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ORIGINAL RESEARCH

Characterisation of yoghurt enriched with polyunsaturated fatty acids by using walnut slurry

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This study aimed to evaluate the physicochemical, chemical, rheological, sensorial and microbiological properties of yoghurt enriched with polyunsaturated fatty acids (PUFAs). The PUFA-enriched yoghurt was prepared with walnut slurry (10%−50%) and skimmed milk (50%−90%). Compared with the control yoghurt, it contained a lower content of protein, potassium, sodium and phosphorus, and a higher content of fat, iron, magnesium and zinc. Moreover, it exhibited a lower syneresis value and a higher water-holding capacity value. Its fat was rich in omega fatty acids, mainly linoleic and linolenic acids. These research findings revealed that walnut slurry could be used in yoghurt manufacture to develop fermented milk products as functional foods, especially when enriched with omega fatty acids.

Keywords Fermented milk products, Functional foods, *Juglans regia*, Omega fatty acids.

INTRODUCTION

Yoghurt is one of the most popular fermented milk products as it provides nutritional and health benefits (Lollo *et al.* 2013; Batista *et al.* 2015). Yoghurt contains protein, carbohydrate, fat, minerals and vitamins. It contributes to the development and maintenance of bone health because it is a good source of calcium (121 mg 100 g^{-1}, USDA, 2018a). Yoghurt has the potential to prevent gastrointestinal disorders, such as lactose intolerance and diarrhoeal diseases. It may reduce the risks of colon cancer and inflammatory bowel diseases (Pala *et al.* 2011; Pei *et al.* 2017).

A high intake of saturated fatty acids increases the risk of cardiovascular diseases. For this reason, consumers have begun to demand foods that contain a low level of saturated fatty acids and a high level of unsaturated fatty acids. Foods are enriched with polyunsaturated fatty acids (PUFAs) (Ganesen *et al.* 2014). Polyunsaturated fatty acids (PUFAs), which are essential fatty acids, have been associated with various health benefits, such as protecting against cardiovascular diseases and cancer (Gogus and Chris 2010).

As the fats found in nuts are rich in unsaturated fatty acids, nuts can be used to develop dairy product enriched with unsaturated fatty acids. Nuts (walnut, hazelnut, almond and pistachio) have been incorporated in yoghurt to fortify it (Ozturkoglu-Budak *et al.* 2016). Peanut milk and hazelnut slurry have been used to produce yoghurt with a high level of unsaturated fatty acids (Isanga and Zhang 2009; Ilyasoglu *et al.* 2015). However, no studies have been conducted on the use of walnut slurry in yoghurt manufacture.

Walnut is one of the most popular nuts. It ranks fourth in terms of supply value ($5.554 million) and constitutes 17% of the total supply value of tree nuts (INC, 2017). Its fat is rich in linoleic and linolenic acids as PUFAs. Walnuts are mostly consumed as snacks and are used in food formulations as an ingredient. Walnut milk beverage has been developed (Chen *et al.* 2014). Walnut slurry obtained from wet grinding may be used as an ingredient to develop dairy products enriched with PUFAs.

This study aimed to evaluate the characteristics of yoghurt fortified with PUFAs by using walnut slurry. The proximate composition, physicochemical, rheological and sensorial properties, fatty acid composition, mineral content and starter culture content of the yoghurt samples were determined. The properties of the enriched yoghurt

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samples were compared with those of a control yoghurt prepared from skimmed milk.

MATERIALS AND METHODS

Chemicals

Fatty acid methyl ester (FAME) mix, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Agars and solvents were obtained from Merck (Darmstadt, Germany).

Materials

Skimmed milk (Pınar Co., İzmir, Turkey; according to the manufacturer, protein: 31 g kg^{-1}; carbohydrate: 50 g kg^{-1}), skimmed milk powder (Pınar Co., İzmir, Turkey; according to the manufacturer, protein: 360 g kg^{-1}; carbohydrate: 520 g kg^{-1}), walnuts (Antepsan Co., Gaziantep, Turkey) and starter culture (Chr. Hansen A/S, Horsholm, Denmark) were purchased from supermarkets.

Walnut slurry preparation

Unshelled walnuts were soaked in water at room temperature for 15−16 h, and then the water was drained and the walnuts were washed with water. Walnut slurry was obtained from wet grinding (1:6, m:v) with a laboratory blender (Waring, Conair Corporation, Stamford, CT, USA) for 2 min following filtration through cheesecloth. The walnut slurry obtained had 117 g kg^{-1} total solids content. It contained 80 g kg^{-1} of fat, 26 g kg^{-1} of protein and 9 g kg^{-1} of carbohydrates, as the main constituents.

Yoghurt manufacturing

Five formulations were prepared using walnut slurry with skimmed milk: F1 (10% walnut slurry and 90% skimmed milk), F2 (20% walnut slurry and 80% skimmed milk), F3 (30% walnut slurry and 70% skimmed milk), F4 (40% walnut slurry and 60% skimmed milk) and F5 (50% walnut slurry and 50% skimmed milk). The structure of yoghurt is formed from the coagulation of milk proteins into a gel matrix. For this reason, the maximum amount of walnut slurry in the formulation was set to 50%. The control sample (CY) was prepared from skimmed milk. Skimmed milk powder (40 g L^{-1}) was added to the formulation to enhance the total solids content of the yoghurt samples and the control sample.

