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Discrimination of the autochthonous animal fats by ATR-FTIR-PCA and comparison with vegetable oils

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Abstract

Animal and vegetable lipids are important parts of the human diet, taking into account the constant increasing of the Globe population. The quality and authenticity of such raw materials are always important requests. The goal of this study was the discrimination of the autochthonous animal fats by Fourier-transform infrared spectroscopy coupled with principal component analysis (FTIR-PCA) and comparison with vegetable oils. Lipid fractions from the pork meat of "Landrace" and "Mangalitza" breeds, as well as from the meat samples of "Ţurcană" sheep and "Giant White German" rabbit were compared with non-conventional and classical vegetable oils (black sesame, white mustard and chia seeds, respectively sunflower and palm) through FTIR analysis and the results were used as input data for the PCA classification of animal and vegetable samples. The animal fats and vegetable oils were classified according to the lipid profile by infrared spectroscopy if the differences between the concentrations of saturated and unsaturated fatty components are significant, suggesting the use of these combined techniques to evaluate product quality and to detect possible adulterations in some cases of animal products that have higher costs.

Keywords: animal fat; fatty acid profile; lipid fraction; "Landrace" and "Mangalitza" pork meat; Fourier-transform infrared spectroscopy (FTIR); principal component analysis (PCA); ATR-FTIR-PCA

1. Introduction

Raw materials of animal origin are widely used in the production of more or less processed food products. In the European Union, the population has reached almost 450 million people and an increase of only 3.9% is expected until the year 2040 or even a decrease of 2% until the year 2080. On the other hand, the world's population is constantly growing, currently exceeding 8 billion people, with a non-homogeneous distribution on the Globe. The most populated regions are the most exposed to hunger (almost one billion are below the poverty line). The need for food is therefore obvious and animal raw materials represent an important part of the human food. At the EU level, the herd of pigs, according to the National Institute of Statistics and FAOSTAT for the year 2022 was almost 133.3 million units, followed by cattle (73.9 million), sheep and goats (64.1 million). In Romania, the largest herd in 2022 was for sheep and goats (over 11.7 million units) and pigs (almost 3.34 million). On the other hand, according to the National Institute of Statistics, livestock numbers decreased in 2022 compared to 2021 by approximately 0.7% for cattle, by 7.8% for pigs and increased by approximately 1% for sheep and goats (https://insse.ro/cms/ro/tags/comunicat-efectivele-de-porcine) [1].

Pigs are animals of the species *Sus scrofa domesticus* L. or *Sus domesticus* Erxleben, being some of the most important animals raised for meat.

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There are several breeds that are bred in Romania, starting from the Romanian breeds "Bazna" and "Mangalitza", to those with high productivity such as "Belgian Landrace", "Giant White", "Hampshire", "Duroc" or "Pietrain" [2-7]. The domestic pig comes from the wild pig/boar (*Sus scrofa* L.) and was obtained through various crossings. There are numerous breeds that differ in color, shape and size [2].

Pig fat has a composition that varies a lot depending on the breed, growing conditions, diet, location/region, but also depending on the anatomical part of interest. Pig breeding strategies are aimed at increasing the sensory and nutritional qualities of meat or other pig raw materials. Thus, controlled food is used through the three main components, protein source, terrestrial source of lipids and marine source of lipids [8,9].

