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Utilizing Olive By-Products in the Diet of Bísaro Pigs: Enhancing the Value of the Breed's Dry-Cured Products

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Abstract: This study aimed to evaluate the influence of olive cake on the diet of autochthonous Bísaro pigs in processed products. To this purpose, loins and "cachaços" were used and subjected to a manufacturing flowchart to obtain a dry-cured product. Both products were subjected to the same formulation, ingredients, and curing steps. Regarding the physicochemical composition, there were significant differences between the two products for the parameters of aw ($p<0.001$), moisture ($p<0.001$), total fat ($p<0.001$), protein ($p<0.001$), and haem pigments ($p<0.001$). As for the diet, it only significantly influences the NaCl content ($p<0.05$). Neither the product nor the diet had any influence ($p>0.05$) on the fraction of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acids (PUFA). However, there is a significant difference when it comes to omega 3 ($p<0.05$). These fatty acids increased with the addition of olive cake, and the diet with olive cake centrifuged by 25% had the highest value for both products. The increase in this omega 3 resulted in a decrease in the PUFA n-6/n-3 ratio ($p<0.01$) in the diets with olive cake, compared to the control diets.

Keywords: native Bísaro pig; dry-cured product; olive cake; valorization

1. Introduction

Olive oil production is widely known worldwide, with an average production of 3 million tons of olive oil worldwide, of which 2 million (over 67%) are produced by the European Union (EU). The EU countries with the largest share of this production are Spain, Italy, Greece, and Portugal. More than half of the total produced in the European Union (66%) comes from Spain. Italy and Greece have very similar productions, with 15% and 13% respectively. Portugal is responsible for 5% of the total produced in the EU. Also, the EU is the biggest consumer and exporter of olive oil. The EU is responsible for consuming 50% of annual production, corresponding to 1.5 million tons of olive oil, and exporting 570000 tons a year [1].

As a result of the high production of olive oil, many by-products are generated that are highly toxic to the environment. Efficient management of these by-products is necessary to reduce their environmental impact and ensure economic profitability since most of these raw materials, if not disposed of correctly, result in excessively high treatment costs. According to Molina-Alcaide et al. [2] olive oil by-products can be categorized as olive leaves, olive cake, and other by-products. The olive leaves refer to a mixture of leaves and branches that come from pruning the olive trees and harvesting and cleaning the olives before the oil is extracted [2]. Olive cake comprises olive pulp, skin, stone, and water. Depending on the extraction methods, different terms can be used for olive cake (pressed, extracted, centrifuged). It is important to note that there are three-phase and two-phase

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systems in the case of centrifugal extraction. The main difference is the quantity of oil and moisture, with the two-phase centrifuged olive cake being more efficient and environmentally friendly (with a higher moisture content and lower oil content) [3]. As far as pressed olive cake is concerned, extraction is achieved by a discontinuous press process, a more traditional extraction method. The nutritional valorization of these by-products has become a prime target for various research sectors. It is widely used in ruminant feed but is still rarely used in other animal breeds. It is also used as an energy resource due to its lignin content, which provides high calorific value with a low ash content [4]. Another use for this product is through composting, mainly as fertilizer or as a component of agricultural substrates [5]. Olive cake has had various uses but remains an under-exploited resource [2]. The markets for commercializing raw materials for animal feed have been severely constrained by price volatility. Using this by-product to feed Bísaro pigs could be another alternative to minimize its harmful effects. Incorporating this olive oil by-product into the diet of Bísara pigs could influence the quality of the processed products and be an alternative to the raw materials that normally form part of the diet of this type of animal in extensive farming.

Furthermore, incorporating this by-product makes perfect sense in the northern region of Trás-os-Montes (Portugal), given the amount of olive cake that is produced and the number of farms with Bísara animals. In addition, the time of year when these by-products are obtained coincides with the finishing phase of these animals. Therefore, transporting this by-product is more accessible due to the proximity of the farms and oil mills, and storage is also easier. Bísaro pigs offer products characterized by high quality [6]. Due to the characteristics of their meat, native breeds are highly valued for producing high-quality dry-cured products [7]. Therefore, the main objective of this work is to evaluate the potential of olive cake (pressed and centrifuged) for use in the animal diet of Bísaro pigs and the effects produced in the processed product obtained at an industrial scale.

2. Materials and Methods

2.1. Experimental Diets and Slaughter Procedure

The animals used were indigenous Bísaro pigs, kept in an extensive system on the farm belonging to the company Bísaro-Salsicharia Tradicional, Lda® (Gimonde-Bragança, Portugal). The first diet consisted of a traditional diet typical of these native breeds in an extensive system, thus considered the control diet. The second diet concerns the Base diet + 15% of centrifuged olive cake (BgCf15). The third diet consists of a Base diet + 25% of centrifuged olive cake (BgCf25). The fourth diet consists of a Base diet + 15% of pressed olive cake. Table 1 shows the chemical composition of the diet and its fatty acid profile.

Table 1. Ingredient composition of the experimental diets (g/kg, as fed basis) and fatty acids composition (g/100 g).

	Diets			
	Control	**BgCf15**	**BgCf25**	**BgPr15**
Chemical composition of the diet				
DM	86.35	83.76	90.77	88.40
OM	94.73	95.35	95.03	95.00
NDF	20.06	27.47	36.63	27.50
ADF	7.62	12.31	23.67	13.97
ADL	2.49	4.78	9.62	5.75
PB	12.31	11.18	13.78	10.94
GB	5.74	6.75	4.51	5.86
Fatty acids (g/100 g)				
ΣSFA	15.27	12.88	16.28	11.91
ΣMUFA	23.80	59.32	41.71	56.69
ΣPUFA	22.25	9.18	25.11	7.27
n-6/n-3	17.18	7.77	14.08	7.57

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DM – dry-matters; OM – organic matter; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL: acid detergent lignin; PB – crude protein; GB – crude fat. C – control; BgCf15 – Base diet + 15% olive cake two-phases; BgCf25 – Base diet + 25% olive cake two-phases and BgPr15 – Base diet + 15% crude olive cake.

