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# Using Olive Cake as a Sustainable Ingredient in Diets of Lactating Dairy Cows: Effects on Nutritional Characteristics of Cheese

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**Abstract:** This study aimed to investigate the chemical composition, fatty acid profile and polyphenol content of Provola cheese made with cow's milk from cows fed a diet incorporating olive cake. Cheese samples were analysed in different months in order to test diet and diet×season effects. The results show that the cheese composition was influenced by both factors. The most beneficial cheese from a human health point of view was produced with milk from cows fed the treatment diet in the spring. Supplementing the diet of dairy cows with olive cake reduced the atherogenic and thrombogenic indices while increasing the total polyphenols in the cheese product. With a 32.9% increase in polyphenols, the cheese from the TEST group has greater functional nutrients and properties than the cheese from the CTR group. The data show that, combining the benefits of a more sustainable production process with a better final product, the supplementation of dried and stoned olive cake in the dairy cow diet improves the nutritional and health composition of the cheese.

**Keywords:** olive cake; cheese; fatty acids; chemical composition; functional foods; polyphenols



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## 1. Introduction

A wide variety of healthy foods with functional properties are available on a global scale, consumed mainly according to cultural context. The consensus document of the EC FUFOSE project describes that "Foods with functional properties are those for which there is sufficient evidence of beneficial effects on one or more target functions of the body, beyond adequate nutrition, that are relevant to improving health and well-being and/or reducing the risk of disease" [1]. According to their expected effects, these foods can be divided into two main categories: (1) those intended to improve physiological functions and (2) those intended to reduce the risk of specific pathologies. The functional properties of foods intended for human consumption can be manipulated in a variety of ways, including feeding animals [2] with diets rich in antioxidants (tocols, e.g., vitamins E and C; carotenoids; flavonoids and polyphenols) and fatty acids (e.g., omega-3 fatty acids, GLA and CLA) to enhance the functional nutrients or compounds in the final edible product.

Olive cake (OC), the dry fraction produced as a bulky by-product of the olive oil industry, is known to be rich in functional molecules such as polyphenols [3]. Several studies have focused on exploring the properties of olive cake or cake extracts when incorporated into animal feeds to extend the shelf life of certain foods or to develop food-related products

with improved nutritional profiles [4]. Previous research confirms that the quality of milk and dairy products can be easily manipulated through diet formulation [5,6]. Thus, the incorporation of by-products rich in bioactive compounds should theoretically represent a cost-effective way to improve the quality and health-promoting aspects of milk and dairy products [7–9] and also contribute to the implementation of "Transforming our world: the 2030 Agenda for Sustainable Development" as well as the European Commission's Circular Economy Action Plan [10,11]. Indeed, the circular approach increases the value of the by-product when it is transformed from waste into a resource, with financial benefits for the olive oil supply chain as well. European consumers have recently started to show more interest in functional foods [2], with the leading category being dairy products (46%), followed by cereals (28%).

Europeans have the highest per capita consumption of cheese, estimated at 18.39 kg (FAS-USDA, FAO and Eurostat data processed by www.clal.it (accessed on 6 March 2024)). Provola, a typical pasta filata cheese, is traditionally produced in several southern regions of Italy. There are several types of Provola cheese on the market, often bearing the quality logo of the Protected Designation of Origin (PDO). This type of cheese is in great demand throughout Italy and is often used as an ingredient in various gastronomic preparations. In this context, consumers would welcome a healthier choice of fortified Provola cheeses, with an enriched nutritional profile, produced in a sustainable way and respecting the principles of circularity.

The present study was therefore aimed at investigating the chemical composition, fatty acid profile and polyphenolic content of Provola cheese produced with milk obtained from cows fed on a diet containing olive cake. To the best of our knowledge, the determination of the polyphenol content is a parameter that has not been evaluated in any previous study on this type of cheese, and therefore represents the main novelty of the study.

## 2. Materials and Methods

### *2.1. Animals and Diet*

The study was performed on a commercial dairy farm in Ragusa (Sicily, Italy), at an altitude of about 500 metres above sea level, with a herd of four hundred and sixty healthy, multiparous Holstein Friesian dairy cows. The animals were separated into two groups (CTR and TEST) of two hundred and thirty cows each. The animals were randomly assigned so that each treatment group was homogeneous in terms of body condition score ($3 \pm 0.5$), calving interval (90–120 d) and milk performance ($27 \pm 3$ kg/d). The two groups were managed according to local traditional practices. The cows in both groups were housed in a free-stall barn and kept on "deep litter" straw bedding. The barn was equipped with an automatic cooling system with fans and sprinklers that were activated during hot periods. Animals were given access to pasture for at least 6 h during the day (08:00 to 14:00 CEST). Both groups were given isoenergetic and isoproteic diets as a total mixed ration of 20 kg dry matter (DM)/head twice daily at 08:00 and 14:00 CEST. Diets were based on grass hay (crude protein: 110.9 g/kg dry matter, ether extract: 25.0 g/kg dry matter and neutral detergent fibre: 521.9 g/kg dry matter) supplemented with a concentrate pellet, whose nutrient characteristics are shown in Table 1.

The control group (CTR) received a concentrate without OC inclusion, while the experimental group (TEST) received a specially formulated concentrate containing 8% on a DM basis of olive cake (OC). The addition of OC within the diet formulation is in line with an approved UE disciplinary "QS Sicilia" aimed at the recovery of agro-industrial by-products. All animals had free access to drinking water. A flow chart of the olive cake production is shown in Figure 1.

