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Fermentation Enhances the Anti-Inflammatory and Anti-Platelet Properties of Both Bovine Dairy and Plant-Derived Dairy Alternatives

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Abstract: Within the present study, the effects of fermentation on the anti-inflammatory and anti-platelet properties of both homemade and commercially purchased bovine dairy and almond, coconut, and rice-based dairy alternatives were evaluated. The extracted total lipids (TL) from homemade and commercially purchased fermented and unfermented bovine, almond, coconut, and rice-based products were further separated into their neutral lipids (NL) and polar lipids (PL) fractions by counter current distribution. The TL, PL, and NL of each sample were assessed in human platelets against the inflammatory and thrombotic mediator, platelet-activating factor (PAF), and the well-established platelet agonist, adenosine 5′ diphosphate (ADP). In all samples, the PL fractions showed significantly stronger inhibitory effects against human platelet aggregation induced by PAF or ADP, in comparison to the TL and NL, with higher specificity against PAF. PL of all fermented products (bovine yogurt and fermented dairy alternatives from almond, rice, and coconut), exhibited the strongest anti-inflammatory and anti-platelet potency, in comparison to PL from their initial pasteurized materials (bovine milk and rice, almond, and coconut-based dairy alternative drinks). PL of the pasteurized rice-based drink and, especially PL from the novel homemade rice-based fermented product (HMFRD), showed the strongest anti-PAF and anti-ADP potency compared to all samples, with anti-PAF activity being most potent overall. The unfermented pasteurized coconut-based drink showed the lowest anti-inflammatory and anti-platelet potency, and the bovine and almond-based fermented products showed an intermediate effect. Further lipidomics with LC-MS analysis of all these PL fractions revealed that fermentation altered their fatty acid content in a way that decreased their degree of saturation and increased the content of unsaturated fatty acids, thus providing a rationale for the stronger anti-inflammatory and anti-platelet potency of the more unsaturated PL fractions of the fermented products. This study has shown that fermentation alters the fatty acid content and the bio-functionality of the PL bioactives in both fermented bovine dairy and plant-based dairy alternatives, and subsequently improved their anti-inflammatory and anti-platelet functional properties.

Keywords: fermentation; bovine; dairy alternatives; yogurt; anti-inflammatory; anti-platelet; PAF; ADP

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1. Introduction

Chronic disorders, including cardiovascular diseases (CVD), respiratory disease, diabetes, and cancer account for over 70% of global deaths [1]. Chronic inflammation has been implicated as the cause of such diseases. More specifically, inflammatory and thrombotic

mediators like platelet-activating factor (PAF) and thrombin, as well as platelet agonists like adenosine 5′ diphosphate (ADP) and collagen, play a crucial role in the inflammatory and thrombotic activation and response of key cells that are implicated in the onset and development of such chronic disorders [2–4]. Tackling inflammation through pharmaceutical therapies to treat inflammation-related chronic disorders can have unwanted side effects so dietary intervention and especially novel functional foods with anti-inflammatory properties are of particular interest due to the absence of side effects [2,3,5,6]. Bioactive compounds from natural sources with strong anti-inflammatory potency, such as the polar lipid bioactives found in several foods, such as dairy and fermented products, have been found to inhibit the action of PAF and the activities of other inflammatory mediators showing the promise for natural bioactive compounds for the treatment of disease [2,3,5–10].

Bovine dairy and, especially dairy lipids, are sometimes tied to negative perceptions regarding their effects on human health [11]. This is due to the increased degree of saturation and the higher levels of saturated fatty acids in such dairy, the consumption of which may increase serum levels of triglycerides and cholesterol and thus increase the risk for the development of CVD [12,13]. Evidence suggests, however, that the link between dairy fats and negative health outcomes is not well supported, while fermented dairy products like yogurt and kefir have exhibited positive effects on cardiovascular health [14–16]. Nevertheless, the consumption of plant-based alternative drinks has risen due to concerns about dairy consumption on human health, animal welfare, and environmental sustainability [17]. The economic demand for dairy alternatives is expected to reach $14.36 billion [18]. However, there are several limitations to the replacement of dairy products with plant-based alternatives, such as their low protein content and poorer protein quality [19] and differences in nutritional quality, and the presence of anti-nutritional factors [20–22]. Plant-based dairy alternative drinks are produced from legumes, cereals, nuts, or seeds by either pulverizing plant material with water to extract the water-soluble nutrients or by forming an oil-in-water emulsion using plant oils [23]. Many of these products are fortified with vitamins and minerals, including vitamin B12, calcium, and vitamin D, to bring their nutritional composition closer to that of dairy milk before being bottled and subjected to ultra-high temperature treatment (UHT). Additionally, sugars, flavors, and other vegetable oils may be added to improve the sensory properties.

Fermented plant-based dairy alternatives to cheese and yogurt are also widespread. Fermentation of plant material has been shown to improve flavor, texture, and nutritional profile [24,25]. Some common substrates for commercially purchased plant-based fermented products are soy, oat, almond, and coconut, while traditional recipes also exist for rice-based fermented products. Classic fermented dairy products are mainly produced by incubation with monocultures of lactic acid bacteria (LAB) strains [15], while other approaches involving mixed-culture fermentation and utilizing two or more microbial species are gaining ground. Independent of the method and starter culture, fermentation of both bovine dairy and dairy alternatives have been shown to beneficially modulate their lipid content and functionality [6–8,15,16,24,25]. Nevertheless, the research on the anti-inflammatory and anti-platelet potency of such fermented products and especially their lipid content is limited.

The aim of this study was to evaluate the anti-platelet and anti-inflammatory properties of both commercially purchased and homemade, fermented and unfermented, bovine dairy and plant-based dairy alternative products of a high-fat plant source, coconut, an intermediate-fat plant source, almond, and of a plant source, rice, for which fermented products as dairy alternatives do not exist in the market. For the first time, these products were evaluated against PAF-associated inflammatory and thrombotic activation, as well as against the platelet activation effects of a well-established platelet agonist, ADP, in human platelets. Additionally, the most bioactive lipid fractions found in each product were further analyzed for their fatty acid content by LC-MS analysis, in order to evaluate structure–activity relationships. This study will provide valuable insights into the effect of fermentation in dairy and dairy alternatives, as well as the potential use of plant-based

fermented products, as novel functional foods against the development of CVD and other inflammation-related chronic disorders.

2. Materials and Methods

2.1. Materials, Reagents, and Instrumentation

Analytical glass/plastic consumables, reagents, and solvents were purchased from Fisher Scientific Ltd. (Dublin, Ireland). Solvent evaporation was carried out by flash rotatory evaporation (Buchi Rotavapor, Mason Technology, Dublin, Ireland) and nitrogen stream using nitrogen cylinders (BOC, Dublin, Ireland). Platelet aggregation bioassay materials were purchased from Labmedics LLP (Abingdon on the Thames, UK). Blood sampling was carried out using 20G safety needles and evacuated sodium citrate S-monovettes from Sarstedt Ltd. (Wexford, Ireland). Standard PAF and bovine serum albumin (BSA) were purchased from Merk (Wicklow, Ireland) while standard ADP was purchased from CHRONOLOG (Havertown, PA, USA). Platelet aggregation bioassays of human platelet-rich plasma (hPRP) were carried out using a Chronolog-490 two-channel turbidimetric platelet aggregometer (Havertown, PA, USA) coupled to the accompanying AGGRO/LINK software. Centrifugation was performed using Eppendorf 5702R centrifuge (Eppendorf Ltd., Stevenage, UK) and Heraeus Biofuge Stratos centrifuge (Fisher Scientific Ltd., Dublin, Ireland). Spectrophotometric analysis was conducted using the Shimadzu UV-1800 spectrophotometer (Tokyo, Japan) with 1 cm quartz cuvettes. LC-MS analysis was carried out using HPLC (Agilent 1260 series, Agilent Technologies Ireland Ltd., Little Island, Co. Cork, Ireland), Q-TOF mass spectrometer (Agilent 6520), and an Agilent C18 Poroshell 120 column (Agilent Technologies Ireland Ltd., Little Island, Co. Cork, Ireland).

2.2. Sample Preparation

Home-made plant-based dairy alternative drinks were prepared using commercially available rice, desiccated coconut, and almonds. Homemade rice-based dairy alternative drink was prepared by homogenizing 100 g cooked rice with 500 mL water and straining through a muslin cloth. Homemade coconut-based dairy alternative drink was prepared by homogenizing 200 g shredded coconut with 1 L boiling water and straining through a muslin cloth. Homemade almond-based dairy alternative drink was prepared by soaking 100 g almonds overnight to remove the skin, homogenizing with 500 mL water then straining through a muslin cloth. Alternative versions of the almond and rice drinks were prepared using long grain rice and basmati rice and raw and pasteurized almonds.