Skimmed milk powder (40 g L^{-1}) was dissolved in the walnut slurry and skimmed milk mix at 45 °C, stirring for 30 min. The mix was homogenised with a homogeniser (Witeg Labortechnik GmbH, Wertheim, Germany) and pasteurised at 95 °C for 25 min in a water bath (GFL Gesellshaft für Labortechnik GmbH, Burgwedel, Germany). At 45 °C, pasteurised milk was inoculated with the starter culture (30 mL L^{-1}) and then incubated until pH value reached to 4.6−4.7. The yoghurt samples were stored in a refrigerator overnight.

Proximate composition

Proximate composition was determined on the first day in accordance with AOAC methods (2006). Total carbohydrates were calculated by subtracting the total percentages of moisture, protein, fat and ash from 100.

Energy values were calculated by the following equation.

$$\begin{aligned}\text{Energy value (kJ 100 g}^{-1}) = [&(\text{Protein}\% \times 4) \\ &+ (\text{Carbohydrate}\% \times 4) \\ &+ (\text{Fat}\% \times 9)] \times 4,18\end{aligned} \quad (1)$$

Physicochemical properties

The physicochemical properties were determined on the first day. The pH of the sample was determined with a pH meter (Ohaus Europe GmbH, Greifensee, Switzerland, Germany). The acidity of the sample was determined by the alkali titration method. Colour properties (L^*, a^* and b^* values) were determined with a chromometer (Konica Minolta Inc., Tokyo, Japan). To determine the syneresis value, 10 g of the sample was spread on a filter paper in a beaker. Then the beaker was maintained at 4 °C for 5 h, and the whey collected was weighed. The syneresis value was calculated as the whey deducted from the total weight of the sample (Srisuvor *et al.* 2013). The centrifuge method was used to determine the water-holding capacity (WHC). The sample (10 g) stored at 4 °C for 24 h was centrifuged at 5000 *g* for 20 min at 4 °C, and the whey separated from the samples was weighed. The WHC was calculated as the drained yoghurt out of the total weight of the sample (Srisuvor *et al.* 2013).

Fatty acid composition

Fatty acid methyl esters were injected into a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Japan) equipped with a flame ionisation detector, a split/splitless injector and a long capillary column (Teknokroma TR-CN100, Teknokroma Analitica, Spain). The analytical method and instrumental conditions were previously described (Ilyasoglu *et al.* 2015).

Atherogenic (AI) and thrombogenic (TI) indices were also calculated by following equations (Batista *et al.* 2017; Sperry *et al.* 2018).

$$\begin{aligned}\text{AI} = [(\text{C}_{12:0}) + (4 \times \text{C}_{14:0}) + (\text{C}_{16:0})]/[(\Sigma\text{MUFA}) \\ + (\Sigma\text{PUFA}\,(n-6)\,\text{and}\,(n-3))]\end{aligned} \quad (2)$$

$$\begin{aligned}\text{TI} = [(\text{C}_{14:0}) + (\text{C}_{16:0}) + (\text{C}_{18:0})]/[(0.5 \times \Sigma\text{MUFA}) \\ + (0.5 \times \Sigma\text{PUFA}(n-6)) \\ + (3 \times \Sigma\text{PUFA}(n-3)) + ((n-3)/(n-6))]\end{aligned} \quad (3)$$

Mineral analysis

Mineral contents were determined on the first day. Mineral analysis was conducted according to the Nordic Committee on Food Analysis (NMKL) 186 method. A 0.5 g sample

Table 1 Proximate composition and energy values, physicochemical properties and sensorial properties.

Proximate composition (g kg^{-1}) and energy values (kJ 100 g^{-1})

Sample	*Protein*	*Carbohydrate*	*Fat*	*Ash*	*Total solid matters*	*Energy values*
CY	47.3 ± 0.8[a]	62.1 ± 1.2[a]	<1.0[f]	9.3 ± 0.6[a]	119 ± 1[e]	183 ± 1[f]
F1	44.7 ± 0.4[b]	60.2 ± 1.7[a]	10 ± .1[e]	8.6 ± 0.3[b]	123 ± 1[d]	212 + 1[e]
F2	44.4 ± 0.3[b]	54.1 ± 1.1[b]	16 ± 1[d]	8.4 ± 0.2[b]	123 ± 2[d]	224 ± 3[b]
F3	41.2 ± 1.7[c]	58.3 ± 1.0[ba]	21 ± .2[c]	8.6 ± 0.1[b]	127 ± 1[c]	244 ± 4[c]
F4	41.1 ± 0.6[c]	50.7 ± 1.7[c]	31 ± .3[b]	7.8 ± 0.2[bc]	131 ± 3[b]	272 ± 7[d]
F5	38.3 ± 1.0[d]	48.4 ± 2.7[c]	41 ± .3[a]	6.8 ± 0.4[c]	135 ± 1[a]	300 ± 1[a]

