 2002).

Consumption of tiger nuts is alleged to contribute to a lowering of blood cholesterol, prevention of coronary heart disease, controlled blood pressure, weight control, glycemic control and improved gastrointestinal function, probably because of its high fibre content (Anderson et al., 1994). A recent report by Allouh et al. (2015) based on mouse models suggested that tiger nut consumption might be relevant for improving fertility in mammals. Tiger nuts are consumed in some parts of the world because of claims for their diuretic, carminative, emmenagogue and tonic effects (Chopra et al., 1986).

2.2 Tiger nut milk preparation and nutrient composition

Tiger nut milk (TNM) refers to the aqueous extract with milky appearance that is obtained after wet-milling and pressing of tiger nuts. The basic TNM extraction process (**Fig. 2.2**) is well-known in the literature. However, many authors used minor modifications for obtaining either plain TNM or composite beverages using other plant sources. Insoluble solids in the mush that is obtained after the wet-milling process is filtered using either a muslin cloth, cheese cloth, tissue bags, cotton bags or is sedimented under centrifugal force (Adgidzi et al., 2011; Belewu, 2007; Djomdi et al., 2007; Sanful, 2009a; Ukwuru and Ogbodo, 2011). This separation step is necessary for obtaining finely dispersed TNM soluble solids in the aqueous phase. However, most filtering systems show undefined pore size so that it is difficult to compare the properties of TNM in the literature. The centrifugation process has high tendency to compromise the yield on total solids and increase creaming of fats in TNM (Letki, 2000). Press filtration systems or gravitational filtration steps that are documented for TNM extraction might show variable solids transfer from the mush to TNM. Even though most of the aforementioned extraction processes are simple and domestically adaptable, additional possibility to reliably determine the amount of nutrients that are lost to the pressing residue after extraction and the characteristics of TNM obtained is important for

assessing the effectiveness of the extraction process because they impact nutrient quality and product characteristics. Djomdi et al. (2007) and Ejoh et al. (2006) reported that extraction conditions such as the temperature of soaking tiger nuts and grinding time affect the chemical composition of TNM, which also depends on the variety and site of tiger nut cultivation. Asante et al. (2014b) derived models for optimising the yield of dry matter of TNM by modulating the parameters on boiling, intensity of tiger nut crushing and the ratio of tuber to water for wet-milling.

Figure 2.2: Tiger nut milk extraction (Sanful, 2009a)

Reports have shown that steam blanching or irradiation of tiger nut has influence on the physicochemical and sensory properties of TNM (Adgidzi et al.,

2011; Okyere and Odamtten, 2014). Currently, information on the effects of the extraction method on the physical properties of TNM such as the colloidal stability and colour is limited although these parameters impact TNM quality.

Table 2.2 shows the composition of TNM which, freshly prepared, has a pH of 6.2 - 7.0 (Corrales et al., 2012; Okyere and Odamtten, 2014). Depending on the extraction method, total solids content (soluble and insoluble) of TNM may vary (Asante et al., 2014b; Djomdi et al., 2007; Ejoh et al., 2006). The composition of TNM in **Table 2.2** shows considerable variability. This might partly be attributed to limited scientific literature on methods for extracting TNM reproducibly. Tiger nut milk shows a lower content of protein compared to regular vegetable milk extracts from, for example, soy bean and coconut. On the other hand, tiger nut milk is known to have a higher content of essential amino acids (Ladokun and Oni, 2014; Ugochi and Chukwuma, 2015).

Table 2.2: Nutrient composition of tiger nut milk (g/100 g TNM)

Nutrient composition	Wakil et al. (2014)	Ukwuru & Ogbodo (2011)
Moisture	86.56 - 90.36	77.0 - 80.0
Protein	0.79 - 1.66	6.4 - 7.7
Fat	0.76 - 3.02	5.2 - 5.5
Ash	0.32 - 0.42	0.70
Insoluble fibre	-	-
Soluble fibre	-	-
Carbohydrate	7.77 - 8.34	6.6 - 11.0

- not determined

2.3 Colloidal characteristics of tiger nut milk

A colloid is a two phase system consisting of a dispersed, homogenously distributed phase of sub-microscopic particles (from 1.0 nm to 1.0 µm) in a continuous phase. A suspension contains larger insoluble particles than colloids and therefore, particles may float or sediment in a continuous medium over a specific period of time (Tadros, 2009). Emulsions refer to dispersions of

immiscible liquids in which one component droplet is dispersed in a continuous liquid medium. Emulsions maybe be categorised as water-in-oil w/o (water is the dispersed phase and oil is the continuous phase), oil-in-water o/w (oil is the dispersed phase and water is the continuous phase) or oil-in-oil o/o (for example, a polar oil dispersed in a non-polar oil) (Sjöblom, 2006). Considering that TNM is composed mainly of carbohydrate, fats, protein and minerals that are dispersed in an aqueous milieu, such a complex dispersion may show characteristics similar to colloids, suspensions and o/w emulsion systems. The fraction of soluble and insoluble solids in the form of carbohydrate, protein, fat and minerals may as well influence the characteristics of TNM dispersion (Djomdi et al., 2007; Sjöblom, 2006).

2.4 Factors accounting for the dispersion stability of tiger nut milk

Creaming and sedimentation phenomena in TNM were recently confirmed in the literature (Codina-Torrella et al., 2016). This destabilisation process occurs through a time-dependent build-up of a concentration gradient, where larger droplets or particles migrate either to the top layer (creaming) or bottom layer (sedimentation), forming a closely packed array in the system that results in a semi-transparent continuous liquid phase (Reddy and Fogler, 1981; Tadros, 2009). Tiger nut milk might undergo rapid creaming, partly because of the considerable proportion of droplets of fats, which is non-polar and has a lower density than the continuous phase (Ekeanyanwu and Ononogbu, 2010; McClements, 1999). Sedimentation in TNM might result from insoluble solids that contribute to differences in the particle size distribution (Codina-Torrella et al., 2016; Tadros, 2009). Low viscosity TNM suspensions might show high propensity towards phase separation because of a higher downward or upward gravitational effects on droplets than Brownian rapid random particle migration (McClements, 1999). Other destabilisation phenomena such as flocculation (aggregation of droplets into larger units), coalescence (droplet membrane thinning, diffusion and fusion of droplets into larger ones), Ostwald ripening

(deposition of smaller droplets onto larger droplets) and/or phase inversion (exchange between the disperse phase and the continuous phase) (**Fig. 2.3**) might also contribute to phase separation in TNM (Tadros, 2013).

Tiger nut protein molecules might be relevant for stabilising the emulsion by forming thin films around oil droplets, thereby reducing the surface tension at water-oil boundaries (as emulsifier), and promoting interfacial stability (Kinsella, 1979; Sun and Gunasekaran, 2009). Recently, Codina-Torrela et al. (2015) conducted an *in situ* fractionation of tiger nut proteins and showed that albumin constitutes the largest fraction whilst globulin, prolamin and glutelin form a minor component. However, the low concentration of protein in most tiger nuts (**Table 2.1**) and, consequently, in the milk extract (**Table 2.2**) do not provide any clear evidence of the contribution of tiger nut proteins to TNM stability.

Thus, isolation and characterisation of globular tiger nut proteins might be of practical relevance for studying their effect on the dispersion characteristics of TNM or on the fermented system. Other colloidal polymers such as polysaccharides in TNM may adsorb at the oil-water interface to stabilise o/w emulsions (Binks, 2002).

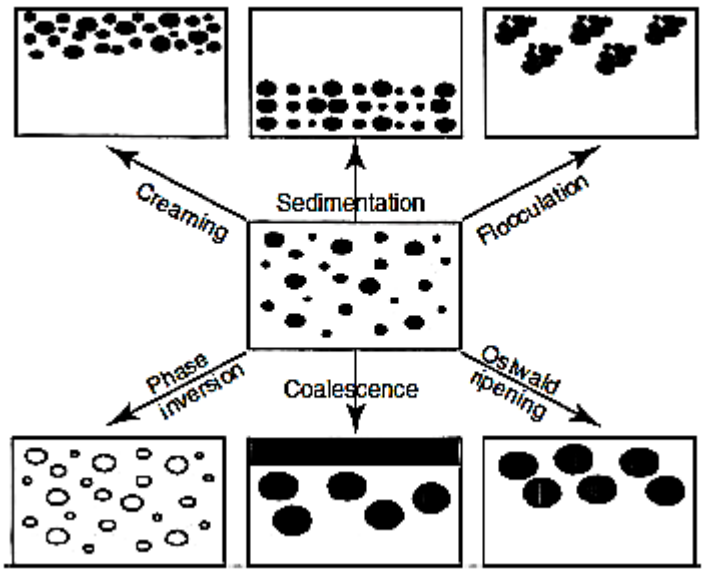


Figure 2.3: Possible emulsions break down processes in tiger nut milk (Tadros, 2013).

For producing TNM beverages or products thereof, phase separation must be curtailed to ensure products with stable appearance and textural quality. Typically, phase separation is more likely to increase during lactic acid fermentation of TNM because of the acidification process, which affects the solubility and distribution of proteins, and alters ionic characteristics of polysaccharides at the oil-water boundaries, thereby enhancing droplet aggregation and emulsion break down (Akoma et al., 2000; Belewu et al., 2010; Binks, 2002; Sanful, 2009b; Sun and Gunasekaran, 2009).

2.5 Enhancing tiger nut milk stability

Emulsions are thermodynamically unstable systems because of the unfavourably high energy required to maintain oil and water contact, which is expressed as free energy (ΔG^{form}, J) given by

$$\Delta \mathrm{G}^{\mathrm{form}} = \Delta \mathrm{A}_{\gamma} - \mathrm{T}\Delta \mathrm{S}^{\mathrm{conf}} \quad (1)$$

where $\Delta \mathrm{A}$ (m^2) is the change in interfacial droplet area if the bulk oil with surface area A_1 is dispersed into large number of droplets with total area A_2 in an emulsion, γ (J/m^2) is the interfacial tension, T (K) is the temperature and $\Delta \mathrm{S}^{\mathrm{conf}}$ (J/K) is the entropy of the dispersion (Tadros, 2013). Emulsion stabilisation might be accomplished by using a surfactant, an emulsifier or a polymer that forms an energy barrier and creates a kinetically stable system. London dispersion forces, which are van der Waals interactions that arise from charge fluctuations in emulsion droplets, are the main attractive forces that influence emulsion stability; the closer droplets approach each other, the stronger the van der Waals attraction forces that lead to flocculation and emulsion breakdown. Fundamentally, electrostatic and steric forces can be introduced to counteract attractive forces in emulsion droplets. Firstly, electrostatic forces are induced on droplet membranes by adding adsorbing ionic surfactants, which create double-layer repulsion barriers. High surface potential, low electrolyte concentration and valency leads to high repulsion energy in emulsion droplets, which enhance emulsion stability (Tadros, 2007).

Secondly, addition of non-ionic adsorbing surfactants/polymers/hydrocolloids in emulsions under good solvation conditions lead to polymer interpenetration or compression (**Fig. 2.4**). In the polymer interaction region, increase in local segment density results in osmotic repulsion, described by a free energy of interaction (G_{mix}, J) and a reduction in configurational entropy (decrease in polymer chain volume due to interpenetration or compression), known as elastic interaction, which is described by a free energy of interaction (G_{el}, J). The cumulative effect of high free energy of interaction and a low energy of elastic interaction increases the steric interaction free energy (G_s, J), which results in steric stabilized emulsions (Tadros, 2007):

$$G_s = G_{mix} + G_{el} \quad (2)$$

To enhance TNM colloidal stability, a number of mechanisms may be employed: Firstly, by decreasing the particle size at droplet interface until a given critical limit, the droplet sizes decrease, which enhances emulsion stability (Binks and Horozov, 2006). Secondly, particle polydispersity is known to show adverse effects on emulsion stability. In the literature, polydispersed fine particles are known to disrupt surface coverage of droplets in water, resulting in a barrier that is less resistant to coalescence (Tambe and Sharma, 1994).

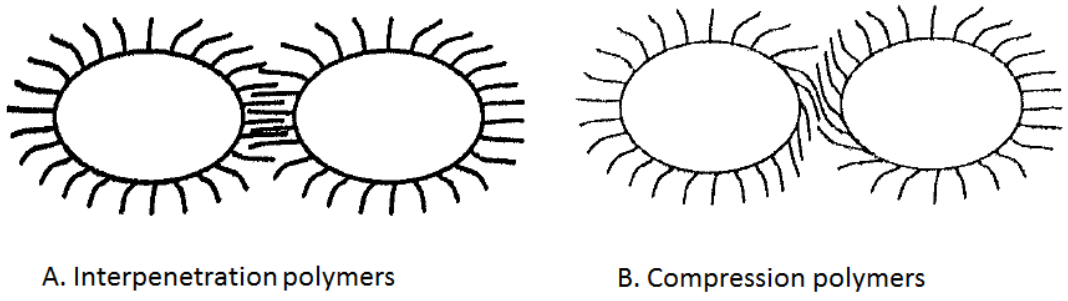


Figure 2.2: Interaction between particles containing adsorbed polymers (Tadros, 2013).

A recent report confirmed that, by reducing particle size and improving particle homogeneity in TNM dispersion, the colloidal stability was enhanced (Codina-Torrella et al., 2016). Fundamentally, Tadros (2013) explained that, by reducing particle size, Brownian particle motion becomes higher than the gravitational force on particle droplets, preventing sedimentation or creaming. The Stokes equation relates hydrodynamic force and gravitational force by

$$V_s = 2r^2\Delta\rho g/9\eta_o \quad (3)$$

where V_s is Stokes sedimentation or creaming velocity, r is droplet radius, $\Delta\rho$ is density difference between droplet and the continuous medium, g is the force of gravity and η_o is the viscosity. Particle size reduction leads to a lower hydrodynamic velocity because of increases in the hydrodynamic particle interaction. At maximum droplet packing fraction (where hydrodynamic particle interaction exceeds the critical limit, φ_p), Stokes velocity reaches zero (eqn 4), given by

$$V_r = V_s(1 - K\emptyset) \quad (4)$$

where V_r is the reduced Stokes velocity, K is the constant for hydrodynamic interactions, and $\emptyset$ is the hydrodynamic volume. This shows that the stability of emulsions partly depends on the concentration of the dispersed droplets (Tadros, 2013). Although particle size reduction and concentration improves TNM stability, for use as base substrate in lactic acid fermentation, the effect of acidification on the TNM stability is crucial. In the literature, it has been shown that pH affects steric and electrostatic properties of colloidal polymers, thereby altering their stability in dispersions (Abdolmaleki et al., 2016; Hunter, 1998). However, studies that relate microbial acidification to the physical properties of fermented TNM are limited in the literature although such a study is relevant for improving fermented TNM quality.

The addition of natural emulsifiers such as hydrocolloids or protein polymers to TNM might be advantageous for producing beverages with enhanced colloidal properties (Bahrami et al., 2013). Hydrocolloids on one hand interact extensively with the bulk milieu because of its hydrophilic property and might

contribute to thickening, gelling or act as stabilising agents. Proteins on the other hand are surface active and can contribute to colloidal stabilisation through electrostatic and hydrophobic-hydrophobic interactions. Fundamentally, non-covalent interactions such as electrostatic, H-bonding and van der Waals, and hydrophobic bonds dominate protein-polysaccharide interactions, and influence structural and textural properties of colloidal food systems (Gosh and Bandyopadhyay, 2012). Polysaccharides such as xanthan gum, carrageenan, cellulose derivatives, guar gum, and proteins including soy and dairy proteins are examples of additives that were successfully used to improve the colloidal stability of dispersions (Bouyer et al., 2013; Dickinson, 2011; Kinsella, 1979; Tadros, 2009). It is reported that gum arabic, β-lactoglobulin and xanthan gum enhance emulsion stability through a thickening effect in the continuous phase, interfacial adsorption and formation of interfacial bi-layers on droplets (Bouyer et al., 2013). Interfacial bi-layers that are formed through a combination of carboxymethyl cellulose and casein were reported to be resistant to pH changes and repulsion flocculation in o/w emulsions (Liu et al., 2012). Emulsifying properties of such polymers are influenced by their molecular mass, density, charge, ionic properties, type of protein/polysaccharide interaction and their ability to adsorb at an oil/water interface, their concentration and viscosifying effects (Gosh and Bandyopadhyay, 2012; Tambe and Sharma, 1994; Ye, 2008). Apart from the emulsifying effects of proteins, TNM enrichment with proteins might improve the physical and rheological properties of their fermented product. This could be relevant for tailoring the textural properties of fermented TNM (Jaros and Rohm, 2003; Walstra et al., 2006). However, polysaccharide and protein interactions in colloidal dispersions such as those in TNM may lead to destabilising effects. For example, addition of low concentrations of xanthan gum, succinoglycan, carboxymethyl cellulose or casein resulted in increased creaming in o/w emulsions (Cao et al., 1990; Dickinson et al., 1997). This destabilisation phenomenon is partly explained by a depletion interaction such as bridging flocculation or depletion flocculation (Jenkins and Snowden, 1996; Tuinier et al., 2003). Bridging flocculation is

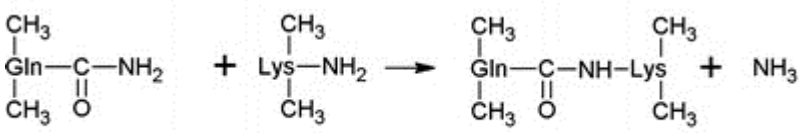
responsible for particle aggregation arising from low polymer concentration. In this phenomenon, polymer chains adsorb and "bridge" two discrete colloidal particles, resulting in particle aggregation. In depletion flocculation, the occurrence of a high polymer concentration in the bulk compared to a low polymer concentration around the immediate zones of colloidal surfaces (depletion zone) creates an osmotic pressure gradient. Overlap of the depletion zone causes anisotropic conditions, leading to a net osmotic force that induces colloidal break down, which is seen by a phase transition that leads to a clear liquid phase and another polymer-rich phase in the colloidal dispersion (Tuinier et al., 2003). The tendency for a polymer such as a polysaccharide to contribute to depletion flocculation is determined by its volume ratio, which is the ratio of the effective volume of the polysaccharide in solution to the actual volume occupied by the polysaccharide chain. The effective volume of a polymer is influenced by the compactness, shape and/or flexibility, and also by the molecular mass (McClements, 2000). Two parameters, namely the critical viscosity concentration (CVC), which is the polymer concentration at which viscosity of the bulk phase reaches infinity, and the critical flocculation concentration, (CFC), which is the minimum polysaccharide concentration required to reduce the interaction potential to the barest minimum, depend on the volume ratio of the polysaccharide. Thus, polysaccharide dispersions with low CVC form stable dispersions with minimal flocculation, whereas those with high CVC but low CFC form highly unstable dispersions (McClements, 2000). This suggests that, for the successful preparation of stable colloidal dispersions, the properties of the polymers and their concentration in the dispersions are relevant factors. A stable TNM dispersion that is relatively resistant to drastic changes in pH and temperature might be important not only for producing milk-like beverages but as a more suitable base substrate for the preparation of fermented TNM with enhanced physical and rheological properties. Thus, experimental data are needed to formulate a stable TNM dispersion that is suitable for producing fermented TNM systems.

2.6 Properties of fermented tiger nut milk

A few authors have reported on the fermentation of TNM using lactic acid bacteria and attempted to generate innovative products with gel-like yogurt properties from tiger nut, which is underutilised as food nutrient in many regions of the world (Adejuyitan, 2011). Belewu (2010) and Wakil et al. (2014) showed that fermented TNM might have relevance due to its chemical, nutritional, sensory and microbiological properties. Fermentation of TNM is important because it might lead to microbiologically stable products with improved shelf life, considering that TNM shows high susceptibility to microbial spoilage (Ocheme et al., 2011; Sebastià et al., 2012). However, Akoma et al. (2000) and Sanful (2009b) noted that lactic acid fermentation of TNM resulted in products with lower sensory scores for sourness, texture, appearance and general acceptance than those from cow milk, coconut milk or their composites. Additionally, drastic increases in browning and phase separation of TNM might be some of the factors that contribute to low sensory scores in texture and appearance (Ocheme et al., 2011). Textural challenges that are generally related to fermented "vegetable plant milk" extracts is reported in the literature (Bernat et al., 2014). However, there is a limitation in studies regarding the physical properties of fermented TNM, even though they impact consumer acceptance (Walstra et al., 2006). Evidently, strategies for improving the textural properties are necessary for enhancing the quality of fermented TNM. Investigating the effects of processes such as TNM enrichment with proteins or polysaccharides on the microbiological, physical, chemical and sensory properties of fermented TNM is fundamental for improving the qualities. Essentially, fermented TNM might be of at least local relevance in many developing countries considering that, worldwide, about 75% of adults experience a decreased lactase activity, and lactose intolerance prevalence rate in Asian and African countries is approximately 90% (Vesa et al., 2000).

2.7 Microbial transglutaminase and properties of fermented tiger nut milk

Transglutaminases (TGase) are a group of enzymes that catalyze protein-glutamine ɣ-glutamyltransferase reactions (Folk, 1980). These enzymes are widely distributed in nature and are found in animal tissues and body fluids, in plants and in microorganisms with a variety of functions (Jaros et al., 2006a). For example, tissue transglutaminase that is found in mammals is known to catalyze dermal matrix re-modeling during wound healing (Raghunath et al., 1996). Of particular interest to food application is microbial transglutaminase, which is mostly obtained from *Streptomyces mobaraensis* (Jaros et al., 2006a). This enzyme has found functional applications in many food industries for enhancing the firmness, elasticity, viscosity, water-binding, gelation, among other textural properties of protein-containing edible foods including dairy and meat products, cheese, fish and bakery products (Kieliszek and Misiewicz, 2014). The enzyme, which is extracellularly secreted by the microorganism, shows Ca^{2+} independent activity and a broad spectrum specificity for the cross-linking and precipitation of various types of proteins including soybean globulin, casein, actin, egg proteins and myosin (Ando et al., 1989; Seguro et al., 1996b). The texturizing effects that the enzymes show on proteins occur by means of a catalyzed acyl transfer of the ɣ-carboxyamide group from a protein-bound glutamine (acyl donor) and primary amines (acyl acceptor), particularly, Ɛ-amino groups of lysine residues in protein-rich systems (**Fig. 2.4**). This enzyme catalyzed reaction results in higher molecular mass protein polymers, consisting of intramolecular and/or intermolecular covalently cross-linked isopeptide bonds, resulting in protein polymers with different physicochemical properties (Gaspar and de Góes-Favoni, 2015).



(5)

Figure 2.3: Microbial transglutaminase modification of protein leading to protein cross-linking

Other possible reaction products of the enzyme have been expounded in the review by Jaros et al (2006a). By introducing peptides or essential amino acids in the protein through the acyl transfer reaction, polymerized cross-linked proteins might additionally lead to protein systems with improved biological value or nutrient properties (Seguro et al., 1996a). The enzyme has an optimum pH 6.0 - 7.0, an isoelectric point of 8.9, and shows a reduced activity at pH 4 and pH 9. The optimum temperature for mTGase activity is 40 °C at pH 5.5, and it is inactivated after a few minutes of heating at 70 °C, making it amendable to a wide range of process conditions in food systems (Ando et al., 1989; Ho et al., 2000). Microbial transglutaminase was observed to show variable activity in various milk proteins, with the highest cross-linking in casein, followed by ultra-filtered skim milk powder, skim milk powder and, to a lesser extent, whey protein isolate (Lorenzen, 2002). In extensively cross-linked caseinate systems, free amino acid groups were observed to have decreased by only 5%, suggesting a small number of cross-linking sites that are necessary for the complete oligomerization of casein (Jaros et al., 2006a; Lorenzen et al., 1998). Microbial transglutaminase cross-linking of caseinate was observed to result in higher polymerization degree than native micellar caseins, suggesting a more amenable mTGase polymerization reaction in unordered caseins than in native casein (Bönisch et al., 2004; Jaros et al., 2006a). In globular whey proteins, mTGase activity shows minimal cross-linking in the native state. However, under denaturation conditions such as pH (8.5 - 9.0), heat treatment or application of pressure, β-lactoglobulin and α-lactoalbumin fractions become susceptible to cross-linking. These treatments induce denaturation in β-lactoglobulin, which exposes relevant amino acid groups for better accessibility by mTGase enzymes, resulting in increased polymerization (Lauber et al., 2003; O'Sullivan et al., 2002). Application of mTGase in dairy milk systems is advantageous for improving the texture and techno-functional characteristics of their products (Jaros et al., 2007, 2006a). Treatment of cow milk with mTGase for set style yogurt production is known to enhance gel strength, improve viscosity and creaminess, and to reduce syneresis (Schey, 2003). Jaros et al.

(2007) observed improvements in viscosity and reduction in syneresis during storage of stirred yogurt pre-treated with mTGase. On one hand, pre-treatment of cow milk with mTGase was reported to prolong fermentation time, increase gel strength, and reduce post acidification in set-style yogurt (Lorenzen et al., 2002; Ozer et al., 2007). On the other hand, Romeih et al. (2014) showed that mTGase had no interference in the acidification rate of Buffalo skim-milk. However, simultaneous addition of mTGase and buttermilk powder to Buffalo skim-milk increased acidification rate and therefore, shortened fermentation time. This implies that the effects that mTGase cross-linked proteins show on the fermentation kinetics of milk might partly be dependent on the substrate characteristics. Isopeptides from ε-(γ-L-glutamy1)-L-lysine cross-linked proteins were found to have equivalent growth promoting effects as non-cross-linked derivatives in rats and chicken, showing that mTGase cross-linking of proteins and untreated proteins might have equivalent growth promoting effects (Waibel and Carpenter, 1972). Furthermore, in vitro digestion experiments that compared untreated casein and mTGase cross-linked casein in gastric conditions of adults and children showed no difference in total and rate of digestion of the proteins (Havenaar et al., 2013). Currently, mTGase is assigned a GRAS status, confirming its safety for use in food processing (Kieliszek and Misiewicz, 2014).

With the view to enhancing the textural properties of fermented TNM, addition of mTGase cross-linked proteins might be novel for reducing syneresis or phase separation in fermented TNM. Currently, there is no information in the literature that substantiates the effects that addition of mTGase cross-linked proteins show on fermented TNM even though they may be relevant for improving the physical qualities of the fermented product. This necessitates investigations on the effects of mTGase cross-linked proteins on the fermentation kinetics, physical properties and storage characteristics of fermented TNM products.

