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Evaluation of sensory and *in vitro* anti-thrombotic properties of traditional Greek yogurts derived from different types of milk

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Abstract

Given that fermented dairy products exhibit high bioactivities against cardiovascular diseases (CVDs), the anti-thrombotic properties, fatty acid profiles and sensory properties of cow, goat and ewe derived Greek yogurts have been assessed and compared. The total lipids (TL), total polar lipids (TPL), total neutral lipids (TNL) were obtained and the polar lipid fractions were further separated by thin layer chromatography (TLC). These lipid samples (TL, TPL and TLC fractions) were subsequently assessed for their biological activity against atherosclerosis based on the *in vitro* inhibition of Platelet Activating Factor (PAF)-induced platelet aggregation. The fatty acid compositions of all yogurts were analyzed by Gas Chromatography with flame ionization detector (GC-FID). Goat yogurt lipids have been found to exert more potent inhibitory activity (i.e. lower IC_{50} values in both TL and TPL samples) in contrast to the corresponding fractions of cow and ewe

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yogurts. The observed sensory data indicates that ewe yogurt was the most palatable of all three Greek yogurts.

Keywords: Food Science

1. Introduction

Despite the fact that milk and dairy products are considered fundamental constituents of the Mediterranean diet and it is thus suggested that they are to be consumed daily; dairy products have been the subject of controversy regarding their impact on human health. According to the recommendations of the World Health Organization (WHO), consumption of dairy products should be limited in order to minimize the intake of cholesterol and saturated fatty acids (SFA) such as palmitic and myristic acid, in order to reduce the incidences of cardiovascular disease risk globally (World Health Organisation, 2002). On the other hand, several clinical studies suggest that the consumption of milk and dairy products may exert a possible protective effect against coronary heart disease, since they contain components such as calcium, magnesium, potassium, vitamin D and certain amino acids (Chrysant and Chrysant, 2013). Some constituents such as peptides, linoleic acid, conjugated linoleic acid (CLA), antioxidants, lactic acid bacteria and probiotic bacteria present in milk and dairy products have been shown to contribute to the functionality of dairy products against chronic diseases (Rogelj, 2000). Dairy products also contain polar lipids such as phosphatidylcholine (PC) and sphingomyelin (SM), which are mainly located in the milk fat globule membrane. Evidence suggests that both PC and SM possess strong anti-inflammatory activities and therefore the capacity to attenuate cardiovascular risk (Liu et al., 2015).

Milk has been found to have moderate cardioprotective properties in comparison to fermented dairy products, especially yogurt. The biologically active peptides derived during bacterial fermentation of milk are thought to have anti-thrombotic properties (Ivey et al., 2015). Moreover, during yogurt fermentation, the bacteria *Streptococcus thermophillus* and *Lactobacillus bulgaricus* seem to enhance the bioformation of lipids capable of inhibiting platelet activation induced by Platelet Activating Factor (PAF) (Antonopoulou et al., 1996). PAF, (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a strong phospholipid intermediary of inflammation (Demopoulos et al., 1979) and it is regarded as a trigger molecule for the onset of atherogenesis (Demopoulos et al., 2003). PAF is involved in the inflammatory generation of plaque and the hindrance of blood vessels, which are the main causes of coronary heart diseases (Demopoulos et al., 2003). Milk and dairy products contain compounds that can inhibit PAF activity, acting as PAF- inhibitors. These dairy components are of particular nutritional merit because they impede platelet aggregation in arteries while at the same time they prevent atheromatosis development (Antonopoulou et al., 1996; Poutzalis et al., 2016; Tsorotioti et al., 2014).

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Recent studies have demonstrated that dairy products may possess cardioprotective and immunomodulatory bioactivities *in vivo*. In one such study, the cardiovascular impact of Minas Frescal cheese consumption was assessed in hypertensive rats. The consumption of this cheese resulted in the significant lowering of blood pressure and an improved lipid profile in the rats (Lollo et al., 2015a). Furthermore, another study examined probiotic fermented milk treated by dynamic high pressure, which was fed to rats for 2 weeks. The fermented milk product was effective in reducing the impact of exercise-induced immunosuppression (Lollo et al., 2015b).

Greek yogurt is produced from three different kinds of milk namely cow, ewe and goat milk. Generally Greek yogurt production does not standardize or homogenize milk resulting in the formation of a crust on the yogurt's surface (Serafeimidou et al., 2012). Cow yogurt is consumed largely due to its low price and less distinctive smell compared to those derived from other ruminant milk sources. The aim of the current study was to evaluate the fatty acid contents, the *in vitro* anti-thrombotic properties and the sensory characteristics of three different yogurts produced from cow, ewe and goat milk respectively.

2. Materials and methods

2.1. Reagents and instruments

All reagents and solvents along with the silica gel G-60 used for Thin Layer Chromatography (TLC) separation were supplied by Merck (Darmstadt, Germany). Bovine serum albumin (BSA), PAF, the fatty acid methyl ester (FAME) standards and the polar lipid standards used for TLC separation (mix standard of hen egg yolk) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Platelet aggregation was measured using a Chrono-Log (Havertown, PA, USA) aggregometer (model 400-VS) coupled to a Chrono-Log recorder (Havertown, PA, USA).