Oleic acid was identified as the most concentrated in both adipose tissue and loin of pigs, with concentrations of 35.8 and 32.8%, respectively. Palmitic, stearic and linoleic acids were also identified in concentrations above 10% [10]. The effect of crossbreeding on the fatty acid composition of the lipid parts was studied in breeds "Large White" (LW), "Duroc" (D), "Pietrain" (P) and "Landrace" (LD). A high intramuscular fat content was found for D×(LR×LW) crossbreds, and P was much more concentrated in polyunsaturated fatty acids (PUFA) and high PUFA/SFA ratios [2]. These techniques have been studied and are widely applied to control the content of "healthy" fatty acids in "Mangalitza" pigs, which have a high content of oleic acid of ~50% [4,7,11]. In an interesting comparative study, the fatty acid composition of domestic pig and wild boar meat was evaluated. Oleic acid in the lipid fraction of wild boar meat was in the range of 35.6-40.6%, while for domestic pork it was in the range of 30.39-51.3% [12]. A comparison of the fatty acid profile of fresh or cooked pork indicated a slight change in fatty acid composition, in some cases with a less significant decrease in polyunsaturated acid concentration upon roasting or baking, but total lipid content increases upon processing, together with the reduction of the content of volatile compounds [5].

Among the other lipid compounds that were evaluated in pork, the most studied was cholesterol. Depending on the anatomical part of the pig studied, the cholesterol content varied widely, from values of 48 mg/100 g in the pork loin or chop to 69-89 mg/100 g in some cooked meat samples [13]. When comparing the cholesterol content depending on the growing conditions of the "Mangalitza" breed pigs, a slight decrease in this concentration was found when growing in free space, from 63.1 mg/100 g to 61.7 mg/100 g. Various breeds of "Mangalitza" show small differences on the composition of this compound [4,11]. Other lipid compounds determined in pork lipid fractions are fat-soluble vitamins, especially tocopherols (vitamin E), which have an antioxidant effect *in vivo* or in pork products (raw material). It was found that vitamin E supplementation for animal feed leads to improvement of the color stability of pork meat, most likely due to the prevention of oxidative processes [14].

Spectroscopic methods can be used separately or by coupling with other techniques for the identification/quantification of components in oils and fats. Many studies use infrared spectroscopy such as Fourier-transform infrared spectroscopy (FTIR), with attenuated total reflectance system (ATR-FTIR), near and mid-infrared spectroscopy (NIR and MIR), Raman spectroscopy, or fluorescence spectroscopy [15-17]. Meat and meat products could be evaluated in terms of fatty acid composition using specific regions in ATR-FTIR analysis and palmitic acid for model prediction [15]. Supplements based on omega-3 oils from marine sources have been effectively determined by ATR-FTIR and FT-NIR techniques [18], as well as edible oils and fats by similar methods (FTIR, NIR, FT-Raman) [19]. The quality and authenticity of fish and meat products could be effectively monitored by using fluorescence spectroscopy, and a modern NIR scanning system allowed the on-line monitoring of fat in batches of meat obtained directly at slaughter [20].

These methods can be coupled with the multivariate statistical techniques such as PCA, PLS-DA and OPLS-DA ("principal component analysis", "partial least squares – discriminant analysis" and "orthogonal projections to latent structures – discriminant analysis"). The coupled methods were used for the successfully detecting of the adulteration with up to 5% pork fat and beef fat in canola oil [21]. The vibrational spectroscopy was coupled with the chemometric methods to determine the authenticity of vegetable oils and animal fats, their falsification or quality involving the PLS, LDA ("linear discriminant analysis"), SIMCA ("Soft

Independent Modeling of Class Analogy") or other similar methods [16].

The goal of the study was the discrimination of the autochthonous animal fats by ATR-FTIR-PCA and comparison with vegetable oils. Lipid fractions from the pork meat of "Landrace" and "Mangalitza" breeds, as well as from the meat samples of "Ţurcană" sheep, "Giant White German" rabbit were compared with non-conventional and classical vegetable oils (black sesame, white mustard and chia seeds, respectively sunflower and palm) through ATR-FTIR analysis and the results were used as input data for the PCA classification of animal and vegetable samples.

