

## NUTRITIONAL CHARACTERIZATION OF SOME ROMANIAN MOUNTAIN PRODUCTS

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Received on 28 September 2022

Revised on 25 November 2022

### Abstract

The consumer demand for healthy and natural products lead to the increased interest of consuming mountain food products. The objective of this work was to evaluate the physical-chemical and sensory characteristics of 8 dairy products and 11 meat products collected from different mountainous pastures from Romania. The protein content of dairy products varied between 37.39-47.90% d.m., while the fat content ranged between 43.63-49.57% d.m. For meat products, the protein content and fat content ranged between 11.69-70.07% d.m., and 17.58-95.92 % d.m., respectively. The fatty acid composition of mountain products was influenced by the pasture location, a better PUFA content being determined for products obtained from farms situated at a higher altitude. This research highlights that the quality of mountain products is strongly influenced by the pasture effects, the type of product analyzed and the technological process.

**Keywords:** mountain products, compositional analyses, fatty acids, dairy products, meat products

### Introduction

Mountain food products express a state of purity and tradition, being perceived by consumers as environmentally friendly and contribute to local and cultural economy and promote at the same time the conservation of their beloved landscapes (Euromontana, 2017).

In order for a product to be named and considered mountain food product, it must meet the conditions formulated by the Regulation (EU) no. 665/2014 of European Commission (2014). The term “mountain product” is used for products intended for human consumption in which the raw materials and animal foodstuffs mainly come from mountain areas, or, in the case of processed products, processing takes place in mountain areas. This concept should be applied to products obtained from animals raised in mountain areas for at least the last two thirds of their lifetimes, if the products are processed in those areas. In addition, the term "mountain product" must be applied to products obtained from transhumant animals, grown for at least a quarter of their lifetime in transhumance and grazed on mountain pastures (European Commission, 2014).

Given the importance of mountain food products, the adoption and implementation of local strategies in order to promote agricultural development in mountain areas can significantly improve the global visibility of these products, reducing the consequences of depopulation, abandonment of territories (Martins and Ferreira, 2017) and increasing the attractiveness of mountain areas. In this respect, one of the priority actions taken was to include mountain areas in the EU's political agendas at both regional and governmental level. In fact, since the adoption in 2014 of EU legislation on mountain products, some efforts have been made, mainly by Euromontana, by some regions and Member States, including Romania, or even by some producers in order to promote sustainable development and visibility of mountain areas. One of the main objectives was to find an appropriate scenario to establish different collaborations between countries and stakeholders responsible for mountain areas at European level (Martins and Ferreira, 2017). All these actions are intended to create a pronounced, effective and beneficial impact on all mountain communities and the environment. In Romania, the National Agency of the Mountain Areas, part of the Ministry of Agriculture and Rural Development, is responsible for awarding the usage of the optional quality term “mountain product” and verifying its compliance with European and national legislation. Therefore, in January 2019, the National Mountain Agency established a national logo to be used for mountain products (Euromontana, 2019).

Mountain food products have specific qualities, being considered special products, representative for a particular region, which offer multiple benefits and unique characteristics, manufactured under singular environmental conditions that are not found elsewhere. Mountain farming offers high-quality foods as a consequence of the specific characteristics of the raw materials and also the traditional processing conditions. Over the last years these one-of-a-kind products have received an increased interest due to their properties (Martins and Ferreira, 2017), which cannot be found in industrially obtained products. Dairy (cheese) products (DP) and meat products (MP) comprise most of the products (Martins and Ferreira, 2017). Mountain food products also have many applications both in food and biotechnology industries, being used even for prophylactic purposes (Martins and Ferreira, 2017).

Studies showed that the chemical and fatty acid (FA) composition of DP and MP is influenced by the conditions in which animals are raised, season, feeding

supplementation, altitude, biodiversity in botanical species, quality of herbage grazed, animal performances and genetics (Farruggia *et al.*, 2014, Bravo-Lamas *et al.*, 2018, Caprioli *et al.*, 2020).

Therefore, the objective of this study was to evaluate the chemical composition, FA profile, and sensory properties of several DP and MP from the mountain pastures of Romania and assess the changes in the macro-nutrient composition determined by the type of products and the feeding area of the animals. The analyzed products are recognized as mountain products by the Mountain Area Agency, the raw materials being obtained in mountain areas and also the processing of these products.

### Materials and methods

Phyto-pastoral reports in four pastures located at altitudes ranging from 280 to 800 meters above sea level, with relatively different climatic conditions is presented in Figure 1.



**Figure 1.** The location of pastures (National Agency for Cadastre and Land Registration of Romania, Esri USGS, NOAA).

Each pasture is located in a different part of Romanian Carpathians and is identified by the following abbreviations: P1 (47°20'46"N 25°21'34"E) Vatra Dornei (Suceava County), P2 (45°32'59"N 22°49'22"E) Peșteana (Hunedoara County), P3

(45°10'23"N 23°20'52"E) Gornăcel (Gorj County), P4 (45°40'45"N 24°20'38"E) Racovița (Sibiu County) (National Agency for Cadaster and Land Registration, 2021). These areas have a superior quality of pastures, rich in hay and spontaneous flora. To have a good representation at the level of the whole mountain range, samples have been collected from the three subdivisions of the Romanian Carpathians: Eastern (the municipality of Vatra Dornei), Southern (Gornăcel and Racovița villages) and Western (Peșteana village).

### *Dairy and meat sampling*

This experiment was carried out in summer 2020. Eight DP and eleven MP samples were studied (Table 1). The fresh samples were taken directly from local producers, transported in sterile containers and stored at 4°C until analyzes were performed. All DP were obtained from cow milk and collected from two farms (P1 and P2). Four pressed cheese samples were collected from the two pastures.

**Table 1.** Codes for mountain products and pastures.

Mountain products	Sample code	Type	Pasture/farm county
<b>Dairy products (DP)</b>	<b>D<sub>1</sub></b>	Calimani Pressed Cheese	P1 (Suceava)
	<b>D<sub>2</sub></b>	Calimani Smoked pressed cheese	
	<b>D<sub>3</sub></b>	Calimani cheese	
	<b>D<sub>4</sub></b>	Calimani Schweizer	
	<b>D<sub>5</sub></b>	Calimani Kneaded cheese	P2 (Hunedoara)
	<b>D<sub>6</sub></b>	Fresh cheese	
	<b>D<sub>7</sub></b>	Ado Pressed cheese	
	<b>D<sub>8</sub></b>	Traditional cheese	
<b>Meat products (MP)</b>	<b>M<sub>1</sub></b>	Angus Pastrami	P3 (Gorj)
	<b>M<sub>2</sub></b>	Angus Burger	
	<b>M<sub>3</sub></b>	Angus Minced meat rolls	
	<b>M<sub>4</sub></b>	Angus Spicy sausages	
	<b>M<sub>5</sub></b>	Angus Sausages	
	<b>M<sub>6</sub></b>	Angus Salami	P4 (Sibiu)
	<b>M<sub>7</sub></b>	Mangalita Smoked breast	
	<b>M<sub>8</sub></b>	Mangalita Smoked chop	
	<b>M<sub>9</sub></b>	Mangalita Smoked sausage	
	<b>M<sub>10</sub></b>	Goose	
	<b>M<sub>11</sub></b>	Mangalita Smoked bacon	

MP were collected from two farms (P3 and P4). With the exception of sausage samples which was common to both pastures, all other samples were different.