The Bísara animals were raised under the care of the University of Trás-os-Montes and Alto Douro in Vila Real, Portugal. They were divided into groups to receive different diets. The control group was fed a traditional diet for this indigenous breed, consisting of local vegetables, cereals, and feed appropriate for each growth stage. The other diets also had the same Base diet as the control diet, plus the type of olive cake with the percentages of 15 and 25 percent (see Table 1). The animals were fed these diets during the finishing phase (90 days) and with an average feed of 3kg per day. Throughout the finalization process, where the different diets were applied, the team from the University of Trás-os-Montes e Alto Douro was responsible for monitoring the animals.

Figure 1. Schematic of the diets applied to the Bísaro pigs.

After the final finishing phase, the animals were slaughtered when they reached 12 months of age, at around 135 kg live weight and 110 kg carcass weight. The slaughter was carried out by the Bragança Municipal Slaughterhouse team. The slaughter and carcass preparation process were previously described by Álvarez-Rodríguez and Teixeira [8]. All animals were cared for and slaughtered in compliance with the welfare regulations and respecting EU Council Regulation (EC) No. 1099/2009 [9]. After slaughter and cutting, the joints obtained were transported to the industry (Bísaro-Salsicharia Tradicional, Lda ®) that will be responsible for processing them.

2.2. Dry-cured Bísaro Loin and "Cachaço"

The curing process was conducted at Bísaro-Salsicharia Tradicional, Lda®, using forty-eight loins and forty-eight "cachaços" from forty-eight slaughtered animals. The ingredients were added in decreasing order: 1.5% salt, 0.5% paprika, 0.5% garlic and 0.1% oregano. Both the loins and "cachaços" were dry-cured for 60 days. After removing the muscles from the animal carcasses, the pieces were chilled in a chamber at 2-5°C, and excess surface fat was removed from each piece. Selected pieces then underwent the curing process. The salting and seasoning phase involved placing the pieces on a rotating drum for about 30 minutes after adding salt, paprika, garlic, and oregano. After mixing, the joints were transferred to a container and stored in a refrigeration chamber at 2-4ºC with approximately 90% relative humidity for 4 days to allow the ingredients to penetrate. Next, the pieces were stuffed into collagen casings. The final phase was drying and curing, during which significant biochemical changes occurred. The temperature and humidity were adjusted as the curing progressed: for the first 15 days, the cuts were kept at 4-8ºC with 80-90% relative humidity.

Subsequently, the temperature was raised to 8-12ºC with 70-80% relative humidity for another 15 days. Finally, for the last 20 days, the product was maintained at 12-18ºC with 60-70% relative humidity. This curing process has been validated by the company Bísaro-Salsicharia Tradicional Lda®, considering all the quality and food safety standards. It should be noted that the company responsible for the entire manufacturing flowchart for this type of product has been certified for over seven years by extremely demanding quality benchmarks (IFS-Food).