2.2. Yogurt samples

Three different types of Greek yogurt, i.e. cow, ewe and goat yogurt were purchased from supermarkets in Athens, Greece. The lipid content of cow, ewe and goat yogurt samples were 3.5%, 4.0% and 7.0%, respectively (i.e. g of lipid/100 g food). All samples were sealed in clay pots, transferred to the laboratory and kept at 4C prior to further analysis.

2.3. Isolation of total lipids (TL), total polar lipids (TPL) and total neutral lipids (TNL)

Total lipids (TL) of all yogurt samples were extracted from 100 g of each sample (cow, ewe and goat yogurt) according to the Bligh-Dyer method (Bligh and Dyer,

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1959) and separated into total polar lipids (TPL) and total neutral lipids (TNL) by countercurrent distribution (Galanos and Kapoulas, 1962).

2.4. Fractionation of TPL by preparative Thin Layer Chromatography (TLC)

The fractionation of TPL was carried out as described by Nasopoulou et al. (2007). About 45 mg of goat and ewe yogurt TPL were applied to the TLC plates. All isolated lipid fractions obtained were evaporated to dryness under nitrogen; the lipids were weighed and re-dissolved in 1 mL chloroform/methanol 1:1 (v/v) and stored at −20 °C until further analysis.

2.5. Biological assay on washed rabbit platelets

TL and TPL of the three types of yogurt (i.e. cow, ewe and goat yogurt) and TLC polar lipid fractions of goat and ewe yogurt samples were tested for their *in vitro* biological activity against 2.5×10^{-11} M PAF (final concentration in the cuvette) towards washed rabbit platelets. Washed rabbit platelets were prepared as described by Demopoulos et al. (1979). The EC_{50}, namely the 50% maximal effective concentration of aggregation, and IC_{50} the inhibitory concentration for 50% inhibition, values were calculated for each biologically active lipid fraction, as described by Nasopoulou et al. (2007).

2.6. Gas chromatographic analysis

Fatty acid methyl esters (FAME) of 35 mg of TPL and 35 mg of TNL of all yogurt samples were prepared and analyzed by GC-FID using the internal standard method, as described by Nasopoulou et al. (2011). A five-point calibration curve (given by the equation $y = 0.00012x + 0.0167$ with $r = 0.99993$) was prepared using five solutions of heptadecanoic (17:0) acid methyl ester and heneicosanoic (21:0) acid methyl ester in various ratios (Poutzalis et al., 2016). The ratio of the area of the analyte peak to that of the internal standard represented the y value in the above equation, and subsequently, the x value represents the analyte concentration of the fatty acid in the unknown mixture. This equation was used in order to quantify the analytes. The GC-FID analysis of FAMEs was carried out as described by Nasopoulou et al. (2011).

2.7. Sensory evaluation of yogurt samples

The training of the panelists in order a) to identify and give scores for the attributes taste, flavor, aftertaste and odour and b) to distinguish and give scores for the basic senses of taste namely sweet, salty, bitter and sour was carried out as described by Sioriki et al. (2015). The panel consisted of 10 assessors (5 females, 5 males), aged 24–45 years, recruited from the University of Athens. Four dairy products (milk;

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cheese; buttermilk and Greek feta cheese) were used for training the assessors to different textures. During the seventh successive session, two samples of cow and ewe yogurt, different from the samples used for the analysis in this study, were used as a frame of reference in order to develop terminology based on the attribute differences (Sioriki et al., 2015). The evaluation of taste, aftertaste and texture was performed according to a list of fifteen attributes: five for taste (fatty, rich, sour, delicious, whey), five for aftertaste (sweet, pleasant, goaty, intense, persistent), and five for texture (creamy, smooth, velvety, grainy, fluid).

2.8. Statistical analysis

All experimental analyses were carried out in triplicate, and the obtained results were expressed as mean value ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed in order to find the significant statistical significant differences. When differences were identified at a significant level of $p < 0.05$ then multiple comparisons of the means were conducted using the Fisher's least significant difference (LSD) test at $p < 0.05$. Statistical analysis of the data was conducted using the statistical software package PASW 18 for Windows (SPSS Inc., Chicago, 215 IL, USA). For the principal component analysis (PCA), XLSTAT 18.06 (Addinsoft, Paris, France), was used.

3. Results

3.1. Lipid contents of cow, ewe and goat yogurt samples

The TL, TPL and TNL levels found in cow, ewe and goat yogurt are shown in Table 1. Cow and goat yogurts have been found to have similar levels of TL and TNL, whereas in the ewe's yogurt sample, the levels of TL levels were found to be statistically higher. The levels of TPL in cow and ewe yogurts were similar, but goat yogurt contained statistically lower levels of TPL.

3.2. Fatty acid profile of TPL and TNL of cow, ewe and goat yogurt samples

The TPL and TNL fatty acid profiles of cow, ewe and goat yogurt are presented in Table 2 and Table 3, respectively. The levels of saturated fatty acids (SFA) were found to be higher in both TPL and TNL of all yogurt samples followed by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Both the TPL and TNL of all yogurt samples contain the following fatty acids: caproic (6:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and a-linolenic (18:3) fatty acid. The most abundant fatty acids in both the TPL and TNL yogurt lipid fractions were palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids. The TPL fraction of ewe yogurt was found to contain statistically higher levels ($p < 0.05$) of caproic (6:0), capric

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Table 1. Content of total lipids (TL), expressed in grams per 100 g yogurt (mean ± SD, n = 3), total polar lipids (TPL) and total neutral lipids (TNL), expressed as percentages of TL in cow, goat and ewe yogurt samples.