The fermented bovine dairy (bovine yogurt) and the fermented plant-based products of all these dairy alternative sources were prepared by inoculation of 200 mL pasteurized bovine milk or each one of the aforementioned home-made purchased plant-based drinks with commercially available yogurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus casei* obtained from bio yogurt. The viability of the starter bacteria was assessed using flow cytometry. All samples were incubated at 37 °C for 24 h. the initial microbial load of all samples was 10^4 CFU/mL. The microbial load was determined using flow cytometry and pH was measured before and after fermentation. Samples were stored at 4 °C before testing.

Commercially purchased pasteurized bovine milk, rice-based drink ("Rice Dream Original Organic", Hain Daniels Group, Leeds, UK), coconut-based drink ("Alpro Coconut No Sugars", Alpro Ltd., Northamptonshire, UK), and almond-based drink ("Alpro Almond No Sugars", Alpro Ltd., UK) were obtained from local grocery stores and stored at 4 °C before analysis. Commercially purchased bovine yogurt and fermented coconut product ("Coconut Collaborative Dairy-Free Natural Coconut Yog", The Coconut Collaborative Ltd., London, UK) and commercially fermented almond products ("Nush Dairy Free Almond M*lk Yog", Nush Foods, London, UK) were also obtained from local grocery stores and stored at 4 °C before analysis. Commercially purchased products were selected based on the purity of ingredients, the most minimal ingredients, and for being produced in similar ways as the homemade products were produced in the lab. Especially for the commercially

purchased bovine yogurt and the fermented plant-based dairy alternative products, these were selected on the basis of purity of ingredients and microbial species present in the final product, as being similar to the microbial species used for the production of the homemade fermented products (*Streptococcus thermophilus* and *Lactobacillus casei*). No fermented rice dairy alternative product was found in the market to be purchased and tested. All other products were analyzed on the same day of their production or their purchase.

2.3. Extraction of Total Lipids and Separation into Polar Lipids and Neutral Lipids

The total lipids (TL) from each sample type were extracted in triplicate (n = 3), as previously described [9,26], using the Bligh and Dyer extraction method [27]. Briefly, each sample was homogenized using a Waring blender (Fisher Scientific Ltd., Dublin, Ireland) with 1:2:0.8 ($v/v/v$) chloroform/methanol/water then filtered using Whatman Grade 1 filter paper (Whatman, Maidstone, UK) with a Büchner vacuum filtration device. Phase separation was induced by transferring the filtrate into a separatory funnel and adding enough chloroform/methanol/water to achieve a 1:1:0.9 ($v/v/v$) ratio. Ten percent of the resulting TL fraction was retained for further analysis, while the remainder was further separated into the neutral lipids (NL) and the polar lipids (PL) fractions as previously described [9,26], using the Galanos and Kapoulas counter current distribution method [28]. Solvents of the obtained lipid fractions were evaporated using a flash rotary evaporator at a maximum of 40 °C and then the lipid samples were re-dissolved in 1:1 (v/v) chloroform/methanol and transferred to pre-weighted small glass vials, where the remaining solvent was further evaporated using nitrogen stream. The remaining evaporated lipid samples were weighed and stored in glass vials at −20 °C for a maximum of 8 weeks before further analysis.

2.4. Platelet Aggregometry Biological Assays

The anti-inflammatory and anti-platelet properties of all TL extracts and PL and NL fractions from the fermented and unfermented homemade and commercially purchased plant-based dairy alternative products from coconut, almond, and rice, as well as those of bovine milk and yogurt, were evaluated in human platelets for their ability to inhibit the aggregation of human platelet-rich plasma (hPRP) induced by the inflammatory and thrombotic mediator, PAF, and the well-established platelet agonist, ADP, using platelet aggregometry biological assays, as previously described [9,26,29].

All platelet aggregation bioassays were carried out in a Chronolog-490 two-channel turbidimetric platelet aggregometer (Havertown, PA, USA) coupled to the accompanying AGGRO/LINK® software (Version Opti8 for performing Aggregation, CHRONOLOG, Havertown, PA, USA) package, for analyses. For the sampling of blood, 20 G safety needles and evacuated sodium citrate S-monovettes were used, which were purchased from Sarstedt Ltd. (Wexford, Ireland), while the isolation of hPRP was performed by centrifugation using an Eppendorf 5702R centrifuge (Eppendorf Ltd., Stevenage, UK), as previously described [9,26,29]. A Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) was used with a quartz 1 cm cuvette for the spectrophotometric analysis. All the other materials, consumables, and reagents for platelet aggregation were purchased from Labmedics LLP (Abingdon, UK) and Chronolog (Havertown, PA, USA), apart from standard PAF and BSA that were purchased from Sigma Aldrich (Wicklow, Ireland).

The anti-inflammatory and antithrombotic potency of all lipid extracts and fractions were expressed as means of their IC_{50} (half-maximal inhibitory concentrations) values ± standard deviation (SD), presented in mass (μg) of the bioactive lipid compounds in the aggregometer cuvette of 0.25 mL that causes 50% inhibition of PAF/ADP-induced platelet aggregation. Briefly, a range of concentrations for each lipid sample was assessed and a linear and dose-dependent relationship of the inhibitory effects of the lipid bioactives with the concentrations of each lipid sample tested was observed, within the 20–80% range of the percentage of inhibition against PAF/ADP induced aggregation of hPRP. In order to derive such inhibitory curves, a range of 10 to 200 μg amount of lipids was assessed for the

most bioactive lipid extracts (PL), while a range of 50 to 800 µg was also utilized for the less bioactive ones (TL and NL). From this derived curve, for each lipid sample assessed, the concentration (µg) of the lipid sample that led to 50% of PAF/ADP induced aggregation of hPRP was calculated as the 50% inhibitory concentration value (IC_{50} value) for each lipid sample. Using blood samples from different donors, all experiments for evaluating the bioactivities of each lipid extract from all dairy and dairy alternative products. were performed several times (n = 6), for each replicate, in order to ensure reproducibility.

2.5. Fatty Acid Composition by LC-MS Analysis

The bioactive PL fractions of all fermented and unfermented dairy and dairy alternative samples against the PAF and ADP pathways of human platelet aggregation were analyzed by LC-MS as previously described [10], in order to elucidate their saponified fatty acid composition (free fatty acids; FFA). Briefly, each of these PL fractions was saponified by adding 1.5 mL of saponification reagent (2.5 M KOH: methanol (1:4, v/v)) and by a gentle vortex. Then the tubes were incubated at 72 °C for 15 min before the addition of 225 µL of formic acid. Then, 1725 µL of chloroform and 375 µL of ultrapure water were added to the tube, and vortexed to separate the content into two layers. The lower chloroform layer containing FFA was carefully transferred to amber gas chromatography vials and evaporated to dryness and stored at 20 °C until used for LC-MS analysis.

For LC-MS analysis, all dried lipids were re-constituted in 500 µL of methanol: dichloromethane (2:1, v/v), centrifuged at 13,000 rpm for 6 min (Heraeus Biofuge Stratos, Fisher Scientific Ltd., Dublin, Ireland) and the content was filtered through 3 kDa ultracentrifuge filters (Amicon Ultra 3k, Merck Millipore Ltd., Carrigtwohill, Co. Cork, Ireland). Then, 10 µL of the filtrate was injected and the fatty acid profiles were obtained from an HPLC (Agilent 1260 series, Agilent Technologies Ireland Ltd., Little Island, Co., Cork, Ireland) equipped with a Q-TOF mass spectrometer (Agilent 6520) and the source type was electrospray ionization (ESI). The column used for the resolution of fatty acids was an Agilent C18 Poroshell 120 column (2.7 µm, 3.0–150 mm). Mobile phase A consisted of 2 mM ammonium acetate in water and mobile phase B consisted of 2 mM ammonium acetate in 95% acetonitrile. Chromatographic separation was performed by gradient elution starting with 60% B for 1 min, then increasing to 90% B over 2.5 min. Subsequently, 90% B was held for 1.5 min and increased afterward to 100% over 5 min. Then, 100% B was held for 4 min, reducing afterward to 60% B over 0.5 min, and held for 1 min until the next run. The mobile phase flow rate was 0.3 mL/min until 5 min elapsed, increasing up to 0.6 mL/min after 10 min, and held at this flow rate until the end of the run. The mass spectrometer was operated in negative ionization mode, scanning from m/z 50–1100. Drying gas flow rate, nebulizer pressure, and temperature were 5 L min^{-1}, 30 psi, and 325 °C, respectively. The fragmentor and skimmer voltages were maintained respectively at 175 V and 65 V, and the capillary voltage was 3500 V. The monitoring reference masses used were 1033.988 and 112.9855 in the negative ion mode. Standard fatty acids such as palmitic (C16:0), oleic (C18:1n-9 cis), and eicosapentaenoic (EPA, 20:5n-3) acids were used to validate the LC-MS protocol by comparing their specific accurate mass and their retention time (RT) (Sigma Aldrich, Wicklow, Ireland). Each FFA from the corresponding lipid fractions was then identified based on their known accurate mass. The peak area of each identified fatty acid was the average of triplicate samples, which was used to determine the relative % of each FFA of the total fatty acids identified in each sample.