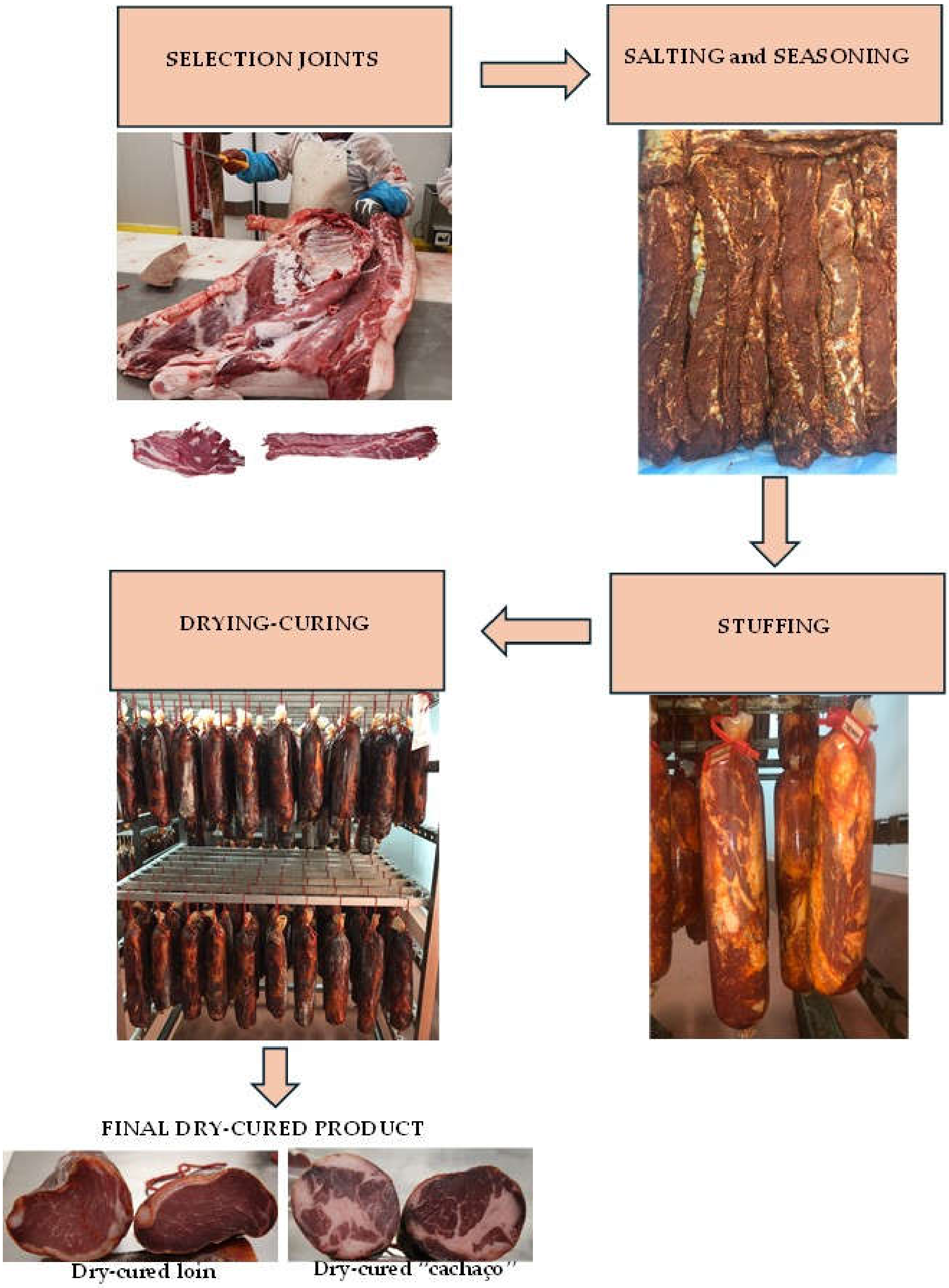

Figure 2. Process of obtaining Bísaro dry-cured loin and dry-cured "cachaço".

2.3. Chemical Composition and Physicochemical Analysis of Dry-Cured Loin and Dry-Cured "Cachaço"

All the physicochemical analyses carried out on these two products were done by the Portuguese standards described below. Water activity was assessed according to AOAC [10] using a probe HigroPalm Rotronic 8303 (Bassersdorf, Switzerland). The determination of moisture was performed according to the Portuguese standard NP 1614 [11]. For this purpose, 3 g of sample was added to 5 mL of ethanol (96% v/v). Stepping forward, the samples were dried in a drying oven (Raypa DO-150, Barcelona, Spain) for 24 hours at 103 ± 2 ºC. The determination of ash was made according to the Portuguese standard [12]. To 3 g of sample, we added 1 mL of magnesium acetate (15% w/v) in crucibles. The following, samples were subjected to 550 ºC ± 25 ºC for 5 hours in a muffle furnace (Vulcan BOX Furnace Model 3-550, Yucaipa, USA). Protein determination was carried out following the Portuguese standard [13] using the Kjeldahl Sampler System (K370, Flawil, Switzerland) and Digest System (K-437, Flawil, Switzerland). In 25 mL of sulfuric acid (97%), two catalyst tablets and 2 g of sample were placed in mineralization tubes. After mineralization completion, the distillation procedure was carried out. At last, the distillate was titrated with a hydrochloric acid solution and the required volume was recorded. The determination of hydroxyproline and collagen content was carried out by Portuguese Standards NP 1987 [14]. The haem pigment content [15] was obtained using the reflectance on the exposed surface by spectroscopy using a Spectronic Unicam 20 Geneys, expressed as mg myoglobin/g fresh muscle. Total chloride content was analyzed according to the recommended methodology in the Portuguese Standard NP 1845 [16].

2.4. Fatty Acid Analysis

Fatty acids in dry-cured loin and dry-cured "cachaço" samples were analyzed in the Laboratory of ESA-IPB. The total lipids were extracted from 25 g of meat sample according to the Folch procedure [17]. The fatty acid profile was determined using 50 mg of fat. The fatty acids were transesterified according to the method described by Domínguez et al. [18]; after adding 4 mL of a sodium methoxide solution and vortexed it for five minutes at a time for 15 minutes at room temperature, 5 mL of H2SO4 solution (in methanol at 50%) was added. Then we add 2 mL of distilled water and vortex once more. The organic phase (with the methyl esters of fatty acids) was extracted with 2.35 mL of hexane. The fatty acid methyl esters separation and quantification were performed using a gas chromatograph (GC-Shimadzu 2010Plus; Shimadzu Corporation, Kyoto, Japan) provided with a flame ionization detector and an automatic sample injector AOC-20i and using a Supelco SP TM - 2560 fused silica capillary column (100 m length, 0.25 mm i.d., 0.2 μm film thickness). The fatty acid contents were calculated using chromatogram peak areas and were expressed as g per 100 g of total fatty acid methyl esters. In addition, the percentage of saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), polyunsaturated fatty acids (ΣPUFA), the ratio PUFA n-6/n-3 and Σtrans were calculated according to Vieira et al. [19]. To assess lipid quality, the atherogenicity index (AI) and the thrombogenicity index (IT) were calculated according to Ulbricht and Southgate [20].

2.5. Statistical Analysis

Data was tested for normal distribution and homogeneity of variance using the Shapiro-Wilk test. Next, the effect of diet and type of product, and the interaction between diet and product, on the physicochemical composition and fatty acid profile were examined using analysis of variance (ANOVA) with the general linear model (GLM) procedure, in which these parameters were defined as dependent variables and diet and type of product as fixed effects. The results were presented in terms of mean values and standard error of the mean (SEM). When there was a significant effect ($p < 0.05$), the means were compared using Student's t-test. To extract a few key combinations (called principal components) from the group of measured variables that capture most of the variability in those variables, we conducted a principal component analysis (PCA). Each principal component was determined by combining the eigenvectors of the correlation matrix linearly. The eigenvalues indicate how much variance each component holds. Also, a multiple factor analysis (MFA) related to principal components analysis (PCA) was performed to produce a table of eigenvalues, summary plots, and a consensus map. All analyses were performed using the statistical package JMP® Pro 17.0.0 by 2023 SAS Institute Inc.© (Cary, NC, USA).