Sample	TL (g/100 g yogurt)	TPL (% TL)	TNL (% TL)
Cow	3.06 ± 0.92[a]	13.4 ± 2.01[a]	83.9 ± 5.56[a]
Goat	3.75 ± 0.90[a]	9.45 ± 1.78[b]	81.6 ± 5.02[a]
Ewe	6.76 ± 1.25[b]	13.2 ± 2.10[a,b]	77.6 ± 4.19[a]

[a,b]Different superscripts indicate significant differences among different yogurt samples within the same lipid class (cow vs. ewe yogurt, goat vs. ewe yogurt and cow vs. goat yogurt, respectively; $p < 0.05$) when means are compared using a Fisher's LSD multiple comparison test.

Table 2. Fatty acid profile of total polar lipids (TPL) of each sample expressed in milligrams per kilogram of each yogurt sample (mean ± SD, n = 3).

Fatty acids	Cow yogurt	Goat yogurt	Ewe yogurt
6:0	22.1 ± 1.99[a]	12.4 ± 0.22[b]	54.7 ± 5.30[c]
8:0	ND	4.34 ± 0.28[a]	5.85 ± 0.27[b]
10:0	3.11 ± 0.16[a]	32.2 ± 1.20[b]	56.2 ± 4.38[c]
12:0	3.04 ± 0.15[a]	14.1 ± 0.52[b]	40.9 ± 1.16[c]
14:0	16.3 ± 0.32[a]	39.4 ± 0.82[b]	126 ± 4.28[c]
15:0	ND	1.00 ± 0.12[a]	3.55 ± 0.01[b]
16:0	59.3 ± 0.25[a]	142 ± 1.29[b]	293 ± 3.12[c]
16:1 (ω-7)	1.05 ± 0.05[a]	2.54 ± 0.21[b]	ND
18:0	24.9 ± 0.01[a]	69.2 ± 0.59[b]	512 ± 9.25[c]
18:1 (cis) (ω-9)	52.6 ± 0.28[a]	173 ± 3.61[b]	243 ± 1.89[c]
18:2 (cis) (ω-6)	12.5 ± 0.03[a]	32.5 ± 0.15[b]	38.8 ± 0.01[c]
18:3 (cis) (ω-3)	ND	5.21 ± 0.04[a]	8.90 ± 0.03[b]
20:0	ND	1.67 ± 0.16[a]	4.23 ± 1.10[b]
20:1 (ω-9)	ND	0.29 ± 0.01[a]	1.52 ± 0.67[b]
20:4	ND	0.83 ± 0.06	ND
24:0	ND	0.83 ± 0.11	ND
SFA	129 ± 0.13[a]	317 ± 0.09[b]	1096 ± 0.03[c]
MUFA	53.7 ± 0.05[a]	176 ± 0.09[b]	245 ± 0.05[c]
PUFA	12.5 ± 0.05[a]	38.5 ± 0.07[b]	47.7 ± 0.04[c]

[a,b,c]Different superscripts indicate significant differences among different yogurt samples within the same row ($p < 0.05$) when means are compared using a Fisher's LSD multiple comparison test. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; ND: non detectable.

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Table 3. Fatty acid profile of total neutral lipids (TNL) of each sample expressed in milligrams per kilogram of each yogurt sample (mean ± SD, n = 3).

Fatty acids	Cow yogurt	Goat yogurt	Ewe yogurt
6:0	182 ± 1.99[a]	211 ± 6.20[b]	340 ± 2.01[c]
8:0	31.8 ± 3.17[a]	118 ± 2.77[b]	9.46 ± 2.33[c]
10:0	200 ± 0.24[a]	748 ± 7.21[b]	198 ± 4.33[c]
12:0	194 ± 7.48[a]	294 ± 0.01[b]	138 ± 2.29[c]
14:0	653 ± 6.56[a]	913 ± 4.29[b]	457 ± 4.77[c]
14:1 (ω-5)	31.3 ± 1.03	ND	ND
15:0	62.4 ± 0.86[a]	35.0 ± 0.13[b]	3.14 ± 0.86[c]
16:0	1959 ± 5.23[a]	3009 ± 2.64[b]	1422 ± 9.59[c]
16:1 (ω-7)	85.6 ± 1.05	ND	ND
17:1 (ω-7)	7.96 ± 0.74	ND	ND
18:0	665 ± 4.74[a]	1917 ± 10.9[b]	2575 ± 4.72[c]
18:1 (cis) (ω-9)	1910 ± 0.27[a]	2399 ± 0.12[b]	1288 ± 4.52[c]
18:2 (cis) (ω-6)	207 ± 0.13[a]	286 ± 0.71[b]	158 ± 0.08[c]
18:3 (cis) (ω-3)	10.7 ± 0.07[a]	62.3 ± 0.18[b]	13.8 ± 9.75[c]
20:0	ND	ND	14.5 ± 0.12
SFA	3947 ± 0.02[a]	7245± 0.07[b]	5157 ± 0.08[c]
MUFA	2035 ± 0.11[a]	2399 ± 0.03[b]	1288 ± 0.06[c]
PUFA	218 ± 0.04[a]	348 ± 0.03[b]	172 ± 0.03[c]

[a,b,c]Different superscripts indicate significant differences among different yogurt samples within the same row ($p < 0.05$) when means are compared using a Fisher's LSD multiple comparison test. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; ND: non detectable.