2. Materials and Method

2.1. Animal and vegetable samples

Lipid fractions were separated from animal sources, respectively some vegetable sources for comparison. Thus, the "Landrace" (*Sus scrofa domesticus* L.) pork meat (code "A-Ld") was obtained from the local farm in the West of Romania (Timiş county, 45°45'35" N, 21°13'48" E), while the "Mangalitza" (*Sus scrofa domesticus* L.) pork meat (code "A-Mg") was achieved from another farm of same region (Timiş county, 45°37'50" N, 21°35'18" E). The "Ţurcană" (*Ovis guineensis* L., code "A-Sp") sheep meat and the "Giant White German" (*Oryctolagus cuniculus* L., code "A-Rb") rabbit meat samples were obtained from farms located in the West and Central regions of Romania, respectively (Arad county, 46°10'30" N, 21°18'45" E, respectively Sibiu county, 45°48'08" N, 24°19'54" E). On the other hand, seeds of black sesame (*Sesamum indicum* L., code "V-Bs"), white mustard (*Sinapis alba* L., code "V-Ms") and chia seeds (*Salvia hispanica* L., code "V-Ch") were obtained from the local market in Timişoara, Romania, being produced in Mexico, Ukraine and Paraguay (the non-conventional vegetable oils were obtained in this study; see below). The sunflower and palm oils (produced in Urziceni, Romania and Malaysia, respectively) were obtained from the market in Timişoara, Romania and were used without other preparations (codes "V-Sf" and "V-Pm").

2.2. Obtaining of lipid fractions

Animal lipid fractions were separated by boiling the finely chopped fresh meat samples with water at normal pressure at a sample mass:water volume ratio of 1:4. The samples of lipid fractions were separated after cooling and keeping at 4 °C, followed by drying with filter paper. The lipid fractions were stored at 4 °C until the analysis. Oleic/palmitic acids were the most concentrated in the "A-Ld" and "A-Mg" lipid fractions of 37.3/21.3 and 36.3/25.6%, respectively. α-Linolenic acid (omega-3) had a concentration of 0.95%. Stearic, palmitic and oleic acids had concentrations of 28.9, 25.0 and 24.2% in the "A-Sp" lipid fractions. On the contrary, linoleic and palmitic acids were the most concentrated in the "A-Rb" lipid fractions (24.3-25.8% each). The detailed analysis of the fatty acid profiles were not subjected to this study.

On the other hand, non-conventional vegetable oils were obtained by semi-continuous multiple extraction using the Soxhlet method. A 250 mL device was filled with 25 g of ground seeds, while the 500 mL extraction flask was filled with 300 mL of petroleum ether (boiling point 30-60 °C, Sigma-Aldrich, Saint Louis, MO, USA). Six extractions were carried out by heating the extraction flask with a water bath. The extract was then cooled at room temperature, the vegetable residue was removed and the raw extract was distilled to dryness. The seeds oil was stored at 4 °C until the analysis. Linoleic and oleic acids were the most concentrated in the "V-Bs" and "V-Ms" oils (36.1 and 32.6, respectively 16,5 and 25,0%), while α-linolenic acid (an omega-3 fatty acid) was the main compound in the "V-Ch" oil of 58% (these analyses were not presented in this study).

2.3. Fourier-transform infrared spectroscopy (FTIR) analysis

Fourier-transform infrared spectroscopy coupled with an attenuated total reflectance system (ATR-FTIR) was used to discriminate samples and identify characteristic FTIR bands for their similarity/dissimilarity assessment and classification. A Bruker Vertex 70 apparatus (Bruker Optik GmbH, Ettlingen, Germany) was used, coupled with an ATR system. The following analysis conditions were set: wavenumber (WN) range 4000-400 cm^{-1} and a resolution of 4 cm^{-1}. The number of scans for each sample, respectively for the "baseline" was set to 64. For the acquisition and processing of ATR-FTIR data, the OPUS program package ver. 7.2 (Bruker) was used. ATR-FTIR analyzes were performed in triplicate.