The products were chosen based on the availability of the products from the market at the time at the sample selection. Samples were selected from mountain pastures

and analyzed during the same period in order to limit season variability or other factors that might change the results.

#### **Chemical composition analyzes**

Moisture, fat, protein and salt contents for DP were determined according to SR EN ISO 5534:2004, SR EN ISO 1735:2005, and SR EN ISO 8968-1/2014, respectively. For the MP, the same parameters were analyzed according to SR ISO 1442:2010, SR ISO 1443:2008, SR ISO 937:2007, and SR ISO 1841-1:2000, respectively. Briefly, moisture content was analyzed using the drying method, protein content using Kjeldahl method, fat content by using Soxhlet extraction with petroleum ether, and salt content with Volhard method. The total carbohydrate content was assessed by subtracting the values of moisture, protein, fat, and ash content from 100. The caloric values were calculated using the following conversion factors: 1 g fat = 9 kcal, 1 g carbohydrates/protein = 4 kcal. Analyzes were made in duplicate per each sample.

The chemical composition was expressed based on the moisture content by using the mean values expressed at 100 g product.

#### **Fatty acids analyzes**

For FA analyzes, two reference standards, F.A.M.E. Mix, C4-C24 (mixture of 37 fatty acid methyl esters (FAME), Bellefonte, PA, USA) and SRM<sup>®</sup>2377 (mixture of 26 FAME, NIST certified, USA) were used. Solvents and reagents used in this study were especially for chromatography. FA composition was determined based on the method previously described (Mihai *et al.*, 2019). For FA quantification, the correction factors method was used.

The FAME profile of analyzed samples was performed by using a GC-MS system (Trace GC Ultra/TSQ Quantum XLS, Thermo Fisher Scientific, USA). FA composition was expressed as weight percentage of FAME/FA individually determined or as sum of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, omega-6, and omega-3 PUFA, also trans FA (TFA) were determined. Results were expressed as triacylglycerol per 100 g fat and presented as mean  $\pm$  standard deviation (SD).

#### **Headspace-electronic nose**

The mountain products were analyzed with a headspace-electronic nose. Air in the headspace of the samples was analyzed with an electronic nose system combined with HS100 auto-sampler together with a Soft version 8.0 software for data processing (Alpha M.O.S. – model FOX 4000, Toulouse, France). The headspace-electronic nose is composed of an array of 18 different metal oxide sensors placed in three controlled temperature chambers. For the sample analyzes, 1 g of ground mountain products was placed in a 10 mL vial, hermetically sealed with a poly(tetrafluoroethylene)/silicone septum and incubated for 600 s at 80°C under agitation (500 rpm) to allow the volatilization of flavor compounds into the headspace. As carrier gas a synthetic air and nitrogen were used with a flow of 150 mL/min. In order to measure the volatile compounds, 1500  $\mu$ L of the mountain products headspace were injected into the measuring chamber of the electronic nose,

with an acquisition time of 120 s. All samples were analyzed in duplicate and the individual signals recorded were used for statistical analyzes.

### **Statistical analyzes**

All analyzes were performed in duplicate and the mean values with the SD were reported. Minitab® 20 Statistical Software (free trial) was employed for statistical analysis of the data.

In order to evaluate if the samples from the same category of products, and from the same pasture have a similar FA composition, data of SFA, MUFA, PUFA, omega-6/omega-3 ratio, industrial TFA, and PUFA/SFA ratio was subjected to principal component analyzes (PCA).

## **Results and discussion**

### **Chemical composition of mountain products**

One of the basic steps in food characterization is chemical analysis. This stage is important both for the scientist and the analysts who work on quality assurance and for the regulation of the production process.

### **Dairy products**

The various DP analyzed were obtained from cow milk, the pressed cheese presenting an extra-hard consistency, while the cheese products had a soft consistency. The chemical composition of DP obtained from the collected Romanian mountain areas is presented in Table 2.

The moisture content of DP varied between 36.91 and 59.35%. As expected, pressed cheese samples had a lower moisture content (36.91-42.98% d.m.) than cheese samples (43.41-59.35% d.m.). Fangmeier *et al.* (2019) found in the cow's cheese a moisture of 58.37%, while Walther *et al.* (2008) realized a classification of cheese, which reflected the variability in the composition of the different types of cheese. Depending on the type of cheese, they found an amount of water of 30-70%.

Cheese contains a high protein content. The protein concentration ranges from approximately 4-40%, depending on the variety (O'Brien and O'Connor, 2004). Cheese products collected from different pastures presented varied levels of protein concentration, ranging between 37.39-47.90% d.m. Different results are due to the formulation recipe, the type of milk and the grazing area.

Most of the DP are rich in fat. The fat content affects cheese firmness, adhesiveness, mouthfeel and flavor. Considering the fat content of the analyzed samples, they can be considered to be creamy products. Compared to pressed cheese samples (43.63-45.97% d.m.), in cheese samples a higher fat content (46.05-49.57% d.m.) was found.

The total carbohydrates ranged between 2.58 (D7) and 7.97% d.m. (D3). Salt content varied between 1.70 (D2) and 4.22% d.m. (D3). Cheese quality depends on the chemical, microbiological and rheological properties of milk, which are influenced by various factors and the cow's diet (Coulon *et al.*, 2004; Martin *et al.*, 2005).

In this study 4 different types of pressed cheese were analyzed, 3 from pasture P1 and 1 from pasture P2. The chemical composition of these samples didn't vary too much, the protein content ranging between 45.11 and 47.90% d.m., the fat content was between 43.63 and 45.97% d.m., while the carbohydrate content varied between 2.58 and 4.37% d.m.

### **Meat products**

Meat has an important place in a healthy human diet, providing high protein content and significant amount of fat. These components have been under the spotlight in recent years in relation to the health of people consuming meat.

The color and the fat content of meat and meat products represent important factors in terms of quality and acceptability of these products by consumers (Bukala and Kedzior, 2001). Animals raised on mountain pastures offer better meat compared to low areas due to the absence of any supplementary feeding or treatment (Ådnøy et al., 2005).

Results for chemical composition of meat products are shown in Table 2.