(10:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) fatty acids than the TPL fractions of goat and cow yogurts (Table 2). The TNL fraction of goat yogurt was found to exhibit statistically higher levels ($p < 0.05$) of caprylic acid (C8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), oleic (18:1), linoleic (18:2) and α-linolenic (18:3) fatty acids in contrast to the TNL fractions of ewe and cow yogurts (Table 3). The rather low levels of fatty acids in the TNL and TPL fractions are in concurrence with similar work on the TPL and TNL of goat dairy products (Poutzalis et al., 2016).

3.3. *In vitro* anti-thrombotic activity of TL, TPL and TLC polar lipid fractions

The TL, TPL and TLC polar lipid fractions of all yogurt samples were assessed for their ability to induce washed rabbit platelet aggregation (EC_{50}) or inhibit the PAF-induced washed platelet aggregation (IC_{50}). The corresponding results for

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biological activity are expressed in micrograms (μg) for the TL and TPL fractions of all yogurt samples and are presented in Fig. 1.

According to Fig. 1, both the TL and TPL fractions of all three yogurt samples have been found to exert inhibitory activity, thus, it could be suggested that these fractions contain lipids that act as PAF inhibitors. The IC_{50} values for the TL and TPL goat yogurt samples (33.0 μg and 15.4 μg, respectively), were found to be significantly lower ($p < 0.05$) compared to the values for ewe yogurt (43.1 μg and 27.2 μg, respectively) and cow yogurt (45.4 μg and 40.8 μg, respectively) (Fig. 1). At this point, it should be emphasized that the lower the IC_{50} value, the stronger the anti-thrombotic potency of the lipid fraction, since less amount of lipid is required to exert 50% inhibition of the PAF-induced washed platelet aggregation. Finally, it should also be stated that the TPL of all three yogurt samples have been found to show stronger inhibitory activity than the TL, within the same yogurt sample (Fig. 1); neither the TL nor the TPL of all samples showed any aggregation.

According to Fig. 1, the TPL of goat and ewe yogurt have been found to exhibit the strongest *in vitro* anti-thrombotic activity; thus these lipid fractions were selected for further separation by preparative TLC, as shown in Fig. 2a and b, respectively. The biological activities of the TLC polar lipid fractions derived from goat and ewe yogurts, expressed in micrograms (μg), are shown in Fig. 3a and b, respectively.

According to Fig. 2a and b, seven bands of polar lipid fractions have been found in both yogurt samples. These results correspond with previously published research on the lipid fractions obtained in goat cheese (Poutzalis et al., 2016).

All TLC polar lipid fractions of goat and ewe yogurt were found to exhibit inhibitory activity against the actions of PAF (IC_{50} value), while only band 2 of the

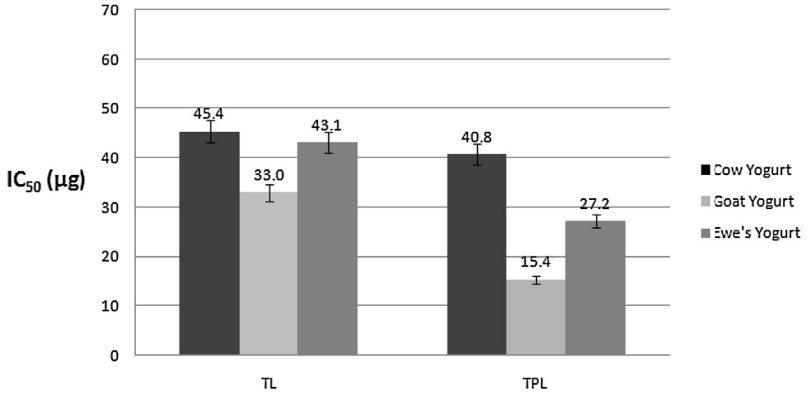

Fig. 1. *In vitro* biological activities of total lipids (TL) and total polar lipids (TPL) of cow, ewe and goat yogurts towards washed rabbit platelet aggregation, expressed in micrograms of TL and TPL. Washed rabbit platelet concentration was approximately 500,000 platelets μL^{-1}. The final concentration of PAF in the cuvette was 2.5×10^{-11} M. The experimental analyses were carried out in triplicate (n = 3).