**Table 1.** Nutritional characteristics of the concentrate (pelleted complete feed) of the two groups, calculated as g/kg of dry matter (DM).

| Diet Chemical Composition (g/kg of DM) | CTR | TEST |
|---|---|---|
| Moisture | 109.0 | 107.0 |
| Starch | 407.0 | 407.0 |
| Crude protein | 194.0 | 196.0 |
| Ether extract | 45.8 | 51.1 |
| Non-fibre carbohydrates | 465.0 | 440.0 |
| Crude fibre | 60.0 | 72.0 |
| Acid detergent fibre | 78.2 | 105 |
| Ash | 64.1 | 70.2 |
| NEL (milk UFL/kf of DM) * | 1.09 | 1.07 |

* NEL: net energy of the lactation. The efficiency of milk production has been calculated according to the net energy system, where one energy unit of milk feed (UFL) is defined as the net energy content of 1 kg of standard barley for milk production, which is equivalent to 1700 kcal.

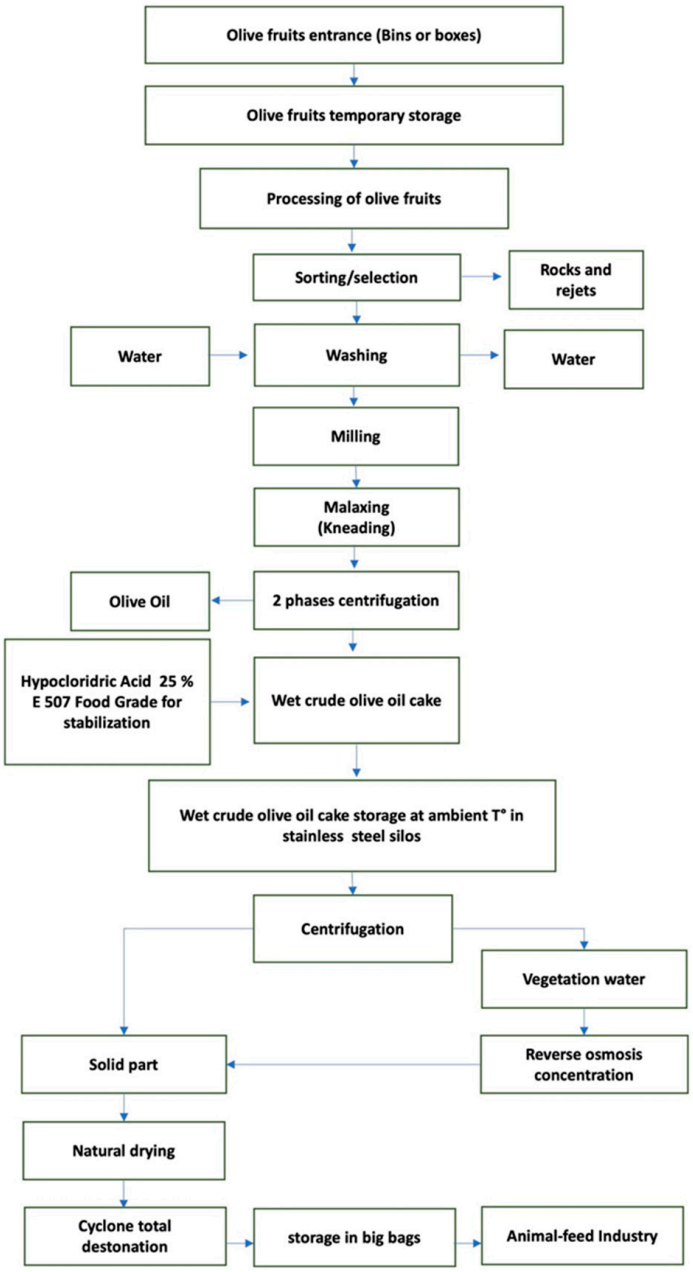


**Figure 1.** Flowchart of the destoned and enrichment olive cake production (Patent N°: 001428707).

### *2.2. Cheesemaking*

After a 3-week adaptation period, the trial started on lactation day 45 (day 0; April) and lasted 45 days (June). All cows were milked twice daily in the milking parlour at 04:30 and 16:30 CEST. The milk from the two groups was collected and kept separate during transport to the dairy plant in refrigerated tanks (4 ± 2 °C). From each treatment group, 400 litres of milk was used to produce Provola cheese according to traditional procedures presented as a flowchart by Calabrese et al. [12]. From each 400-litre batch, 80 Provola cheeses were produced per group. The cheeses were vacuum-packed 24 h after production and transported at +4 °C to the laboratory of the Animal Production Unit of the Department of Veterinary Sciences of the University of Messina, Italy, for further analysis.

### *2.3. Nutritional Analyses*

#### 2.3.1. Chemicals and Reagents

The standard mixture of 37 FAMEs (FAME mix, Supelco, Bellefonte, PA, USA) was supplied by Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Nitrogen of 99.9990% purity and helium of 99.9995% purity were supplied by Rivoira gases (Rivoira S.p.A., Milan, Italy).

#### 2.3.2. Moisture and Protein Contents of OC and Provola

The moisture content in OC and Provola was determined following the procedure described by the official method AOAC 934.01 (2001), whereas protein content was determined according to the Kjeldahl method (AOAC method 992.15) (2020). Moisture and protein analysis for both OC and Provola cheese were performed in triplicate.

#### 2.3.3. Polyphenols Content

The Folin–Ciocalteu method, with some modifications, was used for the spectrophotometric determination of total polyphenols, following the method by Calabrese et al. [12]. Approximately 1 g of the homogenized samples was added to 2.5 mL of 95% ethanol and left at 0 °C for 48 h, and then 1 mL of the upper layer was transferred to a test tube and mixed with 95% ethanol and ultrapure water. Further, Folin–Ciocalteu reagent (50%) and Na2CO3 (5%) were added to it. Using 95% ethanol as an analytical blank, the absorbance was read at 760 nm on a UV-visible spectrophotometer.