Physicochemical properties

Sample	*pH*	*Acidity (g kg^{-1})*	L* *value*	a* *value*	b* *value*	*Syneresis (g kg^{-1})*	*WHC (g kg^{-1})*
CY	4.73 ± 0.06[a]	7.2 ± 0.5[b]	84.68 ± 0.47[a]	−3.73 ± 0.03[f]	7.70 ± 0.02[f]	202.3 ± 14.7[a]	357.6 ± 11.16[b]
F1	4.60 ± 0.02[b]	8.0 ± 0.3[a]	80.91 ± 0.07[b]	−1.12 ± 0.01[e]	7.95 ± 0.06[e]	113.7 ± 7.1[c]	388.2 ± 12.5[b]
F2	4.65 ± 0.03[b]	7.8 ± 0.5[a]	79.26 ± 0.04[c]	0.09 ± 0.01[d]	8.16 ± 0.01[d]	107.5 ± 7.2[c]	421.6 ± 11.1[a]
F3	4.72 ± 0.01[a]	6.9 ± 0.2[b]	78.15 ± 0.04[d]	1.01 ± 0.02[c]	8.37 ± 0.01[c]	122.4 ± 9.2[bc]	426.2 ± 19.6[a]
F4	4.70 ± 0.01[a]	7.2 ± 0.2[b]	76.16 ± 0.04[e]	1.87 ± 0.01[b]	8.74 ± 0.02[b]	129.6 ± 11.8[b]	442.3 ± 4.2[a]
F5	4.70 ± 0.02[a]	7.2 ± 0.3[b]	75.68 ± 0.09[f]	2.24 ± 0.01[a]	9.13 ± 0.03[a]	121.3 ± 13.6[bc]	437.6 ± 4.2[a]

Sensorial properties

Sample	*Appearance*	*Consistency*	*Odour*	*Taste*	*General acceptability*
CY	4.4 ± 0.6[a]	4.4 ± 0.6[a]	4.0 ± 0.7[a]	3.9 ± 0.9[a]	4.1 ± 0.6[a]
F1	3.3 ± 0.9[b]	3.3 ± 0.9[b]	3.4 ± 0.9[b]	2.3 ± 0.9[b]	3.0 ± 0.8[b]
F2	2.7 ± 0.8[c]	2.6 ± 0.8[c]	2.9 ± 0.8[c]	2.0 ± 0.8[b]	2.5 ± 0.7[c]
F3	2.4 ± 0.7 [cd]	2.8 ± 0.8[bc]	2.8 ± 0.9[c]	2.4 ± 1.0[b]	2.6 ± 0.7[b]
F4	2.5 ± 1.0 [cd]	3.0 ± 1.0[bc]	2.9 ± 0.9[c]	2.4 ± 1.1[b]	2.6 ± 0.8[b]
F5	2.2 ± 0.8[d]	2.9 ± 1.0[bc]	2.9 ± 0.8[c]	2.4 ± 1.1[b]	2.6 ± 0.8[b]

Different superscript letters within each column present significant difference ($P < 0.05$).

WHC, water-holding capacity.

was placed into a cup, and 1 mL of H_20_2 and 5 mL of HNO_3 were added. The samples were burned in a microwave oven (Milestone Srl, Sorisole, Italy) and analysed with an inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies Inc., Santa Clara, CA, USA). Na, Ca, K, Mg, Fe, Zn and P were determined.

Rheological properties

The rheological properties of the yoghurt samples were determined on the first day according to the method of Rudra *et al.* (2016). A rheometer (Anton Paar GmbH, Graz, Austria, Germany) equipped with a 35-mm parallel plate and a 1 mm gap setting was used for the measurement. The viscosity of the samples was determined as a function of the shear rate at a range of 1–100 s^{-1}. The linear viscoelastic region was set by conducting a strain test. The storage (G′) and the loss (G″) modulus were determined as a function of frequency varying at 1–100 Hz at a fixed strain of 0.005%.

Microbiological analysis

The counts of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were determined on the 1st, 7th, 14th, 21st and 28th day of storage at 4 °C. The starter culture cells were counted using the pour plate technique. The enumerations of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* colonies were conducted in M17 agar (Merck) under aerobic conditions and MRS agar (Merck) under anaerobic or microaerophilic conditions at 37 °C for 48 h, respectively (Trigueros *et al.* 2012). Cell count was expressed as colony forming units per gram (cfu g^{-1}) of the product.

Sensorial properties

The sensorial properties (appearance, consistency, odour, taste and overall acceptability) of the samples were analysed after overnight storage. The samples (15 g) placed into the cups were coded randomly with three random digit numbers and served to the panellists, who were inside booths. Twenty-seven panellists (students of the Department of Nutrition and Dietetics) who had basic knowledge of sensorial analysis and dairy products evaluated the samples using a five-point scale, ranging from 1 (dislike extremely) to 5 (like extremely). A five-point scale was selected so that the panellists could better understand the items from which they would choose their answer. Water was given to the panellists for rinsing their mouths between samples (Meilgaard *et al.* 1991).

Statistical analysis

Triplicate analysis was conducted, and the mean values and standard deviations were calculated. One-way analysis of variance (ANOVA) and the least significant (LSD) test were applied to evaluate the differences among samples. SPSS version 17.00 (IBM, USA) was used for data analysis.

RESULTS AND DISCUSSION

Proximate composition

The main constituent of the walnut slurry was fat (80 g kg^{-1}) followed by protein (26 g kg^{-1}) and carbohydrate (9 g kg^{-1}). The skimmed milk contained carbohydrate (50 g kg^{-1}) and protein (31 g kg^{-1}) as the main constituents. The proximate composition of the yoghurt samples is presented in Table 1. The samples developed had a lower content of protein and ash than the control sample ($P < 0.05$), but they had a higher content of fat and solid matter as well as a higher energy value than the control sample ($P < 0.05$). The protein, carbohydrate and ash contents of the PUFA-enriched yoghurt samples showed a decreasing trend with increasing walnut slurry in the formulation. However, the fat and solid matter contents and the energy values increased proportionally to the amount of walnut slurry in the formulation. These findings can be attributed to the composition of the walnut slurry. The walnut slurry had lower contents of protein and carbohydrate and a higher content of fat than skimmed milk.