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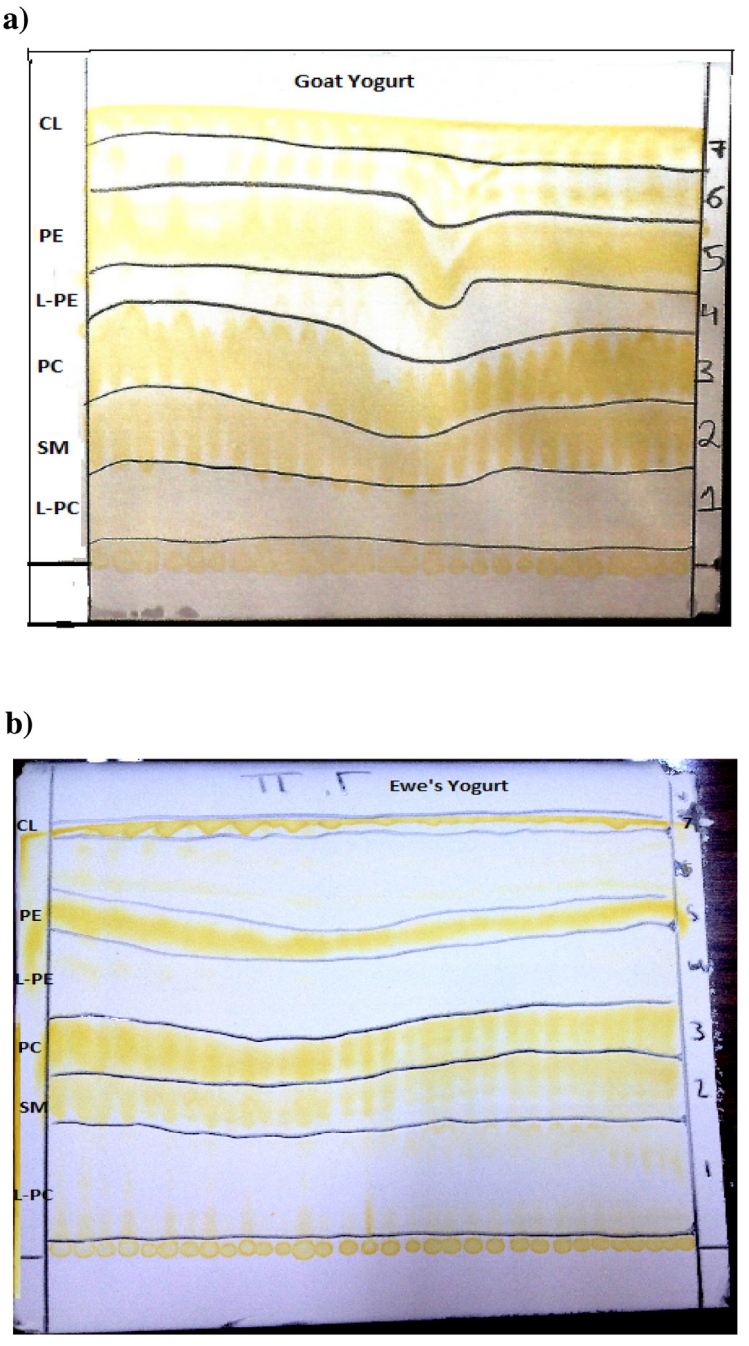

Fig. 2. Typical profile of total polar lipid (TPL) separation on preparative thin-layer chromatography (TLC). a) goat yogurt sample, b) ewe yogurt sample. In the photo, the seven bands obtained are shown on the right whereas on the left, the corresponding elution front of standard compounds is given. L-PC: lyso-phosphatidylcholine, SM: sphingomyelin, PC: phosphatidylcholine, L-PE: lyso-phosphatidylethanolamine, PE: phosphatidylethanolamine, CL: cardiolipin. The preparative plates TLC were stained with iodine vapours.

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TLC polar lipid fractions exhibited aggregatory activity (EC_{50} value) (Fig. 3a and b). The TLC polar lipid fraction 2 has been found to have a similar R_f value corresponding to sphingomyelin (SM). The TLC polar lipid fractions 3 and 7 of the goat yogurt samples exert a greater *in vitro* anti-thrombotic activity in comparison to the respective TLC polar lipid fractions of the ewe yogurt sample (28.4 μg as opposed to 122 μg for fraction 3 and 46.8 μg as opposed to 85.2 μg for fraction 7 (Fig. 3a and b). Fractions 3 and 7 correspond to the R_f values of phosphatidylcholine (PC) and cardiolipin (CL), respectively.