3. Methodology

3.1 Extraction and characterisation of tiger nut milk

3.1.1 Sample collection and preparation

Freshly harvested tiger nuts (brown variety) were directly obtained from farmers in Twifo Praso, Central Region of Ghana. The nuts were rubbed together in a sack to remove sand and root hairs, washed, and dried at room temperature for two months. The sample was then sorted to remove darkened, broken or deteriorated tiger nuts, and the bulk of the nuts was packed and stored in a dry cool room (5 - 6 °C). All batches of tiger nuts were similarly prepared for the study.

3.1.2 Tiger nut milk extraction

Tiger nut milk was prepared from approximately 50 g tiger nuts that were previously dried at room temperature to an initial moisture content of 8.8 ± 0.12 g/100 g. The tiger nuts were washed with demineralised water and hydrated by soaking in a glass beaker with 400 mL demineralised water, placed in a water bath at 40 °C for 24 h. This treatment was to soften the nuts for effective milling and extraction of the milk (Djomdi et al., 2007). The hydrated tiger nuts were then poured into the vessel of a Kult pro mixer (WMF AG, Geislingen, Germany) after washing them with demineralised water until clear wash water was obtained. Exactly 200 g demineralised water was added to the tiger nuts and the mixture was comminuted using the "smoothie mode" for 1, 2 or 3 min to obtain the tiger nut mush. The sample comminuted for 3 min was further dispersed using a T20 ultra turrax mixer (IKA GmbH & CO. KG, Staufen, Germany) at 13,000 rpm for 20 min to obtain finer particles of tiger nut mush. The tiger nut mush was quantitatively transferred by washing with 100 g demineralised water into the hopper reservoir of a pneumatic press with its frit being layered with a Whatman (GE Healthcare Europe GmbH, Freiburg, Germany) 4 µm pore size filter membrane. A pressure of 6.55 x 10^5 N/m^2 was

applied until the flow from the press ceased. The filtrate fraction of the mush (TNM) and the retentate that is the pressing residue (PR) were saved. To prevent microbial proliferation in milk during storage at 5 °C or during analysis, 0.2 g/L sodium azide was added to the tiger nut milk. The soluble and insoluble solids of the milk were determined by filtering exactly 10.0 g extracted TNM under gravity using a pre-calibrated 4 - 7 µm filter paper. The filter paper was dried at 103 °C for 5 h and the mass of the residue was determined. The fractions of insoluble and soluble solids in dry mass (DM) were calculated using the milk solids content.

3.1.3 Nutrient analysis of tiger nuts

The influence of milling intensity on the transfer of nutrients from the tiger nut to the tiger nut milk was investigated to evaluate the amount that is lost through the process. For this, 50 g tiger nuts were soaked as described previously and mopped to remove excess moisture. Next, the mass of the soaked tiger nut (M_{STN}) was recorded. This pre-treatment step was necessary to consider any transfer of compounds from the tiger nuts to the soaking water in the soaking step. Prior to analysis, the moisture content of the pre-soaked tiger nuts was reduced by oven-drying (60 °C, 24 h) to prevent microbial activity on the pre-soaked tiger nuts during nutrient analysis. Subsequently, the mass of soaked, dried tiger nuts (M_{SDTN}) was recorded. The nuts were then dry-milled into a fine powder using an EG100 coffee grinder (Electrolux Hausgeräte GmbH, Nürnberg, Germany) for 2 min to pass a 20 mesh sieve. Portions of the ground samples were then analysed for the nutrient content.

The moisture content, crude protein, crude fat and ash content of the ground samples were determined according to the methods described by Matissek *et al*. (1992) and Nielson (2011). Moisture was gravimetrically determined by drying the sample in an oven at 103 ± 1.0 °C for 5 h to obtain constant mass. The Kjeldahl method was used for determining protein on the basis of nitrogen content (N x 6.25). Fat was determined by acid digestion of samples and

subsequent Soxhlet extraction. To determine ash content, samples were incinerated in a muffle furnace at 550 °C for 6 h. Crude soluble and insoluble fibre were determined using an enzymatic method (Megazyme International, Wicklow, Ireland), with some modifications: a 12 - 14 µm pre-calibrated Whatman filter paper was used for the filtration of insoluble fibres and alcohol-precipitated soluble fibres instead of a celite membrane. The carbohydrate content was estimated as the difference in dry mass (DM) and was analytically confirmed using the sulfuric acid-UV method proposed by Albalasmeh *et al.* (2013).

3.1.4 Analysis of tiger nut products

The moisture content of the pressing residue that was immediately obtained, called the wet pressing residue (W_{WPR}, g/g), the mass of the wet pressing residue (M_{WPR}) and the mass of the extracted tiger nut milk (M_{TNM}) were determined. The material recovery R_m (%), and the relative amount of wet pressing residue RM_{WPR} (g /100 g DM) and dry pressing residue RM_{DPR} (g /100 g DM) was calculated using

$$R_m = 100 \cdot \frac{M_{TNM} + M_{WPR}}{M_{STN} + 300g} \tag{3.1}$$

$$RM_{WPR} = 100 \cdot \frac{M_{WPR}}{M_{STN} + 300g} \tag{3.2}$$

$$RM_{DPR} = 100 \cdot \frac{M_{WPR} \cdot (1 - W_{WPR})}{M_{SDTN} \cdot (1 - W_{SDTN})} \tag{3.3}$$

where W_{SDTN} (g/g) refers to moisture content of the soaked and subsequently dried tiger nuts. The wet pressing residue was then analysed to determine the nutrient compounds that remain after the extraction process by using the methods as previously described.

The transfer of total solids and of soluble and insoluble solids from the wet-milled tiger nuts into tiger nut milk was calculated based on the composition of

solids in the pressing residue. The yield of tiger nut milk (Y_{TNM}, g/100g DM) was then calculated from the difference in the relative amount of the dry pressing residue RM_{DPR} (eqn 3.3) up to 100 % using:

$$Y_{TNM} = 100 - RM_{DPR} \tag{3.4}$$

The nutrient content related to total solids of tiger nut milk (NC_{TNM}, g /100 g DM) was estimated for each analysed nutrient compound by using

$$NC_{TNM} = 1 - \frac{NC_{WPR} / (1 - W_{WPR})}{NC_{SDTN} / (1 - W_{SDTN})} \cdot R_m \tag{3.5}$$

where NC_{SDTN} (g/g) refers to the nutrient content in soaked and subsequently dried tiger nuts, NC_{WPR} (g/g) is the nutrient concentration in the wet pressing residue, W_{WPR} (g/g) is the moisture content of the wet pressing residue and R_m is the material recovery from the press (eqn (3.1)).

3.1.5 Particle size distribution

The effects of the milling programme on the starch granular composition was visualized using a light microscope (Carl Zeiss AG, Oberkochen, Germany). To 1 mL sample, 100 µL of Lugol solution (0.007 % I_2, 0.014 % KI) was added to stain the starch granules, and 15 µL of the stained samples were then observed in the microscope using 1,000-fold magnification.

The particle size distribution and droplet formation in the tiger nut milk were evaluated by using a Helos/KR-H2487 laser diffraction unit (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Tiger nut milk droplet and particle size analysis was determined using a relative refractive index of 1.101, which was based on the refractive index of tiger nut oil (1.464) and that of demineralised water (1.330) (Ekeanyanwu and Ononogbu, 2010). For analysing tiger nut milk, samples were diluted to obtain approximately 0.05 % critical optimum particle concentration and the particle size of the milk was reported based on the volume-weighed mean diameter d_{43} using

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{3.6}$$

where n_i is the number of particles with a diameter d_i.

3.1.6 Colloidal stability

The effect of milling intensity on creaming, which is a measure of colloidal stability of the tiger nut milk, was determined according to White *et al*. (2008) with the following modifications; exactly 20.0 g tiger nut milk was transferred into glass tubes with 1.5 cm internal diameter. The tubes were sealed with a silicone stopper and transferred to an environmental chamber for storage at 50 °C for 44 h. Samples were visually analysed for emulsion breakdown by the formation of an upper oily layer. The creaming index CI (%) was determined using

$$CI = 100 \cdot \frac{H_o}{H_e} \tag{3.7}$$

where H_o is the height of the oil layer and H_e is the total height of the emulsion in the tube (White et al., 2008).

3.1.7 Colour measurement

The colour properties of tiger nut milk as influenced by milling were determined during 27 d storage using a LUCI100 CIE Lab colour space colorimeter (Hach Lange GmbH, Düsseldorf, Germany). The instrument was calibrated against black and white surfaces (standard LZM128) and measurements were carried out using D65 xenon light illumination and a 10° standard observer angle. For comparison, pasteurized cow milk that was obtained from the supermarket was included in the analysis. Mean values for lightness L^*, red-green intensity a* and yellow-blue intensity b* were derived from the colour primaries. The Chroma $C^* = [(a^{*2}) + (b^{*2})]^{1/2}$ and the hue angle h_{ab} = arctan (b*/ a*) were additionally calculated (Rohm and Jaros, 1996).

3.2 **Stabilisation of tiger nut milk dispersion**

3.2.1 Tiger nut milk preparation

Tiger nut milk was prepared by adopting the procedure in section 3.1.2 with few modifications. After soaking in demineralised water for 24 h, tiger nuts were washed until clear wash water was obtained. The nuts were then wet-milled using the Kult pro mixer 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. The concentrated TNM was diluted with demineralized water to 10 g/100 g total solids, which served as a reference sample.

3.2.2 Preparation of tiger nut milk enrichments

For investigating the effects of proteins and hydrocolloids on the stability of TNM, various combinations of sodium caseinate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), soy protein isolate (Nutrition Factory Alphacaps GmbH, Augustdorf, Germany), sodium carboxymethyl cellulose (CMC, Fluka Bio Chemika, Buchs, Switzerland), guar gum (Sigma, Steinheim, Germany) or xanthan gum (Cargill, Saint-Germain-en-Laye, France) were experimented. The proteins or hydrocolloids were solubilised in demineralised water on a magnetic stirrer for 12 h. Next, TNM mixtures were prepared according to the experimental set up in **Table 3.1**, which was developed based on a preliminary experiment that was conducted with a model emulsion from sunflower oil, water and the various types of stabilising additives. After adding the proteins and/or the hydrocolloids to TNM, the mixture was homogenised using a T20 ultra turrax mixer (IKA GmbH & CO. KG, Staufen, Germany) at 11,000 rpm for 3 min. To prevent microbial spoilage during analysis, 0.02 g/L sodium azide was added to the mixture and 18 g samples were transferred into glass tubes, which had 1.5 cm internal diameter. The sample was plugged with a silicone stopper and stored in an environmental chamber at 20 °C for 7 d.

3.2.3 Gravitational stability of enriched tiger nut milk

The dispersions were visually observed for emulsion breakdown by the formation of an upper cream layer or a clear serum layer (Wu et al., 2011). The creaming stability (Creaming index, Ci or Serum index, Si) of the mixture was determined by measuring the height of the cream layer (H_C) or the serum layer (H_S), respectively during storage, and expressed as a percentage of the total height of the dispersion (H_T) according to Wu et al. (2011) using

$$Ci = 100 \cdot \frac{Hc}{H_T} \tag{3.8}$$

$$Si = 100 \cdot \frac{H_S}{H_T} \tag{3.9}$$

Thus, a high creaming index depicts a dispersion with low stability and vice versa.

Table 3.1: Experimental set up for tiger nut milk (concentration: 10 g/100 mL) enrichments with various protein and hydrocolloid compounds.

Hydrocolloids (g/100 g)[b]		Soy protein isolate (g/100 g)[a]		Sodium caseinate (g/100 g)		
		+ 1.00	+ 2.00	+ 1.00	+ 2.00	+ 3.00
-	+ 0	x	x	x	x	x
CMC	+ 0.20	x	x	x	x	x
	+ 0.40	x	x	x	x	x
Guar gum	+ 0.15	x	x	x	x	x
	+ 0.30	x	x	x	x	x
Xanthan gum	+ 0.05	x	x	x	x	x
	+ 0.10	x	x	x	x	x

[a] The symbol (x) refer to prepared and analysed dispersions

[b] CMC: Sodium carboxymethyl cellulose

Next, three dispersions from **Table 3.1** that contained sodium caseinate and xanthan gum or guar gum, and that showed relatively higher creaming stability

of less than 5 % of either a creamy phase or a serum phase were selected and analysed with the reference TNM for the effect of pH and temperature according to **Table 3.2**.

For the analysis, pH of the sample was adjusted to 6.8, 7.3 or 7.8, which reflect minor variations in pH of the enriched TNM. For determining the effects of temperature on dispersion stability, samples were adjusted to mimic conditions of cold temperature storage (6 °C), incubation temperature for lactic acid bacteria (45 °C, 3 h) or pasteurization temperature (70 °C, 10 min).

Table 3.2: Tiger nut milk dispersions selected for pH and temperature analyses

Hydrocolloids (g/100 g)		Sodium caseinate (g/100 g)[a]		
		+ 1.00	+ 2.00	+ 3.00
Guar gum	+ 0.30	-	x	-
Xanthan gum	+ 0.10	x	-	x

[a] x and - refer to analysed or not analysed dispersions, respectively.

3.2.4 Accelerated gravitational stability of enriched tiger nut milk

The influence of pH and temperature on the colloidal stability of the TNM mixtures was measured by a more rapid analytical procedure using a LUMiSizer (L.U.M., GmbH, Berlin, Germany). This multisample analytical centrifuge enables measurement of the relative intensity of infra-red light that is transmitted as a function of time and position over the sample length, and provides data on the kinetics of the separation process (Lerche and Sobisch, 2007). Approximately 2 mL TNM mixture that was prepared on the basis of **Table 3.2** was transferred into the sample tubes of the LUMiSizer and centrifuged at 2,300 g for 7.5 h. Measurement of transmission profiles was set at 5 s intervals for 10 min and thereafter, 30 s for 7.5 h. A SEPView software was used for the evaluation of results by determining the instability index (-), which refers to the ratio of the sum of clarification in a given time to that of

the maximum possible clarification after a specific duration of centrifugation (Detloff et al., 2006). Values of the instability index range between 0 and 1, representing stable and instable dispersions, respectively. The instability indices extracted from the transmission profiles represent arithmetic average of duplicate determinations.

3.2.5 Viscosity of TNM mixtures

Apparent viscosity of the enriched TNM was measured by using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) with a cylindrical geometry (inner diameter, 24.66 mm; outer diameter, 26.66 mm; height, 40 mm). TNM mixtures were equilibrated to 20 °C for 5 min before applying a shear rate sweep from 0.01/s to 100/s. To evaluate the effects of enrichment on the continuous phase of the dispersions, enriched samples were centrifuged at 20,000 g for 5 min (SIGMA 3-30K, Laborzentrifugen GmbH, Osterode, Germany), and the viscosity was similarly determined. The Herschel-Bulkley-Model was used to determine the rheological properties of TNM by using

$$\tau = \tau_o + K.\dot{\gamma}^{\eta} \qquad (3.10)$$

where τ (Pa) is the shear stress, τ_o (Pa) is the yield stress, $\dot{\gamma}$(1/s) is the shear rate, K (-) is the consistency of TNM and η (-) is the flow index with values $\eta < 1$, $\eta = 0$ and $\eta > 1$ depicting pseudoplastic, Newtonian or dilatant behaviour, respectively. Experimental results represent arithmetic average from duplicate determinations.

3.3 **Extraction and characterisation of globular tiger nut proteins**

3.3.1 Protein extraction and fractionation

Dry globular tiger nut protein was isolated by ammonium precipitation of aqueous tiger nut extracts that were obtained by using the method in section 3.2.1 with the following modifications: after soaking in demineralized water for

24 h, tiger nuts were wet-comminuted for 3 min and the obtained tiger nut mush was pressed in a pneumatic press. The retentate from the first extraction was re-extracted by adding water and repeating the comminution and pressing procedures to improve protein extraction. Next, the filtrates from the first and second extractions were pooled and centrifuged at approx. 20,000 g at 4 °C for 10 min (SIGMA 3-30K, Laborzentrifugen GmbH, Osterode, Germany). The sediment from starch granules and the creamed fat were removed by carefully decanting the supernatant and filtering the intermediate liquid through a 13 µm pore-size membrane (GE Healthcare Europe GmbH, Freiburg, Germany). Next, approximately 50 % w/v ammonium sulphate was suspended in the filtered extract and stored at 5 °C for 16 h to precipitate the proteins (Wingfield, 2001). After again centrifuging the suspension, the obtained protein-rich pellet was re-suspended and washed four times with diethylether by agitating on a vortex mixer for 10 min, followed by centrifugation. Afterwards, the pellet was re-suspended in demineralized water and dialyzed in a 6 - 8 kDa cut-off membrane tube (Spectrum Laboratories, Inc. Rancho Dominiquez, USA) at 4 °C to remove residual NH_4SO_4. Next, the dialyzed protein was freeze-dried (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) to obtain the dry globular protein isolate.

The mass and moisture content of the protein isolate were measured, and the protein, fat, minerals and carbohydrate content were analyzed as described for the tiger nuts. Two independent analyses of the tiger nut protein isolate were done.

The dry tiger nut protein (TNP) was fractionated to determine the soluble protein composition using the sequential Osborne solvent extraction procedure, which was modified as follows: To determine the albumin fraction, exactly 1.0 g TNP was added to 10 mL demineralized water and solubilized in an ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) at 22 °C for 10 min. Next, the mixture was centrifuged at approx. 20,000 g at room temperature for 10 min to obtain the supernatant, which was saved as the albumin fraction.

Albumin extraction from the weighed protein sample was repeated three times, each time with 10 mL fresh aqua demin. The globulin fraction was obtained by adding 10 mL 0.5 mol/L NaCl to the protein residue from the aqueous extraction, and similarly extracted by sonication and centrifugation. The remaining protein pellet was re-suspended and solubilized in 10 mL 70 % ethanol in three consecutive steps to obtain the prolamin fraction. Similarly, the glutelin fraction was obtained by repeating the extraction procedure using 10 mL 0.1 mol/L NaOH. The protein remnant from the glutelin extraction was re-suspended in demineralized water and stored as the protein residue (R).

The effect of the alkaline extraction procedure on the susceptibility of the glutelin fraction to thermal denaturation was investigated in a separate TNP fractionation procedure, where the use of NaOH in the last solubilization step for extracting the glutelin fraction was avoided. Protein remnants between solvent changes were washed using demineralized water. After the extraction, solvents containing similar protein fractions were pooled, and the Kjeldahl method (N x 6.25) was used for determining the protein content in the respective fractions. Procedures for fractionation and quantitative analyses of the proteins were done in triplicate.

Portions of the dilute protein fractions were concentrated by precipitating with trichloroacetic acid (Matsudaira, 1993), centrifugation and washing three times by re-suspending each time in acetone and methanol. The proteins were further washed to remove interfering compounds such as lipids and nucleic acids using the methanol chloroform method (Wessel and Flügge, 1984). Finally, the Bradford method was used to determine the concentration of proteins in the fractions using bovine serum albumin as standard (Bradford, 1976).

3.3.2 Molecular mass of globular tiger nut proteins

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was used for analyzing the molecular mass of globular TNP and its fractions by using a vertical mini-gel

system (CBS Scientific Company Inc., Del Mar, CA, USA) (Laemmli, 1970). Protein samples were dissolved in a buffer composed of 0.0625 mol/L Tris (pH 6.8), 0.07 mol/L SDS, 2.74 mol/L glycerol, 0.025 mol/L EDTA and 0.2 g/L Orange G. Next, DTT was added to the sample buffer in a concentration of 0.286 mol/L and the mixtures were boiled at 100 °C for 5 min to obtain reduced proteins. Samples were immediately cooled on ice water and approximately 0.15 ug proteins were loaded into each well of the gel.

The composition of the separating gel was 1.725 mol/L acrylamide, 3.47 mmol/L SDS and 0.75 mol/L Tris–HCl buffer, pH 8.8, and that of the stacking gel was 0.54 mol/L acrylamide and 3.47 mmol/L SDS in 0.05 mol/L Tris–HCl buffer (pH 6.8). A triple color protein standard III composed of 13 proteins with 5 - 245 kDa molecular mass was used as protein marker to determine the protein molecular mass. The protein bands were observed by silver staining (Rabilloud et al., 1988) after separating at 60 V for 30 min and then at 115 V for 3 h. Proteins were fixed with a solvent mixture that was composed of 25:15:60 ethanol, formaldehyde and demineralized water, respectively. The apparent molecular mass of the fractions were determined by calculating the log molecular mass of the protein marker and their retention factor. SDS-PAGE analysis of protein was repeated three times.

3.3.3 Denaturation temperature of globular tiger nut proteins

To further characterize the extracted proteins, the thermal denaturation temperature of TNP isolate and its protein fractions were determined using a Q100 differential scanning calorimeter (TA Instruments, New Castle, USA). Dry protein isolates of the fractionated protein were obtained by precipitation with ammonium sulphate followed by dialysis in demineralized water and freeze-drying as described for TNP. Next, exactly 0.2 g of TNP or the fractions were mixed in a buffer composed of 1 mL 0.1 mol/L phosphate buffer, pH 7.0 at 22 °C for 4 h. After pipetting aliquots corresponding to 3.30 mg protein into hermetic aluminum pans, the TA Blue DSC sample press was used to

hermetically close the pans. Another pan was filled with an equal volume of buffer and served as the reference. Next, samples were transferred to the sample chamber of the DSC. After equilibrating at 20 °C for 1 min followed by isothermal treatment at 20 °C for 2 min, samples were heated to 100 °C at a

rate of 10 K/min. The sampling and nitrogen gas purging rate was 10 Hz and 50 mL/min, respectively. The heating cycle was repeated to determine whether heat denaturation of tiger nut protein is reversible. Thermographs of the proteins were analyzed using the Universal Analysis Program V. To estimate the transition enthalpy ΔH (J/g), the endothermic zone was integrated after appropriate correction for the base-line (Chiu and Prenner, 2011). Additionally, the denaturation temperature T_d (°C) at the peak maximum of the thermal transition and the onset temperature T_o (°C) were determined. Thermal analyses of the proteins were conducted in triplicate.

3.3.4 Isoelectric point of globular tiger nut protein

The isoelectric point of tiger nut protein was determined according to the method described by Salgin et al (2012). The TNP isolate was suspended in demineralized water at 100 mg/L and solubilized in an ultra-sonic bath at 22 ºC for 10 min. After adjusting the suspensions from pH 3.0 to 8.0 at increments of 0.5 units using 0.1 mmol/L KOH or 0.1 mmol/L HCl, the protein solutions were injected into the sample chamber of the Zeta View analyzer (Particle Metrix GmbH, Meerbusch, Germany) and the zeta potential was determined at 22 ºC. Isoelectric point of the protein was determined at the point of zero zeta potential. The results are based on the arithmetic average of three independent determinations.

3.4 **Properties of fermented tiger nut milk enriched with proteins**

3.4.1 Materials and Reagents

Milk proteins that were used to prepare enriched tiger nut milk were whey protein isolate (< 97 g/100 g protein), which was obtained from Sports Supplements Ltd. (Colchester, UK) and sodium caseinate, which was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Xanthan gum was obtained from Cargill France SAS (Saint-Germain-en-Laye, France). All reagents were of analytical grade.

3.4.2 Preparation of plain and enriched tiger nut milk

Tiger nut milk was prepared according to the procedure in section 3.2.1 with the following modifications: to reduce the development of aroma during the soaking step, tiger nuts were soaked in 1 g/100 mL citric acid for 24 h. Subsequently, the nuts were washed and wet-milled using a Kult pro mixer (WMF AG, Geislingen, Germany) for 3 min. The mush obtained was pressed to obtain the TNM. Next, TNM was concentrated in an R-124 rotational evaporator connected to a B-172 vacuum controller (BÜCHI Labortechnik AG, Flawil, Switzerland) at 70 °C for about 1 h to obtain approximately 30 g/100 g solids. For the reference fermentation substrate, TNM concentrate was diluted with demineralized water to obtain 10 g/100 g solids. TNM enriched with xanthan and sodium caseinate, or xanthan and whey protein isolate were prepared by dispersing the respective compounds in demineralized water and mixing with concentrated TNM to achieve systems with 10 g tiger nut solids, 0.1 g xanthan and 1 g 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.

Globular tiger nut protein (TNP) was prepared from aqueous extracts of tiger nut as described in section 3.3.2. Next, tiger nut protein-enriched TNM substrates were prepared by diluting portions of the concentrated TNM with TNP solubilized in demineralized water to ensure an additional 1 g/100 g (1TNP)

or 2 g/100 g (2TNP) in the system. In a similar set up, TNP solutions were first denatured by heating to 85 °C for 10 min before mixing with TNM to investigate the effects of protein denaturation.

3.4.3 Fermentation of plain and enriched tiger nut milk

Plain and enriched TNM substrates were pasteurized in glass bottles at 70 °C for 15 min in a water bath under continuous agitation on a magnetic stirrer. The systems were allowed to cool and subsequently inoculated with 0.01 g/100 g FVV-211 yogurt starter comprising of *L. delbrueckii* ssp. *bulgariucs* and *S. thermophilus* (DSM Food Specialities, Delft, Netherlands), and fermented at 38 °C for 16.5 h. During fermentation, pH was continuously monitored using an InoLab 730 pH meter (WTW GmbH, Weilheim, Germany). Next, pH/time plots were generated and the lag time λ (h) and maximum pH reduction rate μ (1/h) were graphically estimated on the basis of the modified Gompertz equation for bacterial growth (Soukoulis et al., 2007)

$$pH = pH_0 + (pH_\infty - pH_0)\exp\left\{-\exp\left[{}^{\mu e}/_{(pH_\infty - pH_0)}(\lambda - t) + 1\right]\right\} \qquad (3.11)$$

where pH_0 is initial pH and pH_∞ is final pH.

During fermentation, gel formation was monitored using an ARES RFS3 rheometer (TA Instruments GmbH, Eschborn, Germany) with concentric cylinder geometry of inner diameter, 32 mm; outer diameter, 34 mm and height, 33.5 mm, maintained at 38 °C by a circulator. Immediately after inoculation, approximately 11.2 mL TNM substrate was transferred into the cup and the inner cylinder was lowered into measuring position. To prevent evaporation during the measurement, the sample surface was covered with low viscosity silicone oil. Next, a time sweep was initiated using a strain of γ = 0.003 and an angular frequency of ω = 1 rad/s (Jacob et al., 2011). During fermentation, the dynamic moduli were recorded. Samples were cooled to 6 °C after the fermentation period and stored for analysis. Fermentation of each

substrate and the respective pH/time plots and gel formation measurements were carried out in triplicate.