A calibration curve was constructed using appropriate dilutions of a gallic acid standard solution (95% in ethanol). Determinations were carried out in triplicate, as well as with the blank solutions.

### *2.4. Lipids Analysis of OC and Provola*

#### 2.4.1. Extraction of Lipids

The AOAC method 920.39–1920 was used for the determination of total fat in OC. An aliquot (20.0 g) of dried olive cake was extracted with n-hexane for 6 h using a Soxhlet apparatus. The extract was evaporated, and the dry extraction yield was determined gravimetrically.

The total lipids of Provola were extracted according to the Folch method [13], with modifications. Approximately 5 g of samples were weighed into 50 mL tubes. Folch's solution (chloroform/methanol 2:1 $v/v$), 37% concentrated HCl (to pH 1.00) and 0.73% NaCl were added, and the mixture was vortexed for 1 min and then centrifuged. The bottom layer was collected in a previously weighed flask and dried. The yield was determined gravimetrically.

#### 2.4.2. Preparation of FAMEs

Fatty acid methyl esters (FAME) were prepared by transmethylation of OC and Provola lipid extracts, according to the ISO 5509 2000 method, as follows: 1 mL of a 9:1 $v/v$ methanol/sulphuric acid mixture was added to 1 mL of each extract, and the mixture

was placed in an oven at 110 °C for 1 h. The supernatant was collected and diluted with n-hexane (1:2 *v*/*v*).

#### 2.4.3. Chromatographic Analysis

The fatty acid methyl ester (FAME) organic layer was injected into a gas chromatograph (GC) equipped with a split/splitless injector and flame ionization detector (FID) (Dani Master GC1000, Dani Instrument, Milan, Italy). A Supelco Omegawax 250 was used with the appropriate operating protocol: column temperature from 50 °C (hold time 2 min) to 240 °C (hold time 15 min) at 3 °C/min. Helium was held constant at a linear velocity of 30 cm/s. Injector and detector temperatures were both set at 240 °C. The injection volume was 1 µL with a split ratio of 1:50. Clarity Chromatography Software v4.0.2 (DataApex, Prague, Czech Republic) was used to process the output data. All samples were analysed in quadruplicate with analytical blanks. By direct comparison with the retention times of the compounds present in the reference standard mixture, FAMEs were identified.

#### 2.4.4. Lipid Quality Indices of Provola

The saturated fatty acids were classified as pro-atherogenic because they promote the adhesion of lipids to cells of the immune system, while the unsaturated fatty acids were classified as anti-atherogenic because they inhibit plaque aggregation while reducing the levels of esterified fatty acids, cholesterol and phospholipids, thus preventing the occurrence of coronary heart disease [14].

The results of the fatty acid composition were used to calculate the atherogenicity index (AI) and the thrombogenicity index (TI) using the following equations [15]:

$$AI = \frac{C12{:}0 + 4 \times C14{:}0 + C16{:}0}{\sum MUFA + \sum n6 + \sum n3}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times \sum MUFA + 0.5 \times \sum n6 + 3 \times \sum n3 + (\sum n3 / \sum n6)}$$

where C12:0, C14:0, C16:0 and C18:0 are lauric, myristic, palmitic and stearic acids, respectively, and MUFA is n6 and n3 are the sum of monounsaturated, polyunsaturated n6 and polyunsaturated n3 fatty acids.

### *2.5. Statistical Analysis*

The statistical analyses were performed using JMP® 16 software (SAS Institute Inc., Cary, NC 1989-2021, USA). Descriptive statistics was generated and reported as the mean ± standard deviation (SD). All the measured parameters in Provolas cheese (Y) were modelled using a two-way analysis of variance (ANOVA) with interaction, including the following factors:

$$Yijk = m + Di + Mj + (DM)ij + eijk$$

with m as mean, Di as diet (CTR vs. TEST), Mj as month of cheese production (April/Spring and June/Summer), (DM)ij as diet by month interaction and eijk as random remainder. To identify significantly different levels of significant factors and interactions, the Tukey–Kramer post-hoc test was applied.

## 3. Results and Discussion

### *3.1. Olive Cake Composition*

The results of analytical analyses of the olive cake used in the treatment diet, shown in Table 2, are in agreement with those reported in previous studies. The average protein content was similar to that reported by Molina-Alcaide et al. [16], while the total lipid and polyphenol contents were also in agreement with those reported by Dal Bosco et al. [17]. The fatty acid profiles were in agreement with those described by Vargas-Bello-Pérez et al. [18], with high amounts of C18:1 n-9 (66.63%), followed by C16:0 (16.14%) and C18:2

(10.66%). Other fatty acids present in significant amounts were C16:0 and C18:2, while C18:0 and C18:3 were marginal.

**Table 2.** The chemical composition, fatty acids composition and polyphenols content of the dried and pitted olive cake integrated into the concentrate of the treatment experimental group (TEST). Values are reported as mean ± standard deviation.

| | **Olive Cake** |
|---|---|
| Chemical composition (g/kg dry matter) | |
| Moisture | 36.47 ± 0.57 |
| Ashes | 34.13 ± 0.81 |
| Crude fat | 180.80 ± 0.26 |
| Energy (ME) (Kcal/Kg) | 3535.1 |
| Crude protein (total N × 6.25) | 61.00 ± 1.73 |
| Neutral detergent fibre | 410.33 ± 0.20 |
| Acid detergent fibre | 320.53 ± 0.34 |
| Total polyphenols | 10.18 ± 1.59 |
| Fatty acids (g/100 g fat) | |
| C14:0 | 0.02 ± 0.01 |
| C16:0 | 16.14 ± 0.17 |
| C16:1 | 0.64 ± 0.07 |
| C17:0 | 0.32 ± 0.07 |
| C17:1 | 0.32 ± 0.02 |
| C18:0 | 3.80 ± 0.16 |
| C18:1 n-9 | 66.63 ± 0.19 |
| C18:2 | 10.66 ± 0.16 |
| C18:3 | 0.57 ± 0.05 |
| C20:0 | 0.53 ± 0.05 |
| C20:1 | 0.33 ± 0.03 |
| C22:0 | 0.03 ± 0.01 |
| C24:0 | 0.01 ± 0.01 |