2.6. Statistical Analysis

Kolmogorov–Smirnov criterion was used to test the normality of the yield of extraction, the IC_{50} values, and fatty acid composition obtained for each lipid sample. Subsequently, for comparisons of the lipid content and FA composition of all PL fractions, acquired from the LC–MS analysis, the Kruskal–Wallis nonparametric multiple comparison test was used, while one-way analysis of variance (ANOVA) was used for all comparisons of the IC_{50} values of these lipid bioactives against the ADP-/PAF-induced platelet aggregation. The

differences were statistically significant when the *p*-values were less than 0.05 ($p < 0.05$). The resulting IC_{50} values were expressed as a mean value of the mass of lipid (μg) in the aggregometer cuvette ± standard deviation (SD), while fatty acids content was expressed as the mean % percentage of total fatty acids of each sample (mean ± SD). Analysis of the data was carried out using a statistical software package (IBM-SPSS statistics 26 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Lipid Yield

The mass of recovered TL, PL, and NL of all homemade and commercial samples (expressed as g of lipids/100 g of sample) are given in Table 1. The combination of methods applied for this extraction and separation of lipids into PL and NL fractions in all samples, was the Bligh and Dyer [27] extraction method in conjunction with the Galanos and Kapoulas [28] counter-current distribution technique, as previously described, [9,26], which ensured a minimal loss of yield and retention of integrity of bioactive PL compounds in comparison to traditional methods like Soxhlet extraction that uses heat which threatens to degrade double bonds and other functional groups in the fatty acids and other lipid molecules of the lipid extracts. The methodology in which the lipid extracts and fractions were obtained and stored was thus suitable for the minimum loss of their functionality and their use in assessing their anti-inflammatory and anti-platelet potency using the well-established platelet aggregometry.

Table 1. Lipid extraction yield for homemade plant drinks and fermented plant products.

Samples	TL [1]	NL [1]	PL [1]
BM	2.87 ± 0.25 *	2.01 ± 0.22 *	0.80 ± 0.12 *
HMFBM	2.36 ± 0.41 *	1.55 ± 0.45 *	0.85 ± 0.17 *
HMAD	0.43 ± 0.29	0.32 ± 0.27	0.11 ± 0.07
HMFAD	0.25 ± 0.27	0.19 ± 0.20	0.06 ± 0.07
HMRD	0.20 ± 0.10	0.14 ± 0.04 #	0.06 ± 0.02
HMFRD	0.15 ± 0.05 #	0.09 ± 0.06 #	0.06 ± 0.05
HMCD	2.52 ± 0.81 *	2.47 ± 0.80 *	0.05 ± 0.01
HMFCD	1.88 ± 0.64 *	1.83 ± 0.64 *	0.05 ± 0.01
CPFBM	2.80 ± 0.25 *	2.41 ± 0.25 *	0.38 ± 0.03 *
CPAD	0.62 ± 0.06	0.59 ± 0.07	0.04 ± 0.01
CPFAD	3.42 ± 0.52 *	3.26 ± 0.51 *	0.16 ± 0.01
CPCD	0.77 ± 0.03	0.75 ± 0.03	0.02 ± 0.01 #
CPFCD	7.52 ± 0.47 **	7.39 ± 0.46 **	0.12 ± 0.01
CPRD	0.70 ± 0.08	0.56 ± 0.12	0.14 ± 0.05

[1] Expressed as mean ± SD ($n = 3$) of g of lipids per 100 g of source. ** Denotes the highest yield with a statistically significant difference ($p < 0.01$), while * denotes high yield in comparison to the other samples with lower yield ($p < 0.05$). # Denotes the lowest yields within all the TL, NL, and PL extracts respectively, with statistically significant difference ($p < 0.05$) Abbreviations: TL, total lipid; NL, neutral lipid; PL, polar lipid; BM, bovine milk; HMFBM, homemade fermented bovine milk; HMAD, homemade almond drink; HMFAD, homemade fermented almond drink; HMRD, homemade rice drink; HMFRD, homemade fermented rice drink; HMCD, homemade coconut drink; HMFCD, homemade fermented coconut drink; CPAD, commercially purchased almond drink; CPFAD, commercially purchased fermented almond drink; CPRD, commercially purchased rice drink; CPCD, commercially purchased coconut drink; CPFCD, commercially purchased fermented coconut drink.

As shown in Table 1, the higher TL content was observed in the bovine dairy products (the pasteurized bovine milk BM, and the homemade and commercially purchased bovine yogurts, HMFBM and CPFBM), but mostly in the unfermented and fermented coconut-based homemade and commercially purchased dairy alternative drinks HMCD and CPCD, and their fermented products, HMFCD and CPFCD, with the commercially purchased fermented coconut-based CPFCD product containing the highest TL content (approx 7.5 g of TL per 100 g of source). Apart from the commercially purchased fermented almond-based CPFAD product, all the other homemade and commercially purchased plant-based dairy alternatives, almond-based and rice-based fermented and unfermented HMAD, HMFAD, HMRD, HMFRD, CPAD, and CPRD products showed much lower TL content,

approximately one-fifth of the TL content of the BM, yogurt, and coconut-based products' overall lipid content.

Independently of the overall TL content, all samples contained higher amounts of NL, with their PL comprising a smaller proportion of their TL content, while in some cases, the homemade products' fermentation increased the Pl content in the final fermented product in comparison to the unfermented raw material. For example, BM samples contained ~75% NL and ~25% PL of their TL, while fermentation resulted in a relative reduction of the NL content (65% of TL) and an increase of the PL content (35% of TL) of the homemade HMFBM yogurt. Similarly, the homemade fermented rice-based HMFRD product showed much higher PL content (40% of the TL) in comparison to either the homemade or the commercially purchased unfermented raw rice-based HMRD (PL was 30% of the TL) and CPRD (PL was 20% of the TL) dairy alternative drinks.

However, this was not the case for the commercially purchased CPFBM yogurt, where the NL content was increased and the Pl content was decreased in comparison to the initial raw BM. Fermentation did not alter the % PL content of the homemade HMAD, since this dairy alternative drink and its fermented HMFAD contained a similar proportion of NL (75% of TL) and PL (25% of TL), which were similar also to the proportions of NL and PL observed in the unfermented BM, while both commercially purchased CPAD and CPFAD showed lower content of PL (4–6% of TL) and higher NL content (94–96% of TL), suggesting that fermentation did not seem to influence the ratio of the PL and NL proportions in the almond-based products. Both homemade and commercially purchased coconut-based fermented and unfermented dairy alternatives, HMCD, HMFCD, CPCD, and CPFCD had a significantly higher proportion of NL to PL (97.5% of NL and 2.5% of PL to their TL content, respectively), while it seems that in these coconut-based dairy alternatives, fermentation again did not seem to influence their ratio of the PL and NL proportions. A commercially fermented rice-based dairy alternative drink was not available in the market to be purchased, for any analysis.

Overall, homemade versions had higher PL proportions than commercially available counterparts except for coconut products which had very similar proportions to homemade. Variability between homemade and commercial plant-based beverages and fermented products may be explained by the difference in the plant substrate used. Variability in lipid content is known to occur between varieties of plant substrates [30–32]. Fermentation seemed to only affect the lipid yield of homemade bovine and rice-based dairy alternatives. In terms of lipid content, fermentation did not have a significant effect on commercially purchased samples.

The current study demonstrated that plant-based dairy alternatives were found to contain substantial amounts of PL that differ in yield based on the substrate used. In comparison to dairy, rice-derived products were found to have the most similar or, in some cases (HMFRD), better polar lipid proportion. The PL content of the plant-based dairy alternatives assessed in this study was much lower than other plant substrates like apple pomace [26] and tea [33], but similar to other plant-based fermented products/beverages, such as beer and cider [9,10], indicating that the plant-based alternatives may be good candidates for functional foods containing considerable amounts of PL bioactives per 100 g of food source, with enhanced health-promoting effects.

3.2. Anti-Inflammatory and Anti-Platelet Properties of Fermented and Unfermented Plant Drinks

The anti-inflammatory and anti-platelet bioactivities of the TL extracts and NL and PL fractions from all fermented and unfermented plant-based dairy alternative drinks and bovine dairy were evaluated by assessing their inhibitory effects against aggregation of human platelets induced by the inflammatory pathways of PAF, as well as against platelet aggregation induced by the well-established platelet agonist, ADP (Figure 1). Results depicted in Figure 1A–C are expressed in IC_{50} values (half-maximal inhibitory concentrations; μg of lipid samples needed for 50% inhibition of platelet aggregation). Low IC_{50} values represent a more beneficial inhibitory effect against PAF pathways of inflammation and

ADP-induced platelet aggregation. In all samples assessed in this study (bovine dairy and plant-based alternatives), PL displayed the strongest anti-inflammatory potency against PAF and anti-platelet effect against ADP (Figure 1A) while TL extracts displayed an intermediate potency against both PAF and ADP (Figure 1B) with NL having the lowest potency against both mediators (Figure 1C). The finding that the PL bioactives from both bovine dairy and plant-based dairy alternatives showed the strongest anti-PAF and anti-ADP activities than the NL and TL comes in accordance with similar results observed for the anti-inflammatory properties of PL, TL, and NL in other dairy sources (ovine and caprine), as well as in other plant-based fermented and non-fermented foods, beverages, and their by-products [7–10,26,33]. Both homemade and commercially purchased fermented bovine yogurts (HMFBM and CPFBM) had stronger anti-PAF and anti-ADP effects compared to their unfermented raw material, pasteurized commercially purchased bovine milk (BM). This suggests that fermentation of bovine milk by *Streptococcus thermophilus,* and *Lactobacillus casei* increased the anti-inflammatory and anti-platelet bio-functionality of their PL present.