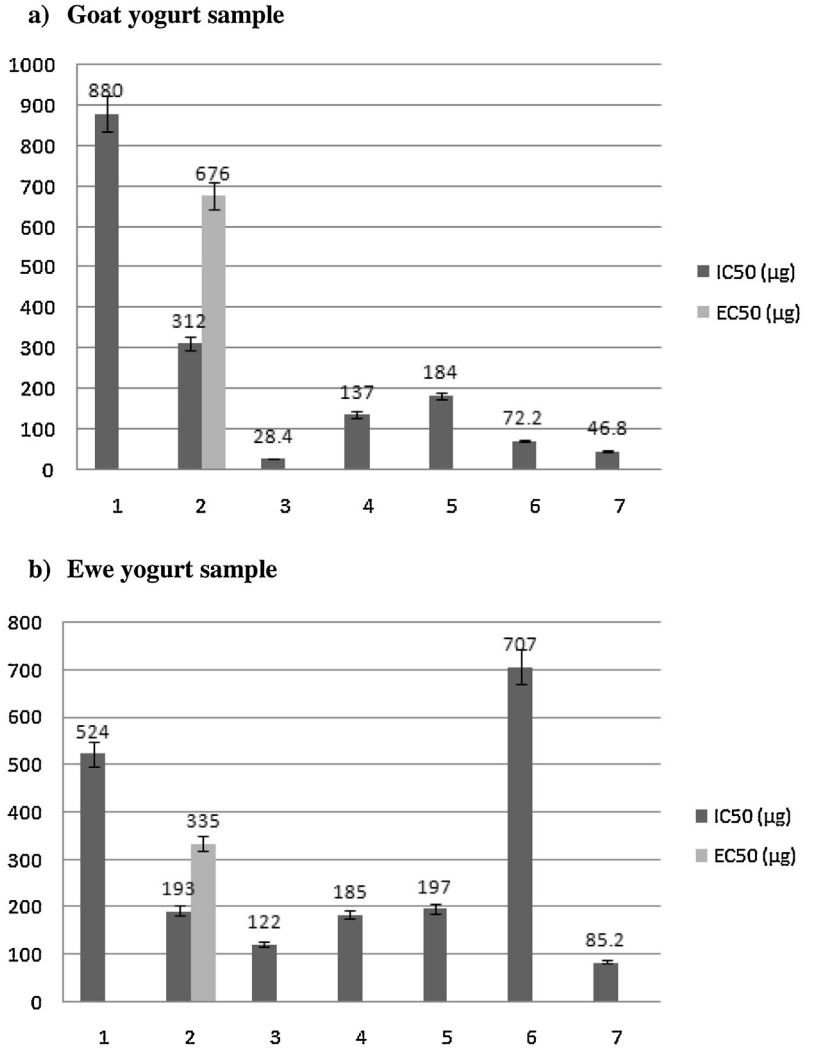

Fig. 3. *In vitro* biological activity of preparative thin-layer chromatography (TLC) polar lipid fractions of a) goat yogurt sample and b) ewe yogurt sample, towards washed rabbit platelet aggregation, expressed in micrograms. Washed rabbit platelet concentration was approximately 500,000 platelets μL^{-1}. The final concentration of PAF in the cuvette was 2.5×10^{-11} M. The experimental analyses were carried out in triplicate (n = 3).

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By comparing the biological activities of the TLC polar lipid fractions of both yogurt samples, it could be said that the TLC polar lipid fractions 3, 4, 5, 6 and 7 of goat yogurt sample have been found to exert statistically lower IC_{50} values ($p < 0.05$). Therefore, the goat TLC polar lipid fractions possess stronger anti-thrombotic activity in comparison to the activity of the ewe yogurt TLC polar lipid fraction (Fig. 3a and b). Conversely, the TLC polar lipid fractions 1 and 2 of the goat yogurt sample have shown statistically higher IC_{50} values ($p < 0.05$) compared to the ewe yogurt sample (Fig. 3a and b). The TLC lipid fraction 3 of both yogurt samples, which has an R_f value corresponding to phosphatidylcholine (PC), also exhibited potent *in vitro* anti-thrombotic activity.

3.4. Sensory analysis

The panelists scored the three yogurt samples using values between zero (0, least liked) and ten (10, most liked) for attributes describing the taste, aftertaste and texture properties. The scores of each yogurt sample (cow, ewe or goat yogurt samples) for each attribute used are given in the form of spider-web plots (Fig. 4). The sensory data was also subjected to principal component analysis in order to detect if there was a pattern in the sensory data (Matera et al., 2014).

The assessors indicated statistically significant organoleptic differences among cow, ewe and goat yogurt. According to Fig. 4, the ewe yogurt sample has been found to rate significantly higher for the taste attributes of “fatty”, “rich”, “delicious”, and “whey” and for the texture attribute “‘grainy”’. On the other hand, the goat yogurt sample has been found to rate significantly higher for the attribute of “sour” for taste, the attributes of “persistent”, “intense”, and “goaty” for the aftertaste and the attributes of “velvety”, “smooth” and “fluid” for the texture (Fig. 4). The cow yogurt samples have been found to rate significantly higher only for the aftertaste attribute of “sweet” (Fig. 4). As it is observed from the PCA (Fig. 5), the first two factors accounted for 25.25% and 19.09% of the variance respectively. Factor one separated most of the samples B (goat yogurt) from samples A and C (ewe and cow yogurts respectively). The goat yogurt was characterised as goaty, rich, smooth, intense and persistent. However, in the case of F2 the separation was not clear. Despite the fact that only 44.34% of the variance was described using the first two factors, we still gain a useful insight into the positioning of the three yogurts in relation to their sensory characteristics.