### *3.2. Provola Cheese Composition*

The composition, including polyphenol content and FA profile, of Provola cheeses from both treatment groups was analysed and evaluated for the effects of diet, month/season and diet×season interaction. The main parameters of interest resulting from this model are presented in Table 3. Cheeses from the milk of animals fed the treatment diet have a higher protein concentration and less moisture content than those produced from the control group. Trends in higher milk protein from animals fed diets supplemented with olive cake have already been reported [19–21]. This could be an effect of (1) a metabolic/physiological process by which cows fed diets with olive cake inclusion produce more milk protein and/or (2) the quality of protein in the milk from animals fed diets with olive cake inclusion has a tendency for higher coagulation within the cheese curd, thereby increasing the concentration of protein in the final product. Russo et al. [22] reported that destoned olive cake supplementation was able to modulate the faecal microbiota by increasing the Firmicutes phylum, which is associated with increased dietary nutrient availability, and decreasing the energetically less beneficial Bacteroidetes. A significant effect of the regime diet on the biosynthesis of carbohydrates, fatty acids, lipids and amino acids was revealed by predicting metabolic pathways [22]. Neither of the two diets had a significant effect on the total fatty acids present in the Provola cheese. However, the FA profile was significantly affected, an observation that is consistent with the findings of several other authors who investigated milk or cheese produced by dairy ruminants fed with olive products [16,19,21,23–25]. Some of the observed changes in the FA profile of the test cheeses can be considered beneficial from a consumer's health point of view. Total MUFAs and PUFAs were higher in cheese made from the milk of cows fed the treatment diet. Similarly, the same cheese had significantly higher levels of the total amount of n-6 and n-3 FAs; however, the ratio of n-6:n-3 remained constant at about 4:1. Since human and porcine

organs are morphologically and physiologically similar, Duan et al. [26] used porcine as a model to investigate optimal dietary proportions of n-6:n-3 PUFAs and concluded that, overall, n-6:n-3 PUFAs have different effects on lipid metabolism and inflammation and that optimal proportions fall within a range of 1:1 to 5:1. Human consumption of PUFAs is associated with reducing LDL cholesterol and thus decreasing the risk of coronary diseases [27].

**Table 3.** Results of the model for the main parameters of interest. The least squares means values of the test and control (CTR) groups and the standard errors of the mean (SEM) are reported, as well as the *p*-value for the three analysed factors.

| | TEST | CTR | SEM | Diet | Season | Diet×Season |
|---|---|---|---|---|---|---|
| Moisture (%) | 42.02 | 43.62 | 0.34 | 0.0025 | <0.0001 | <0.0001 |
| Proteins (%) | 25.02 | 23.49 | 0.31 | 0.0019 | 0.7570 | 0.0004 |
| Total lipids (%) | 20.94 | 20.91 | 0.41 | 0.9598 | 0.1259 | <0.0001 |
| Total polyphenols (mg/kg) | 142.42 | 107.19 | 2.01 | <0.0001 | <0.0001 | <0.0001 |
| Σ FA | 95.34 | 95.02 | 0.18 | 0.2206 | 0.0102 | 0.4615 |
| Σ MUFA | 23.17 | 22.71 | 0.16 | 0.0460 | 0.0073 | <0.0001 |
| Σ PUFA | 3.88 | 3.66 | 0.05 | 0.0028 | <0.0001 | 0.9269 |
| Σ SFA | 68.29 | 68.66 | 0.22 | 0.2517 | 0.0012 | 0.0006 |
| Σ n-3 | 0.72 | 0.65 | 0.02 | 0.0034 | <0.0001 | 0.0489 |
| Σ n-6 | 3.03 | 2.72 | 0.04 | <0.0001 | <0.0001 | 0.0181 |
| Σ n-6/Σ n-3 | 4.26 | 4.20 | 0.09 | 0.6950 | 0.5710 | 0.5962 |
| Undefined FA | 4.66 | 4.98 | 0.18 | 0.2206 | 0.0102 | 0.4615 |
| Atherogenic Index | 2.98 | 3.17 | 0.02 | <0.0001 | 0.0535 | <0.0001 |
| Thrombogenic Index | 3.53 | 3.70 | 0.03 | 0.0004 | 0.0303 | <0.0001 |
| C12:0 | 3.60 | 3.62 | 0.04 | 0.7454 | <0.0001 | 0.6527 |
| C14:0 | 11.40 | 11.54 | 0.06 | 0.1115 | <0.0001 | 0.8423 |
| C16:0 | 31.01 | 32.84 | 0.09 | <0.0001 | 0.0006 | 0.0000 |
| C18:0 | 12.17 | 10.71 | 0.30 | 0.0016 | 0.4862 | 0.8813 |
| C18:1 n-9 | 20.10 | 19.39 | 0.18 | 0.0087 | 0.5608 | <0.0001 |
| C18:2 n-6 cis | 2.74 | 2.44 | 0.03 | <0.0001 | <0.0001 | 0.0053 |
| C18:2 n-6 trans | 0.10 | 0.10 | 0.01 | 0.6852 | <0.0001 | 0.0108 |
| C18:3 n-3 | 0.50 | 0.40 | 0.01 | <0.0001 | <0.0001 | <0.0001 |
| C20:4 n-6 | 0.02 | 0.01 | 0.002 | 0.0759 | 0.0154 | 0.4670 |
| C20:5 n-3 | 0.04 | 0.04 | 0.003 | 0.2410 | <0.0001 | 0.1347 |