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3. Results and Discussion

3.1. Physicochemical Composition

The results of the chemical composition of dry-cured loin and dry-cured "cachaço" are listed in Table 2. This table shows the effect of the product on each of the parameters studied and the effect between the type of product and the diet applied. The product type had a significant influence ($p<0.001$) on the aw, moisture, total fat, protein, and haem pigments parameters. On the other hand, collagen, NaCl content, and consequently ash content were not significantly influenced ($p>0.05$) by the type of product. We can also see that diet did not affect most of the physicochemical parameters studied, except for NaCl content. Regarding the interaction between the product and the diets, the parameters of aw, ash, and NaCl content showed significant differences ($p<0.05$). The other parameters (moisture, total fat, protein, collagen, and haem pigments) did not show any significant results between the type of product and the diet applied.

Table 2. Physicochemical composition of dry-cured Bísaro Loin and dry-cured Bísaro "cachaço".

	Physicochemical composition (g/100g)															
	aw		**moisture**		**ash**		**Total Fat**		**Protein**		**Collagen**		**Haem pigments**		**NaCl**	
	L	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**	**L**	**C**
Control	0.893a	0.857bc	41.27a	31.24b	5.07bc	4.97bc	21.40b	41.09a	32.61a	24.52c	3.44a	3.15a	2.31b	4.17a	3.75cd	4.24bc
Cf15	0.873ab	0.839cd	42.73a	33.43b	4.98bc	5.96ab	19.03b	36.59a	32.24ab	24.99c	3.35a	2.55a	2.15b	4.33a	3.55cd	5.01b
Cf 25	0.866bc	0.862abc	40.02a	31.84b	7.21a	5.36bc	24.62b	40.03a	29.73b	23.40c	3.66a	2.74a	2.72b	4.04a	6.53a	4.01bcd
Pr15	0.896a	0.815d	41.69a	30.99b	4.25c	6.13ab	16.90b	43.37a	34.26a	24.39c	2.83a	2.87a	2.41b	3.84a	2.76d	5.36ab
SEM	0.01		1.37		0.57		3.46		1.13		0.56		0.29		0.53	
Significance product	***		***		ns		***		***		ns		***		ns	
Significance diet	ns		ns		ns		ns		ns		ns		ns		*	
Significance product x diet	*		ns		**		ns		ns		ns		ns		***	

ns – not significant, * $p < 0.05$; *** $p < 0.001$. SEM (Standard Error of the Mean). L – dry-cured loin; C – dry-cured "cachaço"). Haem pigments in mg myoglobin/g fresh muscle. Cf15 – Base diet + 15% olive cake centrifuged; Cf25 – Base diet + 25% olive cake centrifuged; Pr15 – Base diet + 15% olive cake pressed; CT – Base diet.

Regarding water activity (a_w), values between 0.896 and 0.856 for the dry-cured loin and values between 0.862-0.815 for the dry-cured "cachaço" were obtained. The aw value was significantly higher ($p<0.001$) in all diets (including the control) for the dry-cured loin compared to the dry-cured "cachaço". The aw value for the dry-cured loin control was the highest, above the values reported for the same product and without adding olive cake to the diet of the Bísaro pig [21]. On the other hand, the a_w values observed in this study for diets containing olive cake aligne with those obtained in another study [21] (for the same product type and with the addition of olive cake in other percentages). Other authors have reported average values of 0.841 for dry-cured Celta loin [22], 0.830 for dry-cured Korean loin [23], 0.838 for dry-cured Polish neck [24], 0.838 for dry-cured foal loin [25]. Similar aw values have been obtained in other dry-cured products, such as dry-cured shoulder [26-29]. Water activity is a key factor influencing the safety of dry-cured meat products. Therefore, water activity is a Base indicator of the shelf life of dry-cured products, conferring nutritional stability and characterizing the type of microorganisms present in the food [30]. The water, together with salt, creates osmotic changes, causing dehydration which implies a removal of water from the meat [31]. The water content can facilitate the attachment of a radical species and the removal of hydrogen from the fatty acids, thus initiating the oxidation process [53]. Therefore, both products obtained water activity values that make them dry-cured products with the desired microbiological stability, and

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there was no influence (positive or negative) on the addition of olive cake to the Bísaro pig feed. Another important parameter that serves as an indicator of product ripeness is moisture.

The moisture parameter for the dry-cured loin was significantly higher (p<0.001) than that obtained for the dry-cured "cachaço". The average values obtained for dry-cured loin ranged from 40.02 to 42.73%. The average values obtained for the dry-cured "cachaço" ranged from 30.99 to 33.43%. The olive cake used in the animals 'diet also did not influence the moisture parameter. The moisture level of dry-cured products is inversely proportional to the maturation time [32]. The values obtained in this study were higher for both products than those observed by other authors [21]. Other authors have observed values identical to those we observed in dry-cured "cachaço", in a typical dry-cured product from Spain's Mediterranean coast [33], in the traditional Italian product "coppa" [34] As for Iberian dry-cured loin, similar values were obtained (42%) [34], among other dry-cured loins [22, 25, 36].

The changes in ash content are mainly related to the sodium chloride content. The ash values ranged from 4.25 to 7.21 for the dry-cured loin, while for the dry-cured "cachaço" the values ranged from 4.97 to 6.13. For this parameter, there were no significant differences with the introduction of the olive cake diet, nor due to the type of product used. While there was no significance in isolation, we observed that the interaction between the product and the diet was significant (p<0.01). Other authors have obtained higher values for this parameter in dry-cured foal loin [25], "Cecina de León" [72], Bísaro dry-cured shoulder [29], and Celta dry-cured ham [58]. Similar values were observed in the Iberian dry-cured loin [71], and Turkish dried meat [73].