2.4. Classical statistical analysis and principal component analysis (PCA)

The classical statistical analysis was carried using both Microsoft Office Excel 2016 (Microsoft) and Statistica 7.1 (StatSoft) packages. The analysis of variance (ANOVA) in the case of multiple determinations was performed with the *Basic Statistics* and *General ANOVA/MANOVA* modules from the Statistica package, and the values were presented as Mean ± Standard Deviation (SD). Principal component analysis (which only works with independent variables for classification and has the advantage of using intercorrelated variables, because the interferences are canceled by orthogonalization), was performed with the *Principal Components & Classification Analysis* module from the Statistica 7.1 (StatSoft). Centered and normalized values of WN and FTIR band intensities (in combination or separately, based on correlations) were used, while the variances were determined using the ratio of the sum of squares and *n*-1 (*n* represents the number of samples). The scores and loadings plots were used to evaluate the similarity/dissimilarity of the samples, respectively the importance of the variables (characteristic FTIR bands) for classification, as well as the PCs useful in explaining the variance of the data.

3. Results and Discussion

3.1. Fourier-transform infrared spectroscopy analysis of animal lipid fractions

ATR-FTIR analyzes confirmed the presence of significant concentrations of fatty acids from various classes: saturated fatty acids, mono- and polyunsaturated fatty acids in *cis* configuration, respectively unsaturated fatty acids in *trans* configuration in case the separation of lipid fractions led to partial isomerization of some natural unsaturated fatty acids. However, the concentration of *trans* acids was generally reduced, a fact observed by the reduced intensity of the band corresponding to the deformation vibration for C=C bonds in the *trans* configuration, which appears in the range 960-970 cm^{-1}. For the samples of lipid fractions from "Landrace" and "Mangalitza" pork, the ATR-FTIR analysis indicated intense bands at 2921-2922, 2852-2853 and especially 1744 cm^{-1}, corresponding to the asymmetric and symmetric stretching vibrations of the groups CH from the hydrocarbon chain of triglycerides, respectively from the C=O ester group (Figures 1 and 2). It is worth mentioning the presence of the band corresponding to the symmetric stretching vibration for the =CH groups in the *cis* configuration, even if their intensity is reduced, but there is no interference in this case. For pork samples, these bands appear at 3006-3007 cm^{-1}. Also for the HC=CH bonds in the *cis* configuration, the bands corresponding to the stretching vibrations from 1655-1656 cm^{-1} are also identified. Other bands of medium or weak intensity are those corresponding to methyl and methylene groups (bending vibrations), for rocking vibrations of ethylene groups or out-of-plane bending vibrations from ~721 cm^{-1} (solitary band).

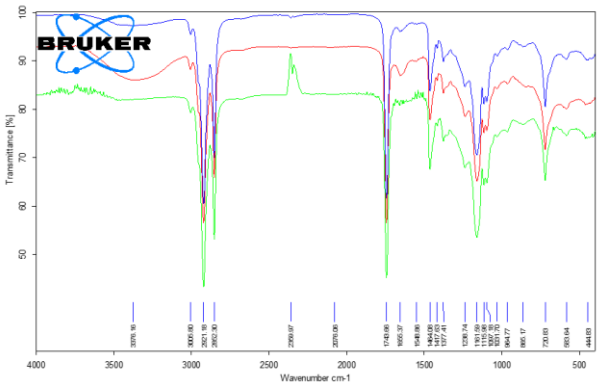

Figure 1. ATR-FTIR analysis of the lipid fractions from the "Landrace" pork meat (triplicate samples)

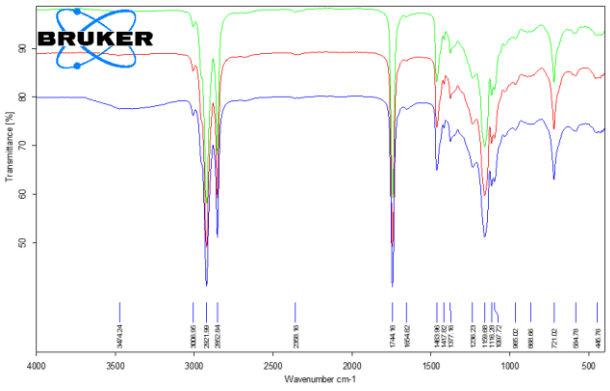

Figure 2. ATR-FTIR analysis of the lipid fractions from the "Mangalitza" pork meat (triplicate samples)