**Table 2.** Chemical composition of dairy and meat products.

Sample	Moisture (%)	Protein (% d.m.)	Fat (% d.m.)	Carbohydrate (% d.m.)	Sugars (% d.m.)	Salt (% d.m.)	Energy (kcal/kj)
<b>Dairy products</b>							
D <sub>1</sub>	41.94	45.42	45.97	3.53	3.53	1.95	354/1471
D <sub>2</sub>	42.98	45.11	43.63	4.37	4.37	1.70	341/1420
D <sub>3</sub>	51.45	37.51	46.05	7.97	7.97	4.22	290/1203
D <sub>4</sub>	36.91	47.04	44.35	3.25	3.25	2.30	379/1575
D <sub>5</sub>	43.41	42.83	48.85	3.18	3.18	1.86	354/1468
D <sub>6</sub>	54.34	37.39	48.66	5.48	5.48	3.77	278/1155
D <sub>7</sub>	42.65	47.90	44.23	2.58	2.34	2.23	344/1429
D <sub>8</sub>	59.35	37.81	49.57	6.10	6.10	2.90	253/1049
<b>Meat products</b>							
M <sub>1</sub>	65.72	53.73	27.83	7.23	1.95	5.40	169/708
M <sub>2</sub>	60.28	46.32	55.60	-	-	3.78	260/1077
M <sub>3</sub>	63.10	40.19	52.90	1.30	0.27	3.79	237/983
M <sub>4</sub>	58.43	44.79	50.11	1.40	0.24	3.13	264/1097
M <sub>5</sub>	57.26	40.20	54.28	0.84	0.23	2.69	279/1157
M <sub>6</sub>	57.28	46.68	47.14	1.85	0.23	3.00	264/1098
M <sub>7</sub>	67.92	70.07	17.58	-	-	7.73	141/591
M <sub>8</sub>	56.72	50.65	42.58	-	-	3.54	254/1055
M <sub>9</sub>	41.27	30.48	63.36	0.37	-	4.95	407/1685
M <sub>10</sub>	44.90	11.69	83.10	-	-	3.52	438/1804
M <sub>11</sub>	6.72	-	95.92	-	-	2.91	805/3310

\*results are expressed as % dry matter

The analyzed MP showed a moisture content ranging between 6.72 and 67.92% d.m., while the protein content varied between 11.69-70.07% d.m. Sheard et al. (1998)

have reported mean protein contents of 16.6%, and 17.9% for beef burger and minced meat respectively which are in broad agreement with those reported here.

The fat content ranged between 17.58-95.92% d.m. Results differed that much due to the variability of MP analyzed. Beef lipidic content is mainly influenced by age, sex, genotype and processing technique (Faria *et al.*, 2012), while Sheard *et al.* (1998) analyzed burger beef and minced meat in term of fat. The results are consistent with those obtained in this study.

The carbohydrate content varied from 0.37 to 7.23% d.m. M2, M7, M8, M10, and M11 samples are free of carbohydrates. This variance comes from the difference in the technological process of MP along with the environmental factors and type of pasture.

The content of salt ranged between 2.69 and 7.73% d.m. Sodium chloride in meat products is an essential ingredient, providing a large number of different functionalities. Preservation is one of them, preventing the spoilage of perishable foods, characteristic flavor and the role to create the desired texture of a processed meat product (Weiss *et al.*, 2010). The results are consistent with those obtained in this study.

In this study two type of sausages from pasture P3 and 1 sausage sample from pasture P4 were analyzed. Comparing the chemical composition, sausages from pasture P3 had a higher moisture, protein and carbohydrate content, and a lower fat and salt content than sample from pasture P4.

### ***Fatty acids composition of dairy and meat products***

#### ***Dairy products***

FA composition of DP is presented in table 3.

All DP samples contained a high content of SFA (66.51-75.13%), followed by MUFA (23.32-29.78%), and a small content of PUFA (1.48-3.71%). Similar results were obtained by Formaggioni *et al.* (2020), who determined for cheese produced from milk from stall, valley pastured and mountain pasture a SFA content of 61.68-68.85%, a MUFA content of 26.47-33.38%, and a PUFA content of 4.66-5.62%.

Palmitic (C16:0), myristic (C14:0), stearic (C18:0), butyric (C4:0), caproic (C6:0), capric (C10:0), and lauric (C12:0) acids are the predominant SFA found in the DP analyzed. As expected, the FA composition of DP from the two pastures was different. Samples from pasture P2 had a higher content of palmitic acid compared to samples from pasture P1. In the case of butyric, caproic, caprylic (C8:0), capric, margaric (C17:0), stearic and arachidic (C20:0) acids, the content was higher for DP obtained from P1 pasture, located at a higher altitude.

Undecanoic acid (C11:0) was only found in samples from pasture P2. Formaggioni *et al.* (2020) compared the FA composition of cheese obtained from milk of cows grazing on mountains, valley pasture or stall and reported similar results showing that the butyric, stearic and arachidic acids were more abundant in cheese from mountains pasture than the ones from valley pasture and from stall. Differences in the FA composition of DP from the two pastures can be correlated with the FA composition of grazed on higher altitude compared to lower altitude.