The values of the major constituents of the PUFA-enriched samples were found to be compatible with the values reported for yoghurt (Tamime and Robinson 1999). Thus, the PUFA-enriched yoghurt could be concluded to provide nutrients and energy as a dairy yoghurt.

Physicochemical properties

The physicochemical properties of the yoghurt samples are presented in Table 1. The F1 and F2 samples had a lower pH value and a higher acidity value than the control yoghurt ($P < 0.05$), and no significant difference was found between the control yoghurt and the other PUFA-enriched samples ($P > 0.05$). These findings may be related to the starter culture counts in the yoghurt samples. The F1 and F2 samples had higher counts than the control yoghurt on the first day. The PUFA-enriched yoghurt had a lower L^* value and higher a^* and b^* values than the control sample ($P < 0.05$). The L^* value of the PUFA-enriched yoghurt decreased with increasing walnut slurry in the formulation, and the a^* and b^* values showed an increasing trend. The addition of walnut slurry into the dairy milk could have reduced the lightness of the yoghurt and enhanced its redness and yellowness. The syneresis level of the PUFA-enriched samples was lower than that of the control sample ($P < 0.05$), and the WHC of the PUFA-enriched samples was higher than that of the control sample ($P < 0.05$). Fat globules are broken down and coated with casein during the homogenisation of milk. The smaller fat globules can act as a copolymer with casein to strengthen the gel network of yoghurt and reduce the syneresis value. Therefore, high-fat yoghurt tends to have a lower level of syneresis than low-fat yoghurt (Vercet *et al.* 2002; Isanga and Zhang 2009). The lower level of syneresis observed in the PUFA-enriched

Table 2 Fatty acid composition (%).

Fatty acid	*F1*	*F2*	*F3*	*F4*	*F5*
Myristic	1.04 ± 0.02^{a}	0.45 ± 0.03^{b}	0.29 ± 0.01^{c}	0.25 ± 0.01^{d}	0.18 ± 0.01^{e}
Palmitic	8.75 ± 0.02^{a}	7.06 ± 0.02^{b}	6.74 ± 0.07^{c}	6.65 ± 0.08^{c}	6.30 ± 0.07^{d}
Stearic	3.13 ± 0.05^{a}	2.70 ± 0.02^{b}	2.48 ± 0.01^{c}	2.53 ± 0.03^{c}	2.35 ± 0.01^{d}
Oleic	15.38 ± 0.64^{a}	13.88 ± 0.01^{b}	14.91 ± 0.06^{a}	14.61 ± 0.06^{ba}	14.19 ± 0.02^{ba}
Linoleic	57.46 ± 0.42^{b}	61.32 ± 0.05^{ba}	61.61 ± 0.12^{a}	61.90 ± 0.30^{a}	62.23 ± 0.12^{a}
Linolenic	14.22 ± 0.13^{ba}	14.60 ± 0.01^{a}	13.97 ± 0.10^{b}	14.07 ± 0.31^{ba}	14.76 ± 0.09^{a}
SFAs	12.94 ± 0.09^{a}	10.22 ± 0.07^{b}	9.51 ± 0.08^{c}	9.43 ± 0.06^{c}	8.84 ± 0.05^{d}
MUFAs	15.38 ± 0.64^{a}	13.88 ± 0.01^{b}	14.91 ± 0.06^{a}	14.61 ± 0.06^{ba}	14.19 ± 0.02^{ba}
PUFAs	71.69 ± 0.55^{c}	75.92 ± 0.06^{b}	75.58 ± 0.02^{b}	75.97 ± 0.01^{b}	76.99 ± 0.03^{a}
AI	0.148 ± 0.001^{a}	0.099 ± 0.001^{b}	0.087 ± 0.001^{c}	0.084 ± 0.001^{c}	0.077 ± 0.000^{d}
TI	0.163 ± 0.000^{a}	0.125 ± 0.001^{b}	0.118 ± 0.000^{c}	0.117 ± 0.000^{c}	0.107 ± 0.000^{d}

Different superscript letters within each row present significant difference ($P < 0.05$).

AI, atherogenic indice; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; TI, thrombogenic indice.

Table 3 Mineral composition (mg kg^{-1}).

Sample	*Fe*	*K*	*Mg*	*Zn*	*Na*	*Ca*	*P*
CY	1.0 ± 0.2^{e}	1022.2 ± 1.5^{a}	123.7 ± 0.3^{e}	5.3 ± 0.2^{c}	419 ± 1.0^{b}	1666.5 ± 23.4^{a}	1489.7 ± 1.0^{a}
F1	2.5 ± 0.1^{c}	1033.8 ± 4.2^{a}	141.5 ± 0.3^{d}	8.6 ± 0.5^{b}	427.2 ± 0.8^{a}	1666.5 ± 26.2^{a}	1321.2 ± 3.4^{c}
F2	2.5 ± 0.0^{c}	938 ± 2.5^{b}	154 ± 0.7^{c}	7.1 ± 0.4^{bc}	403.1 ± 0.9^{c}	1666.5 ± 13.0^{a}	1338.4 ± 1.3^{c}
F3	1.8 ± 0.0^{d}	940.8 ± 5.7^{b}	166.5 ± 0.7^{b}	7.4 ± 0.5^{b}	371.9 ± 1.4^{d}	1664.6 ± 38.8^{a}	1390.7 ± 11.0^{b}
F4	3.6 ± 0.1^{a}	822 ± 1.2^{c}	174.6 ± 0.4^{a}	11.0 ± 0.5^{a}	341.7 ± 1.0^{e}	1666.5 ± 20.8^{a}	1337.2 ± 7.4^{c}
F5	3.1 ± 0.0^{b}	726.3 ± 7.2^{d}	175.2 ± 1.0^{a}	9.0 ± 0.6^{b}	313 ± 1.1^{f}	1666.5 ± 19.6^{a}	1226.6 ± 7.2^{d}