For the "Țurcană" sheep meat samples, the bands corresponding to the stretching vibrations for the CH and C=O groups are intense and appear at 2919, 2851 and 1742 cm^{-1}, but the band at 3008 cm^{-1} is much less intense in comparison with pork samples (Figure 3). This fact is in agreement with the gas chromatographic analysis (not presented in this study), where only oleic acid was identified in

higher concentration, as an unsaturated fatty acid. The proportion of saturated fatty acids is much higher (almost 60%). This fact is also supported by the intensity of the other bands corresponding to the stretching and rocking vibrations at 1647 and 1408 cm^{-1}, which are weaker. The other bands studied do not show obvious differences, even if some samples did not lead to relevant FTIR spectra (too high water content, as in the case of the triplicate sample highlighted in red, Figure 3).

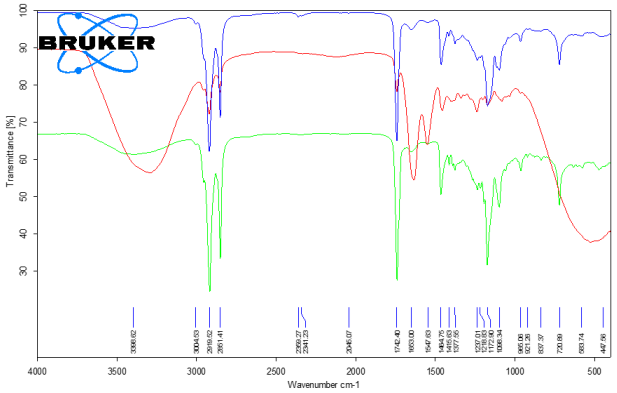

Figure 3. ATR-FTIR analysis of the lipid fractions from the "Ţurcană" sheep meat (triplicate samples)

The samples from rabbit meat presented FTIR spectra in which the relatively high intensities of the bands corresponding to the stretching vibrations of the =CH and C=C groups in the *cis* configuration were noted, especially for the representative band at 3010 cm^{-1}. The bands corresponding to the stretching vibrations for the CH and C=O bonds from the hydrophobic chain, respectively from the ester group of triglycerides appear intense at 2922, 2853 and 1744 cm^{-1}, respectively (Figure 4). The same observation can be made in this case, for the presence of a higher water content (humidity) in some samples, which did not allow the clear identification of some bands.

3.2. Fourier-transform infrared spectroscopy analysis of vegetable oils

FTIR analyzes for vegetable oils from non-conventional seeds clearly indicated the presence of high concentrations of unsaturated fatty acids, especially polyunsaturated ones revealed by the band corresponding to the valence vibration for the =CH bond in the *cis* configuration from 3005-3011 cm^{-1} (Figures 5 and 6). These differences are much more obvious for the white mustard seed oil and especially for the chia seed oil, which showed a very high content of α-linolenic acid, an omega-3 polyunsaturated fatty acid with three *cis* double bonds. Also, the bands corresponding to the asymmetric and symmetric stretching vibrations for the methylene and methyl CH bonds in the hydrocarbon chains, respectively the stretching vibrations of the carbonyl bonds in glycerides appear very intense (especially in the latter case) at 2921-2924, 2852-2854 and 1742-1744 cm^{-1}.