**Table 3.** Fatty acid composition of the dairy products.

Fatty acid	D1	D2	D3	D4	D5	D6	D7	D8
C4:0	5.50 ± 0.14	4.94 ± 0.25	3.73 ± 0.18	4.52 ± 0.97	3.47 ± 0.34	2.43 ± 0.04	3.24 ± 0.10	2.82 ± 0.11
C6:0	2.66 ± 0.05	2.81 ± 0.11	2.42 ± 0.37	2.50 ± 0.07	2.64 ± 0.26	1.69 ± 0.03	1.98 ± 0.13	1.83 ± 0.04
C8:0	1.31 ± 0.06	1.56 ± 0.01	1.31 ± 0.17	1.27 ± 0.03	1.40 ± 0.14	1.00 ± 0.02	1.15 ± 0.10	1.10 ± 0.02
C10:0	3.03 ± 0.14	3.75 ± 0.10	2.26 ± 0.13	2.96 ± 0.07	3.40 ± 0.30	3.52 ± 0.30	3.00 ± 0.26	2.96 ± 0.04
C11:0	-	-	-	-	-	0.11 ± 0.01	0.06 ± 0.01	0.09 ± 0.00
C12:0	2.12 ± 0.05	3.85 ± 0.23	2.67 ± 0.16	3.42 ± 0.12	3.85 ± 0.23	3.09 ± 0.06	3.71 ± 0.29	3.81 ± 0.11
C13:0	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.17 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
C14:0	10.60 ± 0.17	12.39 ± 0.20	12.23 ± 0.68	11.83 ± 0.69	9.98 ± 0.71	11.59 ± 0.16	12.08 ± 0.41	12.23 ± 0.05
C14:1n5	1.32 ± 0.15	1.49 ± 0.18	1.34 ± 0.17	1.24 ± 0.05	1.27 ± 0.13	1.19 ± 0.06	0.78 ± 0.05	0.92 ± 0.06
C15:0	1.52 ± 0.03	1.49 ± 0.27	1.72 ± 0.22	1.78 ± 0.06	1.45 ± 0.31	1.59 ± 0.08	0.96 ± 0.05	1.29 ± 0.07
C15:1n5	-	-	-	0.16 ± 0.01	0.18 ± 0.02	-	-	-
C16:0	26.15 ± 1.23	30.91 ± 1.14	28.83 ± 0.57	25.79 ± 1.10	25.83 ± 2.25	37.64 ± 0.12	39.42 ± 0.60	37.97 ± 0.34
C16:1n7	1.07 ± 0.06	1.63 ± 0.17	1.10 ± 0.14	1.01 ± 0.04	1.15 ± 0.14	1.60 ± 0.07	1.16 ± 0.06	1.23 ± 0.08
C17:0	1.06 ± 0.07	0.89 ± 0.10	0.73 ± 0.09	0.76 ± 0.03	0.84 ± 0.11	0.48 ± 0.02	0.39 ± 0.02	0.39 ± 0.02
C18:0	12.64 ± 1.40	10.34 ± 0.10	11.48 ± 1.07	12.37 ± 0.32	13.17 ± 0.68	9.15 ± 0.05	8.96 ± 0.28	9.03 ± 0.11
C18:1n9t	0.34 ± 0.04	0.29 ± 0.03	0.27 ± 0.04	0.25 ± 0.01	0.28 ± 0.03	0.17 ± 0.01	0.12 ± 0.01	0.15 ± 0.00
C18:1n11t	3.02 ± 0.19	2.43 ± 0.16	3.30 ± 0.15	2.95 ± 0.15	2.92 ± 0.02	0.64 ± 0.02	0.66 ± 0.03	0.71 ± 0.01
C18:1n9	22.24 ± 0.76	17.08 ± 1.50	21.82 ± 0.90	22.31 ± 0.93	23.22 ± 0.24	21.37 ± 0.32	20.03 ± 0.62	20.82 ± 0.21
C18:1n11	0.75 ± 0.03	0.40 ± 0.02	0.76 ± 0.00	0.84 ± 0.00	0.56 ± 0.01	0.78 ± 0.00	0.64 ± 0.03	0.83 ± 0.01
C18:2n6t	0.33 ± 0.03	0.40 ± 0.05	0.33 ± 0.04	0.27 ± 0.01	0.32 ± 0.04	0.15 ± 0.01	0.09 ± 0.00	0.13 ± 0.01
C18:2n6	1.95 ± 0.16	1.58 ± 0.02	2.11 ± 0.10	2.10 ± 0.03	2.12 ± 0.17	1.42 ± 0.08	1.23 ± 0.06	1.29 ± 0.06
C18:3n3	1.14 ± 0.10	1.19 ± 0.12	1.06 ± 0.14	0.98 ± 0.04	1.06 ± 0.14	0.07 ± 0.00	0.11 ± 0.00	0.08 ± 0.00
C20:0	0.32 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.00	0.19 ± 0.03	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
C20:1n9	0.30 ± 0.03	-	-	0.19 ± 0.01	0.20 ± 0.03	0.07 ± 0.00	-	0.07 ± 0.00
C21:0	0.06 ± 0.00	-	-	-	-	-	-	-
C20:4n6	0.06 ± 0.01	0.05 ± 0.00	-	-	0.05 ± 0.01	-	0.05 ± 0.00	0.05 ± 0.00
C22:0	0.13 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	-	-	-
C20:5n3	0.07 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	-	-	-
C23:0	0.06 ± 0.01	-	-	-	0.05 ± 0.01	-	-	-
C24:0	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.07 ± 0.01	-	-	-
C22:5n3	0.07 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.00	0.10 ± 0.01	-	-	-
SFA, % fat	67.34 ± 0.33	73.30 ± 1.89	67.76 ± 0.41	67.56 ± 0.88	66.51 ± 0.48	72.54 ± 0.41	75.13 ± 0.50	73.72 ± 0.02
MUFA, % fat	29.04 ± 0.64	23.32 ± 1.68	28.59 ± 0.70	28.95 ± 0.96	29.78 ± 0.10	25.82 ± 0.32	23.39 ± 0.56	24.73 ± 0.09
PUFA, % fat	3.62 ± 0.31	3.38 ± 0.20	3.65 ± 0.29	3.49 ± 0.08	3.71 ± 0.38	1.64 ± 0.09	1.48 ± 0.07	1.55 ± 0.07
omega 3, % fat	1.28 ± 0.12	1.35 ± 0.13	1.21 ± 0.15	1.07 ± 0.04	1.22 ± 0.16	0.07 ± 0.00	0.11 ± 0.00	0.08 ± 0.00
omega 6, % fat	2.01 ± 0.17	1.63 ± 0.02	2.11 ± 0.10	2.10 ± 0.03	2.17 ± 0.17	1.42 ± 0.08	1.28 ± 0.06	1.34 ± 0.06
<i>Trans</i> natural, % fat	3.02 ± 0.19	2.43 ± 0.16	3.30 ± 0.15	2.95 ± 0.15	2.92 ± 0.02	0.64 ± 0.02	0.66 ± 0.03	0.71 ± 0.01
<i>Trans</i> industrial, % fat	0.67 ± 0.07	0.69 ± 0.08	0.60 ± 0.08	0.52 ± 0.02	0.60 ± 0.08	0.32 ± 0.02	0.21 ± 0.01	0.28 ± 0.01
Omega 6/omega 3 ratio	1.57	1.21	1.74	1.96	1.78	20.29	11.64	16.75
PUFA/SFA ratio	0.05	0.05	0.05	0.05	0.06	0.02	0.02	0.02

It is known that mountain vegetation has a higher content of PUFA which affects the proportion of long chain SFA from the milk (C20:0, C22:0, C23:0, C24:0) (Bravo-Lamas *et al.*, 2018) and the results showed that the DP from pasture P1 presented

these acids compared with samples collected from lower altitude which did not contain such acids.

The major quantitative MUFA present in these products were oleic (C18:1n9), trans-vaccenic (C18:1n11t) and myristoleic (C14:1n5) acids. In general, DP from the first manufacturer had higher content of myristoleic, elaidic (C18:1n9t), trans-vaccenic, and eicosanoic (C20:1n9) acids than those from the second manufacturer. With the exception of D2 sample, all other samples from the first manufacturer had a higher content of MUFA than the ones from the second manufacturer.