Different superscript letters within each column present significant difference ($P < 0.05$).

yoghurts could have been related to their higher fat content compared to the control yoghurt. The WHC of yoghurt is related to the ability of protein to retain water. The ability of WHC of proteins depends on the composition, conformation and surface properties of protein (Vareltzis *et al.* 2015). Walnut slurry proteins may have a higher WHC than milk proteins. For this reason, the PUFA-enriched yoghurt could have exhibited a higher WHC. The PUFA-enriched samples generally had similar syneresis and WHC values. An increase in the proportion of walnut slurry could not have affected the syneresis and WHC values.

Fatty acid composition

The fatty acid composition of the yoghurt samples is presented in Table 2. The main fatty acid found in the PUFA-enriched yoghurt samples was linoleic acid (57.5%−62.2%) followed by linolenic acid (14.0%−14.8%), oleic acid (13.9%−15.4%), palmitic acid (6.3%−8.8%), stearic acid (2.4%−3.1%) and myristic acid (0.2%−1%). The saturated fatty acid content of the PUFA-enriched yoghurt samples decreased with increasing amounts of walnut slurry in the formulation, whereas the PUFA content increased. Skimmed milk powder was used to enhance the total solids of the yoghurt samples, and this contained 1.25% fat. Milk fat is rich in saturated fatty acids such as myristic, stearic and palmitic acids (Junior *et al.* 2012), whereas walnut oil is rich in unsaturated fatty acids such as oleic, linoleic and linolenic acids (Bada *et al.* 2010). The reduction in the level of saturated fatty acids could be related to the increase in the walnut slurry in the formulation. The replacement of saturated fatty acids with unsaturated fatty acids was previously proposed as helping to lower the incidence of cardiovascular diseases (Ros and Mataix 2006). The fat from PUFA-enriched yoghurt contained a lower content of saturated fatty acids and a higher content of unsaturated fatty acids than the fat from dairy milk yoghurt. The fat from dairy milk yoghurt contained more than 60% of saturated fatty acids (Junior *et al.* 2012). The consumption of PUFA-enriched yoghurt can be proposed to increase the intake of PUFAs. The PUFA-enriched yoghurt may exhibit potential health benefits, especially for cardiovascular health, because of its fatty acid composition. The ratio of linoleic acid (omega-6 fatty acid) to linolenic acid (omega-3 fatty acid) in the fat from the PUFA-enriched sample was comparable with the optimal ratio (4:1−5:1)

Table 4 Microbiological analysis results.

Sample	*Day 1*	*Day 7*	*Day 14*	*Day 21*	*Day 28*
Lactobacillus delbrueckii subsp. *bulgaricus* (log cfu g^{-1})					
CY	$7.84 \pm 0.02^{b,c}$	$8.21 \pm 0.16^{a,b}$	$8.01 \pm 0.02^{b,c}$	$8.68 \pm 0.01^{a,a}$	$8.73 \pm 0.03^{a,a}$
F1	$8.20 \pm 0.07^{a,ba}$	$7.55 \pm 0.77^{a,b}$	$7.76 \pm 0.03^{c,ba}$	$8.39 \pm 0.04^{c.ba}$	$8.48 \pm 0.06^{c,a}$
F2	$8.41 \pm 0.07^{a,ba}$	$8.14 \pm 0.13^{a,b}$	$7.31 \pm 0.02^{d,c}$	$8.56 \pm 0.07^{b,ba}$	$8.70 \pm 0.06^{a,a}$
F3	$8.02 \pm 0.02^{ab,b}$	$8.10 \pm 0.14^{a,ab}$	$8.17 \pm 0.02^{a,ab}$	$8.41 \pm 0.04^{c,a}$	$8.35 \pm 0.04^{d,a}$
F4	$8.04 \pm 0.02^{ab,b}$	$7.58 \pm 0.02^{a,c}$	$8.21 \pm 0.02^{a,a}$	$8.26 \pm 0.02^{d,a}$	$8.23 \pm 0.02^{e,a}$
F5	$8.12 \pm 0.02^{ab,a}$	$7.63 \pm 0.89^{a,a}$	$8.17 \pm 0.01^{a,a}$	$8.03 \pm 0.02^{e,a}$	$8.15 \pm 0.06^{e,a}$
Streptococcus thermophilus (log cfu g^{-1})					
CY	$7.17 \pm 0.12^{b,d}$	$8.47 \pm 0.06^{a,c}$	$8.73 \pm 0.07^{a,b}$	$9.11 \pm 0.06^{a,a}$	$9.13 \pm 0.03^{a,a}$
F1	$8.43 \pm 0.04^{a,a}$	$8.07 \pm 0.52^{ab,a}$	$8.50 \pm 0.02^{b,a}$	$8.52 \pm 0.05^{b,a}$	$8.68 \pm 0.07^{ab,a}$
F2	$8.49 \pm 0.04^{a,b}$	$8.04 \pm 0.06^{ab,c}$	$8.13 \pm 0.03^{c,c}$	$8.44 \pm 0.01^{b,b}$	$8.76 \pm 0.07^{ab,a}$
F3	$7.55 \pm 0.78^{ab,b}$	$8.01 \pm 0.15^{ab,ab}$	$8.21 \pm 0.02^{c,ab}$	$8.56 \pm 0.07^{b,a}$	$8.87 \pm 0.14^{ab,a}$
F4	$8.22 \pm 0.20^{ab,b}$	$7.75 \pm 0.07^{ab,c}$	$8.14 \pm 0.07^{c,b}$	$8.54 \pm 0.10^{b,a}$	$8.61 \pm 0.07^{b,a}$
F5	$8.41 \pm 0.03^{a,c}$	$7.70 \pm 0.01^{b,e}$	$8.16 \pm 0.01^{c,d}$	$8.60 \pm 0.12^{b,b}$	$8.76 \pm 0.04^{ba,a}$

First different superscript letters within each column present significant difference among the samples ($P < 0.05$).