A similar effect was observed in homemade almond, coconut, and rice-based fermented dairy alternatives, whereby the PL-bioactives of these plant-based fermented products showed much stronger anti-PAF and anti-ADP potency in comparison to their unfermented raw materials (almond, coconut, and rice dairy alternative drinks). This relationship was also seen in commercially purchased fermented almond and coconut products (CPFAD and CPFCD), the PL of which had higher bioactivities than the commercially purchased unfermented almond and coconut drinks. Since this effect can be seen in both bovine and plant-based products, it can be reasonably suggested that fermentation by species of *Lactobacillus casei* and *Streptococcus thermophilus* led to the increase in bio-functionality of PL fractions. Although it should be noted that neither plant-based fermented product contained the specific species *Lactobacillus casei* and the CPFAD stated the use of "live vegan cultures" including *L. acidiphilus* and *Bifidobacterium*, therefore, the complete microbial composition is unknown and may differ from other samples. Additionally, CPFCD listed *L. bulgaricus* and *L. acidiphilus* in addition to *S. thermophilus* and *Bifidobacterium* being present in CPFBM.

It should be stressed that the strongest anti-PAF and anti-ADP effects of PL fraction from all products were observed in the novel homemade fermented rice-based product (HMFRD), which surprisingly had stronger anti-inflammatory and anti-platelet effects than the ones observed in this study for the PL of the commercially purchased bovine yogurt and previously studied fermented dairy [7,8]. Such a rice-based fermented product was not available in the market to purchase and assess as a base for a comparison with the observed activities of the homemade novel rice product. Nevertheless, the very potent anti-inflammatory and anti-platelet potency of the PL from this novel homemade fermented rice-based dairy alternative product further suggest new perspectives for novel fermented plant-based foods and dairy alternatives as functional foods with anti-inflammatory and antithrombotic potential. Next to the HMFRD, the PL from the commercially purchased CPFCD and CPFAD also had strong anti-inflammatory and anti-platelet properties, with a potency that was similar to that of the PL from the CPFBM.

Interestingly, the PL bioactives in all these rice, almond, and coconut-based fermented dairy alternative products, as well as the PL from the bovine fermented dairy yogurt, showed strong inhibition of the PAF-induced and inflammatory activation and ADP-induced platelet aggregation, but with significantly higher anti-inflammatory specificity against the PAF-pathway than their anti-platelet potency against the ADP-induced platelet aggregation. This result also follows previously reported results for PL bioactives from several sources, since these PL have higher specificity as direct antagonists and agonists for the G-coupled protein PAF-receptor in cell membranes, due to structural homology to PAF, they inhibit strongly the binding of PAF to its receptor and subsequently the PAF-induced inflammatory activation [2,3,34]. Such PL bioactives can indirectly affect other pathways of platelet activation, like that of the platelet agonist ADP [34], which provides a rationale for

the higher specificity of the PL bioactives from both dairy and dairy alternative-fermented products against the PAF-pathway.

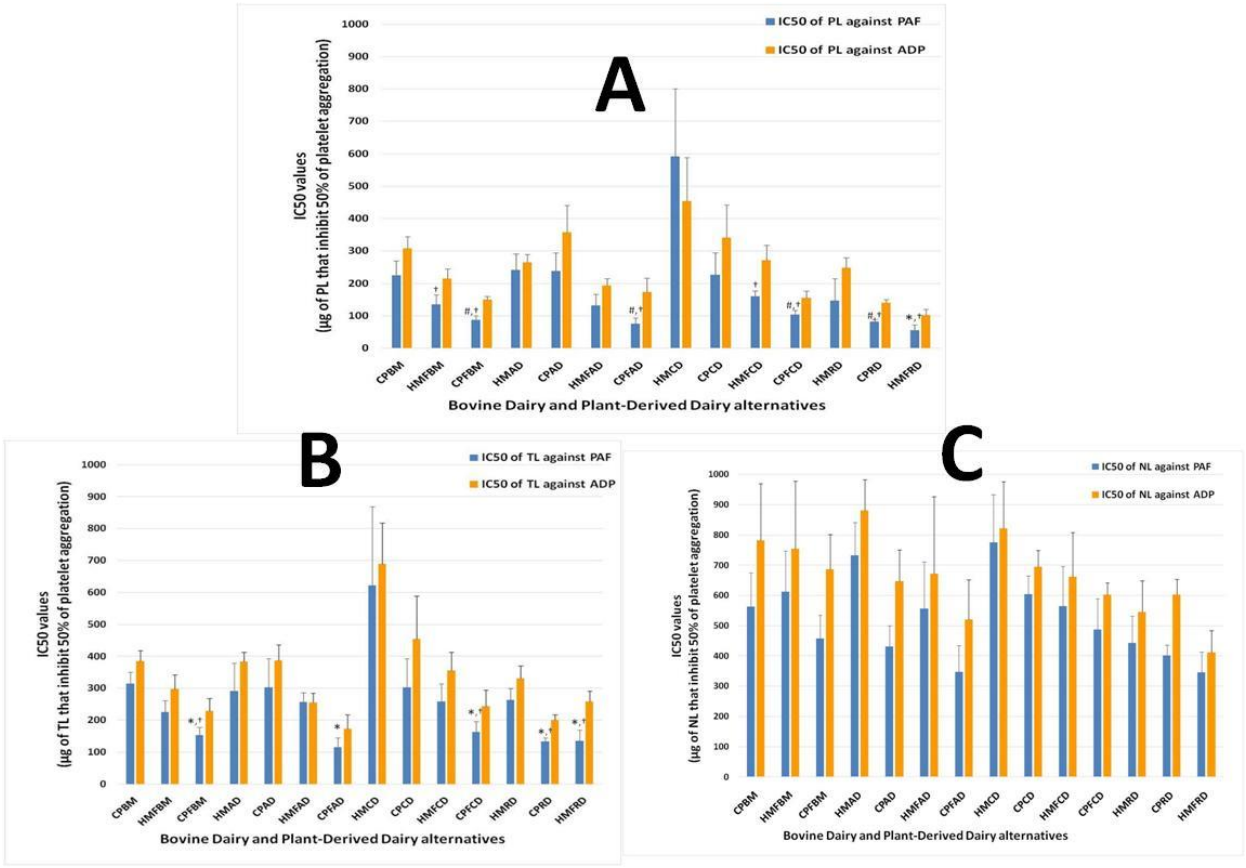

Figure 1. The anti-inflammatory and anti-platelet potency of bioactive PL (**A**), TL (**B**), and NL (**C**) from fermented and non-fermented plant-derived dairy alternatives, versus bovine milk and yogurt. The anti-inflammatory and anti-platelet potency of lipid bioactives from all samples were assessed against human platelet aggregation induced by the inflammatory and thrombotic mediator PAF (anti-PAF effects in blue bars) or by a classic platelet agonist, ADP (anti-ADP effects in yellow bars). Results are expressed as means of the IC50 values (half-maximal inhibitory concentrations) in µg of lipid extract in the aggregometer cuvette that causes 50% inhibition of PAF/ADP-induced platelet aggregation (the lower the IC50 value for a lipid extract the higher its inhibitory effect against the specific agonist of platelet aggregation). * Denotes statistically significant difference ($p < 0.05$) when the strongest anti-PAF potency (IC50 value) of the most bioactive lipid extracts were compared with those of all the other samples. # Denotes statistically significant difference ($p < 0.05$) when the intermediate anti-PAF effects of the PL bioactives from the samples assessed were compared with those showing the lowest anti-PAF effects. † Denotes statistically significant difference ($p < 0.05$) when the anti-PAF potency (IC50 values against PAF) of the lipid bioactives from a sample was compared to its anti-ADP potency (IC50 values against ADP) of the same sample. Abbreviations: PL: polar lipids; TL: total lipids; NL: neutral lipids; PAF: platelet-activating factor; ADP: adenosine 5′ diphosphate; CPBM: Commercially Purchased Bovine Milk; HMFBM: Homemade Fermented Bovine Milk (Homemade Yogurt); CPFBM: Commercially Purchased Fermented Bovine Milk (Commercially Purchased Yogurt); HMAD: Homemade Almond Drink; CPAD: Commercially Purchased Almond Drink; HMFAD: Homemade Fermented Almond Drink; CPFAD: Commercially Purchased Fermented Almond Drink; HMCD: Homemade Coconut Drink; CPCD: Commercially Purchased Coconut Drink; HMFCD: Homemade Fermented Coconut Drink; CPFCD: Commercially Purchased Fermented Coconut Drink; HMRD: Homemade Rice Drink; CPRD: Commercially Purchased Rice Drink; HMFRD: Homemade Fermented Rice Drink.