The total fat content was significantly different (p<0.001) between the dry-cured loin and dry-cured "cachaço", 16.90-24.62% and 36.59-43.37%, respectively. As far as diet is concerned, it did not influence the total fat content of processed products. Although these two muscles come from the same *Longissimus thoracis Lumborum* (LTL) muscle, they are obtained from different sections. The "cachaço" is obtained from the proximal part of the LTL muscle and the loin is obtained from the lumbar part of the LTL muscle. The high complexity of the meat matrix means that products from the same muscle obtain significantly different values for the total fat parameter.

It is expected that if the fat content is high, the protein content will be lower. As can be seen in Table 2, the protein content was significantly higher (p<0.001) in the dry-cured loin, with average values between 29.73 and 34.26%. In the dry-cured "cachaço", we obtained lower protein values, between 23.40 and 24.99%. The diets applied with the olive by-product did not influence the protein content of the products studied. This difference between dry-cured loin and dry-cured "cachaço" has also been observed in other studies [21]. Similar values to dry-cured "cachaço" were observed in a product designated dry-cured coppa [34]. Values like those we obtained for the dry-cured loin have been reported by other authors on Turkish dried meat [73], dry-cured Celta ham [58], and Bísaro dry-cured shoulder [29]. Higher values were observed in dry-cured foal [25].

No significant differences (p>0.05) were found between diets with olive cake or between the two types of products for collagen content, ranging from 2.83-3.66% and 2.55-3.15% in the dry-cured loin and dry-cured "cachaço", respectively.

Total haem pigments expressed as myoglobin concentration were significantly different among the two products (Bísaro dry-cured loin and dry-cured "cachaço"). The myoglobin content for these products ranged from 2.15-2.72 mg/g and 3.84-4.33 mg/g in dry-cured loin and dry-cured "cachaço", respectively. The inclusion of pressed and centrifuged olive cake did not influence this parameter, as observed by other authors [21].

Significant differences were observed in the chloride content for the addition of olive cake (p<0.05) and the interaction between diet and type of product (p<0.001). The NaCl content varied between 2.76-6.53% in the dry-cured loin and 4.01-5.36% in the dry-cured "cachaço". The highest salt content was observed in the dry-cured loin with the centrifuged olive cake diet (25%). This value explains the ash value observed for the Cf25 diet in the dry-cured loin. Similar NaCl content values were observed in Salame Milano (4.3%), Coppa (5.9%), and Parma Ham (6.1%) [74], and in dry-cured foal "Cecina" [75].

3.2. Fatty Acids Composition

The fatty acid (FA)composition of the dry-cured loin and dry-cured "cachaço" is shown in Table 3. For both products, most fatty acids were oleic acid as monounsaturated fatty acid (MUFA), palmitic acid, stearic acid as saturated fatty acids (SFA), and linoleic acid as polyunsaturated fatty acid (PUFA). These four acids account for more than 93% of the total fatty acids in dry-cured loin and dry-cured "cachaço". These results are in line with the typical fatty acid composition of pork and are consistent with the fatty acid profile described by Leite et al., [52]. A similar trend of FA distribution has been documented in various types of dry-cured products [21, 34, 42-46]. Except for the C18:0 fatty acid, there were no significant differences in the other predominant fatty acids between the two products studied, nor was there any influence from adding olive cake to the animal diets. Concerning the product, a significant difference was obtained for the following FA. C15:0, C17:0, C18:0, C20:0, C18:3n-6, C22:0, C23:0 and n-3. As for the diet, the inclusion of olive cake in the animal diet of Bísaro pigs had a significant influence on the following FA: C16:1n-7, 9t-C18:1, C20:1n-9, C18:3n-3, C20:2n-6, n-3 and ratio n-6/n-3. The content of the FA fractions did not influence the type of product studied. Similarly, the addition of centrifuged and pressed olive cake in percentages of 15 and 25% did not influence the lipid quality of dry-cured loin and dry-cured "cachaço". Therefore, although numerous factors can affect the lipid composition of meat, such as diet, and genetic lineage, among others, the fatty acid profile of pork does not deviate from the typical pork profile.

Table 3. Effect of muscle and diet in fatty acids profile in Bísaro dry-cured Loin and dry-cured "cachaço".