3.4.4 Viable counts of starter cultures in fermented tiger nut milk systems

The capability of plain and enriched TNM to allow the growth of the starter cultures was investigated by determining the viable counts of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in the freshly fermented products. After appropriately diluting the samples using peptone water, viable counts of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* were enumerated after incubating for 48 h at 37 °C or 25 °C on MRS or M-17 media, respectively (IDF, 2003). Determinations were done in triplicate.

3.4.5 Chemical analysis of unfermented and fermented tiger nut milk

The composition of TNM was analyzed according to section 3.1.3. Specifically, total carbohydrate was determined according to Albalasmeh, Berhe, & Ghezzehei (2013) and fat content according to IDF (2008).

Sucrose, glucose and fructose content of TNM were determined by HPLC and refractive index detection. After clarification using the Carrez procedure and 0.45 µm filtration, samples were separated by applying 0.5 mL/min isocratic elution using a 300 x 7.8 mm Rezex™ RPM-Monosaccharide cationic lead, Pb^{2+} (8 %) monosaccharide analysis column (Phenomenex Ltd., Aschaffenburg, Germany). Sugars were detected by light scattering at a reference cell temperature of 8 °C. Triplicate determinations were carried out for each sample.

Titratable acidity (TA) of the fermented systems was determined after diluting 10.0 g samples to 40 g using demineralized water and titrating with 0.1 mol/L NaOH against phenolphthalein. The lactic acid equivalent was determined based on the volume of NaOH required to neutralize the analyte (Sadler and Murphy, 2014).

3.4.6 Physical analysis of fermented tiger nut milk products

Phase separation after 16.5 h incubation of the tiger nut milk systems at 38 °C was visually measured according to the procedure in section 3.2.3. The fermented TNM systems were subjected to accelerated gravitational separation to determine their susceptibility to syneresis (Jaros et al., 2007). After fermentation of exactly 30.0 g TNM samples in pre-weighed sterile falcon tubes, samples were stored at 6 °C for 24 h and subsequently centrifuged at 600 g at same temperature for 10 min. After removing the separated liquid serum, the mass of the serum related to the initial mass of the sample was determined and expressed as percent syneresis (%).

To measure the apparent viscosity of fermented TNM systems, a Physica MCR 301 rheometer with a cylindrical geometry (inner diameter, 24.66 mm; outer diameter, 26.66 mm; height, 40 mm) was used as described in section 3.2.5. Fermented TNM with a smooth texture was obtained by homogenizing the samples using a T20 ultra turrax mixer (IKA GmbH & CO. KG, Staufen, Germany) at 11,000 rpm for 40 s. Apparent viscosity was measured after storing the homogenized samples at 6 °C for 24 h. Each of the syneresis and viscosity measurements was carried out in triplicate.

Gel firmness of the fermented TNM was determined by penetration using the RSA 3 solids analyzer (TA Instruments GmbH, Alzenau, Germany). Approximately 20.0 mL TNM substrates were fermented in screw-top glass bottles with an inner diameter of 39 mm and the fermented products were stored at 6 °C for 24 h. Where applicable, the gels were penetrated by a cylindrical plunger (diameter, 15 mm; height, 10 mm) mounted on the RSA analyzer at 0.5 mm/s. Gel firmness was determined from the initial slope of the force/distance curves (Jaros et al., 2006b). Quadruplicate measurements were done.

3.4.7 Sensory analysis of fermented tiger nut milk products

Sensory analysis of fermented tiger nut milk products was conducted by using Flash profiling according to Delarue & Sieffermann (2004). Modifications were made based on the procedure of Thamke et al (2009). A 13 member panel (mean age: 31 y, 9 females) was selected for the study. The reference sample, which is the fermented plain tiger nut milk, and four samples composed of sodium caseinate or whey protein enrichments were presented for sensory assessment. A duplicate of the sample 1WPX was included to evaluate the discriminatory quality of the test. After encoding with 3-digit numbers, samples were simultaneously served in a counterbalanced order using 20 mL transparent plastic cups in a standard sensory laboratory. Principal component and generalized procrustes analyses were used to analyze raw data on the attributes and their respective intensities using the Senstools.Net software (OP&P Product Research BV, Utrecht, Netherlands). Experiments were carried out in duplicate.

3.5 **Microbial transglutaminase and fermented tiger nut milk property**

3.5.1 Preparation of plain and enriched tiger nut milk

Tiger nut milk (TNM) was prepared according to section 3.4.2 by wet-milling, mush separation through pressing and filtration and concentration of the obtained TNM in an R-124 rotational evaporator to obtain approx. 30 g/100 g tiger nut solids. A concentration of 10 g/100 g TNM was prepared and used as the reference fermentation substrate.

Protein or xanthan dispersions were prepared by dispensing the appropriate amount in demineralized water and agitating on a magnetic stirrer at 25 °C for 2 h to obtain sodium caseinate (8 g /100 g), whey protein isolate (8 g/100 g) or xanthan gum (1 g /100 g). Next, the protein dispersions were heated in a water bath at 80 °C for 10 min for denaturation where applicable and cooled to room temperature. After dividing the protein dispersions into two parts, one part was treated with microbial transglutaminase Activa MP (mTGase) (Ajinomoto Foods

Deutschland GmbH, Hamburg, Germany) according to Jaros et al. (2014a, 2014b). For protein cross-linking, 3 U mTGase per g protein was added to the protein dispersion after equilibrating to 40 °C in a water bath. The mixture was incubated for 2 h and the enzyme was subsequently inactivated by heating to 80 °C for 10 min and cooled in ice water. The protein solution without mTGase treatment was similarly subjected to the heating and cooling steps to prevent effects due to the heat treatment. Protein-enriched TNM were prepared for fermentation by appropriately mixing with the necessary amount of xanthan gum to result in 10 g tiger nut solids, 0.1 g xanthan gum and 3 g sodium caseinate or 3 g whey protein isolate without mTGase treatment (3CnX, 3WPX) or with mTGase treatment (3CnXe, 3WPXe) per 100 g substrate.

3.5.2 Fermentation of plain and enriched tiger nut milk

Plain or enriched TNM substrates were pasteurized in 500 mL plastic jars under continuous agitation, cooled and inoculated with 0.01 g /100 g FVV-211 yogurt starter and fermented at 38 °C for 16.5 h as described previously in section 3.4.3. To determine the fermentation kinetics during fermentation, an InoLab 730 pH meter was used to continuously monitor the pH. Subsequently, pH/time plot, lag time λ (h) and maximum pH reduction rate μ (1/h) were determined using the Gompertz model as described in section 3.4.2. Semi-solid gel products that were produced after the fermentation were homogenized at 11,000 rpm for 20 s using an ultra turrax to enhance smooth texture of the products. After filling 120 mL sterile plastic jars with samples, jars were firmly sealed with lids and stored for 24 h. Samples were analyzed after 0, 5, 10 and 15 d storage at 6 °C. TNM substrates were prepared and fermented in triplicate.

3.5.3 Analysis of the enzymatically cross-linked proteins

The effect of mTGase cross-linking on the proteins was assessed using a liquid chromatography system (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 of pH 6.8 was composed of 1 g/L CHAPS, 6 mol/L Urea, 0.1 mol/L NaCl, and 0.1 mol/L Na_2HPO_4. Protein solutions were diluted in the elution buffer to dissociate the protein aggregates and for reducing disulphide bonds, proteins were treated with DTT to a concentration of 0.15 g/L. A Superdex 200 increase 10/30 column (GE Healthcare, Uppsala, Sweden) was used to separate samples by applying 0.5 mL/min isocratic elution at room temperature. After acquiring the chromatographic data using ClarityChrom v.3.07 (Knauer Wissenschatlische Gerate GmBh, Berlin, Germany), the corresponding peak areas (A) were analysed for the fraction of monomers, dimers and polymers. Degree of polymerisation (DP, %) was computed according to Bönisch et al (2004) by

$$DP[\%] = \sum \frac{A(dimers+trimers+polymers)}{A(monomers+\ dimers+trimers+polymers)} . 100\ \% \qquad (3.12)$$

3.5.4 Viable counts

The effect of mTGase cross-linking of protein on the starter viable counts in the fermented products was determined by pour plating during 16 d storage. Samples were appropriately diluted in peptone water. Subsequently, *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* were enumerated using MRS or M-17 media, respectively as described in section 3.4.4. Viable counts were determined in triplicate.

3.5.5 pH and titratable acidity

pH of the fermented products was measured at 20 ± 1 °C. Titratable acidity was determined according to section 3.4.5 by titrating diluted samples with 0.1 mol/L NaOH and using the average titre to calculate the lactic acid equivalence according to Sadler and Murphy (2014).

3.5.6 Syneresis and viscosity

The effects of mTGase cross-linked protein on the syneresis of fermented TNM products were analyzed under accelerated gravity as described by Jaros et al. (2007) with the following modifications: after transferring 15.0 g fermented product into pre-weighed falcon tubes, samples were centrifuged at 1,400 g at 4 °C for 20 min. The separated serum was removed using a Pasteur pipette and the amount of removed liquid related to the initial amount of sample subjected to centrifugation was calculated and expressed as syneresis (%).

Apparent viscosity of fermented TNM was measured using a Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria). Samples stored at 6 °C were transferred into a cylinder geometry (inner diameter, 24.66 mm; outer diameter, 26.66 mm; height, 40 mm) and adjusted to 20 °C for 5 min. Subsequently, a shear rate sweep from 0.01/s to 100/s was applied. Results on syneresis and viscosity are based on triplicate experiments.

3.5.7 Colour of fermented tiger nut products

The effects of mTGase cross-linked proteins on the colour attributes of the fermented product was analyzed during 16 d storage using LUCI100 CIE-Lab colour space colorimeter equipped with D65 xenon lamp and 10° standard observer as described in section 3.1.7. After calibration against black and white standard surfaces (LZM128), mean values of lightness (L*), yellow-blue intensity, b* and red-green intensity, a* were derived from the colour primaries. Next, The Chroma $C^* = [(a^{*2}) + (b^{*2})]^{1/2}$ and the hue angle h_{ab} = arctan (b*/ a*) were calculated according to Rohm and Jaros (1996). Results are based on triplicate determinations.

3.6 Statistical analysis

Data were evaluated using one-way analysis of variance (ANOVA). The t-Test, Tukey HSD or Games-Howell post hoc analysis was used to compare the mean

values when necessary. For these, SPSS software package version 16.0 was used for performing the analysis (SPSS Inc., Chicago, IL, USA). All significant values refer to $P < 0.05$.

4. Results and discussion

4.1 Extraction and characteristics of tiger nut milk

4.1.1 Material recovery, mass transfer and yield of tiger nut solids

The extraction procedure for tiger nut milk (TNM) was standardised by defining parameters such as the temperature and duration of tiger nuts soaking, milling duration, filtration pore size and magnitude of applied pressure during mush pressing. This allowed the effects of milling duration and intensity on the material transfer from tiger nut to tiger nut milk and on the physical properties of the obtained TNM to be studied. During soaking, tiger nuts absorbed water, which resulted in swelling of tiger nuts, weakening of cell walls and mass increase from 50.21 ± 0.08 g to 75.66 ± 1.16 g, representing about 50.70 % increase in mass. Water uptake during soaking is known to be related to the temperature of the soaking milieu and average size of soaked tiger nuts (Djomdi et al., 2007). After soaking, tiger nuts were wet-milled using a cutting mill by adding 300 g demineralised water to obtain the mush, which was then filter pressed. This resulted in a corresponding mass of wet pressing residue, M_{WPR} (g) and tiger nut milk, M_{TNM} (g) from each extraction sample of 43.09 ± 1.21g and 326.8 ± 0.7 g, respectively, representing an average material recovery of the extraction process, R_m = 97.86 - 98.97%. The marginal loss in the extraction process is due to the limitation in recovery of material from the filter paper. Neither the milling duration nor intensity showed any significant influence on the material recovery, which implies that the extraction process was reproducible.

The mass of the wet pressing residue obtained from the mush with 2 min of milling (Mush.2) and that with 3 min milling plus ultra turrax dispersion (Mush.3) was significantly lower than that with 1 min milling (Mush.1; **Table 4.1**). Prolonged milling reduced the size of tiger nuts more effectively and enhanced solids transfer across the filtration membrane during pressing, resulting in mass reduction of the wet pressing residue. Additionally, it can be observed in **Table 4.1** that milling affected the moisture content of the pressing residue, W_{WPR}

significantly. Specifically, the moisture content of the pressing residue from Mush.2 (PR.2) was higher than that of the pressing residue from Mush.1 (PR.1) and Mush.3 (PR.3). This is probably because of the contribution that originates from the soluble fibre content of the pressing residue. **Table 4.2** shows that PR.2 had a higher content of soluble fibre, which is known to have a high water retention capacity (Chen et al., 2014; Elleuch et al., 2011). This demonstrates that milling intensity can be regarded as useful for modulating the moisture content in high fibre food products from tiger nuts.

Table 4.1: Influence of wet-milling duration and intensity on mass transfer from tiger nuts mush to the pressing residue and the milk

Wet-milled tiger nuts [1]	M_{WPR} (g/100 g)[2]	W_{WPR} (g/g)	Y_{TNM} (% of DM)	Milk solids (%)	
				Soluble	Insoluble
Mush.1	11.83 ± 0.03[a]	0.445 ± 0.002[a]	35.06 ± 0.14[a]	89.30 ± 0.20[a]	10.70 ± 0.20[a]
Mush.2	11.39 ± 0.01[b]	0.456 ± 0.004[b]	37.87 ± 0.17[b]	86.13± 0.74[a]	13.87 ± 0.74[a]
Mush.3	11.16 ± 0.09[b]	0.438 ± 0.003[a]	41.23 ± 0.43[c]	74.46 ± 1.49[b]	25.54 ± 1.49[b]

[1] Pre-soaked tiger nut wet-milled for 1 min (Mush.1), for 2 min (Mush.2), or for 3 min plus ultra turrax dispersion (Mush.3).

[2] Values in the same column with different superscripts differ significantly ($p < 0.05$). Arithmetic means ± standard deviations are based on triplicate experiments. M_{WPR}: Mass of wet-pressing residue, W_{WPR}: Moisture content of wet pressing residue, Y_{TNM}: Yield of tiger nut milk, DM: Dry matter.

The increase in milling intensity resulted in a progressively higher yield of TNM solids from 35.1 % to 41.2 % (**Table 4.1**). In the literature, a maximum yield of 30 % TNM solids was reported (Djomdi et al., 2007). The difference in yield of TNM solids is attributable to the variety of tiger nuts, type of extraction method, the degree of size reduction, the pore size of the filter membrane and the applied pressure (Ejoh et al., 2006). The increase in yield was, however, accompanied with a significant reduction of the fraction of soluble TNM solids from 89.3 % to 74.5 %. Ejoh et al (2006) attributed the decrease in the soluble TNM fraction to the increase in the content of fibre and lipids. In this study,

microscopic observation of TNM showed an increase in the proportion of insoluble starch granules (data not shown), which might additionally account for the decrease in the fraction of soluble solids. In this extraction procedure, a higher ratio of soluble to insoluble solids than that reported in the literature was observed (Ejoh et al., 2006). The high amount of insoluble solids influences the content of unadsorbed polysaccharide granules, which is known to contribute to flocculation through a depletion mechanism, resulting in phase separation in dispersions (Chanamai and McClements, 2001; Jenkins and Snowden, 1996). Therefore, even though high intensity of tiger nut milling improves the yield on milk solids, the corresponding increase in insoluble solids might contribute to phase separation, which influences the physical properties of the extracted product.

4.1.2 Nutrient composition of tiger nut products

Tiger nuts have been reported as being rich in energy and minerals (Sánchez-Zapata et al., 2012). However, little is known about the efficiency of nutrient transfer during extraction of tiger nut milk. To evaluate the effect of the extraction procedure on the distribution of nutrients from tiger nuts to the pressing residue and the tiger nut milk, the composition of the tiger nuts, the pressing residue and that of TNM was determined.

Equivalent mass of base tiger nuts for TNM extraction that was soaked and subsequently dried for the analysis of nutrient composition resulted in mass and moisture content of 49.6 ± 0.2 g and 8.56 ± 0.02 g/100 g, respectively. The dry matter-related gross composition of the pre-soaked tiger nuts shown in **Table 4.2** was within the reported range (Adejuyitan et al., 2009; Coskuner et al., 2002; Ejoh et al., 2006; Oladele et al., 2009).

Results on the composition of the pressing residue in **Table 4.2** show that the fraction of fat and salt was comparable to what has been reported for residues from a local horchata process (Sánchez-Zapata et al., 2009). The distribution of individual nutrients from wet-milled tiger nuts to the milk or pressing residue is affected by the duration or intensity of tiger nut milling,

maceration and the number of re-extraction or washing steps applied in the process (Ejoh et al., 2006).

Table 4.2: Influence of wet-milling duration or intensity on nutrient retention (g /100 g dry matter) in the pressing residue during extraction of tiger nut milk.

Nutrient compound	Pre-treated tiger nuts[3]	Tiger nut pressing residue (PR)[1,2]		
		PR.1	PR.2	PR.3
Protein	4.95 ± 0. 01	1.72 ± 0.46[a]	1.78 ± 0.16[a]	1.61 ± 0.09[b]
Fat	21.60 ± 0.12	10.32 ± 0.11[a]	8.78 ± 0.16[b]	8.15 ± 0.11[c]
Ash	1.75 ± 0.01	0.74 ± 0.01[a]	0.74 ± 0.01[a]	0.69 ± 0.01[b]
Insoluble fibre	18.37 ± 0.20	28.58 ± 0.23[a]	30.96 ± 0.48[b]	31.03 ± 0.11[b]
Soluble fibre	2.56 ± 0.15	0.85 ± 0.03[a]	1.48 ± 0.15[b]	0.56 ± 0.03[c]
Carbohydrate	55.08 ± 0.13	57.79 ± 0.25[a]	56.26 ± 0.28[b]	57.96 ± 0.05[a]

[1] Tiger nut pressing residue after 1 min milling (PR.1), after 2 min milling (PR.2), or after 3 min milling plus ultra turrax dispersion (PR.3).

[2] Values in the same row with different superscripts differ significantly ($p < 0.05$). Arithmetic means ± standard deviations are based on triplicate experiments.

[3] Pre-soaked, oven-dried tiger nuts

Table 4.2 further shows that PR.3 had a significantly lower protein content than PR.1 and PR.2, which was probably due to solubilisation of tiger nut proteins because of the high milling intensity, and depicts that tiger nuts might have a high fraction of water-soluble proteins (Codina-Torrella et al., 2015). Fat and ash content in the pressing residues was also reduced significantly when milling time was increased. As milling was accompanied by partial mush warming, this might have enhanced lipid hydration and the subsequent transfer to the aqueous fraction during pressing (Cevc and Marsh, 1985; Nielsen, 2003). Milling affected the soluble and insoluble fibre content of the pressing residue and was highest in PR.2 and PR.3, respectively. Milling of tiger nuts for 2 min was most effective for extracting carbohydrates but a more intense milling programme was necessary for extracting proteins, ash and fat but not necessarily fibre.

Table 4.3 shows the effect of wet-milling on the individual nutrient

compounds that are transferred from tiger nut into the tiger nut milk. The material recovery, R_m (eqn 3.1) was factored during calculation and accounts for the difference to 100 %. Generally, it can be observed that the fractional distribution of nutrients from tiger nut into the milk or pressing residue depended not only on the milling intensity but also on the solubility and concentration of compounds in the tiger nut.

Table 4.3: Effect of wet-milling intensity on the transfer of nutrient compounds into tiger nut milk during extraction of tiger nut milk (%).

Nutrient compound	TNM.1[1,2]	TNM.2	TNM.3
Protein	80.34 ± 0.58[a]	80.48 ± 1.51[a]	81.71 ± 0.67[a]
Fat	73.49 ± 0.17[a]	77.89 ± 0.75[b]	79.14 ± 0.54[b]
Ash	76.42 ± 0.47[a]	77.32 ± 0.45[a]	78.18 ± 0.41[a]
Insoluble fibre	16.63 ± 1.03[a]	14.54 ± 2.61[a]	13.19 ± 1.83[a]
Soluble fibre	80.71 ± 1.72[a]	68.85 ± 4.41[b]	86.97 ± 0.11[c]
Carbohydrate	38.57 ± 0.36[a]	43.31 ± 0.63[b]	40.68 ± 0.51[a]

[1] Tiger nut milk after 1 min milling (TNM.1), after 2 min milling (TNM.2), or after 3 min milling plus ultra turrax dispersion (TNM.3)

[2] Values in the same column with different superscripts differ significantly ($p < 0.05$). Arithmetic means ± standard deviations are based on triplicate experiments.

Exemplarily, whilst the fractional distribution of protein, fat, salts and soluble fibre between the pressing residue and the milk showed approximately 1:4, the distribution of insoluble fibre was 6:1 and that of carbohydrates was 2:3.

The composition of TNM was as follows: moisture, 79.38 - 82.47 g/100 g; protein, 1.53. - 1.76 g/100 g; fat, 6.14 - 7.42 g/100 g; ash, 0.52 - 0.58 g/100 g; insoluble fibre, 1.04 - 1.18 g/100 g; soluble fibre, 0.68 - 0.95 g/100 g; and carbohydrates, 7.54 - 8.95 g/100 g. The protein, carbohydrate and ash composition was comparable to the results of Wakil et al (2014), while the moisture and fat content was consistent with the report of Ukwuru and Ogbodo (2011). The results imply that, during extraction of tiger nut milk, a greater proportion of protein, fat, ash and soluble fibre are transferred into the tiger nut milk, whereas carbohydrate and insoluble fibre form a greater fraction of

retained nutrients in the pressing residue. By increasing the milling intensity, the transfer of nutrients such as proteins, minerals and fat into tiger nut milk can be enhanced. However, the influence the milling process shows on the colloidal characteristics is relevant because it affects the physical properties, which can impact consumer acceptance of TNM products (Walstra et al., 2006).

4.1.3 Physical properties of tiger nut milk

The effects that milling intensity shows on the physical properties of TNM was investigated by using light microscopy, which generally showed some deposits of starch granules (data not shown). The milk that was extracted after 3 min milling plus ultra turrax dispersion (TNM.3) depicted starch granules with a less defined crystal structure, suggesting that the starch granules were partially hydrated. These hydrated granules might show a higher solubility and may increase the milk density, which may improve milk stability (Tadros, 2009). Li et al. (2013) reported that starch granule size and their concentration in dispersions affect the emulsion stability. Tiger nuts that were milled for 1 min (TNM.1) or 2 min (TNM.2) showed relatively intact granules and appeared in a lower concentration. TNM.3 may show a tendency towards a more stable colloidal suspension compared to TNM.1 and TNM.2 based on the concentration and the degree of hydration of the starch granules. However, differences in particle size and the particle distribution in the dispersion are necessary for determining the physical stability of TNM (Binks and Horozov, 2006).

4.1.3.1 Particle size distribution of extracted tiger nut milk

Fig. 4.1 shows the particle size distribution of TNM and depicts that increasing the milling intensity caused the production of finer particles. This was evident in the change in particle size distribution from bimodal (TNM.1, TNM.2) to monomodal (TNM.3). It was observed that higher milling intensity decreased the mean volumetric diameter of TNM. Exemplarily, ninety per cent of tiger nut milk particles (D_{90}) showed a diameter smaller than 13.6 µm (TNM.1), 6.46 µm

(TNM.2) and 2.45 µm (TNM.3) with a corresponding D_{50} median value of 3.94 µm, 1.64 µm and 1.40 µm. A range of 0.72 - 0.79 µm was recorded for the D_{10} values. The results show that TNM.3 had the most uniform size distribution of the tiger nut milks.

In summary, increase in milling intensity affected TNM properties such as increased concentration of milk solids, improved hydration of starch granules and uniformity of dispersed particles, and reduced average particle size, which might have relevance for enhancing the stability of tiger nut milk (Tadros, 2009).

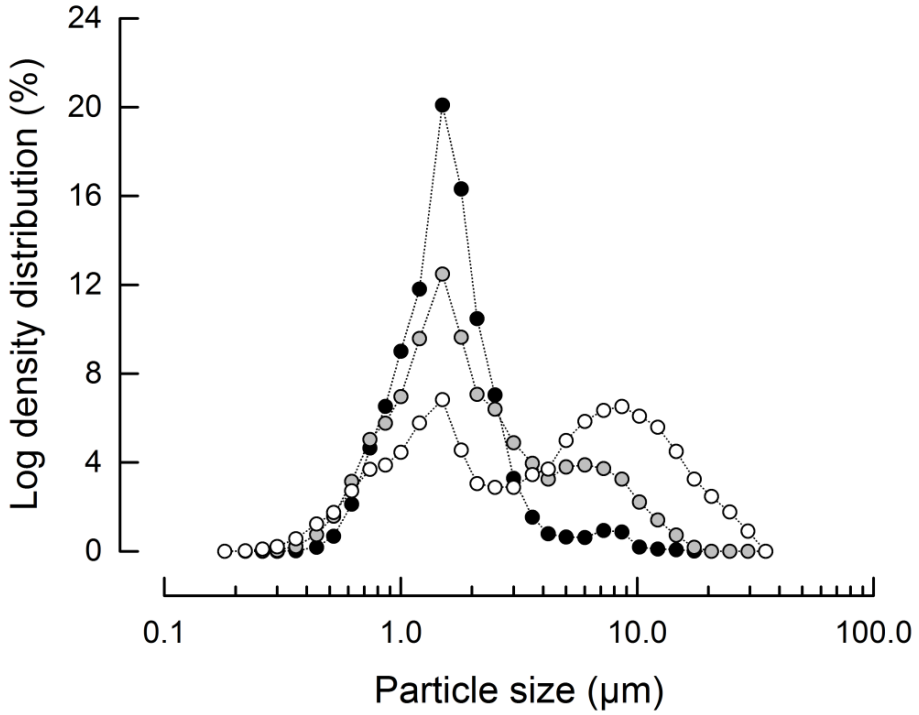


Figure 4.1: Effect of wet-milling intensity on particle size distribution of tiger nut milk. Milk was extracted from pre-soaked tiger nuts comminuted with cutting mill for 1 min (TNM.1, open circle), 2 min (TNM.2, grey circle) or 3 min plus dispersion using an ultra turrax (TNM.3, dark circles)

4.1.3.2 Colloidal stability of tiger nut milk

The effect of milling on creaming was determined during storage as indicator for emulsion breakdown of tiger nut milk, which is illustrated in **Fig. 4.2.** Two

stages of dispersion destabilization were observed: Firstly, tiger nut milk formed a creamy lipid-rich top layer during 2 - 16 h storage. After 16 h storage, a second type of phase separation that was characterized by the aggregation of milk solids leading to a two-layer system of biological polymer-rich upper layer and a polymer-depleted lower serum layer was observed. The increase in milling intensity delayed initial creaming of the tiger nut milk as seen in TNM.2 and TNM.3 (**Fig. 4.2**). These samples with higher milling intensity had a smaller average particle size (**Fig. 4.1**) and a higher concentration of TNM solids, which were mainly carbohydrate polymers, fats and proteins **(Table 4.1, Table 4.3)**.