DP collected from producers located at a lower altitude had a reduced content of PUFA (1.48-1.64%) compared to the products collected from higher altitudes (3.38-3.71%), these results being associated to a lower content of linoleic (C18:2n6- LA), and linolenic (C18:3n3- ALA) acids in the samples from pasture P2. Similar results were obtained by Bravo-Lamas *et al.* (2018) who found a lower content of PUFA for milk collected from valley farms compared with the one collected from mountain farms. Agradi *et al.* (2020) analyzed the FA composition of milk and cheese from two different farming system and showed that a higher content of PUFA was obtained for products from grazing farms compared with the ones with no grazing farm.

LA and ALA were the predominant PUFA. It is well known that LA present pro-inflammatory properties and reduces the risk of cardiovascular disease (Wang, 2018), while ALA has anti-inflammatory properties and antioxidant activity. All DP contained a higher content of omega-6 than omega-3 PUFA. Between the FA present in the DP analyzed, LA and ALA can be used to classify a product as belonging to one of the pastures from which the products originate. Similar results were obtained by Agradi *et al.* (2020) who could classify milk and cheese coming from grazing or no grazing system.

Omega-3 PUFA, and in particular ALA have a higher content in DP from pasture P1 compared with the ones from pasture P2 where the raw materials are from lower altitude. Coppa *et al.* (2015) also showed that there is a difference in milk FA composition according to altitude, an increase of LA concentration being correlated with a higher altitude. Results also shown that DP from pasture P1 had a higher content of long chain PUFA such as C20:5n3 (eicosapentaenoic acid– EPA), a nutritionally omega-3 PUFA, and C22:5n3, results being in accordance with the one reported by Bravo-Lamas *et al.* (2018) who found a correlation for the milk produced in mountain farms and valleys and these FA. EPA presence can be the result of the elongation and desaturation of ALA that takes place in the ruminants' tissues, the composition of fresh pastures having an effect on the content of these FA (La Terra *et al.*, 2010).

The technological process to obtain cheese and pressed cheese is different. As expected, the FA composition of these samples from the same farm and from different pastures varied. The European Commission (based on Regulation (EC) no. 1924/2006) set several nutritional and health claims regarding the FA composition of food products which can be considered a source of omega-3 PUFA if it contain at least 0.3 g ALA per 100 g and per 100 kcal. Based on this statement, DP collected

from pasture P1, with the exception of D4, can be considered sources of omega-3 PUFA as a result of the ALA content, confirming its nutritional potential with beneficial health effects.

TFA can be found in food products being produced naturally or industrially. Natural TFA are formed through bacterial transformation of unsaturated FA in the digestive tract of ruminant animals (Ferlay *et al.*, 2017). Industrial TFA can be formed as a result of industrial hydrogenation, deodorization of unsaturated vegetable oils and through exposure of oils to high temperature (>220°C) (EFSA, 2018). The main natural TFA present in DP and MP is trans-vaccenic acid. The industrial TFA are mainly represented by elaidic acid which represents around 20-30% of total of trans isomers of C18:1 (EFSA, 2018). As a consequence of the fact that consumption of diets containing TFA has an adverse effect on blood lipids and increase the risk of coronary heart disease, Romania set the level for industrial TFA as lower than 2% fat content of food products (EFSA, 2018, Commission Regulation (EU) 2019/649). According to WHO (2019), the TFA intake should be limited to less than 1% of total energy intake, which represents less than 2.2 g/day for a balanced diet of 2000 calories.

The DP analyzed had an industrial TFA content lower than 2%, the mountain products from pasture P1 having higher content of TFA than the ones from pasture P2. Also, DP from higher altitude had a higher content of natural TFA (2.43-3.30%) than of the products from lower altitude (0.64-0.71%).

None of the analyzed samples exceeded the minimum recommended value of PUFA/SFA ratio of 0.4, better ratios being obtained for the samples from pasture P1.

#### *Meat products*

The MP analyzed had a content of SFA ranging between 30.01 and 57.13%, MUFA between 40.95 and 63.92%, and a content of PUFA between 1.72 and 10.44%. There are differences between the FA composition of samples from the two pastures. The SFA content of MP from the pasture P4 was lower than that of products from pasture P3, being influenced by the geographic origin of pastures. The high amount of SFA in the MP is the result of the high proportion of C14:0, C16:0 and C18:0. Mountain MP from pasture P3 presented a higher content of C14:0, C16:0 and C17:0. In the study realized by Lorenzo *et al.* (2010), the SFA content of *Longissimus dorsi* muscles from mountain foals also recorded a high content of these FA. Pentadecanoic acid (C15:0) was only found in samples from pasture P3.

With the exception of M11 sample, the MUFA content was higher for samples from the second manufacturer. The main MUFA constituent was oleic acid, having the highest content (53.90%) in goose sample (M10), and the lowest (30.94%) in burger sample (M2). Meat products from pasture P3 presented in their composition C14:1n5, C15:1n5, C18:1n11t MUFA which were not determined in samples collected from pasture P4. A higher content of cis-vaccenic acid was found in MP from pasture P4, ranging between 3.42 and 6.66% compared with samples from pasture P3 who presented a content of 0.9-2.14%.

**Table 4.** Fatty acids composition of the meat products.

Fatty acid	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
C10:0	0.08	0.08	0.05	0.09	0.06	0.08	0.07	0.11	0.06	0.03	0.04
	±	±	±	±	±	±	±	±	±	±	±
	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.01	0.00	0.00	0.00
C12:0	0.10	0.10	0.07	0.11	0.07	0.11	0.05	0.09	0.05	0.03	0.06
	±	±	±	±	±	±	±	±	±	±	±
	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.00
C14:0	2.65	3.97	3.31	3.35	3.27	3.39	0.98	1.76	1.08	0.86	1.24
	±	±	±	±	±	±	±	±	±	±	±
	0.04	0.16	0.19	0.14	0.19	0.16	0.32	0.09	0.09	0.11	0.06
C14:1n5	0.80	0.77	0.87	0.83	0.68	0.77					
	±	±	±	±	±	±	-	-	-	-	-
	0.01	0.03	0.06	0.04	0.05	0.04					
C15:0	0.33	0.65	0.45	0.56	0.62	0.56					
	±	±	±	±	±	±	-	-	-	-	-
	0.01	0.03	0.04	0.03	0.04	0.03					
C15:1n5	0.08		0.11	0.09		0.10					
	±	-	±	±	-	±	-	-	-	-	-
	0.00		0.01	0.01		0.01					
C16:0	28.27	31.07	26.63	29.07	30.17	29.40	21.94	23.92	24.7	20.58	26.48
	±	±	±	±	±	±	±	±	±	±	±
	0.66	0.02	1.08	0.28	0.43	1.00	0.21	0.05	0.14	0.65	0.05
C16:1n7	4.73	2.89	3.09	3.2	2.66	2.67	3.47	4.7	2.19	2.01	1.93
	±	±	±	±	±	±	±	±	±	±	±
	0.08	0.01	0.07	0.02	0.05	0.05	0.02	0.06	0.13	0.37	0.13
C17:0	0.60	1.42	0.91	1.04	1.28	1.41	0.07	0.08	0.11	0.14	0.19
	±	±	±	±	±	±	±	±	±	±	±
	0.04	0.03	0.13	0.08	0.04	0.03	0.02	0.01	0.00	0.03	0.02
C18:0	11.36	19.77	16.35	15.99	18.07	15.94	8.07	12.51	11.06	8.22	11.31
	±	±	±	±	±	±	±	±	±	±	±
	0.02	0.05	0.02	0.06	0.45	0.35	0.10	0.73	0.16	0.04	0.11
C18:1n9t	0.09	0.07	0.14		0.09	0.06	0.05	0.06	0.06	0.07	0.05
	±	±	±	-	±	±	±	±	±	±	±
	0.00	0.00	0.02		0.01	0.01	0.02	0.00	0.00	0.01	0.01
C18:1n11t	0.90	5.30	1.46	2.26	1.91	5.74					
	±	±	±	±	±	±	-	-	-	-	-
	0.00	0.03	0.04	0.06	0.04	0.10					
C18:1n9	45.97	30.94	42.81	41.52	38.05	35.89	53.06	46.21	49.22	53.90	43.93
	±	±	±	±	±	±	±	±	±	±	±
	0.43	0.39	0.49	0.70	0.25	1.17	0.19	0.94	0.22	0.64	0.32
C18:1n11	2.14	0.90	1.61		1.14	1.09	6.66	4.84	3.83	3.99	3.42
	±	±	±	-	±	±	±	±	±	±	±
	0.04	0.00	0.04		0.03	0.01	0.03	0.17	0.03	0.03	0.00
C18:2n6t	0.14	0.12	0.12	0.15	0.09	0.20					
	±	±	±	±	±	±	-	-	-	-	-
	0.01	0.00	0.02	0.02	0.02	0.02					