	L				C							
Fatty acids	**Control**	**Cf15**	**Cf25**	**Pr15**	**Control**	**Cf15**	**Cf25**	**Pr15**	**SEM**	**P**	**D**	**DxP**
C10:0	0.02a	0.02a	0.02a	0.02a	0.02a	0.01a	0.02a	0.01a	0.004	ns	ns	ns
C12:0	0.05a	0.05a	0.05a	0.05a	0.04a	0.05a	0.04a	0.05a	0.003	ns	ns	ns
C14:0	1.14	1.15	1.13	1.11	1.13	1.13	1.12	1.11	0.03	ns	ns	ns
C14:1	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.02	0.005	ns	ns	ns
C15:0	0.05a	0.05a	0.05a	0.07a	0.02b	0.03b	0.02b	0.01b	0.008	***	ns	ns
C16:0	26.12ab	25.98ab	26.05ab	25.48b	26.38a	26.17ab	26.37a	25.80ab	0.30	ns	ns	ns
C16:1n-7	2.50ab	2.32bc	2.29bc	2.36abc	2.53a	2.34abc	2.32abc	2.15c	0.08	ns	**	ns
C17:0	0.17bc	0.20ab	0.21ab	0.12c	0.24a	0.23a	0.24ab	0.22ab	0.02	**	ns	ns
C17:1n-7	0.23ab	0.22ab	0.22ab	0.19b	0.24a	0.23ab	0.23ab	0.20ab	0.02	ns	ns	ns
C18:0	13.16ab	13.41a	13.30ab	12.96ab	12.68b	12.72b	12.93ab	12.84ab	0.32	*	ns	ns
9t-C18:1	0.21abc	0.23a	0.19bc	0.21abc	0.20abc	0.22ab	0.18c	0.22ab	0.01	ns	**	ns
C18:1n-9	47.82a	47.40a	47.49a	48.43a	47.97a	48.01a	47.43a	48.49a	0.55	ns	ns	ns
C18:2n-6	6.50a	6.79a	6.76a	6.74a	6.62a	6.81a	7.02a	6.90a	0.26	ns	ns	ns
C20:0	0.21ab	0.19bc	0.21ab	0.22a	0.17d	0.17d	0.18cd	0.18cd	0.01	***	ns	ns
C18:3n-6	0.011a	0.011ab	0.013a	0.006abc	0.003c	0.006bc	0.004c	0.003c	0.002	***	ns	ns
C20:1n-9	0.77c	0.80bc	0.91a	0.82bc	0.76c	0.80bc	0.86ab	0.79bc	0.03	ns	***	ns
C18:3n-3	0.20c	0.24a	0.24ab	0.22abc	0.21bc	0.24a	0.24a	0.25a	0.01	ns	***	ns
C20:2n-6	0.24bc	0.24c	0.28a	0.25abc	0.23c	0.24c	0.28ab	0.24c	0.01	ns	**	ns
C22:0	0.04abc	0.05a	0.03bc	0.05ab	0.03c	0.04abc	0.03c	0.03abc	0.03	*	ns	ns
C20:3n-6	0.05ab	0.05ab	0.05ab	0.06a	0.04b	0.05ab	0.05ab	0.04b	0.006	ns	ns	ns
C22:1n-9	0.03bc	0.04ab	0.03abc	0.04ab	0.03bc	0.03ab	0.04a	0.02c	0.004	ns	ns	ns
C23:0	0.35abc	0.40ab	0.33abcd	0.43a	0.27cd	0.32bcd	0.22d	0.29bcd	0.05	***	ns	ns
C24:1n-9	0.06a	0.07a	0.06a	0.07a	0.07a	0.07a	0.07a	0.06a	0.007	ns	ns	ns
C22:6n-3	0.03a	0.03a	0.02a	0.03a	0.02a	0.02a	0.03a	0.02a	0.005	ns	ns	ns
SFA	41.31a	41.52a	41.38a	40.52a	41.01a	40.88a	41.16a	40.55a	0.56	ns	ns	ns
MUFA	51.65a	51.11a	51.23a	52.15a	51.83a	51.73a	51.15a	51.95a	0.56	ns	ns	ns
PUFA	7.04a	7.38a	7.39a	7.33a	7.16a	7.40a	7.70a	7.49a	0.28	ns	ns	ns
n-6	6.80a	7.10a	7.11a	7.06a	6.90a	7.11a	7.36a	7.18a	0.27	ns	ns	ns
n-3	0.22c	0.27bc	0.28abc	0.26bc	0.26bc	0.28ab	0.33a	0.31ab	0.02	**	*	ns

n-6/n-3	31.75a	27.39bc	27.37abc	28.24abc	30.85ab	26.74c	24.83c	24.64c	1.88	ns	**	ns
IA	0.52a	0.52a	0.52a	0.50a	0.53a	0.52a	0.53a	0.51a	0.01	ns	ns	ns
IT	1.35a	1.36a	1.35a	1.30a	1.33a	1.32a	1.34a	1.30a	0.03	ns	ns	ns
h/H	2.00a	2.01a	2.01a	2.09a	2.00a	2.02a	2.00a	2.07a	0.04	ns	ns	ns

ns – not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SEM (Standard Error of the Mean). Cf15 – Base diet + 15% olive cake centrifuged; Cf25 – Base diet + 25% olive cake centrifuged; Pr15 – Base diet + 15% olive cake pressed; CT – Base diet. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PUFA n-6/n-3 (∑ omega-6)/(∑ omega-3); IA, index of atherogenicity; IT, index of thrombogenicity; h/H= (C18: 1n − 9 + C18: 2n − 6 + C20: 4n − 6 + C18: 3n − 3 + C20; 5 − n3 + C22: 5n − 3 + C22: 6n − 3)/ C14: 0 + C16: 0; only fatty acids which represented more than 0.1% are presented in the table, although all detected fatty acids were used for calculating the totals and the indices.

Concerning MUFAs, the predominant FA was oleic acids, showing levels of around 93% of the total MUFA. This fraction is the majority for both products, which may be an important nutritional aspect since this fraction of fatty acids reduces cardiovascular risk factors [62]. In addition, MUFA reduces plasma levels of LDL cholesterol without harming the anti-atherogenic properties of HDL cholesterol lipoproteins [59]. We obtained values of between 51.11 and 52.15% for the dry-cured loin and 51.15 and 51.95% for the dry-cured "cachaço". This acid had no negative or positive changes with the introduction of olive cake. Although the chemical composition of these two products was significant in terms of total fat content (Table 2), there were no differences between the two products as far as oleic acid was concerned. Similar MUFA were observed in Bísaro dry-cured loin and dry-cured "cachaço" [21]. Other authors have reported higher values of this acid in Iberian ham [56], Iberian dry-cured loin [34], dry-cured "coppa" [44], and Bísaro shoulder [29]. Lower MUFA values were reported in Iberian "lacón" [57], Korean dry-cured loin [23], Italian dry-cured loin [46], and Celta dry-cured ham [42].