4. Discussion

Dairy products have been linked with the increased risk of CVDs, due to their high concentration in SFA and cholesterol (Mann, 2002). However, recent data on the levels of bioactive fatty acids (e.g. short-chain fatty acids and CLA) and other minor components (e.g. phospholipids and sphingolipids) indicate that dairy

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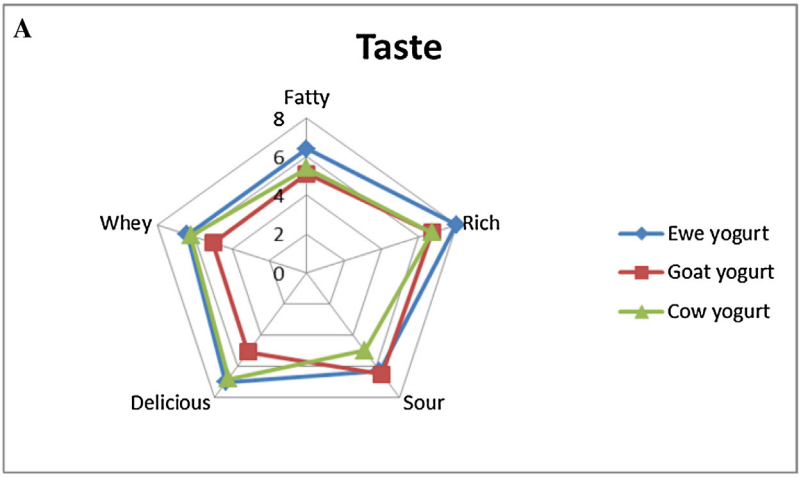

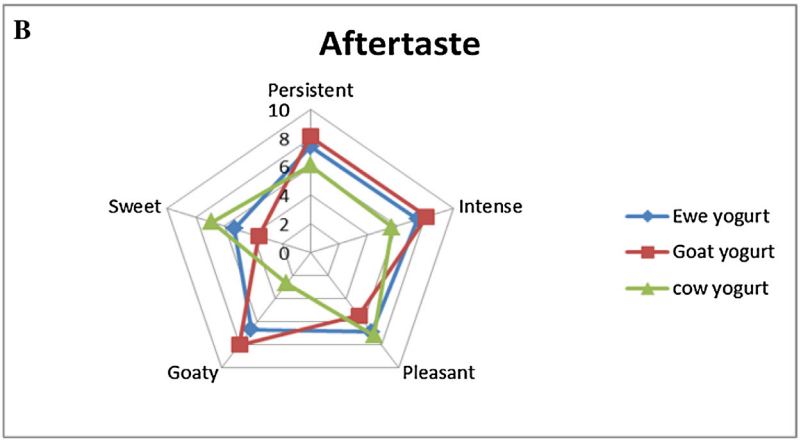

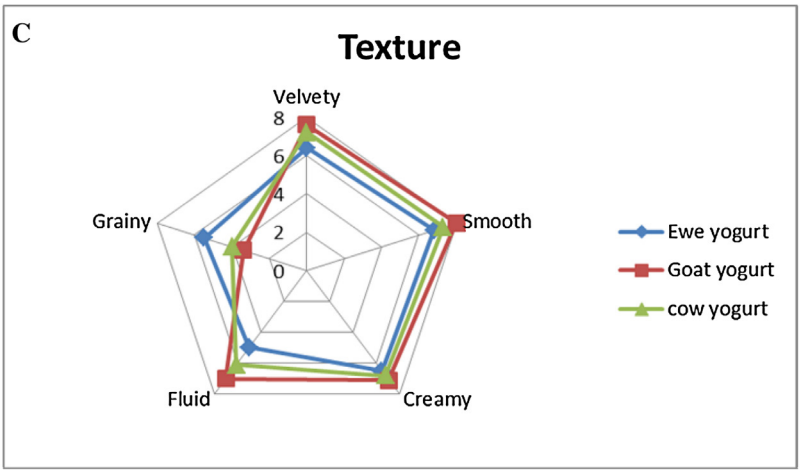

Fig. 4. Comparison of sensory profiles of cow, ewe and goat yogurt samples. The scores for a) taste b) aftertaste and c) texture attributes are given as spider-web plots.

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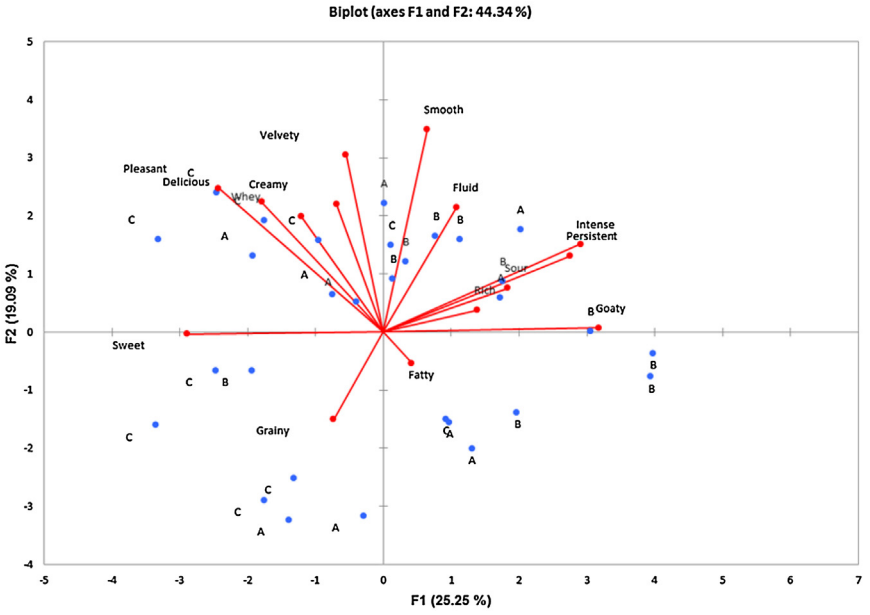

Fig. 5. Principal component analysis (PCA) of the three yogurt samples (A: ewe yogurt, B: goat yogurt, C: cow yogurt).

products may have a positive important impact on cardiovascular and metabolic risk factors (Castro-Gómez et al., 2014).