C18:2n6	1.36 ± 0.11	1.58 ± 0.04	1.61 ± 0.03	1.33 ± 0.11	1.47 ± 0.06	2.08 ± 0.26	4.45 ± 0.03	4.54 ± 0.15	6.44 ± 0.04	7.80 ± 0.06	9.89 ± 0.00
C18:3n6	-	-	-	0.01 ± 0.00	-	-	-	-	-	-	-
C18:3n3	0.14 ± 0.01	0.22 ± 0.01	0.22 ± 0.03	0.23 ± 0.23	0.28 ± 0.06	0.31 ± 0.03	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.12 ± 0.04	0.14 ± 0.01
C20:0	-	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.12 ± 0.01	0.12 ± 0.00	0.15 ± 0.06	0.12 ± 0.01
C20:1n9	0.10 ± 0.01	0.08 ± 0.00	0.12 ± 0.02	0.10 ± 0.01	-	0.12 ± 0.01	0.68 ± 0.10	0.76 ± 0.07	0.71 ± 0.00	1.47 ± 0.55	0.79 ± 0.09
C20:2n6	-	-	-	-	-	-	0.12 ± 0.02	0.12 ± 0.01	0.2 ± 0.00	0.48 ± 0.18	0.35 ± 0.04
C20:3n6	0.05 ± 0.00	-	-	-	-	-	-	-	-	-	-
C20:4n6	0.11 ± 0.01	-	-	-	-	-	0.19 ± 0.03	0.09 ± 0.01	0.11 ± 0.00	0.15 ± 0.06	0.06 ± 0.01
SFA, % fat	43.39 ± 0.56	57.13 ± 0.30	47.84 ± 0.68	50.28 ± 0.61	53.63 ± 0.20	50.97 ± 0.86	31.25 ± 0.08	38.59 ± 0.80	37.16 ± 0.08	30.01 ± 0.49	39.44 ± 0.04
MUFA, % fat	54.81 ± 0.41	40.95 ± 0.35	50.21 ± 0.60	48.00 ± 0.73	44.53 ± 0.22	46.44 ± 1.18	63.92 ± 0.13	56.57 ± 0.98	56.01 ± 0.12	61.44 ± 0.27	50.12 ± 0.10
PUFA, % fat	1.80 ± 0.14	1.92 ± 0.05	1.95 ± 0.08	1.72 ± 0.11	1.84 ± 0.02	2.59 ± 0.32	4.83 ± 0.04	4.84 ± 0.18	6.83 ± 0.04	8.55 ± 0.22	10.44 ± 0.06
omega 3, % fat	0.14 ± 0.01	0.22 ± 0.01	0.22 ± 0.03	0.23 ± 0.02	0.28 ± 0.06	0.31 ± 0.03	0.07 ± 0.01	0.09 ± 0.01	0.08 ± 0.00	0.12 ± 0.04	0.14 ± 0.01
omega 6, % fat	1.52 ± 0.12	1.58 ± 0.04	1.61 ± 0.03	1.34 ± 0.11	1.47 ± 0.06	2.08 ± 0.26	4.76 ± 0.03	4.75 ± 0.17	6.75 ± 0.04	8.43 ± 0.18	10.30 ± 0.05
Trans natural, % fat	0.90 ± 0.00	5.30 ± 0.03	1.72 ± 0.04	2.26 ± 0.06	1.91 ± 0.04	5.74 ± 0.10	-	-	-	-	-
Trans industrial, % fat	0.23 ± 0.01	0.19 ± 0.00	0.26 ± 0.03	0.15 ± 0.02	0.18 ± 0.01	0.26 ± 0.04	0.05 ± 0.02	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.05 ± 0.01
Ω 6/Ω 3 ratio	10.51	7.23	7.45	5.70	5.35	6.63	66.07	52.06	83.19	71.94	75.22
PUFA/SFA ratio	0.04	0.03	0.04	0.03	0.03	0.05	0.15	0.13	0.18	0.29	0.26

Additionally, eicosenoic acid (C20:1n9) was in a higher proportion in MP collected from P4 (0.68-1.47) compared with the samples from pasture P3 (0.08-0.12%). The European Commission (based on Regulation (EC) no. 1924/2006) set claims regarding the MUFA content of food products, a product being considered high in MUFA if it contains at least 45% of the FA present in product, and it provides more than 20% of energy of the product. All products from pasture P4 can be considered high in MUFA, also M1, M3, M4 and M6 samples from pasture P3 are sources of MUFA. The major PUFA presented in all analyzed samples was LA, found in a higher proportion in the MP from P4, with a content ranging between 4.45% and 9.89%, compared with products from pasture P3 which presented a content of 1.33-2.08%. In contrast with the LA, the content of ALA was higher in MP collected from pasture P3 compared with the ones from pasture P4. Similarly, Ivanovic *et al.* (2016) found a higher proportion of LA in goat meat from mountain regions compared with the one from hilly and plain regions.