The SFAs that are relevant with regards to the already well-known overall beneficial effects of food and that were registered as significantly affected by the diet in this study were C16:0 and C18:0. Cheese made from milk from cows on the treatment diet registered lower C16:0 and higher C18:0 when compared to cheese from the control group. In addition, the cheese from the treatment group had lower concentrations of palmitic and myristic acids, both of which have been shown to increase LDL cholesterol, and higher concentrations of stearic acid (C18:0), which did not appear to be associated with adverse health effects [28]. The MUFAs, n-3 and n-6 and C16:0 and C18:0 all contribute towards the calculations of the atherogenic and thrombogenic indexes of food. In this study, the incorporation of olive cake in the diets of dairy cows had an overall lowering effect on both the atherogenic and thrombogenic indexes, making the cheese from the milk of cows fed OC supplements a healthier option. The C18:2 cis and C18:3 n-3 acids both registered significantly higher in the test cheeses. These essential FAs are also precursors of other FAs with beneficial properties: linoleic acid is essential for the synthesis of arachidonic acid (C20:4, n-6), the precursor of prostaglandins and prostacyclins (involved in the reproductive function) or thromboxanes (playing a role in the haemostasis function). Conjugated linoleic fatty acid, which is made up of a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds, has been reported to have a wide range of beneficial effects, including anticarcinogenic [29], antiatherogenic [30] and antiobesity activities [31]. α-linolenic acid is the precursor of eicosapentaenoic acid, which in turn is a precursor of

several compounds essential for the heart, retina, brain and immune system functions [32]. It is possible that this increase in CLA content is the result of a decreased biohydrogenation rate of oleic and linoleic intermediates by *Butyrivibrio genus* and *B. proteoclasticus* [5]. These changes in the cheeses' FA profile are in partial agreement with those reported by Castellani et al. [21]. These authors observed an increase in MUFAs and a decrease in SFAs in milk and cheese, whereas no effect of OC on PUFAs was found. They claim that dietary inclusion of olive cake reduces palmitic acid and atherogenic and thrombogenic indices while increasing oleic, vaccenic, stearic and CLA in both milk and cheese.

In agreement with Vasta et al. [23], the polyphenols recorded throughout the study period in the cheese from the test group were significantly higher than those detected in the cheese from the control group. With a 32.9% increase in polyphenols, the cheese from the TEST group has greater functional nutrients and properties than the cheese from the CTR group. The consumption of these bioactive compounds is considered beneficial to human health as they enhance the natural defence system by decreasing the formation of free radicals and harmful oxidative events in metabolism [33]. While changes in the FA profile and polyphenol content can have positive health implications for the consumer, the same observation can influence consumer acceptance due to a potentially altered taste. Thus, the cheeses are currently being evaluated on their organoleptic properties, and related results will be published.

### *3.3. Season and Diet×Season Effects*

The present trial extended over the spring and summer seasons in Ragusa (Sicily). Having a typical Mediterranean climate during which the thermal comfort zone is frequently challenged and, at times, surpassed during the summer months, animals are prone to experiencing heat stress due to the combined effect of elevated environmental temperatures coupled with high relative humidity. Heat stress affects the physiological, metabolic, endocrine and molecular mechanisms of the animal, leading to reduced productivity. Two key factors generally trigger these mechanisms: (1) a reduction in feed intake and (2) an increase in water intake. Such a situation tends to negatively affect milk production and its composition [34]. Indeed, milk production in dairy cows subjected to chronic heat stress is closely related to dry matter and water intake [35]. Such environmental conditions have a significant influence on the organic and inorganic composition of milk. They also affect cheese yield and quality [36]. Bernabucci et al. [37] report a slightly lower casein concentration in milk during the summer when compared to winter, which may account for the lower cheese production generally observed during the summer period, while according to Bouraoui et al. [38], cheeses made from milk produced in the summer have a reduced fat and protein content when compared to that manufactured in the spring.

The data obtained in this study indicate that C12:0, C14:0, C18:2 n-6 cis, C18:2 n-6 trans, C18:3 n-3, C20:4 n-6, C20:5 n-3, undefined FAs, Σ n-3, Σ n-6 and Σ PUFA were recorded to be significantly higher during the cooler spring period, whereas C16:0, C18:3 n-6, Σ FA, Σ MUFA and Σ SFA registered significantly higher in summer. The effects of heat stress on milk fat content are still not fully understood, and controversial results have been reported in the literature. Bernabucci et al. [37] show a substantial and significant decrease in milk fat during the summer as compared to the spring. A study by Summer et al. [36] also observed a decrease in milk fat content during the summer period compared to the autumn. In the case of Cowley et al. [39], they found no significant differences between cows under normal conditions or under heat stress. During the summer period, it has been registered that cheeses have significantly higher moisture (%), thrombogenic index and total polyphenols (mg/kg). Contrary to the conventional school of thought that dictates a lowering of protein content during the summer period, in this study, changes in protein did not register as significant.

The statistical model showed that the diet×season interaction had a significant effect on a number of parameters of importance, namely, moisture (%), proteins (%), total lipids (%), total polyphenols (mg/kg), Σ MUFA, Σ SFA, Σ n-3, Σ n-6, AI and TI, C16:0, C18:2 n-6

cis, C18:2 n-6 trans and C18:3 n-3. The interaction plots for these parameters are reported in Figures 2–4.