Lower anti-inflammatory and anti-platelet properties were observed in unfermented plant-based dairy alternatives drinks which were in line with anti-PAF and anti-ADP effects of PL from CPBM apart from CPRD and HMCD. Interestingly, the unfermented rice-based dairy alternative drinks, and especially the commercially purchased ones (CPRD) showed the strongest anti-PAF and anti-ADP effects within the unfermented products, which was comparable to that of the fermented products and higher than that of the PL from bovine milk. In contrast, the homemade coconut drink (HMCD) displayed the lowest bioactivity against both PAF and ADP of all samples assessed. Both commercially purchased and homemade almond-based drinks (HMAD and CPAD) showed an intermediate potency, similar to that of the BM.

Moreover, there did not appear to be an overall difference in the bioactivity of commercially purchased samples versus the homemade samples of the same source, therefore, it can be suggested that UHT treatment (above 135 °C for 2–5 s) did not result in the reduction of the bioactivity of their PL against the PAF and ADP-induced platelet aggregation.

While TL extracts had intermediate potency, they followed a similar trend relative to their PL bioactivities. More specifically, TL from fermented bovine products (CPFBM and HMFBM) had greater anti-PAF and anti-ADP than the unfermented CPBM. The same effect of fermentation can be seen with CPFAD and CPFCD which had greater bio-functionality compared to unfermented CPAD and CPCD. In homemade products, only HMFRD and HMFCD had stronger anti-PAF and anti-ADP than their unfermented drinks (HMRD and HMCD). Again, a higher specificity for PAF-pathway over ADP-pathway of TL fractions was seen with fermented bovine yogurts (AMFBM, CPFBM), some fermented plant-based products based in almond and coconut (CPFAD and CPFCD but, especially in the rice-based fermented product (HMFRD) and the unfermented rice-based dairy alternative drink (CPRD). Lastly, all NL extracts showed very low bioactivity against both PAF and ADP. A similar potency was observed for all NL samples assessed.

There are previous studies showing anti-platelet effects of coconut, almond, and rice-based products, however, these studies were mostly based on oils and/or extracts from these plant-derived sources and against the activity of classic platelet agonists, such as ADP and collagen, and not against prothrombotic inflammatory mediators like the PAF. For example, in a study conducted in 2010, it was found that rats fed a diet of rice bran oil, sesame oil, or a blend of either with coconut oil reduced platelet aggregation induced by ADP and collagen compared to coconut oil alone [35]. Moreover, a sweet almond (*Prunus amygdalus*) extract with beneficial effects against blood lipid biomarkers, such as lowering the total cholesterol, triacylglycerol, LDL-C, and VLDL-C while increasing HDL-C levels, also showed anti-coagulant properties, by increasing the prothrombin time, partial thromboplastin time, and clotting time [36]. The effects of rice bran (*Oryza sativa*) extract on platelet aggregation and adhesion induced by ADP and collagen were investigated by oral administration to male rats. Significant inhibition of aggregation and adhesion was observed [37]. Another study found that rats fed a hyperlipidemic diet supplemented with anthocyanin extracts from black rice (AEBR) had reduced serum TAG levels and improved platelet function [38].

Within the present study, the anti-inflammatory and anti-platelet potency of lipid bioactives from plant-based dairy alternatives and dairy products, fermented and unfermented, commercially purchased and homemade products, were studied against the inflammatory mediator for the first time, along with their anti-platelet potency against the well established classic platelet agonist ADP. The initial results of the present study further support the potential anti-inflammatory and anti-platelet functional properties of plant-based dairy alternatives, especially those from rice and almond, with similar or even better potency to that of the bovine dairy, which further suggests their use as novel functional foods with protective properties against inflammation, platelet aggregation, and associated disorders. Nevertheless, more research is needed to support this notion.

3.3. Fatty Acid Composition of the Fermented and Unfermented Dairy and Plant-Based Dairy Alternatives

The alteration of the FA profile of plant substrates by fermentation is particularly interesting in developing novel products, functional foods, and nutraceuticals. Fermented foods are also of increasing interest due to their probiotic and prebiotic properties, specifically the production of short-chain fatty acids (SCFAs), which are beneficial to gut health [39]. In addition, the alteration of FAs by fermentation was found to influence bio-functionality in dairy and non-dairy fermented products and, especially increased the anti-inflammatory potency of specific PL bioactives of these products [6–10,40]. This is because FAs and other bioactive lipids survive the digestion process, enter the blood through the intestinal epithelium, and participate in metabolic pathways. Specific FAs, including the monounsaturated fatty acid (MUFA) oleic acid, and the polyunsaturated fatty acids (PUFA) linoleic, linolenic eicosapentaenoic (EPA), and docosahexaenoic (DHA) fatty acids, have been found to possess anti-inflammatory properties and cardioprotective effects, while foods and diets rich in them have been associated with lower disease incidence and mortality [34,41]. A similar effect has been seen in legume-based dairy alternatives subjected to fermentation by lactic acid bacteria. For example, lactic acid fermentation of white beans resulted in increased oleic acid and decreased palmitic acid, indicating an increased health benefit from fermentation [30].

Even though there are studies on the FA composition of plant-based alternative dairy drinks [42], limited research compares the FA composition of several plant-based substrates before and after fermentation. The FA composition of the PL bioactives from the fermented and unfermented bovine dairy and alternative dairy products, either homemade or commercially purchased, were identified within the present study using LC-MS analysis as previously described [10]. The results of this analysis on homemade samples are shown in Table 2, while the results for commercially purchased samples are shown in Table 3. BM has been included in both tables for comparison purposes.

Concerning the dairy samples assessed in the present study, in BM, saturated fatty acids (SFA) were the dominant class of FAs in BM PL, with the most abundant being medium- and long-chain SFAs like lauric (12:0), myristic (14:0), and palmitic (16:0) acids, followed by unsaturated fatty acids (UFA), mainly MUFA, especially the omega-9 (n-9) oleic acid (19:1c9), and lower amounts of PUFA, such as linoleic (18:2) and linolenic (18:3) acids. Compared to the FA content of PL from BM, PL from HMFBM yogurt had decreased oleic acid content and increased lauric acid content. This difference explains the increase in the SFA content and a decrease in the UFA content, even though the PUFA content and its representatives linoleic, linolenic, docosapentaenoic acid (DPA), and EPA increased. Similar outcomes were observed for the PL from the HMFBM, where SFA was again the dominant class of FAs.

In contrast, PL from the CPFBM increased PUFA, especially the linoleic, linolenic, DPA, DHA, and EPA, increased the overall UFA content, while the MUFA content remained stable. The SFA content was substantially reduced, which resulted in UFA content equalizing the SFA content, increasing the UFA/SFA ratio, compared to the value for the same ratio of the BM. It should also be stressed that the PL of the CPFMB had the highest omega-3 (n-3) PUFA content of all the other bovine dairy and plant-based dairy alternative samples tested.

It has been suggested that substitution of SFA by UFA and thus an increase in the UFA/SFA ratio in food, either by specific processing or by fortification of the initial food matrix with bioactive UFA (MUFA and especially PUFA) from sidestreams, to design functional foods that prevent specific nutrient deficiencies and promote population health [43,44], by reducing the risk of chronic disorders. It seems that the benefits of the increase of the UFA content in the novel products seem to be associated mainly with their anti-inflammatory potency [34] rather than any other proposed effects on dyslipidemia and body composition since there is no substantial evidence that the replacement of SFA with UFA may benefit lipid profiles in metabolically healthy adults with overweight and obesity on markers of dyslipidemia and body composition [44], due to null results and a small number of studies.

Table 2. Fatty acid composition of home-made dairy and dairy alternative products.