$\Gamma$ -linolenic acid (C18:3n6) was only found in sample M4, spicy sausages, while dihomogamma-linolenic (C20:3n6) was found just in pastrami sample (M1). All samples from pasture P4 presented eicosadienoic acid (C20:2n6) which was not present in MP collected from pasture P3. Arachidonic acid (C20:4n6), an essential FA, was found in all samples from pasture P4, while in samples from pasture P3 was found just in sample M1. The FA composition of the sausage samples from pasture P3 and the one from pasture P4 varied in terms of SFA, MUFA, and PUFA content. In all sausage samples, the predominant FA were oleic, palmitic, stearic acids, followed by myristic acid in case of sausages from pasture P3 and LA for M9 sample. Also, Leite *et al.* (2015) showed that the most abundant FA of sausages studied were the one presented for M9 sample.

In general, MP from pasture P4 had a better FA profile than MP from pasture P3 in terms of PUFA, TFA, and PUFA/SFA ratio.

Natural TFA were only present in MP collected from pasture P3. This difference could be in part a result of the effect of farm location in the botanical composition of pastures in these two areas from Romania, which affects the meat composition and in consequence the FA composition of MP. C18:2n6t was found just in MP from the pasture P3. Industrial TFA were significantly present in products from pasture P3 (0.15-0.26%) compared to products from pasture P4 (0.05-0.07%), but the content was below 2%. Industrial TFA have been associated with the development of cardiovascular diseases, inflammation and endoplasmic reticulum stress (Oteng and Kersten, 2020).

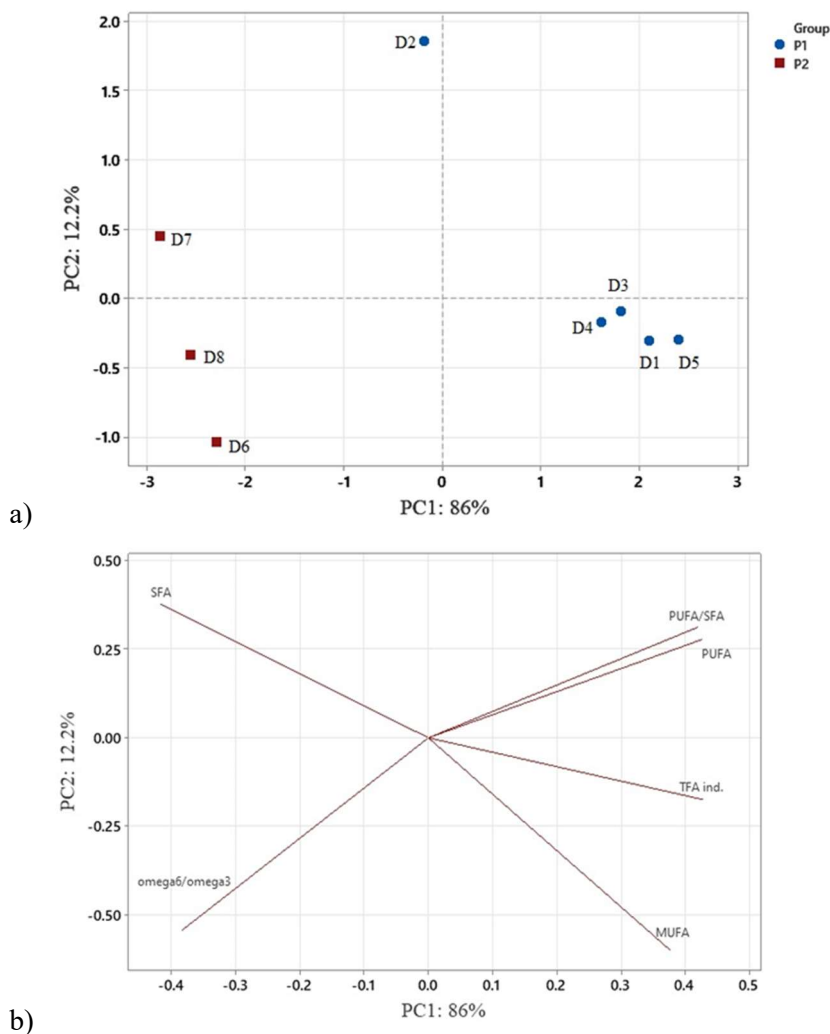
In terms of PUFA/SFA ratio, better proportions were obtained for samples from the pasture P4 as a result of the lower content of SFA and higher content of PUFA.

#### **Principal Component Analysis**

Multivariate statistical analyzes by PCA was applied in order to verify the discrimination according to the origin of production of DP and MP in terms of FA profile.

### Dairy products

Figure 2 shows the possibility to discriminate between samples from pastures P1 and P2, with the exception of sample D2 which is different from the other samples, being the only smoked cheese analyzed.



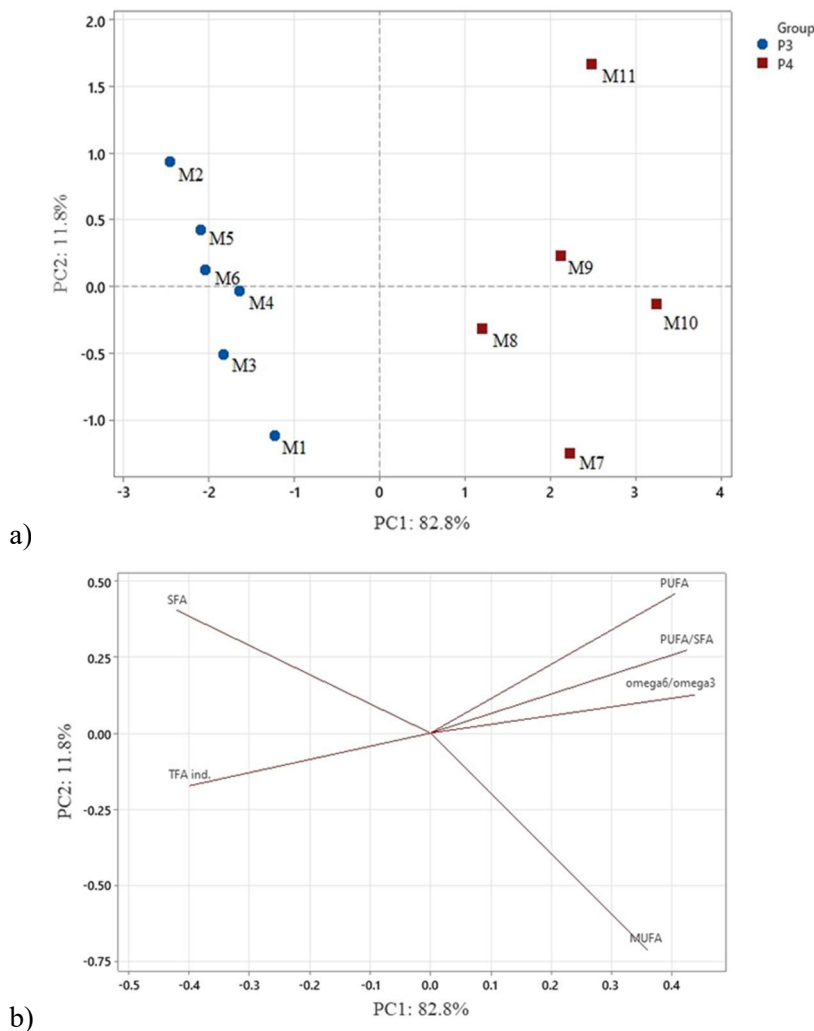
**Figure 2.** PCA of dairy products: a) score plot of first and second principal component (PC1 and PC2) describing the variability among dairy products analyzed; b) loading plot of PC1 and PC2 describing the variation between the fatty acid composition of dairy products.