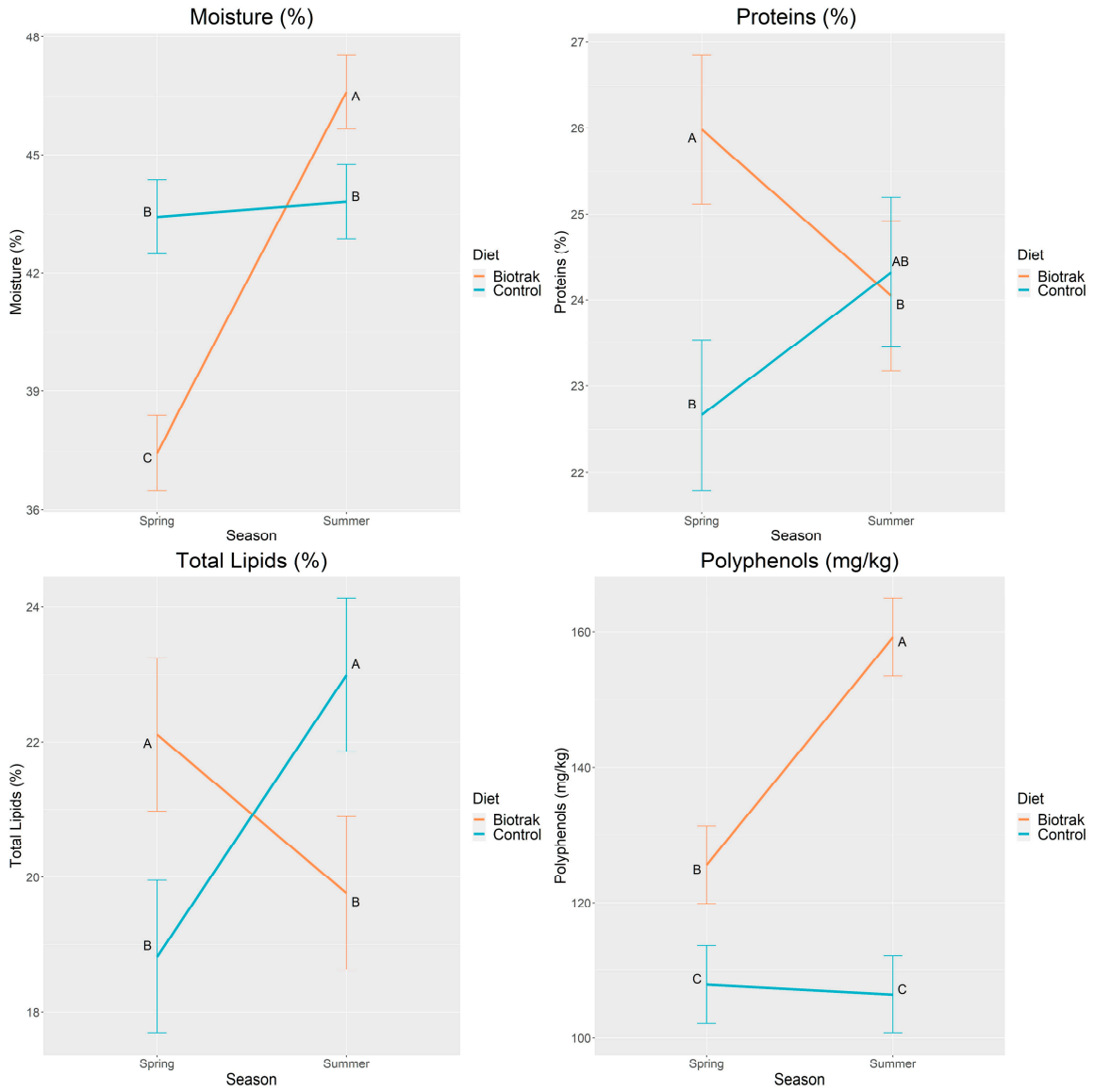


**Figure 2.** Diagrams of the interaction diet×season for moisture (%), protein (%), total lipids (%) and total polyphenols (mg/kg). Different letters indicate significantly different values according to the Tukey–Kramer test.

The treatment diet registered the following overall highest values for spring: proteins, Σ n-3, Σ n-6, C18:2 n-6 cis, C18:3 n-3 and C18:3 n-6, while it registered the lowest values for C16:0, atherogenic and thrombogenic indexes, moisture and Σ SFA. With regards to the summer period, the treatment diet registered the overall highest for moisture and total polyphenols and the overall lowest for Σ n-3.

The control diet registered the following overall highest values for spring: C16:0, C18:2 n-6 trans, atherogenic and thrombogenic indexes and Σ n-6, while registered the lowest value for proteins, total lipids, polyphenols and Σ MUFA. With regards to the summer period, the treatment diet registered the overall highest total lipids, moisture and Σ MUFA and the overall lowest for C18:2 n-6 trans, C18:3 n-3, polyphenols, Σ n-3 and Σ n-6.