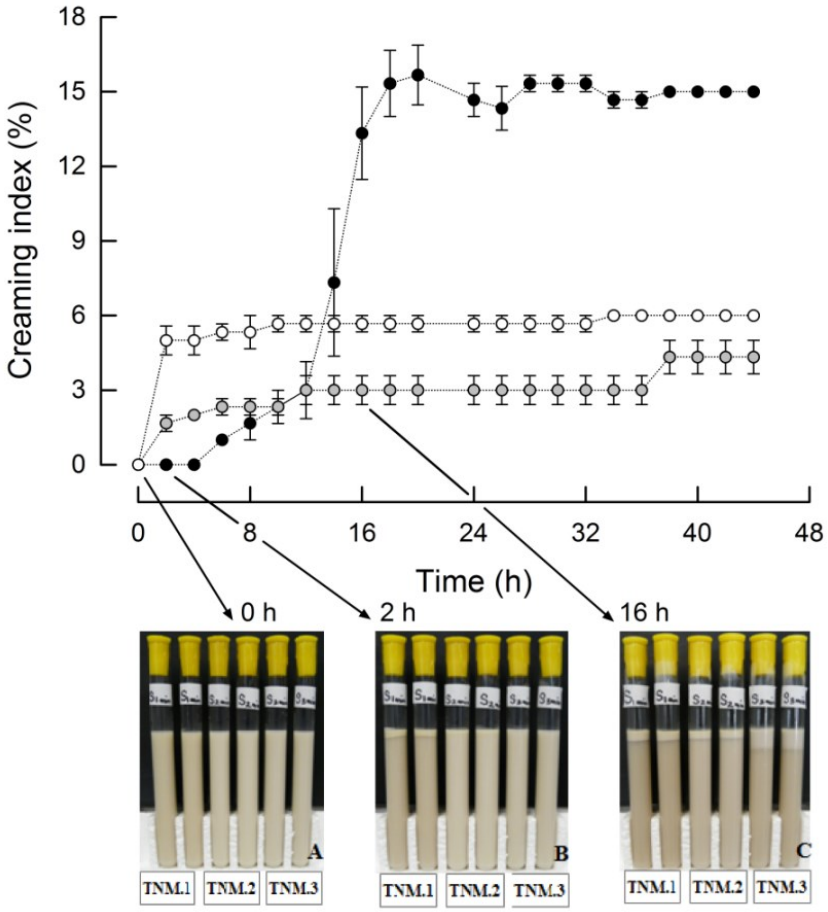


Figure 4.2: Effect of wet-milling intensity and storage on the creaming rate of tiger nut milk. Milk was extracted from pre-soaked tiger nuts comminuted with cutting mill for 1 min (TNM.1, open circles), 2 min (TNM.2, grey circles), or 3 min plus dispersion using an ultra turrax (TNM.3, dark circles) and stored at 50 °C. Photographs of milk were taken at 0 h, 2 h and 16 h.

Homogenisation of TNM probably enhanced the hydrophobic surface characteristics of the proteins through molecular unfolding and produced finer TNM particles, enhancing adsorption of proteins or polysaccharides on droplet molecules in the emulsion, thereby reducing the interfacial tension, which improved creaming in the dispersion (Dickinson, 2011; Ye, 2008).

In the second stage of phase separation (≥ 16 h), TNM.3 showed the highest formation of aggregates at the biological polymer-rich upper layer compared to TNM.1 and TNM.2. Aggregation of the polymers might be ascribed to an increase in interactions of the partially unfolded protein (due to the energy intake during milling and dispersion), the lipid and carbohydrates. Aggregation of polymers leading to phase separation in thermodynamically unstable colloids was ascribed to a stronger interaction in the biological polymers compared to the biological polymer-aqueous phase (Cheetangdee and Fukada, 2014; Dickinson et al., 1997; Doublier et al., 2000). Therefore, to produce TNM that shows improved resistance to aggregation and phase separation, further optimization of the tiger nut milling programme might be appropriate.

4.1.3.3 Colour stability of tiger nut milk

The intensity of milling during TNM extraction did not show any significant influence on the average lightness L^* of freshly prepared TNM, which was 66.2 ± 0.30 for TNM.1, 67.6 ± 0.29 for TNM.2 and 66.2 ± 0.21 for TNM.3. For comparison, commercial full fat cow milk showed a higher value of 76.9 ± 0.35. A higher range of lightness for horchata products has been reported in literature (Mosquera et al., 1996). The initial colour intensity increased from C* = 6.0 (TNM.1) to 6.7 (TNM.3), showing that milling intensity, which affects particle size distribution, contributes to the colour intensity. The initial h_{ab} ranged between 1.4° and 1.5°. Milling intensity showed different effects on L* of TNM products, which significantly decreased during 27 d storage, whereas, C* and h_{ab} remained constant. **Fig. 4.3** illustrates the effect of milling intensity and

storage on the lightness of tiger nut milk.

TNM.3 showed a lower decrease of lightness during storage probably due to inactivation of the enzymes such as polyphenol oxidase, which catalyse browning reactions by the higher milling intensity (Queiroz et al., 2008).

On one hand, enzymatic browning of milk or non-enzymatic processes, which can additionally lead to formation of Maillard products might cause a reduction in milk lightness with a corresponding increase in colour intensity (Cheetangdee and Fukada, 2014). On the other hand, protein-sugar conjugates from Maillard products have been reported to enhance colloidal stability through enhanced inter-facial droplet properties, and as potent antioxidants, which might lead to reduction in the rate of further lightness decrease at lower storage temperature (Cheetangdee and Fukada, 2014; Codina-Torrella et al., 2016).

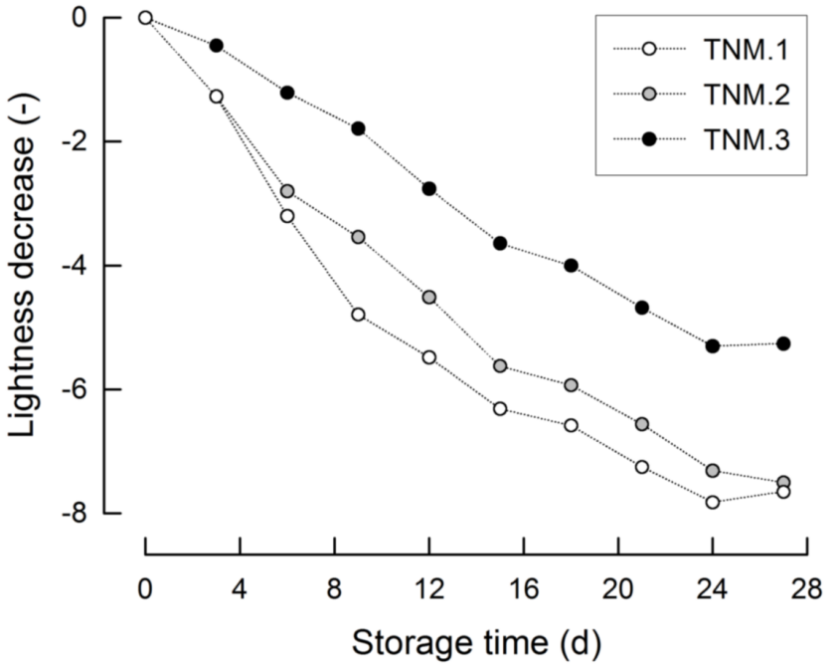


Figure 4.3: Effect of wet-milling intensity and storage period on lightness changes of tiger nut milk. Milk extracts from pre-soaked tiger nuts comminuted with cutting mill for 1 min (TNM.1), 2min (TNM.2), or 3 min plus dispersion using an ultra turrax (TNM.3).Storage conditions: 5 °C, 27 d

The rate of change in TNM attributes from the characteristic nutty, sweet and vanilla flavour to brown, earthy and bitter taste during storage was reduced through heat treatment of TNM (Mosquera et al., 1996).

In this study, optimization of the milling programme for soaked tiger nuts might have additional relevance for improving the lightness and for reducing browning of TNM during storage, which is important for enhancing the overall shelf life quality of tiger nut milk.

4.2 Stabilisation of tiger nut milk

4.2.1 Effects of enrichments on the stability of tiger nut milk

Tiger nut milk shows limited dispersion stability. For producing TNM with enhanced stability, and also suitable as substrate for generating lactic acid fermented products with acceptable properties, addition of various types and concentrations of proteins and/or hydrocolloids to tiger nut milk were investigated. **Table 4.4** shows the effects of the additives on phase separation (creamy phase, %) during storage of enriched TNM at ambient temperature for 7 d.

Table 4.4: Phase separation (creamy phase, %) of stabilized tiger nut milk samples stored at 20 °C for 7 d. Creamy phase of plain tiger nut milk: 5.38 %.

Hydrocolloids[1] (g /100 g)		Soy protein isolate (g /100 g)		Sodium caseinate (g /100 g)		
		+ 1.00	+ 2.00	+ 1.00	+ 2.00	+ 3.00
	+ 0	4.70	3.77	19.20	26.76	37.78
CMC	+ 0.20	0.00	0.00	0.00	0.00	0.00
	+ 0.40	0.00	0.00	0.00	0.00	0.00
Guar gum	+ 0.15	0.00	0.00	0.00	0.00	0.00
	+ 0.30	0.00	0.00	0.00	0.00	0.00
Xanthan gum	+ 0.05	0.00	3.86	0.00	0.00	2.94
	+ 0.10	0.00	0.00	2.94	3.48	3.42

[1]CMC; carboxymethylcellulose, TNM; tiger nut milk. Arithmetic means are based on triplicate determinations.

Plain TNM, which served as the reference sample, showed a creamy phase of 5.4 %. Enrichment of TNM with protein and/or hydrocolloids reduced creaming successfully, which can be attributed to the viscosity enhancing and/or emulsifying effects of the polymers on the continuous phase or droplets (Cao et al., 1990). Enrichment of TNM with only proteins was not effective to impede creaming, probably because proteins show slow unfolding rate, diffusion and alignment at oil-water interfaces, and lower viscosity enhancing effect than their mixtures with the hydrocolloids (Lam and Nickerson, 2013). It was observed that, when a higher amount of sodium caseinate was added, TNM creamy phase increased. This might be ascribed to depletion flocculation, whereby unadsorbed sodium casein molecules form casein submicelles, which creates an osmotic potential difference in the continuous phase, and which leads to droplet aggregation and creaming (Dickinson et al., 1997).

The effects of the additives on serum phase (solids depleted layer, %) formation during storage is shown in **Table 4.5**. Plain TNM exhibited the highest emulsion breakdown with a serum phase of 69.9 % during 7 d storage.

Table 4.5: Phase separation (serum phase, %) of stabilized tiger nut milk samples stored at 20 °C for 7 d. Serum phase of plain tiger nut milk: 69.9 %.

Hydrocolloids[1] (g /100 g)		Soy protein isolate (g /100 g)		Sodium caseinate (g /100 g)		
		+ 1.00	+ 2.00	+ 1.00	+ 2.00	+ 3.00
	+ 0	6.27	10.07	3.06	3.67	3.61
CMC	+ 0.20	57.30	36.16	67.27	53.98	27.27
	+ 0.40	39.69	21.38	37.27	22.12	16.31
Guar gum	+ 0.15	30.96	28.79	62.47	32.55	19.59
	+ 0.30	50.79	21.68	8.47	1.79	5.75
Xanthan gum	+ 0.05	57.90	46.86	33.67	8.53	6.49
	+ 0.10	5.58	13.00	0.00	0.00	0.00

[1]CMC: carboxymethyl cellulose. Arithmetic means are based on triplicate determinations.

Enrichment of TNM with proteins and/or hydrocolloids considerably decreased serum formation in TNM. Addition of only sodium caseinate to TNM resulted in a lower serum phase compared to that of soy protein isolate. Sodium caseinate with its lower molecular mass, flexible and open structure probably uncoiled more readily and elaborately interacted with TNM droplets (Lesmes et al., 2010) resulting in a more reduced phase separation than soy protein isolate, which has a larger molecular mass and more closely packed globular subunits, that limits its emulsifying effects (Chen et al., 2014).

The amount of serum phase formed by enriching TNM with protein and hydrocolloids depended on the concentration of the components, particularly, the hydrocolloids in the mixture. For example, addition of hydrocolloids at a low concentration mostly resulted in higher serum phase than mixtures without hydrocolloids or with higher hydrocolloids content. This phenomenon, whereby addition of low hydrocolloid content to a dispersion increases phase separation much faster than in their absence or at sufficiently high concentration was attributed to flocculation of the suspended particles both by adsorption at particle surface and by bridging flocculation (McClements, 2000).

TNM mixtures with sodium caseinate and xanthan (0.1 g/100 g) or with guar gum (0.30 g/100 g) revealed highly stable dispersions, and systems with xanthan showed the highest stability, whereas mixtures with carboxymethyl cellulose exhibited the lowest stability compared to that with xanthan and guar gum. Xanthan gum is known to have a high molecular mass and a rod-like structure, which favours a high volume ratio (Holzwarth, 1978). Hydrocolloids with high volume ratio show a low critical viscosity concentration and a high critical flocculation concentration that are relevant for reducing emulsion breakdown (McClements, 2000). Similarly, Cao et al. (1990) observed that caseinate-based emulsions with xanthan gum had higher stability than those from carboxymethyl cellulose at a comparable concentration and neutral pH, which was attributed to a higher low-shear viscosity of xanthan systems than those of carboxymethyl cellulose. The results show that TNM mixtures

containing sodium caseinate and 0.1 g/100 g xanthan or 0.30 g/100 g guar gum, have a high potential for generating dispersions with enhanced stability.

4.2.2 Effects of pH and temperature on the stability of enriched TNM

The stability of four tiger nut milk samples composed of 10 g tiger nut solids (TNM, reference) or 10 g tiger nut solids mixed with 0.1 g xanthan and 1.0 g sodium caseinate (1CnX) or 3.0 g sodium caseinate (3CnX) per 100 g substrate, and 10.0 g/100 g tiger nut solids, 0.3 g guar gum and 2.0 g/100 g sodium caseinate (2CnG) per 100 g substrate were analysed by using an analytical centrifuge.

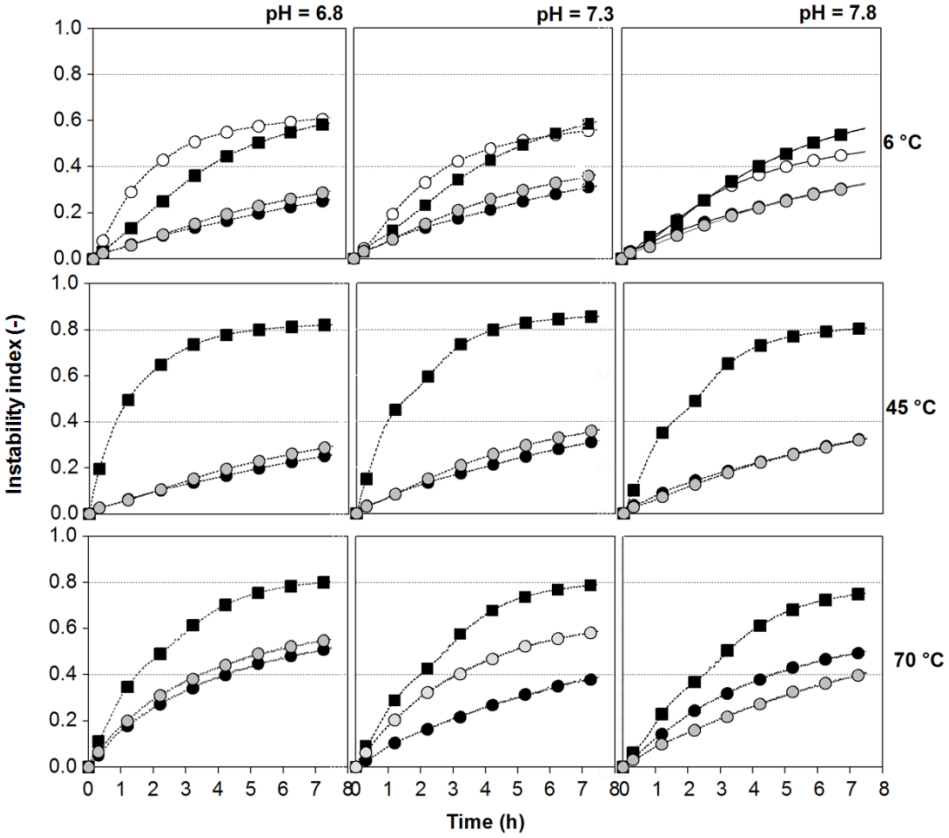


Figure 4.4: Effect of pH on the instability index of tiger nut milk enriched with protein and hydrocolloids at 6 °C, 45°C or 70°C. Open circles, 10.0 g/100 g tiger nut milk; grey and dark circles, 10.0 g tiger nut solids enriched with 0.1 g xanthan and 1.0 g or 3.0 g sodium caseinate (CnX) per 100 g sample, respectively; dark squares, 10.0 g tiger nut enriched with 0.3 g guar gum and 2.0 g sodium caseinate (CnG) per 100 g sample. Each curve represents the arithmetic mean of duplicate measurements. Measurement of instability index was continuous, only selected data points are displayed.

The effects of pH and temperature on the instability indices shown in **Fig. 4.4** imply that plain TNM, stored at 6 °C, exhibited low emulsion stability that improved when pH was increased. Increase in pH probably improved solubility of tiger nut proteins, which show a pI of 4.9, resulting in enhanced surface protein coverage, reduction in surface tension and improvement in TNM stability (Zayas, 1997).

Increase in pH of enriched TNM did not show any clear effect on the stability at 6 °C. However, it was clear that 2CnG had the lowest stability compared to the xanthan-based additives at 6 °C. The mixture with guar gum, which is a neutral polysaccharide, and sodium caseinate probably led to weaker polymer interactions than the mixture containing xanthan, which is anionic and which might form stronger electrostatic interaction with sodium caseinate, leading to a more stable membrane boundary at oil-water interface (Nor Hayati et al., 2016).

Treatment of the samples at 45 °C resulted in a clear decrease in the stability of TNM enriched with CnG, whilst the mixtures from CnX remained relatively unchanged. This means that enrichment with CnG resulted in emulsions with a more temperature-sensitive stability than that of CnX. Guar gum is known to be susceptible to heat treatment, leading to the detachment of galactose from the main mannose chain. This loss of branching and, consequently, diminished steric stabilization and network forming capability might account for the decrease in stability (Rao et al., 1981). Still at a higher temperature (45 °C), the effect of pH on the stability of the dispersions from CnX enrichment was not evident.

Generally, treatment of TNM-enriched samples at 70 °C for 10 min, a condition that could be used for pasteurization of TNM, caused a decrease in stability. Decrease in CnX stability might be caused by the reversible change from the xanthan helix structure to a disordered single-stranded chain at the higher temperature (Paoletti et al., 1983). In comparison to pH 6.8, the stability of 3CnX increased at pH 7.3, whilst that of 1CnX increased at pH 7.8. This shows that, at higher temperature, pH affects the colloidal stability of TNM

enriched with CnX. Dispersion stability of TNM enriched with 2CnG at 70 °C did not differ significantly from 45 °C, probably because it might have reached its lowest point of stability.

The results mean that the enrichment of TNM with sodium caseinate and xanthan gum might be relevant for enhancing the colloidal stability of TNM during cold storage or at temperature that is relevant for fermentation with lactic acid bacteria, but might show emulsion breakdown during regular pasteurization.

4.2.3 Effects of enrichments on the rheology of tiger nut milk

Plain TNM showed almost Newtonian flow at pH 7.3 and 7.8 **(Fig.4.5)**, whilst the corresponding continuous phase, which is the dispersion obtained after centrifugation, showed a pseudoplastic flow. The occurrence of partially dissolved solids (for example; starch granules, lipids, proteins) in plain TNM might have affected the flow properties. Enrichment of TNM with CnX or CnG caused a pronounced shear thinning, which was similar to that of their corresponding continuous phase.

Generally, all samples showed a higher viscosity than that of their corresponding continuous phase. Enrichment of TNM with CnX at, for example, pH 7.3, resulted in higher viscosity (1CnX, 240 ± 2.0 mPa.s; 3CnX, 270 ±1.0 mPa.s) than that of CnG (190 ± 3.0 mPa.s), which was on the other hand higher than that of plain TNM (3.0 ± 0.10 mPa.s) at shear rate of 1/s. The difference in viscosity might partly account for the disparity in stability (Tadros, 2009). pH did not significantly affect the viscosity of plain TNM or its mixtures at 6 °C.

The viscosity of TNM enriched with 2CnG at 45 °C was, at a pH 6.8 and shear rate of 1/s, approximately 113 mPa.s lower than that at 6 °C, which confirms the effect of temperature. On the other hand, TNM enriched with CnX did not show any significant effect in viscosity even when the pH or temperature was increased.

At 45 °C, the viscosity of TNM enriched with 2CnG was higher at pH 7.3 (110.0 ± 6.0 mPa.s) than at pH 6.8 (71.0 ± 3.0 mPa.s) or pH 7.8 (93.0 ± 3.0

mPa.s), but lower for the corresponding continuous phase (~ 43 mPa.s), meaning that pH probably affects solubility of the solids in TNM enriched with 2CnG. The results show that the stability of TNM enriched with 2CnG is more sensitive to changes in pH and temperature than that with CnX. There was no significant effect of heat treatment on the flow properties of TNM enriched with 3CnX.

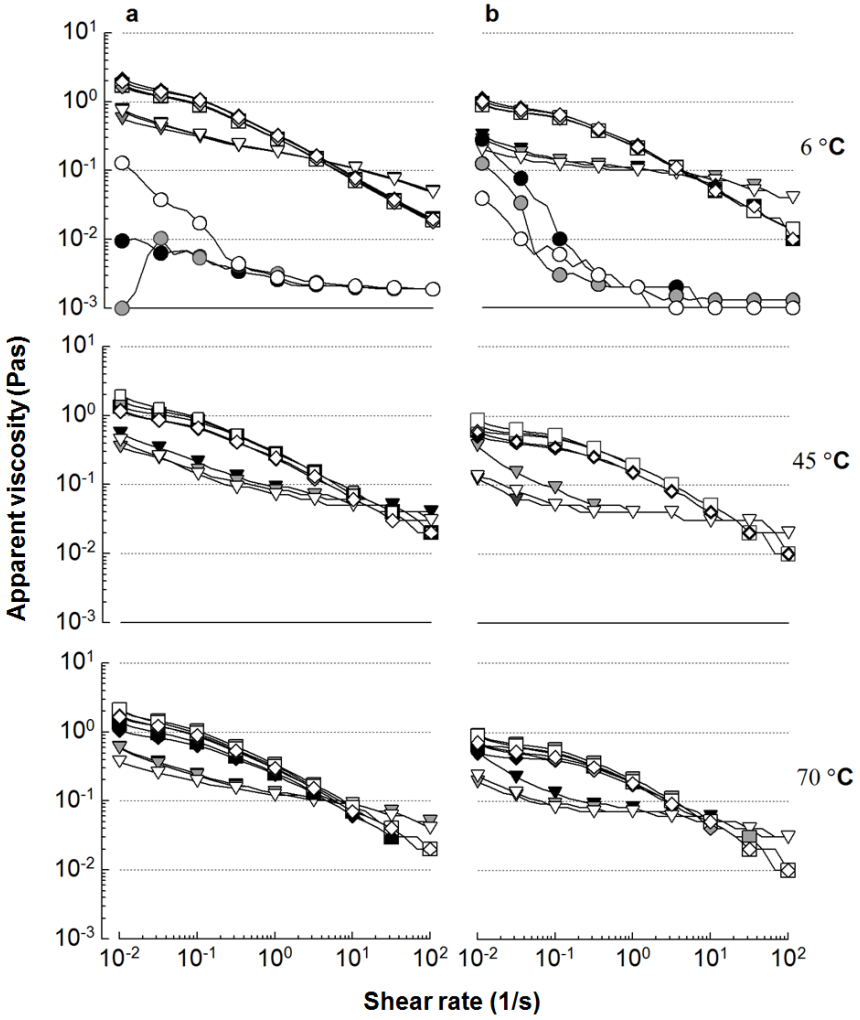


Figure 4.5: Effects of pH on the apparent viscosity of tiger nut milk (TNM) with different compositions at 6 °C, 45°C or 70°C. (a) without centrifugation, (b) after centrifugation; open, grey and dark shapes represent pH 6.8, 7.3 and 7.8, respectively; circles, 10. 0 g/100 g tiger nut milk; kites and squares, 10.0 g tiger nut solids enriched with 0.1 g xanthan and 1.0 g or 3.0 g sodium caseinate (CnX) per 100 g sample, respectively; triangles, 10.0 g tiger nut enriched with 0.3 g guar gum and 2.0 g sodium caseinate (CnG) per 100 g sample. Each curve represents the arithmetic mean of duplicate measurements. Measurement of viscosity was continuous, only selected data points are displayed.

However, at a higher temperature (70 °C), the viscosity at pH 7.8 (0.253 ± 0.018 Pas) was lower than at pH 6.8 (0.317 ± 0.01 Pas). The results suggest that adding CnX to TNM leads to dispersions that are more resistant against emulsion breakdown compared to CnG during pasteurisation.

4.3 Tiger nut protein extraction and characterisation

4.3.1 Protein extraction and fractionation

Tiger nuts and their milk extracts show low protein content but a high fraction of essential amino acids (Salau et al., 2013), which might be vital for the techno-functional exploitation of tiger nut protein, and as a source of food nutrients (Deng et al., 2011; Siebert, 2003). Tiger nut proteins were isolated and characterised for its potential application as a functional ingredient in tiger nut food products. **Table 4.6** shows the composition of the base tiger nuts, which had a protein content of 4.7 g/100 g dry mass. The isolation procedure resulted in a protein purity, which is the mass fraction of protein relative to the mass of the isolate, TNP, of 83.5 % and fat content of 14.2 %. Probably, the presence of lipid-protein complexes in tiger nuts contributed to the relatively low purity of the protein isolate. Similarly, Manamperi et al. (2011) reported interference of lipids during protein isolation from canola meal. Isolation of protein from potatoes, which show comparable content of carbohydrate and protein, resulted in a lower purity of 26 - 43 % (Maloney et al., 2012), and suggests that isolation of globular plant protein using ammonium sulphate precipitation might be useful for obtaining higher-purity proteins.