In the current study, it was demonstrated that the TL and TPL fractions of all yogurt samples tested (cow, ewe and goat yogurts) contain PAF inhibitors (Fig. 1), which is in accordance with the literature (Antonopoulou et al., 1996; Poutzalis et al., 2016). The TL and TPL of the goat yogurt samples exhibited the most potent biological activity against PAF (Fig. 1), suggesting that goat yogurt contains more potent PAF inhibitors than the cow or ewe yogurts and thus stronger *in vitro* anti-thrombotic properties. The *in vitro* anti-thrombotic properties of these yogurts could be attributed to the fact that during yogurt fermentation, bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* seem to augment the biosynthesis of lipids capable of inhibiting PAF activity (Antonopoulou et al., 1996; Antonopoulou et al., 2006).

The TLC polar lipid fractions 3 and 7 of both goat and ewe yogurts exhibited potent inhibitory activity against PAF-induced platelet aggregation (Fig. 3) and were found to have similar R_f values to those of PC and CL. The concentrations of PC and CL in goat and ewe milk are considerably high (Castro-Gómez et al., 2014). It could therefore be suggested that the strong *in vitro* anti-thrombotic activities found in goat and ewe yogurts could be due to biologically active lipids that are derivatives of PC and CL (Zabetakis, 2013; Nasopoulou et al., 2014).

The TLC polar lipid fraction 2 of both yogurt samples (goat and ewes), were found to contain lipid micro constituents inducing platelet aggregation (Fig. 3), thus

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acting as PAF agonists. The presence of such PAF agonists (i.e. lipids with aggregatory activity) is beneficial because PAF agonists have been found to exhibit stronger *in vivo* antiatherogenic activity than PAF-inhibitors (Nasopoulou et al., 2010; Tsantila et al., 2007). This can be explained on the basis that these PAF agonists have a higher affinity than PAF to the PAF G-protein coupled receptors, hence they compete with PAF for its receptors; this biochemical competition results in antiatherogenic activity (Nasopoulou et al., 2010).

The results of the present study indicate that the lipid fractions (TPL or TLC polar lipid fractions) of all yogurt samples exhibited either aggregatory or inhibitory actions, suggesting that these lipid fractions are biologically active against PAF activity and consequently against thrombosis.

The results of the extracted TL of all yogurt samples reflected their reported fat concentration. The ewe's yogurt sample (7% fat) contained the highest TL levels. The TNL of all three yogurt samples were found to be the dominant lipid class whereas the TPL were present in lower levels (Table 1).

SFAs were found to be higher in both the TPL and TNL of all yogurt samples followed by MUFAs and PUFAs, while the predominant fatty acids identified were palmitic (C16:0), oleic (C18:1), and stearic (C18:0) fatty acids (Table 2a and b), which is in agreement with the literature (Poutzalis et al., 2016; Serafeimidou et al., 2012). A number of metabolically valuable short and medium carbon chain FAs that are associated with the characteristic yogurt flavour and taste were identified. These include caproic (C6:0), caprylic (C8:0), capric (C10:0) and lauric (C12:0) fatty acids. The presence of these FAs was significantly higher in goat and ewe yogurts as opposed to cow yogurt (Table 2a and b). The differences in fatty acid levels among these yogurt samples is species specific and can vary due to the different types of milk; the breed, age, stage of lactation and the nutrition of the animal (Serafeimidou et al., 2012; Michaelidou 2008; Park et al., 2007).

The sensory evaluation of the yogurt samples demonstrated that the goat and ewe yogurts have been found to rate significantly higher for specific taste, aftertaste and texture attributes (Fig. 4). Such organoleptic variations may be related to the observed differences in lipid content and fatty acid profile of the yogurt samples. The evaluation also identified that the ewe yogurt sample was the most palatable.

5. Conclusions

The *in vitro* anti-thrombotic activities, the fatty acid levels and the organoleptic properties of traditional Greek yogurts made using three different types of milk, were determined. The results highlight the biological properties of Greek yogurt lipids against thrombosis, suggesting a potential cardio-protective capacity for these yogurts. The fatty acid contents along with the organoleptic properties varied

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according to the type of milk used in the production of these yogurts. Our aggregometric data suggests that goat and ewe yogurt have stronger anti-thrombotic properties as opposed to cow yogurt. It is proposed that the activities evident in goat and ewe yogurts could be linked to lipids that are PC derivatives since they exhibit similar R_f values to PC. Further mechanistic studies are required in order to elucidate the structure of these potent anti-thrombotic yogurt derived lipids.

Declarations

Author contribution statement

Kalliopi Megalemou, Eleni Sioriki: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ronan Lordan, Maria Dermiki: Analyzed and interpreted the data.

Constantina Nasopoulou, Ioannis Zabetakis: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Competing interest statement

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Additional information

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