Second different superscript letters within each raw present significant difference among the storage days ($P < 0.05$).

for the balanced intake of omega fatty acids (Gomez-Candela *et al.* 2011). It may be concluded that consumption of the developed product may help provide a balanced intake of omega fatty acids.

The PUFA-enriched yoghurt presented low atherogenic (AI: 0.08–0.15) and thrombogenic indices (TI: 0.11–0.16). The AI and TI values of the PUFA-enriched yoghurt decreased with increasing walnut slurry in the formulation ($P < 0.05$). The addition of the walnut slurry to the yoghurt formulation could contribute to the health benefits of yoghurt, improving its fatty acid composition and reducing AI and TI. Low AI and TI values may help prevent coronary diseases (Batista *et al.* 2017).

Mineral content

The mineral content of the yoghurt samples is presented in Table 3. The control sample had higher contents of potassium, sodium and phosphorus than the PUFA-enriched yoghurt samples. However, the PUFA-enriched samples had higher contents of iron, magnesium and zinc than the control sample. The calcium content of the samples showed no significant difference ($P > 0.05$). The potassium, sodium and phosphorus contents of the PUFA-enriched samples decreased with the increasing ratio of walnut slurry from 10% to 50% in the formulation, whereas the iron, magnesium and zinc contents of the PUFA-enriched samples increased. These results can be attributed to the mineral contents of milk and walnut slurry. Walnut is known to be a good source of minerals, especially iron, magnesium and zinc, in comparison with milk (USDA 2018b, 2018c).

The calcium, phosphorus, iron, zinc and magnesium contents of the PUFA-enriched yoghurt samples were higher than the standard reference values set for plain yoghurt made from whole milk (USDA 2018a, 2018b). However, the contents of sodium and potassium were lower than the standard reference values. The mineral analysis showed that the PUFA-enriched yoghurt sample was a good source of minerals for a dairy yoghurt, especially calcium, phosphorus and magnesium.

The low sodium content of the PUFA-enriched yoghurt sample may make it superior to regular dairy yoghurt. A low sodium intake is recommended because a high sodium intake increases blood pleasure, which is a risk factor for cardiovascular diseases (Noubiap *et al.* 2015).

Starter culture counts

The counts of lactic acid bacteria during four weeks of storage at 4 °C are presented in Table 4. On the first day, the F1 and F2 samples exhibited a higher count of *L. delbrueckii* subsp. *bulgaricus* than the control sample ($P < 0.05$), and no significant difference was observed in the other samples ($P > 0.05$). In the first week, the yoghurt samples showed no significant difference ($P > 0.05$). In the second week, the F3, F4 and F5 samples had a higher count of *L. delbrueckii* subsp. *bulgaricus* than the control sample ($P < 0.05$), whereas the F1 and F2 samples had a lower count ($P < 0.05$). In the third week, the control sample showed a higher count of *L. delbrueckii* subsp. *bulgaricus* than the PUFA-enriched samples ($P < 0.05$). In the fourth week, it also had a higher count of *L. delbrueckii* subsp. *bulgaricus* except F2.

On the first day, the F1, F2 and F5 samples exhibited a higher count of *S. thermophilus* than the control sample ($P < 0.05$), and the other samples presented no significant difference ($P > 0.05$). In the first week, the F5 sample had a lower count than the control sample. In the second and third weeks, the control sample had a higher count of