To analyze the characteristics, the tiger nut protein isolate was fractionated using various solvents to determine the soluble and insoluble compositions, to quantify their fractions, to determine their average molecular mass, and their susceptibility to thermal denaturation.

The fractional TNP contents were for glutelins, 47.5 %; albumins, 31.8 %; globulins, 4.7 % and prolamin, 3.8 % and refer to a ratio of approximately

13:8:1:1, respectively. Codinella et al. (2015) conducted *in situ* analysis of tiger nut proteins and reported that albumin contributed by approximately 82-92 %, whilst the remaining 3-7% was from glutelins, globulins and prolamins. Differences in the results is attributable to the procedures applied for protein fractionation and imply that, even though ammonium sulphate precipitation was effective for obtaining pure protein isolate, it was not able to isolate all the proteins in the tiger nut.

After fractionation of TNP, an insoluble fraction consisting of 12.2 g/100 g protein was obtained, suggesting that the solvents were only able to solubilize 87.8 g/100 g of the protein. The results show differences in the protein composition of tiger nut root tubers compared to other tuber crops such as potato, which contains 49 % albumin, 26 % globulin, 4 % prolamin and 9 % glutelin (Lisinska, 1989). Functionally, albumin might influence the foaming and emulsifying properties of proteins (Deng et al., 2011), whereas glutelin and globulin might contribute to gelling properties (Shabbir et al., 2011).

Processing of the tiger nut liquid extract into fermented systems has gained much interest. However, the physical or textural attributes of these products present a considerable challenge (Akoma et al., 2000; Wakil et al., 2014). Probably, the enrichment of the fermented systems with tiger nut protein might be relevant.

Table 4.6: Composition of base tiger nuts and tiger nut protein isolate (n=2)

Components	Protein	Fat	Ash	Total fibre	Carbohydrate
Base tiger nuts[a]	4.70 ± 0.02	21.15 ± 0.39	1.80 ± 0.01	24.64 ± 0.47	48.12 ± 0.47
Tiger nut protein isolate[b]	83.48 ± 0.35	14.24 ± 0.08	0.01 ± 0.00	-	2.28 ± 0.43

[a] g/100 g dry mass of tiger nuts

[b] g/100 g dry mass of tiger nut protein isolate

4.3.2 Molecular mass of tiger nut protein

The molecular mass of globular tiger nut proteins was determined using gel electrophoresis. **Fig 4.6** shows the electropherograms of TNP isolate and its globular fractions in non-reducing (a) and reducing (b) buffers. These electropherograms depict that aqueous TNP extracts comprise complex proteins as it shows a wide range of apparent molecular mass, m^m. **Fig 4.6a** shows that TNP isolate had 15 protein bands with a range of m^m from approximately 5.3 to 88 kDa. The more prominent fractions depict m^m of 16.1, 19.2, 21.2, 24.0, 26.5, 31.4, 33.1, 35.2 and 42.1 kDa. A cluster of proteins with bands at 8.7 - 5.3 kDa was additionally detected. Tiger nut protein molecular bands ranging from 18 to 78 KDa and a single band of 106 KDa was reported by Codinella et al. (2015). In this study, two sets of closely migrating groups of proteins of m^m = 16.1 - 19.2 kDa and m^m = 31.4 - 33.1 kDa were repeatedly identified. Protein doublets which show similar band density and thickness might portray similarities in structure, which is known to be characteristic for isoforms that were also reported in potato protein (Leubner-Metzger and Meins, 1999).

Each of the albumin and globulin fractions comprised 13 protein bands with m^m = 5.3 - 73 kDa. In the prolamin fraction, 10 protein bands in the range of m^m = 5.3 - 46 kDa were identified. At least three major protein bands with 5.3 - 30 kDa and 5.3 - 27 kDa were identified in the glutelin and the residual fraction of TNP, respectively. Furthermore, doublets that were identified in TNP with m^m 16.1 and 19.2 kDa re-occurred in the globulin and prolamin fractions only, whereas only one unit of the doublets was repeated in the albumin, glutelin or residual fraction, emphasizing the effects of solvents on tiger nut protein extractability. Moreover, TNP showed prominent clusters of protein bands (8.7 - 5.3 kDa), which re-occurred in all the protein fractions but for albumin, which showed two sharp doublets of 8.9 and 6.9 kDa. Protein bands that correspond to 29.7 and 35.2 kDa were unique to the albumin fraction, whereas a 73.3 kDa protein band was more prominent in the globulin fraction. The protein band that occurred with m^m = 46.2 kDa was unique to the prolamin fraction only. Glutelin

and the residual fractions were similar in the pattern of their bands, which means that, probably, the insoluble remnant is composed of glutelin. This observation implies that the mass composition of glutelin in the globular TNP might be greater than 47.5 g/100 g. Observation of these fractions under reducing conditions **(Fig 4.6b)** revealed higher numbers of polypeptide bands, especially in albumin, prolamin and glutelin fractions with a clearer distinction in their band patterns.

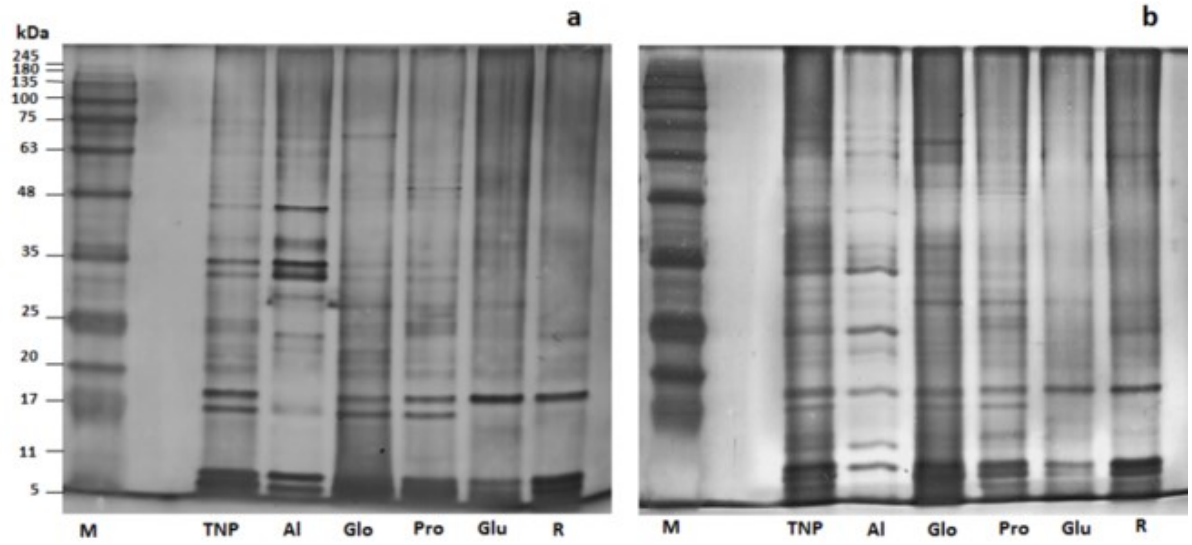


Figure 4.6: Electropherograms of tiger nut protein isolate and its fractions in non-reducing (a) and reducing conditions (b). TNP, Tiger nut protein; Al, Albumin; Glo, Globulin; Pro, Prolamin; Glu, Glutelin; R, Residue; M, Molecular mass marker.

4.3.3 Thermal denaturation of tiger nut protein

The results on thermal susceptibility and thermodynamic properties of the globular tiger nut protein isolate and its soluble fractions are shown in **Table 4.7**. Tiger nut protein isolates showed a broad thermal transition, probably because it is composed of proteins with a broad distribution of thermodynamic states (Bhambhani and Kumah, 2007). Thermal disruption of hydrophobic core peptides in aqueous milieu is known to predominantly account for the endothermic denaturation enthalpies (Urry et al., 1991). Therefore, the endothermic TNP thermograph in **Fig 4.7** might suggest that tiger nut proteins

contain hydrophobic peptides. Functionally, hydrophobicity of a protein is important for its emulsifying property (Zayas, 1997).

The conformational transition of the globular tiger nut protein isolate during thermal treatment showed an onset temperature (T_o) of 54.40 ± 0.97 °C. The denaturation temperature (T_d) derived from the peak of endothermic transition was 69.61 ± 0.56 °C, which is comparable to that reported for patatin of potato (Pots et al., 1998). The denaturation enthalpy, which was 5.43 ± 0.07 J/g, indicates the thermodynamic state of various molecular interactions that account for the native folding of the protein (Makhatadze, 2001).

A similar range of transition enthalpy of rice proteins was reported in the literature (Ju et al., 2001). When the thermal regime was repeated, no denaturation curve was observed (data not shown), which suggests that TNP undergoes irreversible heat denaturation (Gill et al., 2010). Thermal treatment of the protein fractions resulted in a broad range of T_o (42 - 64 °C) and T_d (50 - 78 °C) **(Table 4.7),** which might account for the broad thermal transition in total TNP. Prolamin showed the highest T_o and T_d, followed by albumin, globulin and, finally, glutelin. Albumin showed two denaturation peaks that correspond to 66.67 °C and 76.36 °C**.**

Table 4.7: Thermodynamic properties of globular tiger nut proteins and its fractions (n=3).

Protein	Thermal denaturation parameters		
	Onset T_o (°C)	Denaturation temperature T_d (°C)	Enthalpy ΔH (J/g)
Tiger nut protein	54.40 ± 0.97	69.61 ± 0.56	5.43 ± 0.07
Albumin	54.37 ± 0.50	66.67 ± 0.08	6.27 ± 0.19
	-	76.36 ± 0.72	2.15 ± 0.11
Globulin	48.30 ± 0.13	63.16 ± 0.79	1.73 ± 0.12
Prolamin	64.53 ± 0.41	78.19 ± 0.46	0.8 ± 0.39
Glutelin	42.02 ± 1.80	50.27 ± 0.85	1.27 ± 0.07
Glutelin -NaOH*	64.56 ± 0.66	81.94 ± 0.34	1.01 ± 0.01

* Protein remnant after fractionation using all solvents except NaOH. All values are based on at least triplicate determinations.

Based on the T_o and T_d values, prolamin and glutelin might show the least and highest susceptibility to thermal denaturation, respectively. Furthermore, the thermal response of prolamin showed the least transition energy, whilst albumin revealed the highest energy, followed by globulin and glutelin. It is reported that a high enthalpy of transition usually correlates with a large area of internally folded hydrophobic proteins (Demarest and Verna, 2015; Urry et al., 1991). This implies that albumin might show a higher surface area of buried hydrophobic groups than prolamin in its native state. Thus, thermal denaturation of proteins, which leads to protein unfolding and exposure of hydrophobic groups and, possibly, enhancement of the hydrophobic characteristics of proteins might be more effective in albumin-rich TNP suspensions.

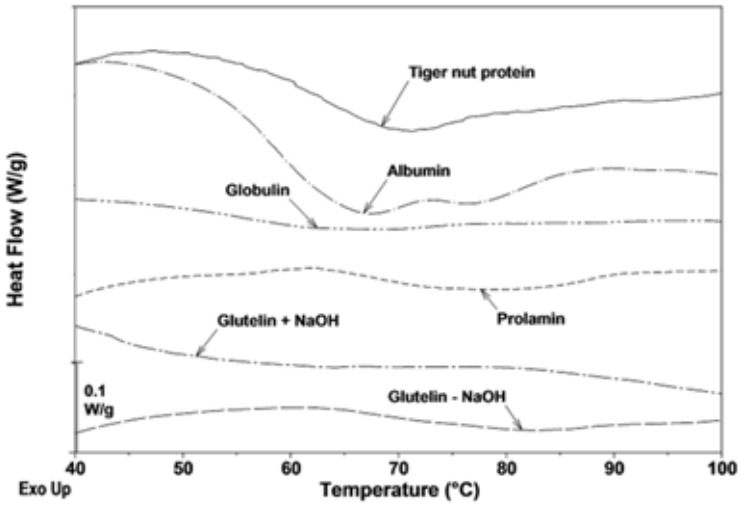


Figure 4.7: Base-line corrected denaturation thermographs of globular tiger nut protein and its fractions in 0.1 mol/L phosphate buffer, pH 7.02, concentration: 20 % w/v, scan rate: 10 K/min. Glutelin + NaOH and Glutelin-NaOH are Osborne fractions obtained with or without NaOH extraction, respectively.Fig 4.7 Base-line corrected denaturation thermographs of globular tiger nut protein and its fractions in 0.1 mol/L phosphate buffer, pH 7.02, concentration: 20 % w/v, scan rate: 10 K/min. Glutelin + NaOH and Glutelin-NaOH are Osborne fractions obtained with or without NaOH extraction, respectively.

The use of NaOH for extracting the glutelin fraction resulted in a lowering effect on the T_o and T_d during thermal treatment. The glutelin fraction without

NaOH treatment revealed a T_o and T_d of 64.56 °C and 81.94 °C, respectively **(Table 4.7),** opposed to 42.02 °C and 50.27 °C in glutelin plus NaOH extraction. Based on the assumption that the residual fraction is composed of glutelin **(Fig 4.6),** the decrease in the thermal stability of glutelin might be attributed to the effect of the NaOH extraction caused by alkaline hydrolysis of peptide groups (Warner, 1942). This observation might confirm the hypothesis on the effect of alkaline extraction on the thermal properties of canola protein (Manamperi et al., 2011).

Processing of most tiger nut products involve thermal treatment steps to ensure microbiological safety and modification of the sensory and techno-functional quality of the product. Knowledge on the thermal properties of tiger nut proteins is fundamental for optimising processing parameters, particularly when the functional quality of such products depends on the physico-chemical characteristics of the protein.

4.3.4 Isoelectric point of tiger nut proteins

In food systems, isoionic properties are relevant because, through acidification or alkalinisation, the proteins might flocculate, precipitate or coagulate to form solid aggregates that may result in the alteration of food structure (Vaclavik and Christian, 2014).

Fig 4.8 shows that the isoelectric point, pI of the tiger nut protein isolate was 4.9. Pearsall and Ewing (1924) reported a similar range of pI for plant proteins such as those from wheat (5.2) and potato (4.3 - 4.5). The pI range of TNP isolate suggests that globular tiger nut proteins may have a higher contribution from carboxylate groups of amino acids as reported by Henriksson et al (1995); a high arginine content of tiger nut protein was also reported by Bosch (2005). The pI of TNP suggests that these proteins might, through isoionization, show minimal solubility at pH 4.9, which can lead to changes in the functional properties of the protein (Bryan, 1978).

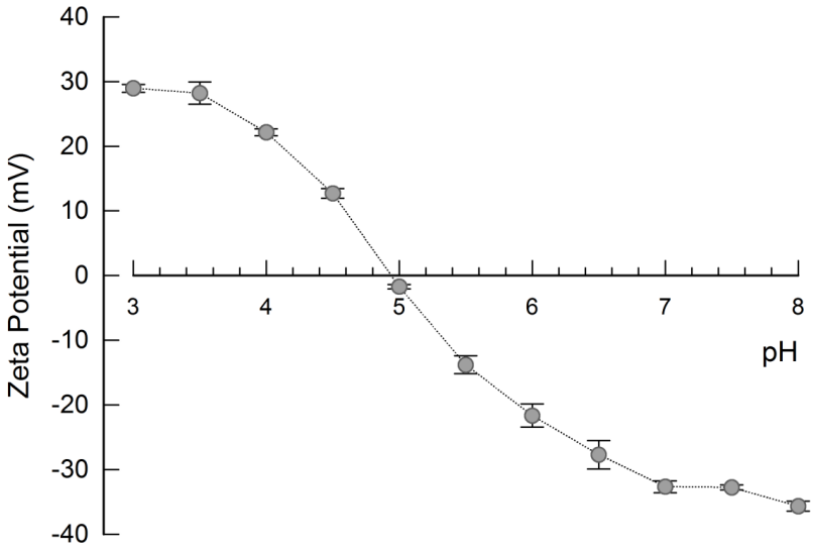


Figure 4.8: Zeta potential of globular tiger nut protein as a function of pH. Error bars are based on triplicate determinations

Fundamentally, these results might be relevant for the techno-functional optimization of food products from tiger nuts.

4.4 Properties of fermented tiger nut milk enriched with proteins

4.4.1 Acidification and gel formation during fermentation

Tiger nuts milk (TNM) that was enriched with proteins or in combination with hydrocolloids to enhance the dispersion stability was investigated for producing lactic acid fermented TNM products with improved physico-chemical and acceptable sensory properties. The effect of substrate enrichment on the pH reduction during microbial fermentation is shown in **Fig. 4.9,** which depicts that the acidification profiles of both plain and enriched systems followed profiles that are similar to that of milk fermentation (Soukoulis et al., 2007). The addition of tiger nut protein reduced pH of the plain TNM from 6.23 to 5.98, which was probably because tiger nut proteins comprise more acidic amino acids than basic amino acids (Aremo et al., 2015). On the contrary, addition of sodium caseinate increased initial pH of TNM to 6.40. The Gompertz lag time parameter λ for plain TNM and systems enriched with dairy proteins was

approximately 1.6 h. However, when TNM was enriched with tiger nut protein, a significantly higher lag time (λ = 3.2 h) was recorded. Öztürk & Öner (1999) reported a similar effect of an initially reduced pH on lactic acid bacteria lag time during lactic acid fermentation. Plain TNM and TNM enriched with whey protein isolates showed a range of maximum pH reduction rate of 0.43/h – 0.45/h. This was lower than μ ~ 0.65/h – 0.70/h that was reported for cow milk yogurt (De Brabandere and De Baerdemaeker, 1999). The enrichment of TNM with 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 type of fermentable sugars in TNM might partly account for the general decrease in the pH reduction rate. It was observed in **Table 4.8** that, apart from traces of fructose, approximately 6 g/100 g sucrose serves as carbohydrate source, which was reported to have delayed growth of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* (Amoroso et al., 1989).

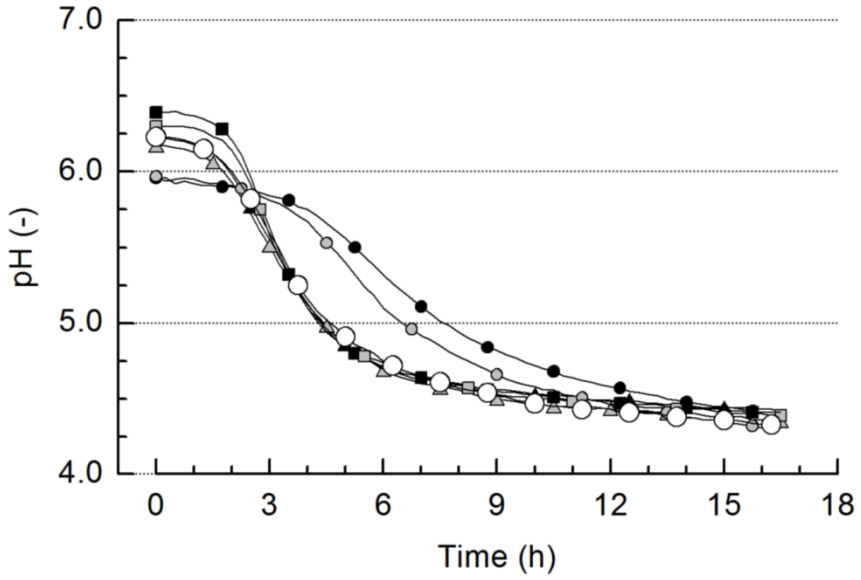


Figure 4.9: Acidification profiles during fermentation of plain (TNM) or enriched tiger nut milk. Open circles, plain tiger nut milk; grey and dark circles, TNM enriched with 1.0 g or 2.0 g/100 g tiger nut protein, respectively; grey or dark squares, TNM enriched with 0.1 g/100 g xanthan and1.0 g or 3.0 g/100 g sodium caseinate respectively; grey and dark triangles, TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g/100 g whey protein isolate, respectively. pH measurement was continuous, only selected data points are displayed.

These results indicate that the type of protein influences the time to reach a specific pH during fermentation of TNM. A pH of 4.4, which is comparable to that of dairy yogurt, was achieved for all systems after approximately 15 h fermentation. Systems enriched with tiger nut proteins remained liquid during fermentation as opposed to dairy protein enriched systems, which formed semi-solid gel products. This allowed the monitoring of gel formation in dairy-enriched systems using small amplitude oscillatory shear during fermentation, with the results being shown in **Fig. 4.10**. The addition of a higher concentration of both types of dairy protein to TNM resulted in a pH of approximately 0.2 to 0.3 units higher at gelation onset, which refers to the point when a storage modulus (G') of 1 Pa was achieved (Jacob et al., 2011). Consequently, a shorter time was required for the onset of gelation because of the similar pH reduction rate.

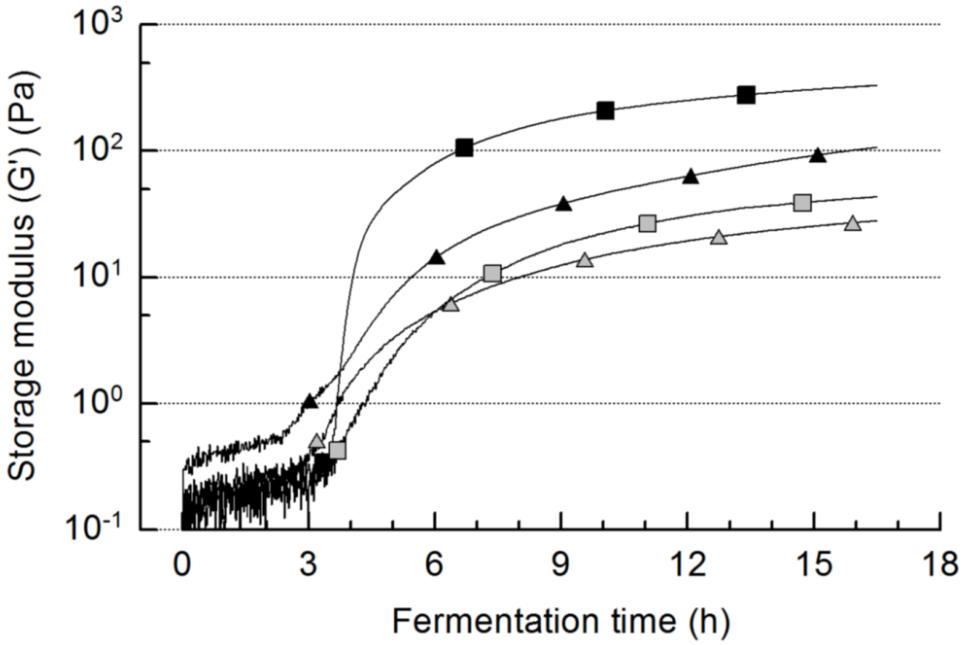


Figure 4.10: Development of gel stiffness during acidification of enriched tiger nut milk. Grey or dark squares, TNM enriched with 0.1 g/100 g xanthan and1.0 g or 3.0 g/100 g sodium caseinate respectively; grey and dark triangles, TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g/100 g whey protein isolate, respectively. Stiffness measurement was continuous, only selected data points are displayed. Each curve represents the arithmetic mean of triplicate measurements.

Moreover, pH at gelation onset of TNM enriched with dairy proteins was 0.04 to 0.52 units lower than the range reported for cow milk (Lee and Lucey, 2006).

Fig 4.10 shows that the amount of added proteins significantly influenced gel stiffness, and TNM systems enriched with sodium casein resulted in stiffer gels. Fermented systems from TNM enrichments with higher concentrations of casein showed a storage modulus of approximately 300 Pa, which is comparable to that of set yogurt from skimmed cow milk with 12 g/100 g dry matter (Jaros et al., 2002). It is well known that heat-denatured whey proteins form gels mainly through disulphide interactions whilst casein forms gels through hydrophobic, van der Waals, hydrogen bonding and electrostatic interactions (Alting et al., 2000; Dalgleish, 1997). Therefore, the observed variations in gel stiffness in enriched fermented TNM might be ascribed to differences in rearrangement mechanisms during gelation. The reported gel stiffness of only sodium caseinate or whey protein gels was higher than that of acid gels from TNM enriched with dairy proteins at comparable concentration in this study (Alting et al., 2004; Lucey et al., 1997). Reasons for the disparity include effects of substrate composition, the rate of acidification and fermentation temperature (Lucey et al., 1997; Nguyen et al., 2014). Fundamentally, the broad variation of gel stiffness achieved by varying type and content of dairy proteins is functionally beneficial for modulating the textural properties of the tiger nut product.

4.4.2 Microbiological properties of fermented enriched tiger nut milk

Plain tiger nut milk allowed the development of lactic acid bacteria with only a minor influence. Analysis of lactic acid bacteria viable counts in all fermented fresh products resulted in *S. thermophilus* range from 2.8 to 5.6 x 10^8 cfu/g, which was higher than that of *L. delbrueckii* ssp. *bulgaricus* (2.4 to 5.0 x 10^6 cfu/g). The differences in the lactic acid bacteria is probably due to the initial composition of the commercial starter, which was determined to be 1:1.7 *L. delbrueckii* ssp. *bulgaricus and S. thermophilus*, respectively. These viable counts were slightly higher than the reported values from lactic acid

fermentation of TNM using wild starter cultures (Wakil et al., 2014). The viable counts for both *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* from sodium caseinate or whey protein isolate enrichments were slightly higher than those from the plain fermented TNM and TNM enriched with tiger nut protein, which suggests that dairy protein enrichment might be more suitable for supporting starter development in fermented TNM.

4.4.3 Physico-chemical properties of fermented enriched tiger nut milk

The composition of tiger nuts is relevant for determining the nutrient content of TNM, which serves as fermenting substrate for starter culture development. Dry matter-related analysis of the base tiger nut composition resulted in 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. The content of protein, ash and carbohydrate were slightly higher than that obtained in the previous batch of tiger nuts in **Table 4.6,** probably because of the different harvest period. Plain TNM used as the reference fermentation substrate had total solids composition of 10.40 g/100 g, and comprised 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 4.8** shows that sucrose is the main fermentable carbohydrate in the substrate. Generally, the sugar content in tiger nuts can widely vary based on the cultivar and the ripening stage (Coskuner et al., 2002).