Fatty Acid	Lipid Number	BM	HMFBM	HMRD	HMFRD	HMAD	HMFAD	HMCD	HMFCD
Caprylic	8:0	0.04 ± 0.011	0.04 ± 0.001	0.26 ± 0.163	0.15 ± 0.035	ND	ND	0.02 ± 0.005	0.13 ± 0.004
Pelargonic	9:0	0.02 ± 0.001	ND	ND	ND	ND	ND	ND	ND
Capric	10:0	0.69 ± 0.010	0.41 ± 0.010	0.09 ± 0.038	0.19 ± 0.043	ND	0.11 ± 0.010	0.36 ± 0.018	1.01 ± 0.026
Undecylic	11:0	0.16 ± 0.001	ND	ND	ND	ND	ND	ND	0.04 ± 0.000
Lauric	12:0	7.33 ± 0.086	35.11 ± 0.835	1.97 ± 0.110	9.82 ± 0.037	0.67 ± 0.023	9.12 ± 0.602	20.35 ± 0.096	46.95 ± 0.206
Tridecylic	13:0	0.38 ± 0.004	0.08 ± 0.004	0.06 ± 0.000	0.05 ± 0.001	0.04 ± 0.001	0.03 ± 0.002	0.06 ± 0.000	0.07 ± 0.001
Myristic	14:0	16.78 ± 0.113	15.92 ± 0.230	11.54 ± 0.305	11.02 ± 0.195	1.71 ± 0.034	4.78 ± 0.281	9.35 ± 0.084	21.82 ± 0.022
Pentadecylic	15:0	4.18 ± 0.015	0.65 ± 0.007	0.44 ± 0.007	0.33 ± 0.003	0.84 ± 0.670	0.22 ± 0.012	0.23 ± 0.003	0.07 ± 0.000
Palmitic	16:0	27.26 ± 0.442	18.53 ± 0.085	47.73 ± 0.142	34.18 ± 0.294	31.71 ± 0.280	15.57 ± 1.076	14.42 ± 0.412	9.68 ± 0.078
Palmitoleic	16:1(9)	4.02 ± 0.020	1.10 ± 0.010	0.64 ± 0.017	0.64 ± 0.008	0.74 ± 0.011	0.97 ± 0.059	1.31 ± 0.012	0.08 ± 0.000
Palmitelaidic	16:1(9t)	ND	ND	ND	ND	ND	ND	ND	ND
Margaric	17:0	3.18 ± 0.115	0.6 ± 0.005	0.35 ± 0.019	0.27 ± 0.011	0.39 ± 0.019	0.32 ± 0.016	0.19 ± 0.012	0.08 ± 0.003
Stearic	18:0	0.65 ± 0.046	0.44 ± 0.009	0.62 ± 0.012	0.38 ± 0.018	1.37 ± 0.019	4.80 ± 4.954	1.12 ± 0.135	0.38 ± 0.034
Oleic	18:1(9)	33.09 ± 0.270	21.77 ± 1.049	29.28 ± 0.260	18.52 ± 0.766	61.79 ± 0.269	49.07 ± 2.335	47.48 ± 0.507	17.11 ± 0.113
Elaidic	18:1(9t)	ND	ND	ND	ND	ND	ND	ND	ND
Linoleic	18:2(9,12) n-6	1.18 ± 0.004	3.88 ± 0.038	6.58 ± 0.119	23.41 ± 0.316	0.36 ± 0.004	14.06 ± 0.768	4.50 ± 0.050	2.40 ± 0.019
Linolenic ($\alpha + \gamma$)	18:3(9,12,15) n-3/ 18:3(6,9,12) n-6	ND	0.39 ± 0.004	0.02 ± 0.001	0.86 ± 0.009	ND	0.19 ± 0.011	0.10 ± 0.000	0.08 ± 0.000
Stearidonic	18:4(6,9,12,15) n-3	ND	ND	ND	ND	ND	ND	ND	ND
Nonadecylic	19:0	0.02 ± 0.001	ND	ND	ND	ND	ND	ND	ND
Arachidic	20:0	0.48 ± 0.128	0.23 ± 0.108	0.23 ± 0.162	ND	0.38 ± 0.231	0.21 ± 0.150	0.21 ± 0.150	ND
Gadoleic	20:1(9)	0.26 ± 0.047	0.14 ± 0.015	0.20 ± 0.007	ND	ND	0.24 ± 0.018	0.31 ± 0.011	0.08 ± 0.010
Gondoic	20:1(11)	ND	ND	ND	ND	ND	ND	ND	ND
DihomoLinoleic	18:2(10,12) n-6	0.04 ± 0.002	ND	ND	ND	ND	0.05 ± 0.001	ND	ND
Dihomolinolenic	18:3(8,11,14) n-6	0.01 ± 0.000	0.16 ± 0.002	ND	ND	ND	0.01 ± 0.001	0.01 ± 0.000	ND
Mead Acid	20:3(5,8,11)	ND	ND	ND	ND	ND	ND	ND	ND
Arachidonic	20:4(5,8,11,14) n-6	ND	0.24 ± 0.005	ND	ND	ND	0.03 ± 0.006	ND	ND
Eicosatetraenoic	20:4	ND	ND	ND	ND	ND	ND	ND	ND
EPA	20:5(5,8,11,14,17) n-3	ND	0.12 ± 0.002	ND	ND	ND	0.05 ± 0.004	ND	ND
Heneicosylic	21:0	ND	ND	ND	ND	ND	ND	ND	ND
Behenic	22:0	ND	ND	ND	ND	ND	ND	ND	ND
Erucic	22:1(13)	0.16 ± 0.111	ND	ND	ND	ND	0.1 ± 0.074	ND	ND
Docosadienoic	22:2(13,16) n-6	ND	ND	ND	ND	ND	ND	ND	ND
Eranthic	22:3(5,13,16) n-6	ND	ND	ND	ND	ND	ND	ND	ND
Ardenic	22:4(7,10,13,16) n-6	ND	0.03 ± 0.003	ND	ND	ND	ND	ND	ND
DPA	22:5(4,7,10,13,16) n-3	ND	0.16 ± 0.001	ND	ND	ND	ND	ND	ND
DHA	22:6 (4,7,10,13,16,19) n-3	0.01 ± 0.002	ND	ND	ND	0.02 ± 0.008	0.05 ± 0.007	ND	ND
Tricosylic	23:0	ND	ND	ND	0.19 ± 0.264	ND	ND	ND	ND
Lignoceric	24:0	0.06 ± 0.080	ND	ND	ND	ND	ND	ND	ND
SFA		61.23	72.02	63.29	56.57	37.10	35.16	46.30	80.24
MUFA		37.53	23.01	30.11	19.17	62.53	50.39	49.10	17.27
PUFA		1.24	4.97	6.60	24.26	0.37	14.45	4.60	2.49
UFA		38.77	27.98	36.71	43.43	62.90	64.84	53.70	19.76
UFA/SFA		0.63	0.39	0.58	0.77	1.70	1.84	1.16	0.25

Values are expressed as the mean % percentage of total fatty acids of each sample (mean ± standard deviation (SD), $n = 3$). Abbreviations: TL, total lipid; NL, neutral lipid; PL, polar lipid; BM, bovine milk; HMFBM, homemade fermented bovine milk; HMAD, homemade almond drink; HMFAD, homemade fermented almond drink; HMRD, homemade rice drink; HMFRD, homemade fermented rice drink; HMCD, homemade coconut drink; HMFCD, homemade fermented coconut drink; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; ND, not detected.

Table 3. Fatty acid composition of commercially purchased dairy and dairy alternative products.