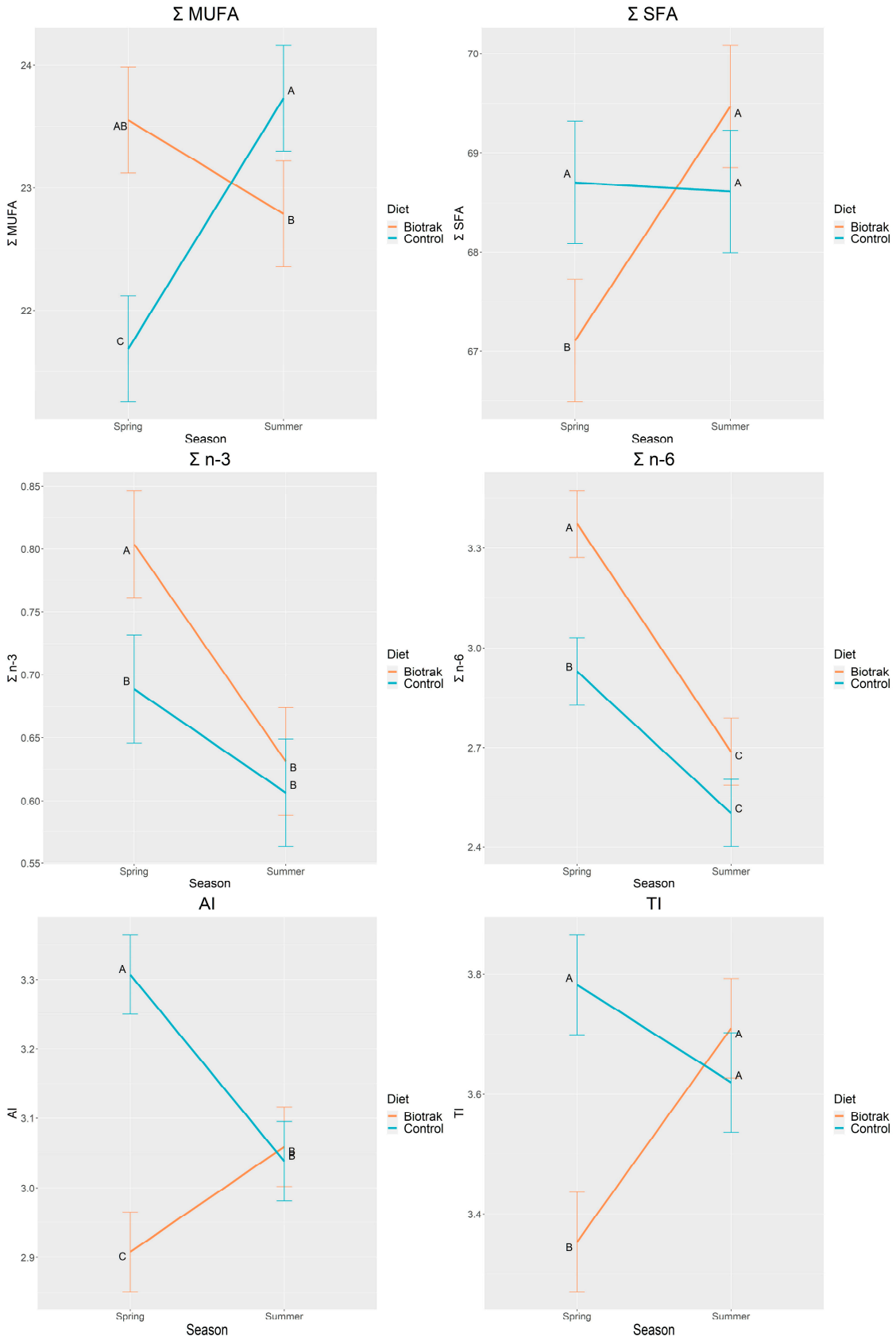


**Figure 3.** Diagrams of the interaction diet×season for Σ MUFA, Σ SFA, Σ n-3, Σ n-6 and atherogenic and thrombogenic indices (AI and TI). Different letters indicate significantly different values according to the Tukey–Kramer test.