Analysis of the sugar composition of the plain TNM showed a higher fructose content in the fermented system than in the unfermented system, presumably because of microbial sucrose hydrolysis and the further utilization by the starter microorganisms, which prefer glucose to fructose for growth (Amoroso et al., 1989). The content of lactic acid that was generated because of starter culture development was higher than that reported by Akoma et al. (2000).

Enrichment of TNM with proteins significantly increased the lactic acid content, which was higher in the fermented products with higher protein content.

The pH at the end of the fermentation time was similar, even though the products showed differences in lactic acid content, which is clearly attributable to the buffering capacity especially of the milk proteins. The results show that increased protein content in fermented TNM improves yogurt starter development, which might enhance the production of microbial by-products such as lactic acids as flavor active compounds (Sadler and Murphy, 2014).

Table 4.8: 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	Lactobacill	Sucrose	Fructose	
TNM	-	-	6.01^{a} ± 0.02	0.07^{a} ± 0.01	-
Fermented TNM	340.0^{a} ± 34.0	2.40^{a} ± 0.47	5.58^{bd} ± 0.10	0.14^{b} ± 0.01	0.54^{a} ± 0.00
1TNP	280.0^{a} ± 52.0	2.50^{a} ± 0.36	5.92^{b} ± 0.02	0.18^{c} ± 0.02	0.69^{b} ± 0.00
2TNP	358.0^{a} ± 26.0	2.95^{a} ± 0.19	5.70^{c} ± 0.06	0.15^{b} ± 0.01	0.73^{c} ± 0.01
1CnX	380.0^{a} ± 49.0	3.25^{b} ± 0.21	5.49^{d} ± 0.01	0.13^{b} ± 0.01	0.76^{d} ± 0.00
3CnX	553.0^{c} ± 36.0	5.08^{d} ± 0.44	5.31^{e} ± 0.06	0.13^{b} ± 0.00	1.08^{f} ± 0.00
1WPX	460.0^{b}± 47.0	3.78^{c} ± 0.31	5.28^{e} ± 0.63	0.14^{b} ± 0.02	0.70^{b} ± 0.01
3WPX	498.0^{bc} ± 41.0	4.88^{cd}± 0.82	4.87^{f} ± 0.65	0.13^{b} ± 0.02	0.88^{e} ± 0.01

[a] TNM, tiger nut milk; 1TNP and 2TNP represent TNM enriched with 1.0 g or 2.0 g/100 g tiger nut protein, 1CnX and 3CnX are TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g /100 g sodium caseinate, respectively; 1WPX and 3WPX are TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g/100 g whey protein isolate, respectively.

[b] Results are arithmetic mean ± standard deviation from (n=3) determinations. Values in the same column marked by a different superscript differ significantly at $P < 0.05$.

Analysis of the effects of lactic acid fermentation on phase separation of TNM and the enriched substrate shown in **Fig. 4.11** revealed that, during fermentation, TNM formed a semi-transparent lower liquid part and an opaque upper part. It was shown that differences in solute properties such as particle size, molecular shape and charge contribute to phase separation in dispersions (de Jong and van de Velde, 2007). Emulsifying proteins are known to form thin membranes around fat droplets in emulsions, leading to lower interfacial

energy and surface tension, and a more stable emulsion.

During fermentation, phase separation might increase in low protein emulsions such as TNM because of the decrease in pH, which affects protein solubility and distorts the protein film, and facilitates oil droplet contact leading to flocculation (Kinsella, 1979).

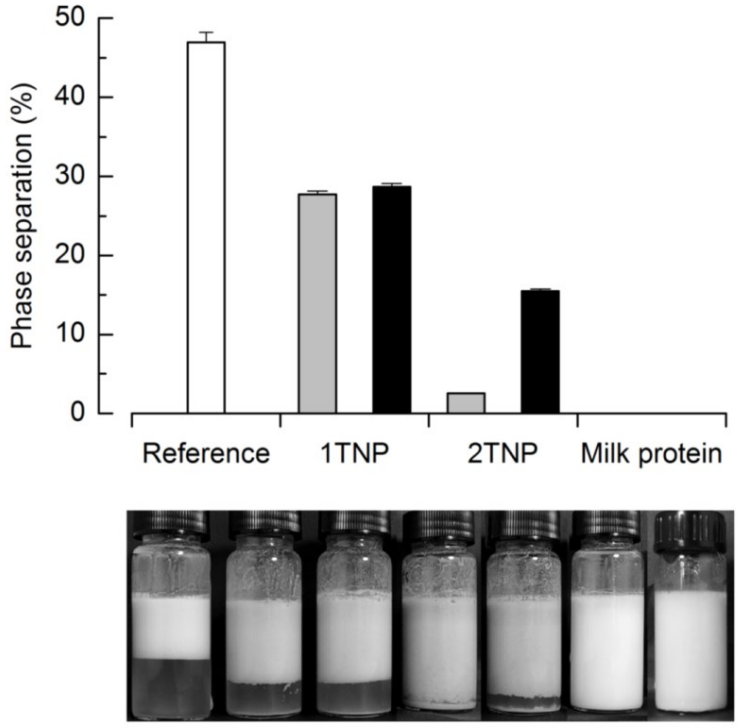


Figure 4.11: Phase separation, expressed as percentage transparent lower layer, of fermented tiger nut milk (TNM) with different composition. 1TNP and 2TNP, tiger nut milk was enriched with 1.0 g or 2.0 g/100 g tiger nut protein. Black bars indicate that tiger nut protein was heated to 85 °C for 10 min prior to fermentation. Samples "Milk protein" contained 1.0 g/100 g sodium caseinate or whey protein isolate.

Fig 4.11 depicts that the increase in tiger nut protein content resulted in a more stable fermented TNM dispersion, probably because of enhancement in the interfacial protein membrane and a consequent decrease of the surface tension (Sun and Gunasekaran, 2009). Denaturation of tiger nut proteins by thermal treatment was not appropriate to inhibit phase separation. However, inclusion of CnX and WPX resulted in a more stable system during fermentation. It is known that whey proteins and sodium caseinate exhibit emulsifying properties,

which probably improved the emulsion stability (Amine et al., 2014). Furthermore, milk proteins with their gelation properties may have impeded phase separation by allowing the formation of structures which entrap the liquid phase in a three-dimensional network (de Jong et al., 2009).

Results on the flow properties show that all TNM fermented systems exhibited shear thinning behavior (**Fig. 4.12).** It was observed that fermented TNM systems with added TNP exhibited a lower viscosity than the plain fermented TNM at shear rate of 1.0/s, which was probably due to surface tension reduction through protein coating of TNM lipid droplets, which reduces the lipid packing fraction and their contribution to product viscosity (Zayas, 1997).

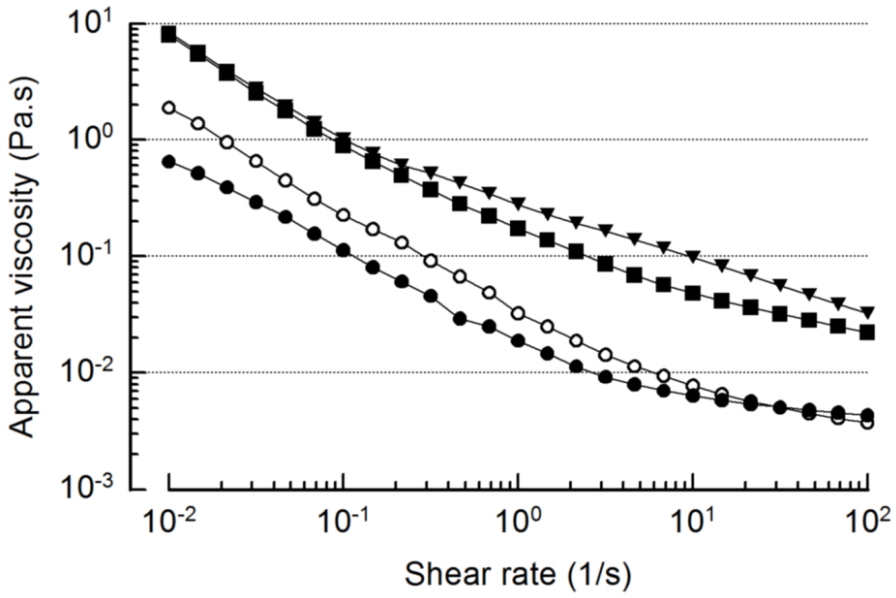


Figure 4.12: Apparent viscosity of fermented tiger nut milk (TNM) with different composition. Open circles, plain tiger nut milk ; dark circles, TNM enriched with 2.0 g/100 g tiger nut protein ; dark triangles, TNM enriched with 3 g/100 g sodium caseinate and 0.1 g g/100 g xanthan; dark squares, TNM 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

Enrichment of TNM with 3CnX or 3WPX resulted in increased viscosity of the fermented system, which might originate from the protein gels or soluble protein aggregates from the heat denaturation treatment. Akalın et al (2012)

reported a similar increase in yogurt viscosity on addition of sodium caseinate or whey protein concentrate.

Increasing the shear rate applied to the samples resulted in a more pronounced viscosity difference, which suggests that WPX systems have a higher shear resistance and viscosity than CnX systems. It was observed that smooth texture of the fermented enriched TNM products improved after homogenization, although the mixing process, which was necessary to dissolve lumps, may have attenuated the effects of intact gels on the viscosity. The results show that enrichment of TNM with dairy proteins before fermentation leads to products with improved uniformity and more viscous characteristics.

Enriched TNM samples, which formed gels during fermentation, were analyzed for gel firmness and susceptibility to syneresis. It was observed that the firmness of gels from TNM enriched with dairy proteins was higher when protein enrichment increased, probably because of a denser protein-protein network that was generated during gelation **(Table 4.9**).

Table 4.9: Effect of enrichment with dairy proteins on gel properties and whey drainage 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 and 3CnX indicate TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g/100 g sodium caseinate, respectively. 1WPX and 3WPX are TNM enriched with 0.1 g/100 g xanthan and 1.0 g or 3.0 g/100 g whey protein isolate.

[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 $P < 0.05$.

Furthermore, TNM enrichment with sodium caseinate resulted in higher gel firmness of the fermented system than when whey proteins were used. This observation is similar to results from regular yogurt fortified with either sodium caseinate or whey proteins (Modler et al., 1983; Rohm and Schmidt, 1993).

Susceptibility of fermented enriched TNM products to syneresis was determined to assess the physical quality of the protein gels (Rohm and Kovac, 1994). The results in **Table 4.9** clearly show that syneresis was more reduced the more protein was added to TNM, and that the enrichment with sodium caseinate resulted in a more pronounced effect. Gel firmness and syneresis are known to be inversely related as long as they are generated by differences in protein content (Jaros et al., 2002). However, this inverse relationship might differ from that of protein enrichment, if comparisons are made between yogurts produced by different starter cultures (Rohm and Kovac, 1994).

4.4.4 Sensory properties of fermented tiger nut milk products

After fermentation, flash profiling was used to evaluate the sensory attributes and to assess the impact of the enrichments on TNM products. The consensus matrix was evaluated using principal component analysis. The results show that dimension 1 and dimension 2 accounted for 83.59 % of the total variance and an additional 11.14 % by dimension 3. In addition, the coordinates of the two identical samples clustered together in the same quadrant (**Fig. 4.13**), which is a strong indicator for the discriminative reliability of the test (Díaz-Maroto et al., 2002). Panelists generated 34 different descriptors for the products indicating that protein enrichment leads to the development of a broad spectrum of sensory properties in fermented TNM. The most emerging descriptors used for fermented TNM were *sweet*, *watery*, *brown*, *almond*, *phase separation*, *woody* and *nutty*. These attributes are coherent with the physical properties related to phase separation and viscosity of the fermented plain TNM. Those for 1WPX were *fruity*, *sweet* and *oil droplets on surface* whilst 3WPX were *fruity*, *banana*, *viscous*, *earthy*, *sour*, *adstringent*, *creamy*, *lightness* and *homogenous*. Frequently used descriptors for 1CnX were *musty*, *foamy*, *oil droplets on*

surface and *nutty*, and for 3CnX were *graininess*, *viscous*, *balanced*, *sour*, *foamy* and *mushroom*. When lower protein concentrations were used, TNM products commonly showed *oil droplets on surface* whilst enrichment with higher protein concentrations resulted in *viscous* and *sour* products. The results show that, each product generated key sensory attributes that can be exploited for producing fermented tiger nut milk with acceptable sensory and textural properties.

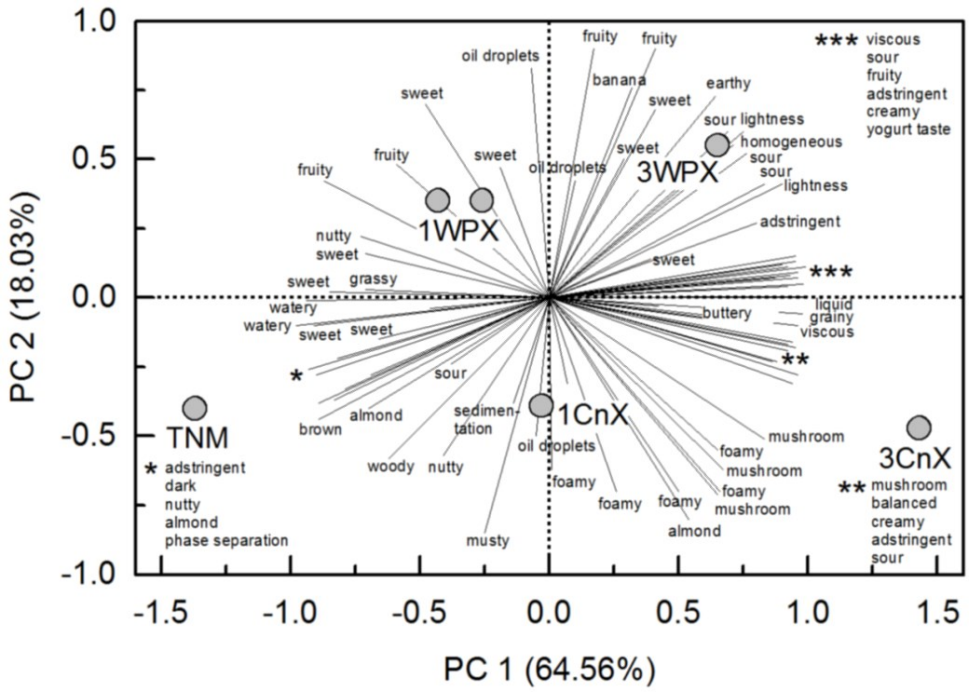


Figure 4.13: GPA group average plots for descriptors of fermented tiger nut milk (TNM) with different compositions. In the consensus space are CnX, enrichment with sodium caseinate and 0.1 g/100 g xanthan; WPX, enrichment with whey protein isolate and 0.1 g/100 g xanthan. Numbers in the code refer to addition of sodium caseinate or whey proteins isolate (g/100 g). Plots are based on duplicate experiments

4.5 Microbial transglutaminase and fermented tiger nut milk property

4.5.1 Effects on tiger nut milk fermentation

Fermentation of plain TNM or TNM enriched with mTGase treated or untreated protein resulted in pH profiles (**Fig. 4.14**) comparable to that in **Fig. 4.9** and those of milk fermentation (Soukoulis et al., 2007). In this study, fermentation

of plain TNM (dry matter, 10.20 ± 0.4 g/100 g; protein content, 0.89 ± 0.02 g/100 g) resulted in an initial pH, pH_0 = 6.35 ± 0.09, and Gompertz maximum rate μ of pH reduction, μ = 0.65 ± 0.11/h, which were slightly higher than those in **Fig. 4.9.** This is probably because of the differences in tiger nut protein content, which varies at different harvest periods (Asante et al., 2014a), and of its higher content of acidic amino acids than basic amino acids (Aremo et al., 2015), that affects pH during fermentation of TNM.

The Gompertz equation lag times λ and maximum rate μ of pH reduction derived from the fermentation of TNM enriched with xanthan and untreated casein (3CnX) was λ = 1.58 ± 0.07 h and μ = 0.69 ± 0.04/h whilst the system from untreated whey protein (3WPX) was λ = 1.24 ± 0.21 h and μ = 0.79 ± 0.07/h.

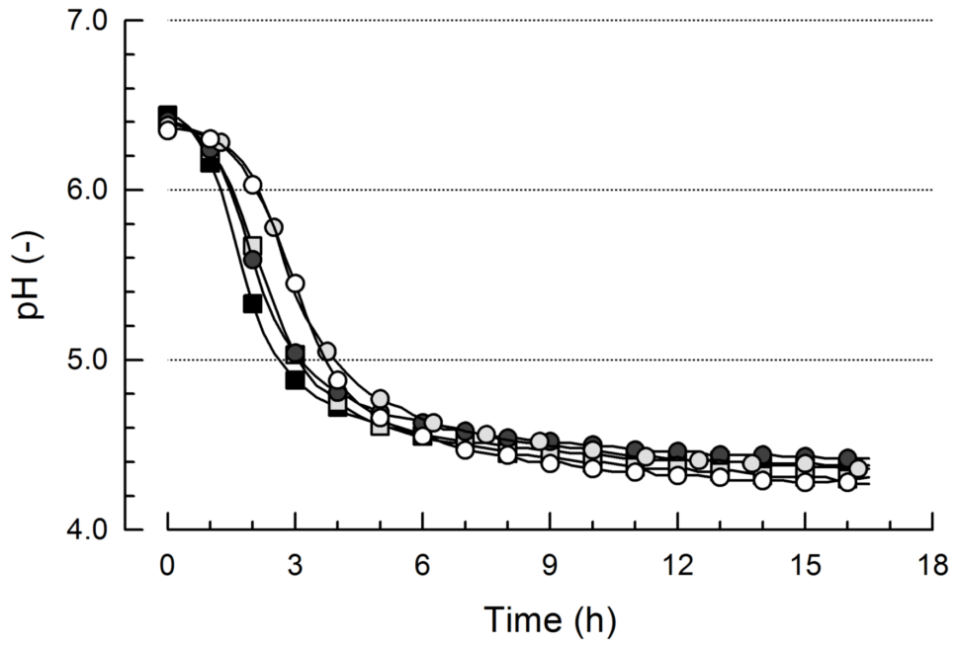


Figure 4.14: Acidification profiles during fermentation of plain (TNM) or enriched tiger nut milk. Open circles, plain tiger nut milk; grey and black circles, TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g sodium caseinate, respectively. Grey and black squares represent TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g whey protein isolate, respectively. Only selected points from continuous pH measurements are displayed.

The addition of mTGase cross-linked casein (3CnXe) or whey protein (3WPXe) resulted in lower microbial lag times of λ ~ 0.95 ± 0.18 h and λ ~ 0.66 ± 0.09 h, respectively, but increased the maximum rate of pH reduction by approximately

0.1/h in both systems during TNM fermentation (**Fig.4.14**). It was reported that mTGase treatment promotes the initial growth of *S. thermophilus* during milk fermentation, which probably accounts for the reduction in microbial lag time (Neve et al., 2001).

A well-known fact is that *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* show protocooperation, where *S. thermophilus* with its little or no proteolytic activity initiates fermentation until pH ~ 5.7, and produces formate, pyruvate, folate, CO_2 and long chain fatty acids. These metabolites stimulate the growth of *L. delbrueckii* ssp. *bulgaricus*, which generates oligopeptides and amino acids that in turn stimulate the growth of *S. thermophilus* (Baglio, 2014; Hill and Kethireddipalli, 2013).

Fig 4.15 confirms that mTGase treatment of the proteins resulted in larger molecular weight polymers, which are known to be covalently cross-linked (Jaros et al., 2006a). The time for LAB fermentation was reportedly prolonged when milk was pre-treated with mTGase (Lorenzen et al., 2002), and was attributed to a decrease in the growth of lactobacilli, assumed to be caused by a limitation in accessible low molecular weight peptides because of the protein cross-linking (Faergemand et al., 1999). Similarly, even though λ decreased and μ increased in products enriched with mTGase treated proteins in this study, a longer fermentation time was required to reach pH 4.5 when mTGase treated casein (3CnXe = 9.8 ± 0.1 h) was used for TNM enrichment than that with the untreated counterpart (3CnX = 8.8 ± 0.1 h). On the other hand, the fermentation time required to reach pH 4.5 in products enriched with 3WPXe, which was 7.2 ± 0.1 h, did not differ significantly from that of the untreated counterpart (3WPX =7.3 ± 0.3 h). This means that the effect of mTGase cross-linking of proteins on the time to reach a specific pH during lactic acid fermentation of milk might be dependent on the nature of milk proteins

(Bönisch et al., 2004). After 15 h fermentation, the pH of plain TNM, 3WPX and 3CnX systems decreased to pH ~ 4.27 ± 0.05, 4.30 ± 0.03 and 4.36 ± 0.03, respectively. The pH of fermented products with mTGase treated proteins was not significantly different from the untreated counterparts, although they showed a trend of slightly higher pH.

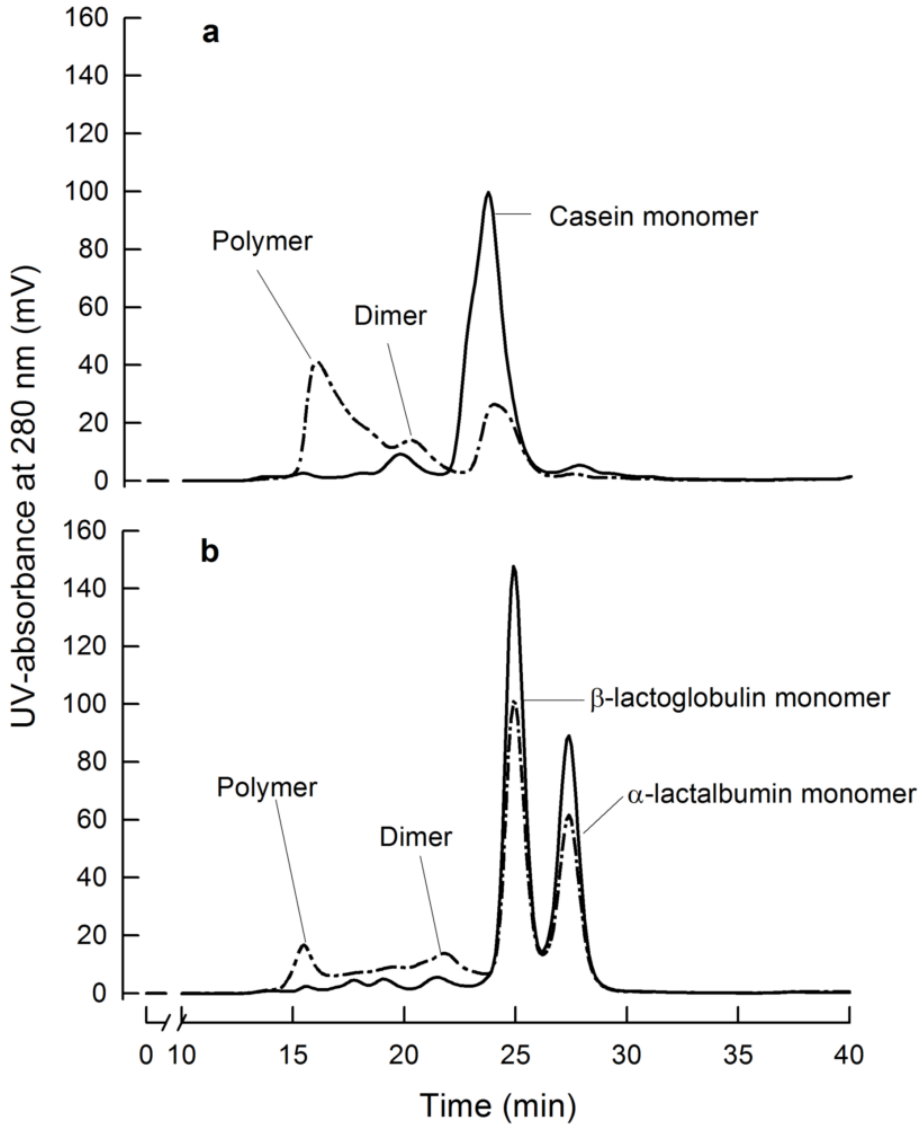


Figure 4.15: Size exclusion chromatogram of microbial transglutaminase cross-linked (a) sodium caseinate or (b), whey protein isolate using 3U mTGase/ g protein at 40 °C for 2 h. Full lines, protein without mTGase treatment; broken lines, protein after mTGase treatment.

4.5.2 Microbiological properties during storage of fermented product

The plain and TNM enriched substrates allowed the development of lactic acid bacteria to various extents. For instance, after homogenization and storage of products at 6 °C for 24 h, (0 d), viable counts of *S. thermophilus* in all products were in the range of 1.6 - 5.8 x 10^8 cfu/g, and were higher than those of *L. delbrueckii* ssp. *bulgaricus* (1.1 - 2.2 x 10^6 cfu/g). Even though the ratio of *S. thermophilus* to *L. delbrueckii* ssp. *bulgaricus* viable counts in this study was similar to those reported in section 4.4.2, viable cell count was lower in the latter study, notably for *L. delbrueckii* ssp. *bulgaricus*.

Products from 3CnXe or 3WPXe enriched systems showed slightly but insignificantly higher viable counts (~ 0.05 log cfu/g and 0.10 log cfu/g, respectively) of *S. thermophilus* than those of their untreated counterparts after 24 h (0 d) storage. Conversely, addition of mTGase treated proteins to the TNM systems consistently diminished the viable count of *L. delbrueckii* ssp. *bulgaricus* by approximately 0.3 log cfu/g compared to the untreated counterparts, pointing to the fact that, enrichment of TNM with mTGase cross-linked proteins might promote the proliferation of *S. thermophilus* but decrease the growth of L. *delbrueckii* ssp. *bulgaricus*, which might result in a decline in post acidification (Xu et al., 2015).

As shown in **Fig. 4.16**, protein enrichment and storage of fermented tiger nut milk during 15 d decreased the viable cell count of *L. delbrueckii* ssp. *bulgaricus* more drastically (**Fig. 4.16a**) than *S. thermophilus*, which exhibited an insignificant reduction in viable cell count in all the fermented systems (**Fig. 4.16b**). Similarly, Neve *et al.* (2001) reported a higher decrease in viable count of *L. delbrueckii* ssp. *bulgaricus* than *S. thermophilus* during storage, even though milk systems were used as fermentation substrates. A higher reduction rate of the viable cell counts of *L. delbrueckii* ssp. *bulgaricus* compared to the plain TNM was observed during 15 d storage when untreated proteins were used for enrichment. Evidently, pre-treatment of proteins with mTGase minimized the rate of decline in the viable cell count of *L. delbrueckii* ssp. *bulgaricus* compared to the untreated proteins **(Fig 16 a).** The results show that

addition of mTGase cross-linked proteins to TNM might reduce protein accessibility for *L. delbrueckii* ssp. *bulgaricus*, leading to weaker growth and less produced lactic acid during storage. This might have relevance for maintaining the microbiological and chemical quality of fermented TNM systems during storage.