Fatty Acid	Lipid Numbers	BM	CPFBM	CPRD	CPAD	CPFAD	CPCD	CPFCD
Caprylic	8:0	0.04 ± 0.011	0.05 ± 0.006	0.02 ± 0.008	0.01 ± 0.001	ND	0.06 ± 0.005	0.31 ± 0.031
Pelargonic	9:0	0.02 ± 0.001	ND	ND	ND	ND	0.01 ± 0.001	ND
Capric	10:0	0.69 ± 0.010	0.49 ± 0.063	0.12 ± 0.006	0.29 ± 0.027	0.01 ± 0.003	0.75 ± 0.021	0.81 ± 0.030
Undecylic	11:0	0.16 ± 0.001	0.18 ± 0.015	ND	ND	ND	0.17 ± 0.009	0.04 ± 0.001
Lauric	12:0	7.33 ± 0.086	8.21 ± 0.342	7.92 ± 0.102	5.24 ± 0.057	1.91 ± 0.011	16.5 ± 1.20	23.76 ± 0.310
Tridecylic	13:0	0.38 ± 0.004	0.32 ± 0.001	0.02 ± 0.000	0.24 ± 0.002	ND	0.21 ± 0.014	0.03 ± 0.001
Myristic	14:0	16.78 ± 0.113	11.72 ± 0.050	5.59 ± 0.031	10.31 ± 0.100	0.96 ± 0.016	12.48 ± 1.010	10.54 ± 0.038
Pentadecylic	15:0	4.18 ± 0.015	2.47 ± 0.031	0.13 ± 0.004	2.68 ± 0.043	0.06 ± 0.000	1.91 ± 0.201	0.06 ± 0.000
Palmitic	16:0	27.26 ± 0.442	20.1 ± 0.466	35.05 ± 0.087	27.49 ± 0.323	13.81 ± 0.248	19.73 ± 1.445	14.41 ± 0.073
Palmitoleic	16:1(9)	4.02 ± 0.020	3.44 ± 0.038	0.70 ± 0.005	2.48 ± 0.031	1.23 ± 0.019	7.26 ± 7.150	0.47 ± 0.004
Palmitelaidic	16:1(9t)	ND	ND	ND	ND	ND	ND	ND
Margaric	17:0	3.18 ± 0.115	4.78 ± 0.111	0.31 ± 0.010	2.66 ± 0.063	0.53 ± 0.036	1.86 ± 0.142	0.12 ± 0.003
Stearic	18:0	0.65 ± 0.046	1.05 ± 0.018	0.78 ± 0.004	1.4 ± 0.369	2.16 ± 0.066	0.97 ± 0.066	1.43 ± 0.172
Oleic	18:1(9)	33.09 ± 0.270	33.7 ± 0.241	24.25 ± 0.132	44.23 ± 0.315	55.41 ± 0.257	29.45 ± 1.915	39.66 ± 0.131
Elaidic	18:1(9t)	ND	ND	ND	ND	ND	ND	ND
Linoleic	18:2(9,12) n-6	1.18 ± 0.004	6.99 ± 0.075	22.97 ± 0.187	1.60 ± 0.028	21.94 ± 0.086	5.13 ± 0.509	7.29 ± 0.051
Linolenic ($\alpha + \gamma$)	18:3(9,12,15) n-3/18:3(6,9,12) n-6	ND	2.37 ± 0.143	1.59 ± 0.025	0.09 ± 0.002	0.54 ± 0.005	1.04 ± 0.069	0.13 ± 0.003
Stearidonic	18:4(6,9,12,15) n-3	ND	0.06 ± 0.002	ND	ND	ND	ND	ND
Nonadecylic	19:0	0.02 ± 0.001	0.03 ± 0.015	ND	0.06 ± 0.002	ND	0.04 ± 0.003	ND
Arachidic	20:0	0.48 ± 0.128	0.37 ± 0.107	0.19 ± 0.138	0.71 ± 0.281	0.65 ± 0.460	1.2 ± 0.930	0.26 ± 0.220
Gadoleic	20:1(9)	0.26 ± 0.047	0.84 ± 0.144	0.30 ± 0.047	0.37 ± 0.009	0.71 ± 0.034	0.48 ± 0.074	0.63 ± 0.077
Gondoic	20:1(11)	ND	ND	ND	ND	ND	ND	ND
DihomoLinoleic	18:2(10,12) n-6	0.04 ± 0.002	0.39 ± 0.015	0.06 ± 0.002	0.04 ± 0.002	0.08 ± 0.003	0.08 ± 0.006	0.04 ± 0.001
Dihomolinolenic	18:3(8,11,14) n-6	0.01 ± 0.000	0.49 ± 0.004	ND	ND	ND	0.1 ± 0.009	ND
Mead Acid	20:3(5,8,11)	ND	ND	ND	ND	ND	ND	ND
Arachidonic	20:4(5,8,11,14) n-6	ND	0.77 ± 0.009	ND	ND	ND	0.17 ± 0.018	ND
Eicosatetraenoic	20:4	ND	ND	ND	ND	ND	ND	ND
EPA	20:5(5,8,11,14,17) n-3	ND	0.41 ± 0.006	ND	ND	ND	0.13 ± 0.008	ND
Heneicosylic	21:0	ND	ND	ND	ND	ND	ND	ND
Behenic	22:0	ND	ND	ND	ND	ND	ND	ND
Erucic	22:1(13)	0.16 ± 0.111	0.15 ± 0.108	ND	0.07 ± 0.049	ND	0.14 ± 0.100	0.02 ± 0.012
Docosadienoic	22:2(13,16) n-6	ND	ND	ND	ND	ND	ND	ND
Eranthic	22:3(5,13,16) n-6	ND	0.02 ± 0.001	ND	ND	ND	ND	ND
Ardenic	22:4(7,10,13,16) n-6	ND	0.08 ± 0.001	ND	ND	ND	0.02 ± 0.001	ND
DPA	22:5(4,7,10,13,16) n-3	ND	0.48 ± 0.004	ND	ND	ND	0.11 ± 0.010	ND
DHA	22:6(4,7,10,13,16,19) n-3	0.01 ± 0.002	0.04 ± 0.028	ND	0.02 ± 0.014	0.01 ± 0.001	ND	ND
Tricosylic	23:0	ND	ND	ND	ND	ND	ND	ND
Lignoceric	24:0	0.06 ± 0.080	ND	ND	ND	ND	ND	ND
SFA		61.23	49.76	50.13	51.10	20.08	55.89	51.77
MUFA		37.53	38.14	25.25	47.15	57.35	37.32	40.78
PUFA		1.24	12.10	24.62	1.75	22.57	6.79	7.46
UFA		38.77	50.24	49.87	48.9	79.92	44.11	48.23
UFA/SFA		0.63	1.01	0.99	0.66	3.98	0.79	0.93

Values are expressed as the mean % percentage of total fatty acids of each sample (mean ± standard deviation (SD), $n = 3$). Abbreviations: BM, bovine milk; CPAD, commercially purchased almond drink; CPFAD, commercial purchased fermented almond drink; CPRD, commercially purchased rice drink; CPCD, commercially purchased coconut drink; CPFCD, commercially purchased fermented coconut drink; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; ND, not detected.

In yogurt's PL, the substitution of the SFA by UFA takes place through the natural process of fermentation of the BM during yogurt production. At the same time, such changes in the FAs' content have been found to increase the anti-inflammatory and anti-thrombotic potency of the PL of yogurt in comparison to the bioactivities of the milk PL [10], suggesting a structure-activity relationship between the FA content of the PL bioactives in fermented dairy and their anti-inflammatory bioactivities. The increase of UFA content and, especially that of the oleic acid and the n-3 PUFA content in the PL of the CPFBM may also explain why these PL bioactives had much stronger anti-inflammatory and anti-platelet potency, especially against PAF, in comparison to the HMFBM. Nevertheless, further research is needed to fully evaluate the fermentation process and the strains needed for increasing the UFA content and anti-inflammatory bio-functionality of yogurt's PL as a natural way of dairy fortification with UFA.

In rice-based samples, in the PL of both the unfermented and fermented homemade products, the SFA was the main class of FAs, with the most dominant being the palmitic acid, followed by the myristic and the lauric acids, with lower but considerable amounts of UFA, mainly by MUFA, especially the n-9 oleic acid, and lower PUFA levels, mainly linoleic acid followed by low but detectable levels of linolenic acids. These results agree with the previously reported FA profile for rice-based products, where oleic acid and linoleic

acid were the dominant UFA [42]. The overall UFA content and the UFA/SFA ratio of the rice-based drink were higher than the BM. Similar to the dairy products, differences were observed in the FA content of the rice-based plant-derived dairy alternatives' PL due to fermentation.

Specifically, in the PL of HMFRD, an increase in lauric acid was observed, accompanied by a decrease in palmitic acid and oleic acid. In contrast, a substantial increase in the linolenic acid was observed, accompanied by an increase in the linolenic acids, compared to the content of these FAs in the PL HMRD. Such changes in specific FAs resulted in decreased SFA, increased UFA content, and increased UFA/SFA ratio in PL of HMFRD. This is due to a substantial increase in their PUFA content, even though MUFA content decreased. Overall, PL from HMFRD and UFA/SFA ratio were increased in comparison to the values of the same ratio for HMRD. This difference seems rational for the stronger anti-inflammatory and anti-platelet properties of HMFRD against PAF, compared to the strong but lower anti-PAF properties of HMRD.

On the other hand, CPRD showed equal levels of SFA with UFA, with dominant SFA being again the palmitic acid followed this time by the lauric acid and with lower amounts of the myristic acid this time. In the CPRD, the UFA content and the UFA/SFA ratio were much higher than the BM, with the n-9 oleic acid being the main MUFA and linoleic acid the main PUFA, followed by lower but considerable amounts of linolenic acids. The high UFA content and UFA/SFA ratio of the CPRD were higher even than that of the homemade one due to the higher PUFA content observed in CPRD. Nevertheless, there was no availability in the market of a fermented rice-based dairy alternative product to be commercially purchased. Thus similar comparisons made for the homemade products could not occur in the present study.

Overall, the higher values of the UFA/SFA ratio of the PL bioactives in the rice-based dairy alternatives, especially the fermented HMFRD, in comparison to the BM, also support the stronger anti-PAF anti-inflammatory potency of the PL bioactives of the rice-based dairy alternatives. Nevertheless, more studies are needed to fully evaluate the FA modifications of the PL bioactives in HMFRD and whether these changes are associated with structure-activity relationships of the anti-inflammatory bio-functionality of these PL from rice-based products.

In contrast to the bovine dairy and rice-based products, in almond-based products, the UFA was dominant in the PL of both the unfermented and fermented homemade and commercially purchased products, with the MUFA being the main class of FAs due to the dominance of the n-9 oleic acid. More specifically, in HMAD, the main SFA was palmitic acid, lower than those of the oleic MUFA. In contrast, only the linoleic acid was detected in very low amounts in this almond-based product from the PUFA. Similar results were also observed in CPAD, where again, the dominant FA was the n-9 oleic acid, followed by the SFA palmitic acid. In contrast, higher amounts were observed for the myristic and lauric acid and the PUFA linoleic and linolenic acids than those observed in HMAD. These results agree with the previously reported FA profile for almond-based products, where oleic acid was the dominant FA [42]. Subsequently, in both these almond-based dairy alternative drinks, their overall UFA content levels were higher than the levels of their SFA content. Thus the ratio of UFA/SFA was higher than that of the relevant unfermented rice-based drink and BM.