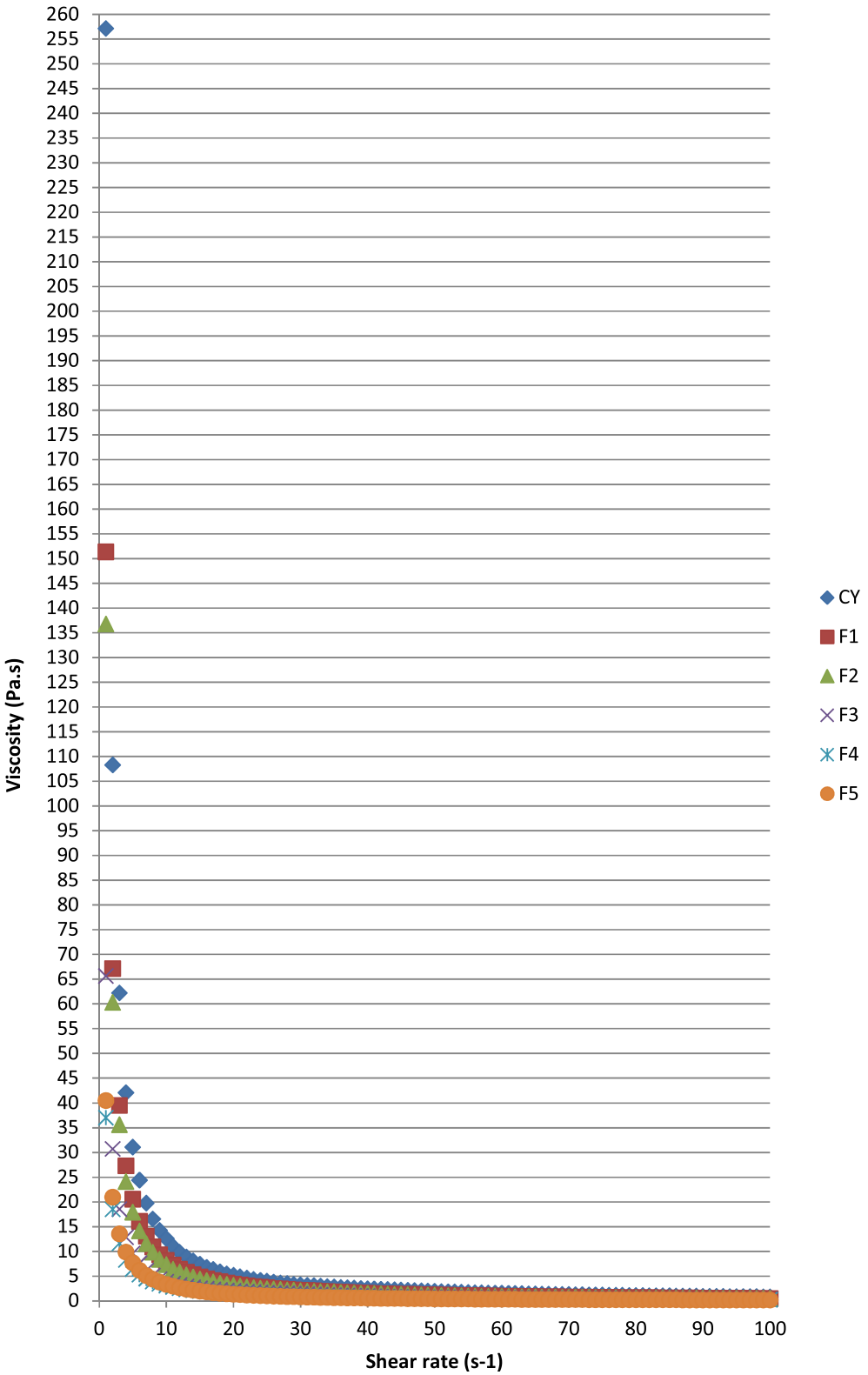

Figure 1 Viscosity values as a function of shear rate. [Colour figure can be viewed at wileyonlinelibrary.com]

S. thermophilus than the PUFA-enriched samples ($P < 0.05$). In the fourth week, the F4 and F5 samples showed a lower count than the control sample.

The F3 and F4 samples had a higher count of *L. delbrueckii* subsp. *bulgaricus* at the end of storage than on the first day. The F2, F3, F4 and F5 samples also