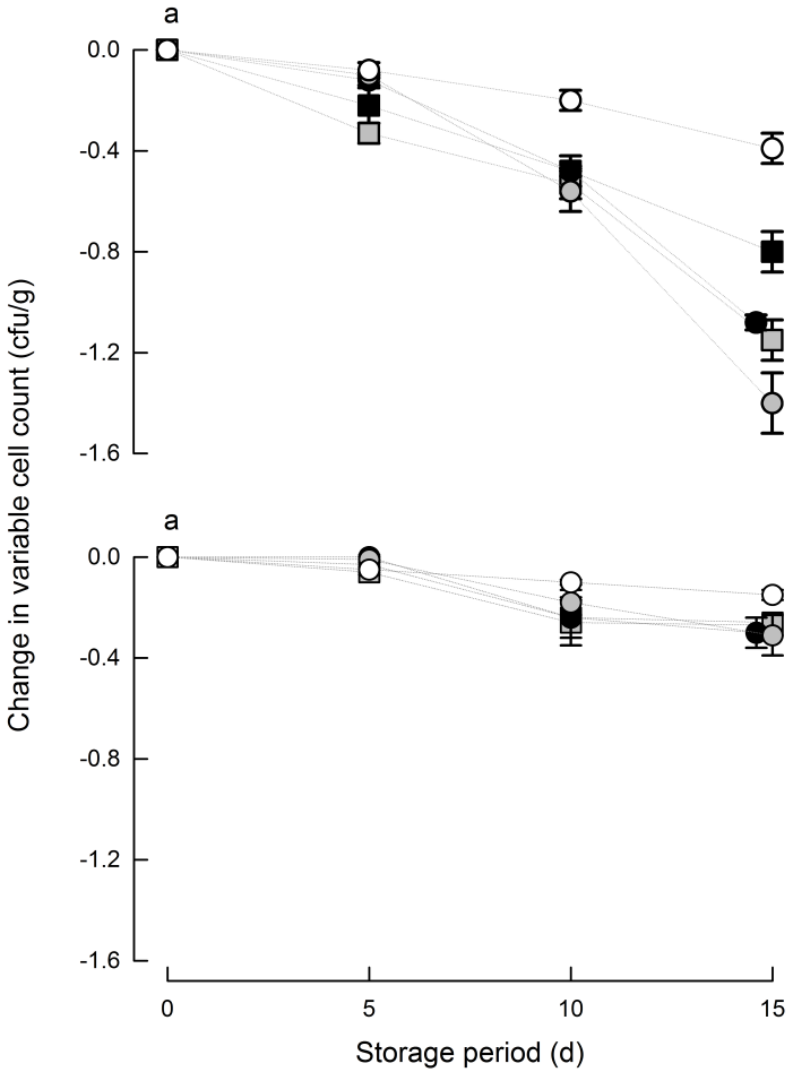


Figure 4.16: Effects of enrichment and storage period on the viable cell count of *Lactobacillus delbrueckii ssp. bulgaricus* (a) and *Streptococcus thermophilus* (b) in fermented tiger nut milk. grey and black circles, TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g sodium caseinate, respectively. Grey and black squares represent TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g whey protein isolate, respectively. Results are based on triplicate measurements

4.5.3 Physico-chemical properties during storage of fermented product

To improve the physico-chemical properties and product quality during storage of fermented TNM, effects of adding enzymatically cross-linked proteins were studied. It was observed that neither pH nor titratable acidity (TA) of plain TNM was significantly affected during storage at 6 °C for 15 d **(Table 4.10**).

Table 4.10: Effects of storage period on the physico-chemical properties of fermented tiger nut milk and the enriched systems.

Parameters	Storage (d)	System[a]				
		TNM[b]	3CnX	3CnXe	3WPX	3WPXe
pH (-)	0	4.23^{a} ± 0.02	4.27^{a} ± 0.01	4.34^{a} ± 0.02	4.28^{a} ± 0.01	4.31^{a} ± 0.02
	5	4.23^{a} ± 0.01	4.22^{a} ± 0.02	4.29^{a} ± 0.01	4.24^{a} ± 0.02	4.25^{a} ± 0.03
	10	4.22^{a} ± 0.01	4.16^{b} ± 0.01	4.15^{b}± 0.01	4.18^{b} ± 0.02	4.16^{b} ± 0.01
	15	4.21^{a} ± 0.01	4.14^{b} ± 0.03	4.15^{b} ± 0.02	4.16^{b} ± 0.03	4.14^{b} ± 0.03
Titratable acidity (g/100 g)	0	0.52^{a} ± 0.01	1.16^{a} ± 0.07	1.07^{a} ± 0.10	0.93^{a} ± 0.06	0.92^{a} ± 0.13
	5	0.52^{a} ± 0.02	1.24^{ab} ± 0.09	1.17^{ab} ± 0.12	1.00^{ab} ± 0.10	0.97^{a} ± 0.10
	10	0.54^{a} ± 0.01	1.29^{ab} ± 0.11	1.26^{ab} ± 0.10	1.04^{ab} ± 0.08	1.00^{a} ± 0.09
	15	0.54^{a} ± 0.03	1.34^{b} ± 0.08	1.30^{b} ± 0.10	1.07^{b} ± 0.05	1.02^{a} ± 0.09
Viscosity (shear rate, 1.0/s (Pa.s)	0	0.02 ± 0.00	0.56^{a} ± 0.01	9.17^{a} ± 1.28	1.40^{a} ± 0.04	5.12^{a} ± 0.16
	5	-	0.61^{a} ± 0.03	9.57^{a} ± 0.54	1.44^{a} ± 0.02	5.14^{a} ± 0.68
	10	-	0.59^{a} ± 0.02	8.93^{a} ± 1.23	1.39^{a} ± 0.07	5.44^{a} ± 1.06
	15	-	0.74^{b} ± 0.08	10.20^{a} ± 0.3	1.47^{a} ± 0.02	6.00^{a} ± 0.86
Syneresis (%)	0	86.2 ± 1.2	38.9^{a} ± 0.1	27.2^{a} ± 0.4	31.5^{a} ± 1.0	20.2^{a} ± 1.60
	5	-	38.0^{b} ± 0.1	24.6^{b} ± 0.3	30.4^{a} ± 1.2	17.9^{ab} ± 2.2
	10	-	36.3^{c} ± 0.4	21.8^{c} ± 0.2	29.8^{a} ± 0.2	17.6^{ab} ± 2.0
	15	-	36.1^{c} ± 0.8	21.5^{c} ± 0.5	27.1^{b} ± 1.2	16.3^{b} ± 0.40

[a]TNM, tiger nut milk; 3CnX (3CnXe), TNM enriched with 3 (3 mTGase-treated) g/100 g sodium caseinate and 0.1 g/100 g xanthan; 3WPX (3WPXe), TNM enriched with 3 (3 mTGase-treated) g/100 g whey protein isolate and 0.1 g/100 g xanthan.

[b]Results are arithmentic mean ± standard deviation from (n=3) determinations. Values in the same column with different superscripts differ significantly at $P < 0.05$.

On the other hand, enrichment with proteins considerably reduced the pH, and accordingly, increased TA of the fermented system after 15 d. Enrichment of

TNM systems with mTGase treated proteins did not show any significant effect on the pH or TA of the fermented product compared to that from their untreated counterparts, although a trend in minor reduction in TA was observed after 15 d storage.

Fig. 4.17 shows that lactic acid fermentation of TNM resulted in products with considerably low viscosity (0.02 Pa.s at a shear rate of 1/s), which is similar to the results in **Fig 4.12**. Addition of sodium caseinate or whey protein to TNM and subsequent fermentation resulted in stirred products with improved viscosity of 0.56 ± 0.01 Pa.s (3CnX) and 1.4 ± 0.04 Pa.s (3WPX) at 1/s after 24 h (0 d) storage. The increase in viscosity of the protein enriched system was similar to that in **Fig. 4.12**, and shows that protein enrichment enhances the formation of firm protein gels during fermentation that impedes phase separation and after homogenization, leads to TNM products with improved smooth texture.

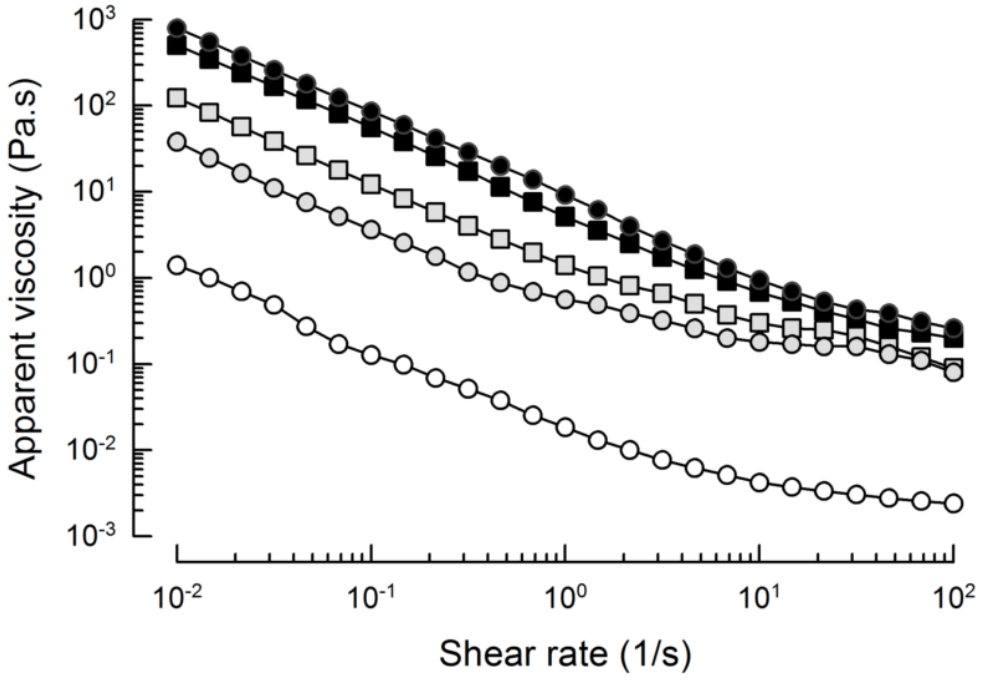


Figure 4.17: Apparent viscosity of fermented tiger nut milk (TNM) with different compositions after 0 d storage at 6 °C. Open circles, plain tiger nut milk; grey and black circles, TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g sodium caseinate, respectively. Grey and black squares represent TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g whey protein isolate, respectively. Only selected points from continuous measurements are displayed.

Furthermore, **Fig. 4.17** shows that addition of mTGase treated casein or whey proteins to TNM and subsequent fermentation caused the viscosity of their homogenized products to increase by a factor of ~ 16.4 and ~ 3.6 compared to their untreated counterparts after 24 h (0 d) storage, respectively. This can be attributed to the increase in protein aggregate size because of the cross-linking effect of mTGase illustrated in **Fig. 4.15**. Assessment of protein polymerization after the enzyme treatment under the applied conditions showed a higher degree of polymerization of casein and whey protein of approximately 68 % and 32 % compared to that of the untreated counterparts of 13 % and 11 %, respectively. Similarly, mTGase cross-linking of ultra-high temperature treated sodium caseinate solution under comparable conditions increased DP from 14 % to 60 % (Bönisch et al., 2004). Additionally, de Jong and Koppelman (2002) showed that mTGase cross-linking was more effective in caseinate systems than in whey protein systems. During storage, a trend towards increase of viscosity of the protein enriched TNM product was evident after 15 d (**Table 4.10**). Similar observations in viscosity increase of stirred yogurt during storage was reported by Jaros et al. (2007) among others, and was ascribed to re-arrangements in the protein network after breaking up during the stirring step.

Although addition of proteins to TNM and lactic acid fermentation successfully improved the viscosity, the fermented products showed considerable susceptibility to syneresis during storage as depicted in **Table 4.10**. Forced syneresis in fermented plain TNM was 86 %. Fermented products obtained from 3CnX enrichments showed syneresis of approximately 39 %, which was higher than when 3WPX (~ 32 %) was used for TNM enrichment. However, a previous study showed that whey drainage in non-homogenized fermented systems from casein-enriched TNM was lower than in similar systems with whey protein enrichments as illustrated in **Table 4.9.**

These results suggest that whey protein aggregates might have higher shear resistance than casein gels. Products that were generated from 3CnXe or 3WPXe showed a considerably different syneresis, being approximately one-third lower than that of their untreated counterparts. This is attributed to a more

elaborate protein network through the mTGase treatment and a consequential decrease in gel pore-size and increase in viscosity (Jaros et al., 2007, 2006a; Lorenzen et al., 2002). A decreasing pattern in the rate of syneresis in the protein-enriched TNM products was observed during storage, and was significant after 15 d. Most likely, the marginal increase in viscosity of the enriched, fermented systems contributed to reduction in syneresis during the storage period.

4.5.4 Effects on colour of fermented tiger nut product

Studies on the sensory properties of fermented TNM suggest that the product might show changes in colour during storage (**Fig. 4.13**). Therefore, the effects of enriching TNM with milk proteins on the colour properties of the fermented products were investigated during storage. Lactic acid bacteria fermentation of plain TNM resulted in an average lightness of L* = 64.2 ± 0.80. Addition of proteins to TNM and subsequent fermentation improved lightness of the products with L*= 69.6 ± 1.10 (3CnX) and L* = 66.5 ± 0.40 (3WPX). However, systems resulting from enrichments with 3CnXe or 3WPXe showed marginally lower L* than their untreated counterparts, which were 67.6 ± 1.63 and 65.7 ± 0.61, respectively.

The colour intensity of the fermented systems were C* = 11.7 ± 1.5 (TNM), 11.5 ±1.2 (3CnX), 10.9 ± 1.5 (3CnXe), 11.3 ± 1.4 (WPX) and 11.2 ± 1.2 (3WPXe), showing that TNM enrichment with proteins or mTGase treated protein did not significantly affect this parameter. The hue angle, h_{ab} of all fermented systems ranged between 1.4° -1.5°. No significant difference in chroma or hue of the fermented systems was observed during storage. On the contrary, fermented plain TNM showed the highest lightness decrease, with L* being approximately 3.8 units lower after 15 d storage (**Fig. 4.18).**

A recent report by Codina-Torrela et al. (2016) showed the presence of peroxidase activity in tiger nut milk. This enzyme together with polyphenol oxidase are known to catalyze the oxidation of phenolic compounds that are

present in tiger nuts (Oladele et al., 2009) to brown quinone products (Queiroz et al., 2008), which contribute to lightness decrease in TNM and many vegetable milk-like extracts.

Fig. 4.18 shows that enrichment of TNM with proteins effectively diminished the lightness decrease, and whey proteins were more effective than caseins during storage of the fermented product. Addition of proteins in fermented TNM might have improved lightness decrease probably because of the colour-imparting effects of the protein powders. The fermented system resulting from 3CnXe showed a slightly lower lightness decrease than the untreated counterpart, whereas no distinct effect was observed for the 3WPXe system.

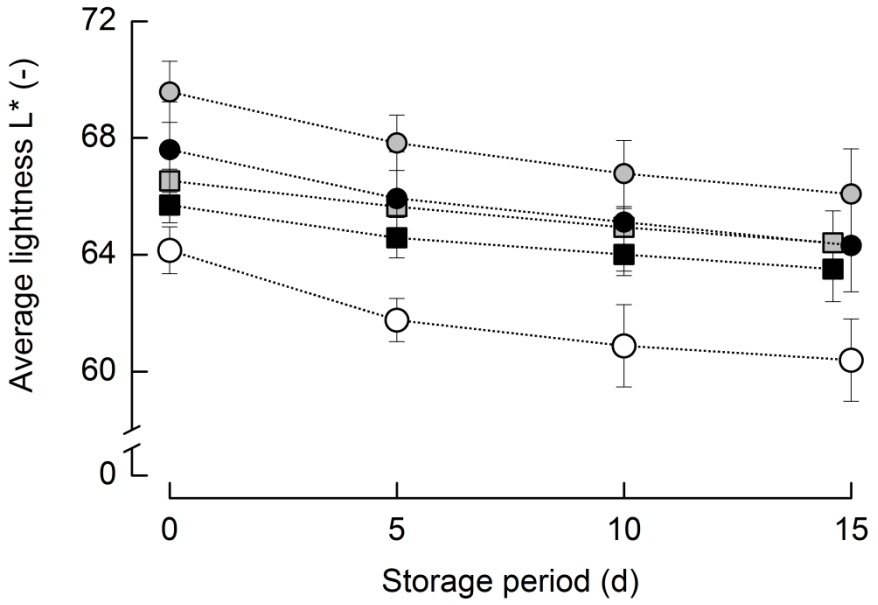


Figure 4.18: Effects of protein enrichment and storage period on lightness of fermented tiger nut milk. Open circles, plain tiger nut milk; grey and black circles, TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g sodium caseinate, respectively. Grey and black squares represent TNM enriched with 0.1 g/100 g xanthan and 3.0 untreated or treated mTGase g/100 g whey protein isolate, respectively.

Ma et al. (2012) showed that mTGase cross-linking of sodium caseinate enhances the stability of the protein against oxidative products, which explains in part, the lessening effect on lightness decrease of enriched, fermented TNM.

5. Conclusions and outlook

Tiger nuts (*Cyperus esculentus* L) grow ubiquitously in tropical and Mediterranean regions including many developing countries in Africa, where the prevalence of lactose intolerance is more than 90 %. However, the use of tiger nuts as a source of food nutrient is fundamentally unexplored, even though many regions have challenges accessing nutritious food. This is partly attributed to limited scientific literature on the technological strategies for generating value-added food products, such as fermented tiger nut milk (TNM) with acceptable physico-chemical and sensory properties. This study investigated strategies for the successful production of yogurt-like products using tiger nut milk.

The extraction process for tiger nut milk was standardized and the influence of the processing parameters on the dynamics of compounds transfer from the tiger nuts into tiger nut milk, and on the physical properties of the extracted milk was studied. Next, TNM was enriched by using various types of proteins and hydrocolloids for improving the physical stability of the fermenting substrate. For this reason, tiger nut proteins were additionally isolated and characterized. Enriched TNM was fermented by using classical yogurt starter cultures, and the fermented product was analyzed for microbiological, physico-chemical and sensory properties. To improve the rheological and storage quality of the fermented products, the use of microbial transglutaminase cross-linked proteins was investigated.

By standardizing the extraction procedure for tiger nut milk, it was evident that higher milling intensity improved yield of tiger nut solids, nutrient composition and colour properties of the milk. However, a more intense milling also generated insoluble solids, which partly affected the colloidal stability during tiger nut milk storage. Milling program only was insufficient to ensure stable substrates during lactic acid fermentation. On one hand, addition of globular tiger nut proteins, which was composed of mainly glutelin and albumin, successfully hindered phase separation even though the fermented

system remained liquid, which means that these proteins have little influence on the textural properties of fermented TNM. On the other hand, addition of milk proteins and xanthan gum resulted in TNM of improved viscosity and stability, even under various pH and temperature conditions. Consequently, fermentation of enriched TNM generated homogenous yogurt-like gel products with enhanced viable starter counts, lactic acid production, viscosity and lightness of products, and different sensory properties. These attributes depict products with improved quality and might lead to higher consumer acceptance than those of fermented plain TNM. Addition of microbial transglutaminase cross-linked proteins to TNM and subsequent fermentation resulted in enhanced viscosity and decreased syneresis of products, thereby, enhancing the quality of the fermented TNM and prolonging the shelf life properties.

These results mean that by optimizing the milling procedure for tiger nut milk extraction, and fermenting using classical lactic acid bacteria after enrichment with xanthan gum and microbial transglutaminase cross-linked sodium caseinate or whey proteins, a nutritious, lactose-free yogurt-like product with acceptable sensory properties and a prolonged shelf life can be developed.

Future experiments on tiger nut milk extraction might be necessary for minimizing nutrient losses through the pressing residue or for valorization of the by-product in food product development. Furthermore, improvement in methods for tiger nut protein isolation might be relevant for studying its functional properties such as antioxidant capability, which might be relevant in plant-based fermented systems. Exploring other types of fermenting micro-organisms, such as *Lactobacillus plantarum* might be important for exploiting their functional characteristics, such as exopolysaccharide production, which can enhance the physical properties of fermented TNM. Lastly, it might be relevant to compare the sensory properties of fermented tiger nut milk enriched with milk proteins with that enriched with enzymatically cross-linked proteins.

Bibliography

Abdolmaleki, K., Mohammadifar, M.A., Mohammadi, R., Fadavi, G., Meybodi, N.M., 2016. The effect of pH and salt on the stability and physicochemical properties of oil-in-water emulsions prepared with gum tragacanth. Carbohydrate Polymers 140, 342–348.

Adejuyitan, J.A., 2011. Tigernut processing: its food uses and health benefits. American Journal of Food Technology 6, 197–201.

Adejuyitan, J.A., Otunola, E.T., Akande, E.T., Bolarinwa, E.A., Oladokun, F.M., 2009. Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from a market in Ogbomoso, Nigeria. African Journal of Food Science 3, 051–055.

Ade-Omowaye, B.I.O., Akinwande, B.A., Bolarinwa, I.F., Adebiyi, A. 0., 2008. Evaluation of tigernut (*Cyperus esculentus*) -wheat composite flour and bread. African Journal of Food Science 2, 87–91.

Adgidzi, E.A., Ingbian, E.K., Abu, J.O., 2011. Effects of hot water and steam blanching on the quality of aqueous extract from tigernuts (*Cyperus esculentus*). Production Agriculture and Technology 7, 90–102.

Aguilar, N., Albanell, E., Minarro, B., Guamis, B., Capellas, M., 2015. Effect of tiger nut-derived products in gluten-free batter and bread. Food Science and Technology International 21, 323–331.

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., Danfulani, S., Akoma, A., Albert, M., 2016. Sensory and microbiological quality attributes of laboratory produced tigernut milk during ambient storage. Journal of Advances in Biology & Biotechnology 6, 1–8.

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.

Allouh, M.Z., Daradka, H.M., Ghaida, J.H.A., 2015. Influence of *Cyperus esculentus* tubers (tigernut) on male rat copulatory behavior. BMC Complementary and Alternative Medicine 15, 851–859.

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., de Kruif, C.G., Visschers, R.W., 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.

Anderson, J.W., Smith, B.M., Gustafson, N.J., 1994. Health benefits and practical aspects of high-fiber diets. The American Journal of Clinical Nutrition 59, 1242S–1247S.

Ando, H., Adachi, M., Umeda, K., Matsuura, A., Nonaka, M., Uchio, R., Tanaka, H., Motoki, M., 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. Agricultural and Biological Chemistry 53, 2613–2617.

Aremo, M.O., Bamidele, T.O., 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 north-east Nigeria. Journal of Biology, Agriculture and Healthcare 5, 213–221.

Asante, F.A., Oduro, I., Ellis, W.O., Saalia, F.K., 2014a. Effect of planting period and site on the chemical composition and milk acceptability of tigernut (*Cyperus esculentus* L.) tubers in Ghana. American Journal of Food and Nutrition 2, 49–54.

Asante, F.A., Saalia, F.K., Oduro, I., Ellis, W.O., 2014b. Modelling of milk solids extraction from tigernut (*Cyperus esculentus* L.) tubers using response surface methodology. Scientific & Academic Publishing 4, 73–79.

Bado, S., Bazongo, P., Son, G., Kyaw, M.T., Forster, B.P., Nielen, S., Lykke, A.M., Ouédraogo, A., Bassolé, I.H.N., 2015. Physicochemical characteristics and composition of three morphotypes of *Cyperus esculentus* tubers and tuber oils. Journal of Analytical Methods in Chemistry 2015, 1–8.

Baglio, E., 2014. Chemistry and technology of yoghurt fermentation, SpringerBriefs in Molecular Science. Springer International Publishing, Cham.

Bahrami, M., Ahmadi, D., Alizadeh, M., Hosseini, F., 2013. Physicochemical and sensorial properties of probiotic yogurt as affected by additions of different types of hydrocolloid. Korean Journal for Food Science of Animal Resources 33, 363–368.

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

Belewu, Y., 2007. Comparative physico-chemical evaluation of tigernut, soybean and coconut milk sources. International Journal of Agriculture & Biology 9, 785–787.

Bernat, N., Cháfer, M., Chiralt, A., González-Martínez, C., 2014. Vegetable milks and their fermented derivative products. International Journal of Food Studies 3, 93–124.

Binks, B.P., 2002. Particles as surfactants-similiarities and differences. Current Opinion in Colloid & Interface Science 7, 21–41.

Binks, B.P., Horozov, T.S., 2006. Colloidal particles at liquid interfaces. Cambridge University Press.

Bönisch, M.P., Lauber, S., Kulozik, U., 2004. Effect of ultra-high temperature treatment on the enzymatic cross-linking of micellar casein and sodium caseinate by transglutaminase. Journal of Food Science 69, E398–E404.

Bosch, L., 2005. RP-HPLC determination of tigernut and orgeat amino acid contents. Food Science and Technology International 11, 33–40.

Bouyer, E., Mekhloufi, G., Huang, N., Rosilio, V., Agnely, F., 2013. β-Lactoglobulin, gum arabic, and xanthan gum for emulsifying sweet almond oil: formulation and stabilization mechanisms of pharmaceutical emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects 433, 77–87.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Bryan, W.P., 1978. The isoionic point of amino acids and proteins. Biochemical Education 6, 14–15.

Cao, Y., Dickinson, E., Wedlock, D.J., 1990. Creaming and flocculation in emulsions containing polysaccharide. Food Hydrocolloids 4, 185–195.

Cevc, G., Marsh, D., 1985. Hydration of noncharged lipid bilayer membranes. Theory and experiments with phosphatidylethanolamines. Biophysical Journal 47, 21–31.

Chanamai, R., McClements, D.J., 2001. Depletion flocculation of beverage emulsions by gum arabic and modified starch. Journal of Food Science 66, 457–463.

Cheetangdee, N., Fukada, K., 2014. Emulsifying activity of bovine β-lactoglobulin conjugated with hexoses through the Maillard reaction. Colloids and Surfaces A: Physicochemical and Engineering Aspects 450, 148–155.