About SFA, the predominant FA was palmitic acid, showing levels of around 63% of the total SFA. As with oleic acid, palmitic acid is not influenced by the type of product or by feeding olive cake. We obtained values of between 40.52 and 41.52% for the dry-cured loin and 40.55-41.16% for the dry-cured "cachaço". The SFA fraction was also not influenced by the addition of olive cake to the processed products. The two products did not obtain significantly different results either. Similar SFA was observed in Bísaro dry-cured loin and dry-cured "cachaço" [21], Iberian dry-cured loin [60]. Lower values for this fraction were obtained in Bísaro shoulder [29], Celta dry-cured loin [43], Iberian lacón [57], Iberian and Serrano Ham, Bayonne and Corsican Ham, Parma, and San Daniele Ham, Jingua Ham [59], dry-cured "coppa" [34]. Other authors have reported higher values of SFA in Korean dry-cured loin [23], and Croatian and Montenegrin dry-cured meat [45]. About SFA, and in agreement with other authors [37, 38], mortality rates were correlated positively with the average percentage of dietary energy coming from saturated fatty acids.

For PUFAs, the predominant FA was linoleic acid, which accounted for more than 95% of the total PUFA. Linoleic acid was also not influenced by the type of product or the use of olive cake in the animal diet. Similar PUFA were observed in Bísaro dry-cured loin and dry-cured "cachaço" [21], Korean dry-cured loin [23], Croatian and Montenegrin Prosciutto [45]. Other authors have reported higher values of PUFA in Bísaro shoulder [29], Celta dry-cured "lacón" [57], Iberian and Serrano dry-cured loin, Bayonne and Corsican Ham, Parma and San Daniele Ham, Jingua Ham [59], Pancetta and Croatian and Montenegrin dry-cured sirloin [45], and dry-cured "coppa" [34]. Lower values for this fraction were obtained in Iberian dry-cured loin [60]. It should be noted that PUFA is highly susceptible to oxidative degradation and is converted into other molecules [63]. For this reason, the curing process reduces the PUFA content in the final product. The addition of a by-product such as olive cake did not counteract this reduction in unsaturated fatty acid.

Compared to the results obtained in previous studies [21], including a higher percentage of olive cake (centrifuged and pressed) does not influence the MUFA, SFA, and PUFA fractions.

Concerning *trans* fatty acids, there were no significant differences between the two types of products ($p > 0.05$), with average values of 0.21 and 0.20 for dry-cured loin and dry-cured "cachaço",

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respectively. The values obtained are lower than the recommended levels [64] as well as lower than values reported by other authors in processed Iberian dry-cured ham [65], and Bísaro shoulders [29]. However, significantly lower values were observed for the diet using 25% centrifuged olive cake. Compared to the control, the 25% olive cake (Cf25) obtained more favorable values for this trans fatty acid. On the other hand, the diet with 15% centrifuged olive cake and 15% pressed olive cake obtained more harmful values for this type of *trans* fatty acid.

The PUFA/SFA and n-6/n-3 ratios, as well as the IT and IA indices, are good markers of the healthiness of fat in food. The recommended PUFA/SFA ratio is less than or equal to 0.4 [47] for a healthy and balanced diet. For the ratio n-6/n-3 the internationally recommended values for a healthy and balanced diet, are 4 [48] and the optimal value is 1 [49-51]. Strategies for genetic alteration and dietary changes have already demonstrated that they are relatively effective in achieving more desirable n-6/n-3 values for this type of product [42]. The n-6/n-3 ratio had no significant effect between the two products studied. On the other hand, the diets applied with olive cake proved to be significantly different ($p<0.05$). From the results shown in Table 3, the addition of olive cake to the animals' diets significantly reduces the n-6/n-3 ratio. This trend was not observed in other studies for Bísaro dry-cured loin a dry-cured "cachaço" with 10% olive cake added [21]. Therefore, we can say that the introduction of a percentage of 15 and 25% olive cake in the animal diet contributes to a decrease in this ratio. Higher values of this ratio were observed in Iberian and Parma dry-cured ham [59]. However, significantly lower values were observed in Serrano dry-cured ham, Bayonne dry-cured ham, and Corsican dry-cured ham [59]. Unsaturated fats, especially PUFAs, are well-known as healthy fats that have important functions in the body, such as cell growth and development and disease prevention [61]. Although linoleic acid is an essential fatty acid, it should be noted that a high intake of this PUFA (in excess) may not be beneficial. Studies [39,40] have shown that excessive intake of this fatty acid has a pro-inflammatory effect. Omega-6 acids promote vasoconstriction and the formation of blood clots, while omega-3 acids have the opposite effect [41]. Among the PUFA n-3, docosahexaenoic acid (C22:6n-3, DHA) plays a preventive and therapeutic role in some chronic inflammatory diseases [54]. In small quantities, these FA are crucial for the correct cerebral and visual development of the fetus, and the maintenance of neural and visual tissues throughout life [54]. This type of ómega-3 FA (DHA) cannot be synthesized by the human body and is therefore only obtained through food [55]. In this study, the value of PUFA n-3 was significantly different with the type of product and the diet applied. The value of n-3 increased with the introduction of diets containing olive cake, with the diet containing 25% centrifuged olive cake having the highest value for both products. For the dry-cured "cachaço", values between 0.26 and 0.33 were obtained, while for the dry-cured loin, lower values were obtained, with results ranging from 0.22 and 0.28.