The PCA provided good separation with 86% of the variation for the PC1, and 12.2% for PC2, respectively. Looking at the first component, the group of pasture P1 are well separated from the group of pasture P2. This is the result of the different FA composition of the DP from the two pastures. The first component was positively

correlated with MUFA, PUFA, PUFA/SFA ratio and industrial TFA, and inversely correlated with SFA and omega-6/omega-3 ratio. The second component was positively correlated with SFA, PUFA, and PUFA/SFA ratio, and negatively correlated with MUFA, industrial TFA and omega-6/omega-3 ratio. The PCA suggested that pasture P1 was characterized by the MUFA, PUFA, PUFA/SFA ratio and TFA industrial, while P2 was characterized by SFA and omega-6/omega-3 ratio.

#### Meat products

The variation between MP coming from the two pastures (P3 and P4) can be seen in Figure 3, results showing that a discrimination between samples can be made.



**Figure 3.** PCA of meat products: a) score plot of first and second principal component (PC1 and PC2) describing the variability among meat products analyzed; b) loading plot of PC1 and PC2 describing the variation between the fatty acid composition of meat products.

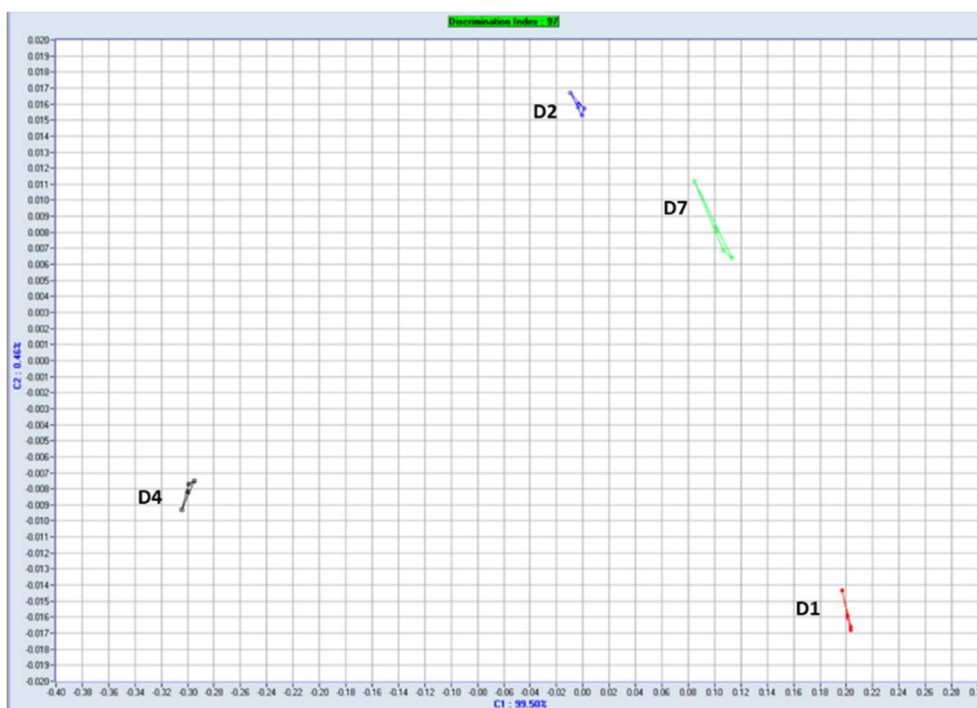


As expected, the group of pasture P3 are well separated from the group of P4, the result of different responses to geographic altitudes.

The PCA described a variance of 82.8% for PC1 and 11.8% for PC2, respectively. The first component was positively correlated with MUFA, PUFA, PUFA/SFA ratio, omega-6/omega-3 ratio, and inversely correlated with SFA and industrial TFA. The second component was positively correlated with SFA, PUFA, PUFA/SFA ratio, and omega-6/omega-3 ratio, and negatively correlated with MUFA and industrial TFA. pasture P3 was characterized by the SFA and TFA industrial, while P4 was characterized by MUFA, PUFA, PUFA/SFA ratio, and omega-6/omega-3 ratio. The altitudes at which farms are located seems to affect FA composition of the analyzed MP.

### *Headspace electronic nose*

Electronic nose analyzes was used to discriminate between the overall volatile compositions of DP and MP. The results obtained show the PCA plot which provides a map of discrimination of the analyzed samples. Electronic nose provided a good separation index with 97% for pressed cheese (D1, D2, D4, D7) (Figure 4), fresh cheese (D3, D6) and cheese (D5, D8).



**Figure 4.** The map of the PCA performed on pressed cheese samples.

The analyses of the PCA performed on MP showed a good separation (92%) demonstrating that the samples are completely different. Also, electronic nose gave

a good separation index of 96% for sausages (M4, M5, M9) (Figure 5). The volatile composition of all DP and MP was very different.



Figure 5. The map of the PCA performed on sausage samples.

## Conclusions

This paper compares the characteristics of mountain food products from different parts of Romanian Carpathians. Studies show that the nutritional quality of dairy and meat mountain products differs depending on the pastures where the animals were raised, the type of product analyzed and the technological process. The chemical composition of dairy and meat products differed as a result of the product variability. The fatty acid composition of dairy and meat products showed that the pasture has an impact on the quality of end products. Dairy and meat products from farms situated at higher altitude had a better PUFA content.

Lately, consumers are concerned and aware of the origin of food products. It is therefore important to use different tools to assess the origin of food products. PCA showed a good discrimination according to the response to the difference geographic altitudes of production of dairy and meat products in terms of FA profile. However, PCA analysis helped us to identify the volatile composition of the analyzed products but does not provide us with an identification of their origin.

Mountain products could promote the economic development of rural areas by offering high-quality food products.

## Acknowledgments

This research was funded by Romanian Agriculture and Rural Development Ministry, grant number ADER 17.1.2. „Mountain product as a model to support the added value of products made by farmers in the mountain area, in order to sustainably develop mountain farms”.

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