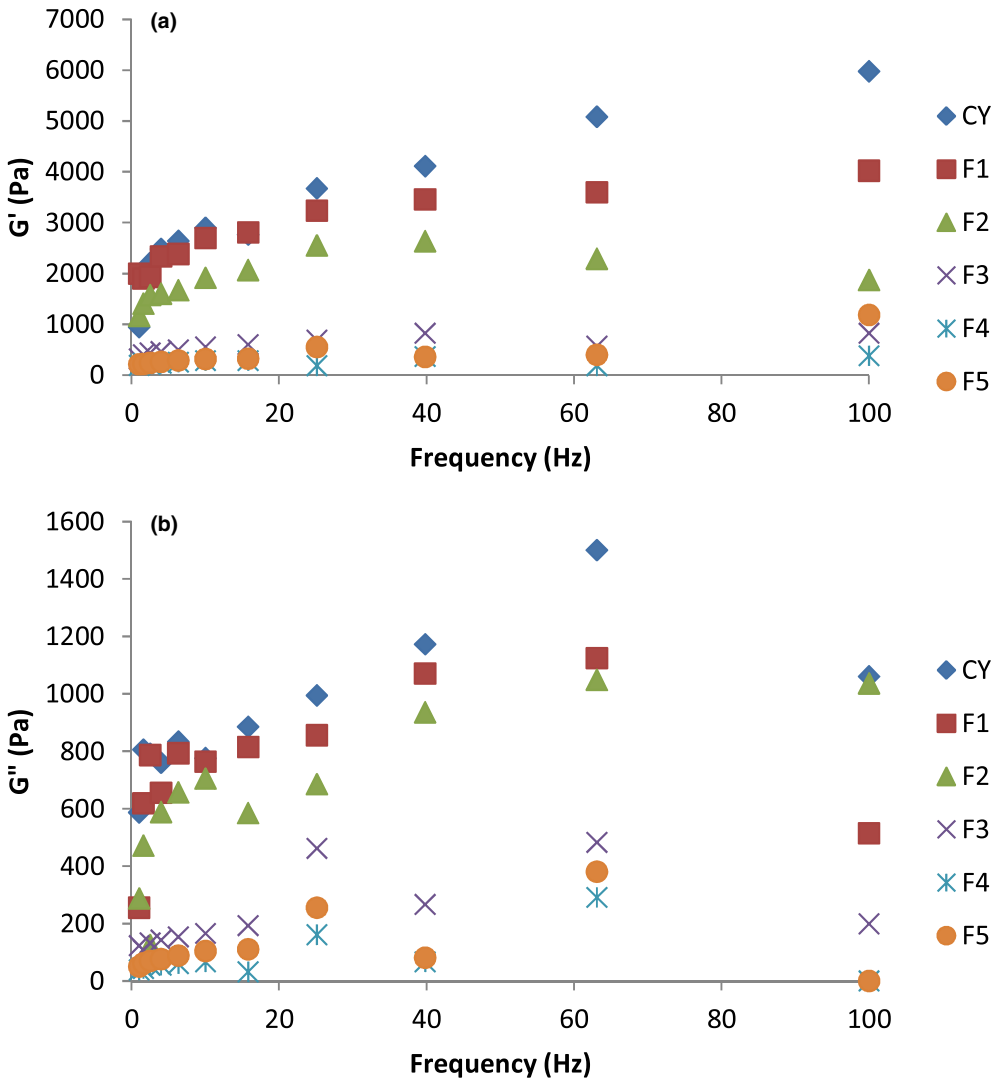

Figure 2 (a) Storage modulus as a function of frequency. (b) Loss modulus as a function of frequency. [Colour figure can be viewed at wileyonlinelibrary.com]

showed a higher count of *S. thermophilus* at the end of storage than on the first day.

The counts of lactic acid bacteria of all the samples increased after four weeks of storage, indicating that lactic acid bacteria could survive in the yoghurt samples. Yoghurt should contain viable bacteria at a concentration of more than 7 log cfu g^{-1}. All samples had viable bacteria of more than 7 log cfu g^{-1}, thus revealing that the PUFA-enriched yoghurt met the criteria suggested for yoghurt (Codex, 2003).

Sensorial properties

The sensorial scores of the yoghurt samples are presented in Table 1. The sensorial scores of the PUFA-enriched yoghurt samples were found to be lower than those of the control sample. The lower sensorial scores of the enriched yoghurt samples could be related to the addition of walnut slurry into the formulation. The appearance, consistency and odour scores of the PUFA-enriched yoghurt samples decreased with the increasing walnut slurry in the formulation. In the colour analysis, the PUFA-enriched yoghurt was found to have a lower lightness value and higher yellowness and redness values than the control sample, which could explain the lower appearance scores of the PUFA-enriched yoghurt samples. Walnut odour could lead to the lower odour scores of the enriched yoghurt samples. Acetaldehyde is responsible for the typical aroma of yoghurt. Walnut aroma was reported to consist of hexanal, nonanal and 2-decenal as the main volatile compounds (Abdallah *et al.* 2015), and these compounds may suppress acetaldehyde. The taste scores of the PUFA-enriched yoghurt samples generally showed no significant difference ($P > 0.05$). Moreover, the general acceptability of the PUFA-enriched yoghurt samples, except the F2 sample, presented no significant difference ($P > 0.05$).

The F1 sample had the highest scores among the samples. The increase in the proportion of walnut slurry in the formulation could have reduced the sensorial scores of the enriched yoghurt samples. The low scores of the enriched yoghurt samples could be related to the fact that this panel was the first experience of the panellists about this product. Flavour agents may be used to suppress the walnut aroma in the PUFA-enriched yoghurt. In this way, the sensorial acceptability of the PUFA-enriched yoghurt can be improved, and this type of yoghurt may obtain the opportunity to be commercialised.

Rheological properties

Viscosity is an important property affecting the texture of foods. The control sample exhibited a higher viscosity value than the PUFA-enriched yoghurt samples (Figure 1). This finding may be attributed to the absence of casein in the walnut slurry. Milk protein is important in the formation of coagulum, and the viscosity of yoghurt is affected by the protein level of the milk (Tamime and Robinson 1999). The viscosity of the yoghurt samples decreased with the increasing shear rate, and thus the samples showed a shear-thinning behaviour. The breakdown of the gel structure could have resulted from the reduction in viscosity as the shear rate increased.

The viscoelastic properties of foods are investigated by the oscillatory test to evaluate the physical structure. The storage modulus (G') shows the deformation energy stored in a sample, and the loss modulus exhibits the energy dissipated (Prasanna *et al.* 2013). For the yoghurt samples, the changes in the storage (G') and loss (G'') modules as functions of frequency were determined (Figure 2a,b). The storage modules of the yoghurt samples were higher than the loss modules, indicating solid-like structure. The control sample had higher G' and G'' values than the PUFA-enriched samples, thus the enriched samples showed a weaker structure than the control sample. A reduction in both modules was observed with the increasing walnut slurry in the formulation. This result indicated that the addition of walnut slurry into formulation could result in a weak structure. Weakness in the structure may be associated with the absence of casein in the walnut slurry, as yoghurt structure is related to the gel formation of milk proteins (Vital *et al.* 2015).

CONCLUSION

This study revealed that the PUFA-enriched yoghurt could provide macronutrients and micronutrients as dairy yoghurt. It had a lower amount of sodium than the control sample. Moreover, it was rich in PUFAs as linoleic (omega-6) and linolenic (omega-3) acids. The PUFA-enriched yoghurt exhibited a comparable value with the optimal ratio of omega fatty acids for a balanced intake. The combination of the walnut slurry with skimmed milk may be utilised as a raw ingredient to develop yoghurt enriched with PUFAs. However, flavouring and thickening agents should be used in the production of PUFA-enriched yoghurt by using walnut slurry to increase the acceptability of the product. Further studies should focus on the use of flavouring and thickening agents to improve the sensorial acceptability of PUFA-enriched yoghurt by using walnut slurry.

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