A lower IA value indicates a reduced ratio of saturated to unsaturated fatty acids. A lower IT value indicates a reduced risk of developing blood clots [69]. For the IA and IT index, there were no significant differences between the two types of products and no influence at the level of the diets with olive cake. No entity/organization has provided any reference value for these indices [66], however, it is known that a lower IA and IT index is indicative of better nutritional quality, which can reduce the risk of coronary heart disease. Lower values of these indices were obtained in dry-cured "coppa" of Nero Siciliano pig [34], and Bísaro shoulder [29]. Similar values were obtained in Bísaro dry-cured loin and "cachaço" whose animals were fed olive cake [21]. Other authors have reported that the intramuscular fat and backfat indices were affected by the inclusion of 10% olive cake in the pig's diet, increasing the MUFA and PUFA content and improving the IA and IT quality indices [67]. According to Cava et al., [68] the higher the h/H ratio, the more nutritionally adequate the fat in the food. In this study, the ratio h/H did not influence the diets applied or the type of product ($p>0.05$). The dry-cured loin obtained values between 2.00 and 2.09 and the dry-cured "cachaço" between 2.00 and 2.07. Lower values of this ratio were obtained in Bísaro dry-cured loin and "cachaço" [21]. Higher h/H values were observed in Celtic ham with a chestnut-based diet [42]. In the case of fresh pork, different cuts of meat obtained different h/H ratio values [70]. Therefore, not only the type of feed influences this ratio, but also the joint obtained. Considering the value obtained for fresh loin (2.170) [70] the curing process does not affect this type of nutritional index.

3.3. Principal Component Analysis (PCA)

Principal component analysis (PCA) is a statistical technique used to simplify a dataset by transforming the original variables into a smaller set of new variables, called principal components, which capture the essential characteristics of the data. These principal components are linear combinations of the original variables that maximize the total variance. The primary graphical output of PCA is often a biplot, which maps the cases using the principal components and includes the original variables to help interpret the distances between case positions [76]. Figure 1 shows the results of the principal component analysis. The graphical result obtained takes the form of a biplot. Figure 1 shows the result of the principal component analysis. The graphical result obtained takes the form of a biplot. The first principal component ($p<0.001$) explained 40% of the total variance, and the second principal component ($p<0.001$) explained 24.7% of the total variance. The two principal components explained the total variance of 64.7%. The positive region of the first factor. Through this principal component analysis, we can see a separation between the dry-cured loin and the dry-cured "cachaço". The physico-chemical composition that best explains the dry-cured loin is salt content, total fat, and haem pigments. On the other hand, water activity, protein content, and moisture are the factors that best explain the dry-cured loin. That said, the principal component analysis shows that although the two products (dry-cured loin and dry-cured "cachaço") come from the same muscle (*Longissimus thoracis Lumborum),* their chemical composition is very different.

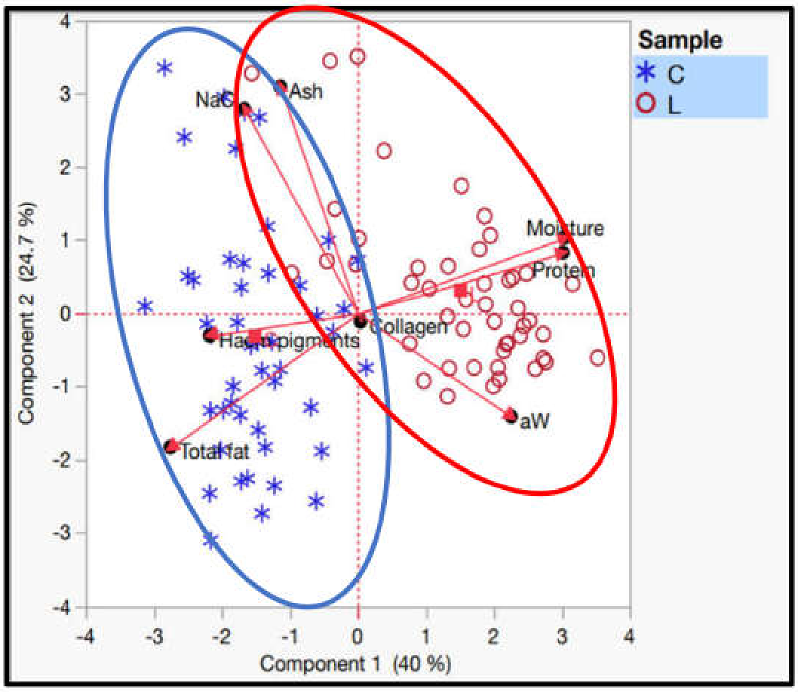

Figure 1. Biplot principal component analysis (C- dry-cured "cachaço"; L – dry-cured loin).

4. Conclusions

The results show that the use of centrifuged and pressed olive cake in different percentages does not affect the physicochemical parameters of the dry-cured products obtained. Regarding the two products, although they are from the same muscle (LTL), significant differences were observed between them, particularly in total fat content, protein, aw, moisture, and haem pigments. This indicates that even though they are from the same muscle (LTL), they are different products, which enhances their appeal from a consumer acceptability perspective. The addition of olive cake significantly improved the n-3 content, with the olive cake with the highest percentage (Cf25) obtaining the highest n-3 value. These values influenced the PUFA n-6/n-3 ratio, showing the same trend, with significantly lower results in olive cake diets. There are no further differences in terms of the different fatty acid fractions and nutritional quality indices, but it would be important in future work to understand how n-3 can influence the stability of the product and how these differences can affect the organoleptic properties of the final product, as well as consumer acceptability.

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Informed Consent Statement: Not Applicable.

Data Availability Statement: All data were presented in the manuscript. Data can be requested from the corresponding author via email.

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Conflicts of Interest: The authors declare no conflict of interest.

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