Chen, W., Li, X., Rahman, M.R.T., Al-Hajj, N.Q.M., Dey, K.C., Raqib, S.M., 2014. Emulsification properties of soy bean protein. Nusantara Bioscience 6, 196–202.

Chen, Y., Ye, R., Yin, L., Zhang, N., 2014. Novel blasting extrusion processing improved the physicochemical properties of soluble dietary fibre from soybean residue and in vivo evaluation. Journal of Food Engineering 120, 1–8.

Chiu, M.H., Prenner, E.J., 2011. Differential scanning calorimetry: an invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. Journal of Pharmacy and Bioallied Sciences 3, 39–59.

Chopra, R.N., Nayar, S.L., Chopra, I.C., 1986. Glossary of Indian medicinal plants (Including the supplement). Counsel of Scientific and Industrial Research, New Delhi.

Codina-Torrella, I., Guamis, B., Ferragut, V., Trujillo, A.J., 2016. Potential application of ultra-high pressure homogenization in the physico-chemical stabilization of tiger nuts' milk beverage. Innovative Food Science & Emerging Technologies. *doi:* 10.1016/j.ifset.2016.06.023.

Codina-Torrella, I., Guamis, B., Trujillo, A.J., 2015. Characterization and comparison of tiger nuts (*Cyperus esculentus* L.) from different geographical origin. Industrial Crops and Products 65, 406–414.

Corrales, M., de Souza, P.M., Stahl, M.R., Fernández, A., 2012. Effects of the decontamination of a fresh tiger nuts' milk beverage (horchata) with short wave ultraviolet treatments (UV-C) on quality attributes. Innovative Food Science & Emerging Technologies 13, 163–168.

Cortés, C., Esteve, M.J., Frígola, A., Torregrosa, F., 2004. Physical and chemical properties of different commercially available types of "horchata de chufa". Italian Journal of Food Science 16, 113–121.

Coskuner, 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 casein, in: Damodaran, S., Paraf, A. (Eds.), Food Proteins and Their Applications, Food Science and Technology. Marcel Dekker, New York, pp. 225–255.

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, G.A.H., Koppelman, S.J., 2002. Transglutaminase catalyzed reactions: impact on food applications. Journal of Food Science 67, 2798–2806.

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.

de Jong, S., van de Velde, F., 2007. Charge density of polysaccharide controls microstructure and large deformation properties of mixed gels. Food Hydrocolloids 21, 1172–1187.

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.

Demarest, D.J., Verna, F., 2015. Differential scanning calorimetry in the Biopharmaceutical sciences, in: Biophysical Characterization of Proteins in Developing Biopharmaceuticals. Elsevier, Amsterdam, Netherlands, pp. 287–310.

Deng, Q., Wang, L., Wei, F., Xie, B., Huang, F., Huang, W., Shi, J., Huang, Q., Tian, B., Xue, S., 2011. Functional properties of protein isolates, globulin and albumin extracted from Ginkgo biloba seeds. Food Chemistry 124, 1458–1465.

Detloff, T., Sobisch, T., Lerche, D., 2006. Particle size distribution by space or time dependent extinction profiles obtained by analytical centrifugation. Particle & Particle Systems Characterization 23, 184–187.

Díaz-Maroto, M.C., González Viñas, M.A., Cabezudo, M.D., 2002. Evaluation of the effect of drying on aroma of parsley by free choice profiling. European Food Research and Technology 216, 227–232.

Dickinson, E., 2011. Mixed biopolymers at interfaces: competitive adsorption and multilayer structures. Food Hydrocolloids 25, 1966–1983.

Dickinson, E., Golding, M., Povey, M.J.W., 1997. Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. Journal of Colloid and Interface Science 185, 515–529.

Djomdi, Ejoh, R., Ndjouenkeu, R., 2007. Soaking behaviour and milky extraction performance of tiger nut (*Cyperus esculentus*) tubers. Journal of Food Engineering 78, 546–550.

Doublier, J.-L., Garnier, C., Renard, D., Sanchez, C., 2000. Protein–polysaccharide interactions. Current Opinion in Colloid & Interface Science 5, 202–214.

Ejoh, R., Djomdi, Ndjouenkeu, R., 2006. Characteristics of tiger nut (*Cyperus esculentus*) tubers and their performance in the production of a milky drink. Journal of Food Processing and Preservation 30, 145–163.

Ekeanyanwu, R.C., Njoku, O., Ononogbu, C.I., 2010. The phytochemical composition and some biochemical effects of Nigerian tigernut (*Cyperus esclentus* L.) tuber. Pakistan Journal of Nutrition 709–715.

Ekeanyanwu, R.C., Ononogbu, C.I., 2010. Nutritive value of Nigerian tigernut (*Cyperus esculentus* L.). Medwell Journals (Agriculture) 5, 297–302.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., Attia, H., 2011. Dietary fibre and fibre-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review. Food Chemistry 124, 411–421.

Ezeh, O., Gordon, M.H., Niranjan, K., 2014. Tiger nut oil (*Cyperus esculentus* L.) : a review of its composition and physico-chemical properties. European Journal of Lipid Science and Technology 116, 783–794.

Faergemand, M., Jörgensen, U., Budolfsen, G., Qvist, K.B., 1999. Transglutaminase: effect on instrumental and sensory texture of set style yoghurt. Milchwissenschaft 54, 563–566.

Folk, J.E., 1980. Transglutaminases. Annual Review of Biochemistry 49, 517–531.

Follak, S., Aldrian, U., Moser, D., Essl, F., 2015. Reconstructing the invasion of *Cyperus esculentus* in Central Europe. Weed Research 55, 289–297.

Galindo Bonilla, P.A., Gomez Torrijos, E., Borja Segade, J., Feo Brito, F., Lombardero Vega, M., 2002. Allergy to tiger nut. Allergy 57, 1083–1084.

Gaspar, A.L.C., de Góes-Favoni, S.P., 2015. Action of microbial transglutaminase (MTGase) in the modification of food proteins: a review. Food Chemistry 171, 315–322.

Gill, P., Moghadam, T.T., Ranjbar, B., 2010. Differential scanning calorimetry techniques: applications in biology and nanoscience. Journal of Biomolecular Techniques 21, 167–193.

Gosh, A.K., Bandyopadhyay, P., 2012. Polysaccharide-protein interactions and their relevance in food colloids, in: Karunaratne, D.N. (Ed.), The Complex World of Polysaccharides. InTech.

Havenaar, R., de Jong, A., Koenen, M.E., van Bilsen, J., Janssen, A.M., Labij, E., Westerbeek, H.J.M., 2013. Digestibility of transglutaminase cross-linked caseinate versus native caseinate in an in vitro multicompartmental model simulating young child and adult gastrointestinal conditions. Journal of Agricultural and Food Chemistry 61, 7636–7644.

Henriksson, G., Englund, A.K., Johansson, G., Lundahl, P., 1995. Calculation of the isoelectric points of native proteins with spreading of pKa values. Electrophoresis 16, 1377–1380.

Hill, A.R., Kethireddipalli, P., 2013. Dairy products: cheese and yogurt, in: Eskin, N.A.M., Shahidi, F. (Eds.), Biochemistry of Foods. Elsevier, Waltham, USA., pp. 348–351.

Holzwarth, G., 1978. Molecular weight of xanthan polysaccharide. Carbohydrate Research 66, 173–186.

Ho, M.-L., Leu, S.-Z., Hsieh, J.-F., Jiang, S.-T., 2000. Technical approach to simplify the purification method and characterization of microbial transglutaminase produced from *Streptoverticillium ladakanum*. Journal of Food Science 65, 76–80.

Hunter, R.J., 1998. Recent developments in the electroacoustic characterisation of colloidal suspensions and emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects 141, 37–66.

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

IDF, 2003. Yogurt – Enumeration of characteristic microorganisms: colony count technique at 37 degrees C. Standard 117. 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., Partschefeld, C., Henle, T., Rohm, H., 2006a. Transglutaminase in diairy products: chemistry, physics, applications. Journal of Texture Studies 37, 113–155.

Jaros, D., Pätzold, J., Schwarzenbolz, U., Rohm, H., 2006b. 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 yogurt, in: Smit, G. (Ed.), Dairy Processing: Improving Quality. Woodhead, Cambridge.

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.

Jaros, D., Schwarzenbolz, U., Raak, N., Löbner, J., Henle, T., Rohm, H., 2014a. Cross-linking with microbial transglutaminase: relationship between polymerisation degree and stiffness of acid casein gels. International Dairy Journal 38, 174–178.

Jaros, D., Schwarzenbolz, U., Raak, N., Löbner, J., Henle, T., Rohm, H., 2014b. Corrigendum to "Cross-linking with microbial transglutaminase: relationship between polymerisation degree and stiffness of acid casein gels" [Int Dairy J 38 (2014) 174–178]. International Dairy Journal 39, 345–347.

Jenkins, P., Snowden, M., 1996. Depletion flocculation in colloidal dispersions. Advances in Colloid and Interface Science 68, 57–96.

Ju, Z.Y., Hettiarachchy, N.S., Rath, N., 2001. Extraction, denaturation and hydrophobic properties of rice flour proteins. Journal of Food Science 66, 229–232.

Karababa, E., Coskuner, Y., Nazlican, A.N., 2001. Chemical composition and technological evaluation of chufa tuber (*Cyperus esculentus* L.). GIDA 26, 243–246.

Kieliszek, M., Misiewicz, A., 2014. Microbial transglutaminase and its application in the food industry. a review. Folia Microbiologica 59, 241–250.

Kinsella, J.E., 1979. Functional properties of soy proteins. Journal of the American Oil Chemists Society 56, 242–258.

Ladokun, O., Oni, S., 2014. Fermented milk products from different milk types. Food and Nutrition Sciences 05, 1228–1233.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lam, R.S.H., Nickerson, M.T., 2013. Food proteins: a review on their emulsifying properties using a structure–function approach. Food Chemistry 141, 975–984.

Lauber, S., Krause, I., Klostermeyer, H., Henle, T., 2003. Microbial transglutaminase crosslinks β-casein and β-lactoglobulin to heterologous oligomers under high pressure. European Food Research and Technology 216, 15–17.

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.

Lerche, D., Sobisch, T., 2007. Consolidation of concentrated dispersions of nano- and microparticles determined by analytical centrifugation. Powder Technology 174, 46–49.

Lesmes, U., Baudot, P., McClements, D.J., 2010. Impact of interfacial composition on physical stability and in vitro lipase digestibility of triacylglycerol oil droplets coated with lactoferrin and/or caseinate. Journal of Agricultural and Food Chemistry 58, 7962–7969.

Letki, A.G., 2000. Centrifugation | Theory of centrifugation, in: Wilson, I.D. (Ed.), Encyclopedia of Separation Science. Academic Press, Oxford, pp. 336–342.

Leubner-Metzger, G., Meins, F., 1999. Pathogenesis-related proteins in plants. CRC Press, Florida.

Li, C., Li, Y., Sun, P., Yang, C., 2013. Pickering emulsions stabilized by native starch granules. Colloids and Surfaces A: Physicochemical and Engineering Aspects 431, 142–149.

Lisinska, G., 1989. Potato science and technology. Elsevier Applied Science : Sole distributor in the USA and Canada : Elsevier Science Pub. Co, London ; New York.

Liu, L., Zhao, Q., Liu, T., Kong, J., Long, Z., Zhao, M., 2012. Sodium caseinate/carboxymethylcellulose interactions at oil–water interface: relationship to emulsion stability. Food Chemistry 132, 1822–1829.

Lorenzen, P.C., 2002. Enzymatic cross-linking of dairy proteins, in: New Processing Technologies for the Future. International Dairy Federation, Brussels, Belgium, pp. 30–36.

Lorenzen, P.C., Neve, H., Mautner, A., Schlimme, E., 2002. Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. International Journal of Dairy Technology 55, 152–157.

Lorenzen, P.C., Schlimme, E., Roos, N., 1998. Crosslinking of sodium caseinate by a microbial transglutaminase. Nahrung 42, 151–154.

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.

Ma, H., Forssell, P., Kylli, P., Lampi, A.-M., Buchert, J., Boer, H., Partanen, R., 2012. Transglutaminase catalyzed cross-linking of sodium caseinate improves oxidative stability of flaxseed oil emulsion. Journal of Agricultural and Food Chemistry 60, 6223–6229.

Makhatadze, G.I., 2001. Measuring protein thermostability by differential scanning calorimetry, in: Coligan, J.E., Dunn, B.M., Speicher, D.W., Wingfield, P.T. (Eds.), Current Protocols in Protein Science. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Maloney, K.P., Truong, V.-D., Allen, J.C., 2012. Chemical optimization of protein extraction from sweet potato (Ipomoea batatas) peel. Journal of Food Science 77, E307–E312.

Manamperi, W.A.R., Wiesenborn, D.P., Chang, S.K.C., Pryor, S.W., 2011. Effects of protein separation conditions on the functional and thermal properties of canola protein isolates. Journal of Food Science 76, E266–E273.

Martín-Esparza, E., González-Martínez, C., 2016. Horchata de chufa: a traditional Spanish beverage with exceptional organoleptic, nutritive, and functional attributes, in: Kristbergsson, K., Ötles, S. (Eds.), Functional Properties of Traditional Foods. Springer US, Boston, MA, pp. 371–375.

Matissek, R., Schnepel, F.M., Steiner, G., 1992. Lebensmittel analytik-Grundzüge. Methoden. Anwendungen. Springer-Lehrbuch, pp. 9–13, 17–19, 32–42,90–102.

Matsudaira, P.T., 1993. A practical guide to protein and peptide purification for microsequencing. Academic Press, San Diego.

McClements, D.J., 2000. Comments on viscosity enhancement and depletion flocculation by polysaccharides. Food Hydrocolloids 14, 173–177.

McClements, D.J., 1999. Food emulsions: principles, practice, and techniques, CRC series in contemporary food science. CRC Press, Boca Raton.

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.

Mosquera, A., Sims, C., A., Bates, R.P., O'keefe, S., F., 1996. Flavor and stability of "Horchata de chufas." Journal of Food Science 61, 856–861.

Neve, H., Lorenzen, P.C., Mautner, A., Schlimme, E., Heller, K., 2001. Effects of transglutaminase treatment on the production of set skim milk yoghurt: microbiological aspects. Kieler Milchwirtschaftliche Forschungsberichte 53, 347–361.

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.

Nielsen, S.S., 2011. Phenol-sulfuric acid method for total carbohydrates, in: Food Analysis Laboratory Manual. Springer US, Boston, MA, pp. 47–53.

Nielsen, S.S., 2003. Food analysis laboratory manual. Springer Science, pp. 29-32. New York, USA.

Nor Hayati, I., Wai Ching, C., Rozaini, M.Z.H., 2016. Flow properties of o/w emulsions as affected by xanthan gum, guar gum and carboxymethyl

cellulose interactions studied by a mixture regression modelling. Food Hydrocolloids 53, 199–208.

Ocheme, O., Eke, M., Banye, T., 2011. Physico-chemical and microbiological changes in tigernut milk under ambient storage conditions. Journal of Agriculture, Forestry and the Social Sciences 8.

Okyere, A.A., Odamtten, G.T., 2014. Physicochemical, functional and sensory attributes of milk prepared from irradiated tiger nut (*Cyperus esculentus* L.). Journal of Radiation Research and Applied Sciences 7, 583–588.

Oladele, A.K., Aina, J.O., 2007. Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). African Journal Biotechnology 6, 2473–2476.

Oladele, K.A., Osundahunsi, F.O., Adebowale, Y.A., 2009. Influence of processing techniques on the nutrients and antinutrients of tigernut (*Cyperus esculentus* L.). World Journal of Dairy & Food Sciences 4, 88–93.

Omode, A.A., Fatoki, O.S., Olaogun, K.A., 1995. Physicochemical properties of some underexploited and nonconventional oilseeds. Journal of Agricultural and Food Chemistry 43, 2850–2853.

O'Sullivan, M.M., Kelly, A.L., Fox, P.F., 2002. Effect of transglutaminase on the heat stability of milk: a possible mechanism. Journal of Dairy Science 85, 1–7.

Ozer, B., Avni Kirmaci, H., Oztekin, S., Hayaloglu, A., Atamer, M., 2007. Incorporation of microbial transglutaminase into non-fat yogurt production. International Dairy Journal 17, 199–207.

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

Paoletti, S., Cesàro, A., Delben, F., 1983. Thermally induced conformational transition of xanthan polyelectrolyte. Carbohydrate Research 123, 173–178.

Pascual, B., Maroto, J.V., LóPez-Galarza, Sa., Sanbautista, A., Alagarda, J., 2000. Chufa (*Cyperus esculentus* L. var. sativus boeck.): An unconventional crop. studies related to applications and cultivation. Economic Botany 54, 439–448.

Pearsall, W.H., Ewing, J., 1924. The isoelectric points of some plant proteins. The Biochemical Journal 18, 329–339.

Pots, A.M., de Jongh, H.H.J., Gruppen, H., Hamer, R.J., Voragen, A.G.J., 1998. Heat-induced conformational changes of patatin, the major potato tuber protein. European Journal of Biochemistry 252, 66–72.

Queiroz, C., Mendes Lopes, M.L., Fialho, E., Valente-Mesquita, V.L., 2008. Polyphenol oxidase: characteristics and mechanisms of browning control. Food Reviews International 24, 361–375.

Rabilloud, T., Carpentier, G., Tarroux, P., 1988. Improvement and simplification of low-background silver staining of proteins by using sodium dithionite. Electrophoresis 9, 288–291.

Raghunath, M., Höpfner, B., Aeschlimann, D., Lüthi, U., Meuli, M., Altermatt, S., Gobet, R., Bruckner-Tuderman, L., Steinmann, B., 1996. Cross-linking of the dermo-epidermal junction of skin regenerating from keratinocyte autografts. Anchoring fibrils are a target for tissue transglutaminase. Journal of Clinical Investigation 98, 1174–1184.

Rao, M.A., Walter, R.H., Cooley, H.J., 1981. Effect of heat treatment on the flow properties of aqueous guar gum and sodium carboxymethylcellulose (CMC) solutions. Journal of Food Science 46, 896–899.

Reddy, S.., Fogler, H.., 1981. Emulsion stability: delineation of different particle loss mechanisms. Journal of Colloid and Interface Science 79, 105–113.

Rohm, H., Jaros, D., 1996. Colour of hard cheese. Zeitschrift für Lebensmittel-Untersuchung und Forschung 203, 241–244.

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., Schmidt, W., 1993. Influence of dry matter fortification on flow properties of yogurt. 1. Evaluation of flow curves. Milchwissenschaft – Milk Science International 48, 556–560.

Romeih, E.A., Abdel-Hamid, M., Awad, A.A., 2014. The addition of buttermilk powder and transglutaminase improves textural and organoleptic properties of fat-free buffalo yogurt. Dairy Science & Technology 94, 297–309.

Sadler, G.D., Murphy, P.A., 2014. pH and titrable acidity., in: Food Analysis. Springer, New York, pp. 219–238.

Salau, R.B., AminuMuhammad, M. T., Bamidele K.G, Bishir, Usman, 2013. Evaluation of amino acids in the milk preparations from tiger nut rhizome and its mixed varieties. OSR Journal of Pharmacy and Biological Sciences 5, 15–19.

Salgın, S., Salgın, U., Bahadır, S., 2012. Zeta potentials and isoelectric points of biomolecules: the effects of ion types and ionic strengths. International Journal of electrochemical science 12404–12414.

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.

Sánchez-Zapata, E., Fuentes-Zaragoza, E., Fernández-López, J., Sendra, E., Sayas, E., Navarro, C., Pérez-Álvarez, J.A., 2009. Preparation of dietary fiber powder from tiger nut (*Cyperus esculentus*) milk ("Horchata") by products and its physicochemical properties. J. Agric. Food Chem. 57, 7719–7725.

Sanful, R.E., 2009a. Production and sensory evaluation of tigernut beverages. Pakistan Journal of Nutrition 8, 688–690.

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

Schey, A., 2003. Texture improvement of fermented dairy products by enzymatic crosslinking with transglutaminase., in: Fermented Milk: Proceedings of the IDF Seminar on Aroma and Texture of Fermented Milk Held in Kolding, Denmark. pp. 371–375.

Sebastià, N., El-Shenawy, M., Mañes, J., Soriano, J.M., 2012. Assessment of microbial quality of commercial and home-made tiger-nut beverages. Letters in Applied Microbiology 54, 299–305.

Seguro, K., Kumazawa, Y., Kuraishi, C., Sakamoto, H., Motoki, M., 1996a. The epsilon-(gamma-glutamyl)lysine moiety in crosslinked casein is an available source of lysine for rats. The Journal of Nutrition 126, 2557–2562.

Seguro, K., Nio, N., Motoki, M., 1996b. Some characteristics of a microbial protein cross-linking enzyme: transglutaminase, in: Parris, N., Kato, A., Creamer, L.K., Pearce, J. (Eds.), Macromolecular Interactions in Food Technology. American Chemical Society, Washington, DC, pp. 271–280.

Shabbir, M.A., Anjum, F.M., Zahoor, T., Nawaz, H., 2011. Electrophoretic and functional mapping of indica rice glutelin protein isolates. International Journal of Food Properties 14, 1375–1385.

Siebert, K.J., 2003. Modelling protein functional properties from amino acid composition. Journal of Agricultural and Food Chemistry 51, 7792–7797.

Sjöblom, J., 2006. Emulsions and emulsion stability. Taylor & Francis, Boca Raton.

Small, E., 2014. North American cornucopia: top 100 indigenous food plants. CRC Press, Taylor & Francis Group, Boca Raton, FL.

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.

Tadros, T.F. (Ed.), 2013. Emulsion formation and stability, Topics in colloid and interface science. Wiley-VCH, Weinheim.

Tadros, T., F., 2009. Emulsion science and technology: a general introduction, in: Emulsion Science and Technology. Wiley-VCH Verlag GmbH & Co.KGaA, Wokingham, United Kingdom, pp. 1–56.

Tadros, T.F. (Ed.), 2007. Colloid stability: the role of surface forces, Colloids and interface science series. Wiley-VCH Verlag, Weinheim.

Tambe, D.E., Sharma, M.M., 1994. Factors controlling the stability of colloid-stabilized emulsions. Journal of Colloid and Interface Science 162, 1–10.

Temple, V.J., Ojobe, T.O., Kapu, M.M., 1990. Chemical analysis of tiger nut (Cyperus esculentis). Journal of the Science of Food and Agriculture 50, 261–263.

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

Tuinier, R., Rieger, J., de Kruif, C.., 2003. Depletion-induced phase separation in colloid–polymer mixtures. Advances in Colloid and Interface Science 103, 1–31.

Ugochi, N.F., Chukwuma, U.M., 2015. Nutrient and sensory quality of soymilk produced from different improved varieties of soybean. Pakistan Journal of Nutrition 14, 898–907.

Ukwuru, M.U., Ogbodo, A.C., 2011. Effect of processing treatment on the quality of tigernut milk. Pakistan Journal of Nutrition 10, 95–100.

Urry, D.W., Luan, C.H., Parker, T.M., Gowda, D.C., Prasad, K.U., Reid, M.C., Safavy, A., 1991. Temperature of polypeptide inverse temperature transition depends on mean residue hydrophobicity. Journal of the American Chemical Society 113, 4346–4348.

Vaclavik, V., Christian, E.W., 2014. Essentials of food science, 3rd ed. Springer, Texas, USA.

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

Waibel, P.E., Carpenter, K.J., 1972. Mechanisms of heat damage in proteins. British Journal of Nutrition 27, 509.

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., Geurts, T.J., Wouters, J.T.M., 2006. Dairy science and technology. CRC/Taylor & Francis, Boca Raton.

Warner, R.C., 1942. The alkaline hydrolysis of egg albumin. Journal of Biological Chemistry 142, 741–756.

Wessel, D., Flügge, U.I., 1984. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Analytical Biochemistry 138, 141–143.

White, D., Fisk, I., Mitchell, J., Wolf, B., Hill, S., Gray, D., 2008. Sunflower-seed oil body emulsions: rheology and stability assessment of a natural emulsion. Food hydrocolloids. 22, 1224–1232.

Wingfield, P., 2001. Protein precipitation using ammonium sulfate, in: Coligan, J.E., Dunn, B.M., Speicher, D.W., Wingfield, P.T. (Eds.), Current

protocols in protein science. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Wu, N., Yang, X., Teng, Z., Yin, S., Zhu, J., Qi, J., 2011. Stabilization of soybean oil body emulsions using κ, ι, λ-carrageenan at different pH values. Food Research International 44, 1059–1068.

Xu, Z., Li, S., Gong, G., Liu, Z., Wu, Z., Ma, C., 2015. Influence of different acidifying strains of Lactobacillus delbrueckii subsp. bulgaricus on the quality of yoghurt. Food Science and Technology Research 21, 263–269.

Ye, A., 2008. Interfacial composition and stability of emulsions made with mixtures of commercial sodium caseinate and whey protein concentrate. Food Chemistry 110, 946–952.

Yoon, S.H., 2016. Optimization of the refining process and oxidative stability of chufa (*Cyperus esculentus* L.) oil for edible purposes. Food Science and Biotechnology 25, 85–90.

Zahra, S., Ahmed, M.S.H., 2014. Exploring the suitability of incorporating tiger nut flour as novel ingredient in gluten-free biscuit. Polish Journal of Food and Nutrition Sciences 64, 27–33.

Zayas, J.F., 1997. Emulsifying properties of proteins, in: Functionality of Proteins in Food. Springer, Berlin, Heidelberg, pp. 134–277.

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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.

Kizzie-Hayford, N., Jaros, D., Zahn, S., Rohm, H., 2016. Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk. LWT - Food Science and Technology 74, 319-324.

Kizzie-Hayford, N., Jaros, D., Rohm, H., (submitted). Enrichment of tiger nut milk with microbial transglutaminase cross-linked protein improves the physico-chemical properties of the fermented system. LWT - Food Science and Technology.

Poster and presentations

Kizzie-Hayford, N., Jaros, D., Zahn, S., Rohm, H., 2016. Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk. 2016. 4TH International ISEKI Food conference, Vienna, Austria.

Kizzie-Hayford, N., Jaros, D., Rohm, H., 2016. Effects of protein enrichment and microbial transglutaminase on the properties of fermented tiger nut milk. 2016. GDL Kongress Lebensmitteltechnologies, Lemgo, Germany.

Kizzie-Hayford, N., Jaros, D., Rohm, H., 2016. Technologische Überlegungen zur Integration von Erdmandeln in der Nahrungskette: Fermentierte jogurt-ähnlische Produkte. GDL-Symposium, Bremerhaven, Deutschland.