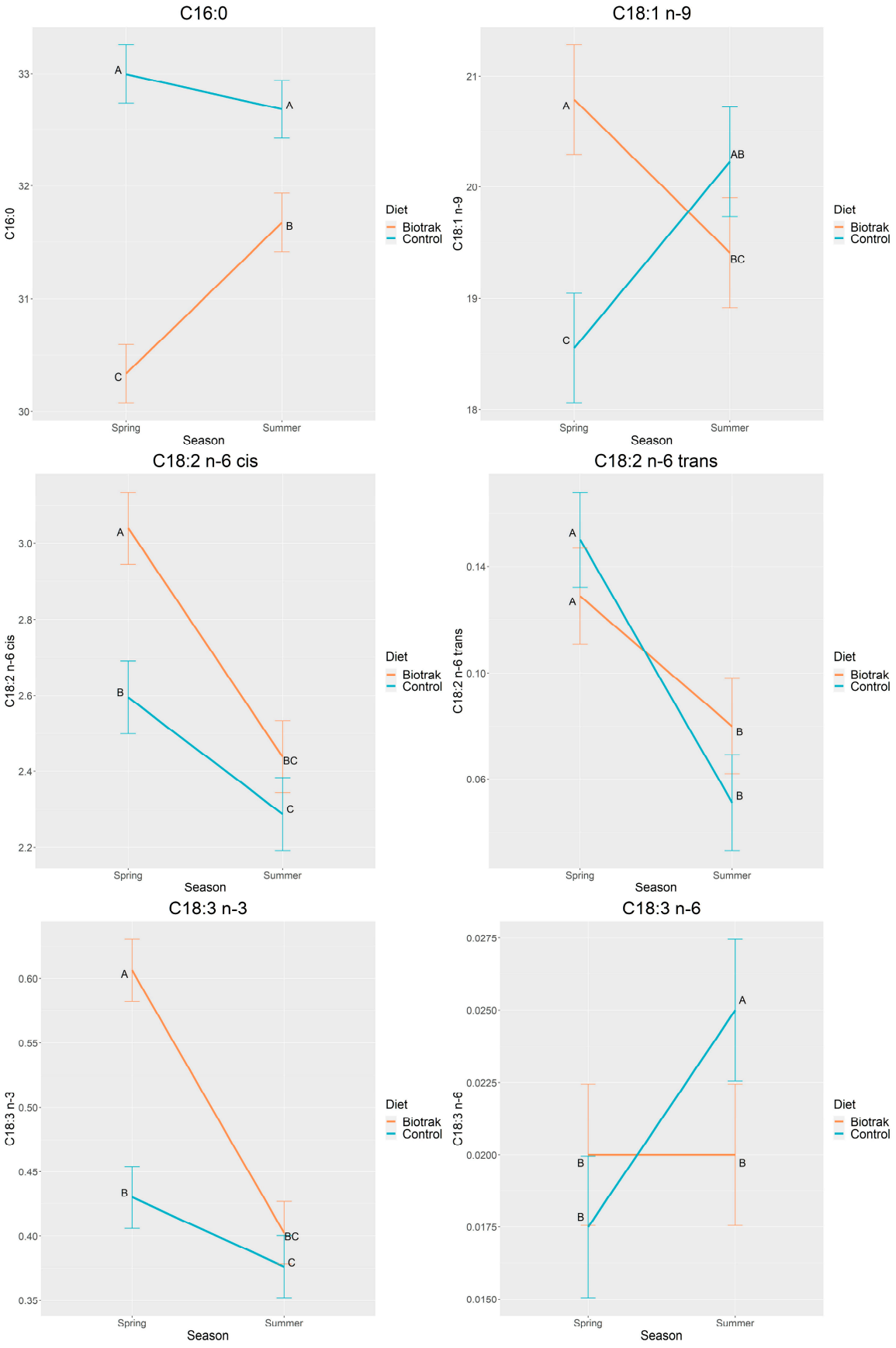


**Figure 4.** Diagrams of the interaction diet×season for C16:0, C18:2 n-6 cis, C18:2 n-6 trans and C18:3 n-3. Different letters indicate significantly different values according to the Tukey–Kramer test.

The literature on the interactions of diets incorporating olive cake with season on dairy cows is scarce. The results presented in Table 4 show an element of inversion between diet type and season. Nonetheless, there tends to be a strong indication that the cheese with the highest nutritional benefit was manufactured from milk produced by the test diet in spring, while the one with the least nutritional benefit is the one from the control group from milk produced in spring. The data further indicate that, irrespective of season, the cheeses with the highest nutritional benefit are those manufactured with milk from cows fed the treatment diet with the incorporation of olive cake.

**Table 4.** Least squares means and standard error of the mean (SEM) of the main parameters of interest together with the respective *p*-value for diet×season interaction effect.

| | TEST | | CTR | | SEM | *p*-Values |
|---|---|---|---|---|---|---|
| | Spring | Summer | Spring | Summer | | |
| Moisture (%) | 37.44 | 46.60 | 43.43 | 43.81 | 0.481 | <0.0001 |
| Protein (%) | 25.98 | 24.05 | 22.66 | 24.32 | 0.444 | 0.0004 |
| Total lipids (%) | 22.11 | 19.76 | 18.82 | 22.99 | 0.578 | <0.0001 |
| Total polyphenols (ppm) | 125.61 | 159.24 | 107.94 | 106.45 | 2.922 | <0.0001 |
| C16:0 | 30.33 | 31.68 | 33.00 | 32.68 | 0.132 | <0.0001 |
| C18:1 n-9 | 20.79 | 19.77 | 18.43 | 19.91 | 0.252 | <0.0001 |
| C18:2 n-6 cis | 3.04 | 2.44 | 2.60 | 2.29 | 0.049 | 0.0053 |
| C18:2 n-6 trans | 0.129 | 0.080 | 0.150 | 0.051 | 0.009 | 0.0108 |
| C18:3 n-3 | 0.606 | 0.403 | 0.430 | 0.376 | 0.012 | <0.0001 |
| Σ MUFA | 23.55 | 22.79 | 21.69 | 23.73 | 0.220 | <0.0001 |
| Σ n-3 | 0.80 | 0.63 | 0.69 | 0.61 | 0.022 | 0.0489 |
| Σ n-6 | 3.37 | 2.69 | 2.93 | 2.50 | 0.052 | 0.0181 |
| Σ SFA | 67.11 | 69.47 | 68.70 | 68.61 | 0.315 | 0.0006 |
| Atherogenic Index | 2.91 | 3.06 | 3.31 | 3.04 | 0.029 | <0.0001 |
| Thrombogenic Index | 3.35 | 3.71 | 3.78 | 3.62 | 0.042 | <0.0001 |

## 4. Conclusions

Increased awareness of the need to reduce environmental impact and make better use of available resources has led researchers to focus on the possibility of identifying by-products of the agro-food chain as potential sources of active compounds with potential health benefits. The diversion of these identified by-products from the waste stream makes a significant contribution to environmental sustainability. As such, their use would benefit both human health and the environment by reducing the need for disposal. In the context of the circular economy, the olive oil processing cycle could serve as an example of the reuse of by-products from agri-food waste. The results of this study indicate that the incorporation of olive cake in the diet of lactating dairy cows has a significant effect on reducing the atherogenic and thrombogenic indexes while also contributing to increasing the total polyphenols in the cheese product. These innovative and functional features are likely to be welcomed and appreciated by consumers, both for their health benefits and for the incorporation and reuse of biomass waste, which also contributes to the well-being of the environment and, ultimately, of all citizens. This study has again highlighted that the qualitative composition of cheeses is significantly influenced by the season. Therefore, cheeses, especially those with a quality guarantee mark, must clearly indicate whether they are summer or winter cheeses in order to facilitate consumer information and ultimately choice. This will be in line with the requirement to include nutrition and health information on the label in accordance with Regulation (EC) No. 1924/2006. This would be the final objective, that is, to provide direct information to the final consumer.

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