Again, differences were observed in the FA content of the almond-based plant-derived dairy alternatives' PL due to fermentation. More specifically, in the PL of both HMFAD and CPFAD, a substantial decrease of the SFA palmitic, myristic, and lauric acids with a subsequent reduction in their SFA content was observed. More importantly, their PUFA content substantially increased due to an increase in the levels of the linoleic acid and an increase in other PUFA like the linoleic acids compared to these FAs in the PL HMAD, and CPAD. There was a difference in the MUFA content changes between the HMFAD and the CPFAD, especially in the oleic acid content, which was decreased during the homemade fermentation process and increased in the commercially purchased fermented products.

However, the substantial increase in the PUFA content of the PL of both almond-based fermented products resulted in a substantial increase in their overall UFA content and their UFA/SFA ratio levels. For example, the high UFA content and the UFA/SFA increased ratio of the PL from the CPFAD was the highest observed within all the samples assessed, followed by that of the PL from the HMFAD. These results seem to provide a rationale for the strong anti-inflammatory and anti-platelet properties of the fermented almond-based dairy alternative products against the inflammatory PAF-associated pathways compared to the lower anti-PAF properties of the non-fermented almond-based dairy alternatives and BM.

Apart from the direct anti-PAF inhibitory effect of these dietary PL bioactives on the PAF-receptor, the high UFA content observed in the PL of the fermented almond-based products seems to provide additional bio-functionality. After digestion in the intestine, they travel on the surface of lipoproteins in the bloodstream. These dietary PL bioactives with UFA in their structure are usually also transferred in cell membranes of target cells due to their amphiphilic properties, where specific phospholipases A2 (PLA_2) exist. From the activities of the UFA, the dietary PL is released intracellularly, affecting several intracellular inflammatory processes [34]. For example, the released free forms of several UFA, such as the n-9 MUFA oleic acid and the PUFA linoleic acid, especially the n-3 PUFA, DHA, EPA, and linolenic acids, have been found to possess strong anti-platelet effects against several mediators, with specific intracellular signaling [34,45,46]. Thus, the increase in the degree of unsaturation, the levels of UFA, and the UFA/SFA content of the fermented products in both the dairy and plant-based dairy alternatives, due to the fermentation process, further enhances the anti-inflammatory and anti-platelet bio-functionality of their PL bioactives

In contrast to all aforementioned samples, the PL from all coconut samples had high SFA and MUFA but very low PUFA levels. The dominant FA was the MUFA oleic acid, in agreement with previously reported results in coconut products [42]. In the SFA of the PL of the HMCD, the more abundant were those of medium-chain SFA and especially lauric acid, followed by palmitic and myristic acid. In contrast, from PUFA, only linoleic acid was present. The high levels of oleic acid present resulted in UFA having similar levels with the SFA content in the PL of the HMCD.

In contrast to all the other samples assessed, a substantial increase in the SFA content was observed in the HMFCD PL after fermentation. Increased SFA content is due to increased lauric acid content, which is the dominant FA in the PL of this product, followed by palmitic and myristic acids that were reduced after fermentation. In addition, both oleic acid and linoleic acids were substantially reduced. Thus, the MUFA, PUFA, and overall UFA content were reduced, resulting in the lowest UFA/SFA ratio levels for the PL of this fermented coconut-based dairy alternative compared to the PL of the other bovine dairy and almond and rice-based dairy alternative samples.

In the PL of CPCD, the oleic acid was the dominant FA. However, this time it was followed by high levels of the SFA palmitic, lauric, and myristic acids. In contrast, linoleic acid was the main PUFA, followed by low but considerable amounts of linolenic acids and very low but detectable amounts of other PUFA, such as the EPA, DHA, and arachidonic acid. Subsequently, the SFA content was higher than the UFA content in the PL of this coconut-based drink. Unlike HMFCD, after fermentation, the oleic acid was substantially increased in the PL of the CPFCD. Thus, it was retained as the dominant FA in this fermented product, followed by lauric acid as the main SFA and palmitic and myristic SFA. In the PUFA, the linoleic acid was increased, and the linolenic acid content was reduced to very low but detectable levels, while no other PUFA was detected. Thus, differently than the PL of the HMFCD, the UFA content of the PL from the CPFCD was higher and at similar levels to their SFA content and subsequently a higher level of the UFA/SFA ratio.

Overall, the increase of the UFA content and the UFA/SFA ratio of the PL of the CPFCD, in contrast to the decreased levels of the UFA content and the UFA/SFA ratio in the PL of HMFCD, seems to be associated with the stronger anti-PAF anti-inflammatory potency observed in the PL of CPFCD, in comparison to the less bioactive PL from HMFCD.

It should also be stressed that higher SFA and lower UFA content of the PL from some coconut-based products resulted in the lowest anti-inflammatory and anti-platelet potency of these coconut-based PL against both the PAF and ADP.

Even though coconut oil has a relatively high medium-chain FA concentration, the clinical benefits of commercial oils based on such FAs cannot be generalized to coconut oil and coconut products. Nonetheless, apart from the potential anti-inflammatory functional properties of the coconut-based products, the abundance of medium-chain SFA lauric acid in this product has been proposed to modify the blood lipid profile by increasing the low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations. Specifically, it plays a main role as a substrate for apolipoprotein (apo)A1 and apoB synthesis, the key molecules in HDL-C and LDL-C particles, respectively [47]. Several studies consistently showed that consumption of coconut products increases mostly LDL-C and could increase adverse cardiovascular health. Until the long-term effects of coconut products on cardiovascular health are established, coconut oil should be considered a saturated fat, and its consumption should not exceed the USDA's daily recommendation (less than 10% of total calorie intake) [47].

Several variations were observed in the FA composition of both the dairy and the plant-based dairy alternatives assessed in the present study. These variations in their FA composition, especially before and after fermentation, agree with other relevant studies where similar variations were detected in the FA composition of bovine dairy and rice, almond, and coconut-based products [7,8,20–22,25,30–32,42]. This variation proposes that other parameters besides FA content play essential roles in the overall functionality of the PL bioactives in such products. Thus, further studies are needed to evaluate how fermentation can increase the functional anti-inflammatory properties of PL bioactives in dairy and dairy alternatives.

4. Conclusions

The present study showed that fermentation could enhance the anti-inflammatory and anti-platelet potency in dairy and plant-based dairy alternatives. The bio-functionality of these products and, especially of their fermented representatives, in both homemade and commercially purchased ones, was mainly attributed to their dietary PL bioactives and the increase of the UFA in these PL. These bioactive PL had potent activity against platelet aggregation initiated by the inflammatory and thrombotic mediator PAF and against that of the well-established platelet agonist ADP but with higher anti-PAF specificity. Comparing plant drink samples, fermentation resulted in PL with more potent bioactivities against platelet aggregation. The dietary PL bioactives of the fermented rice-based dairy alternative had the greatest anti-inflammatory potency against PAF and anti-platelet capacity against ADP, compared to the PL from all the other samples, followed by the PL of the bovine yogurt and the fermented almond-based products. In contrast, the PL from the coconut-based products showed the lowest bioactivities.

The substitution of the SFA by UFA during the natural fermentation process seems to be associated with the increased anti-inflammatory and anti-thrombotic potency of the fermented products, especially against the PAF pathway, in comparison to the bioactivities of the PL of the non-fermented ones. This suggests a structure-activity relationship between the fatty acid content of the PL bioactives in fermented dairy and dairy alternatives, with their potent anti-inflammatory bioactivities. However, there did not appear to be a strong correlation between fermentation and the anti-inflammatory n-3 PUFA content changes. An increase in the bioactive n-3 PUFA, such as linolenic acids, EPA, DPA, and DHA, was found in low levels and very few samples, especially in the bovine CPFBM yogurt, were in smaller amounts compared to other food products. On the other hand, other UFAs like the n-9 MUFA oleic acid and the PUFA linoleic acid were dominant in all samples. At the same time, in most fermented products, their levels were increased, especially those of the linoleic acid, with a subsequent increase in the overall UFA content and the UFA/SFA ratio due to fermentation. Substitution of the SFA content by increasing the UFAs in both bovine dairy

and plant-based dairy alternatives due to the natural process of fermentation is associated with several health benefits. It is also associated with enhancing the anti-inflammatory and anti-platelet bio-functionality of the dietary PL bioactives in these fermented bovine yogurt and dairy alternatives, as observed in the present study.

Nevertheless, more studies are needed to unveil the full benefits of the fermentation process and the strains needed for increasing the UFA content and the anti-inflammatory bio-functionality of both dairy and dairy alternatives, as a natural way of their fortification with UFA and PL bioactives, with a subsequent enhancement of their functional anti-inflammatory properties. These results indicate the potential for developing novel bio-functional sustainable dairy and plant-based dairy alternative products with potent anti-inflammatory, anti-platelet, and cardio-protective properties. However, additional research is needed to thoroughly assess the benefits and application of bioactive PL derived from fermented dairy and plant-based dairy alternatives.

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