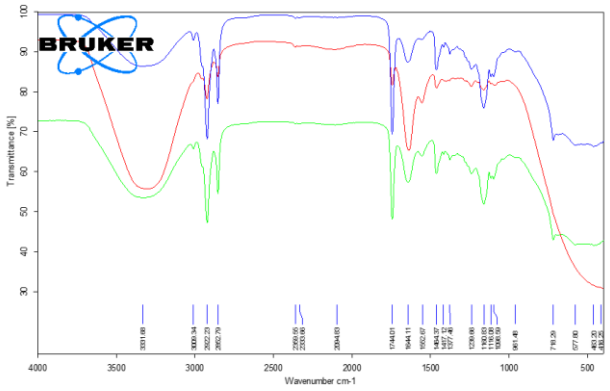

Figure 4. ATR-FTIR analysis of the lipid fractions from the "Giant White German" rabbit meat (triplicates)

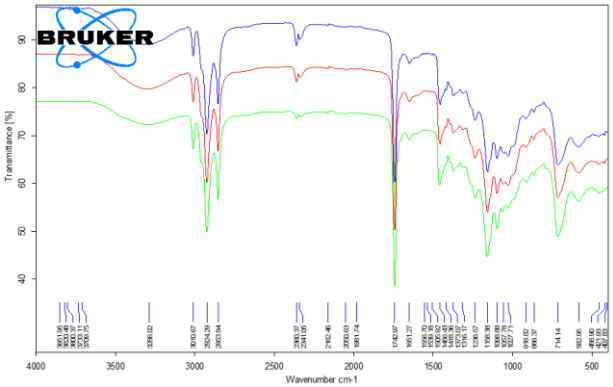

Figure 5. ATR-FTIR analysis of the chia seeds oil samples (triplicates)

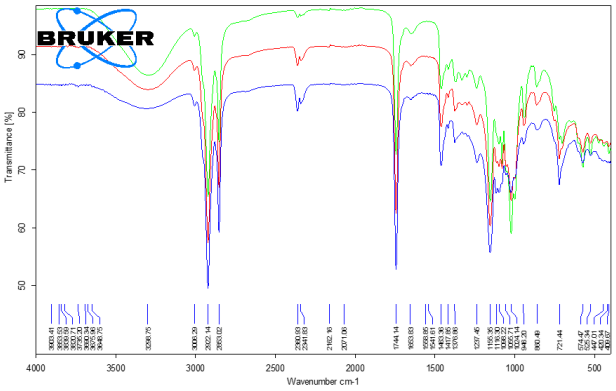

Figure 6. ATR-FTIR analysis of the palm oil samples (triplicates)

For the classic commercial sunflower and palm seed vegetable oils, the FTIR spectrum pattern was

similar to the previous ones, with the band at 3007-3008 cm^{-1} slightly more intense for sunflower oil (higher content of polyunsaturated fatty acids, especially linoleic acid, omega-6), compared to palm oil, which has a higher content of saturated fatty acids. These differences are also observed in the case of the intense bands at 2922-2923 and 2853-2854 cm^{-1}, which correspond only to the stretching vibrations of the methyl and methylene groups (saturated hydrocarbon chains). However, no significant differences were observed for the other lower intensity bands, which suggested the use of a powerful statistical technique to discriminate such samples, based on the ATR-FTIR wavenumbers and intensities of the characteristic bands.

3.3. Discrimination of the animal and vegetable lipid fractions by ATR-FTIR-PCA

The discrimination of animal and vegetable lipid fractions was carried out using the WNs and intensities of the characteristic ATR-FTIR bands for the studied samples. There is a fairly good distribution of the vegetable oil samples along the PC_1 axis and of the lipid fraction samples from animal sources after PC_2, according to the combined ATR-FTIR-PCA analysis when using both types of independent variables, WNs and intensities of characteristic FTIR bands. The mutton samples and the chia seed oil samples are most clearly discriminated in the representation of the PC_3 *vs.* PC_1 (as triplicate samples). The influence of the variables for these classifications is interesting. According to the representations in the loading plots, the WNs are more important for the positive side of PC_1, where the vegetable oils are classified, especially for the stretching vibrations corresponding to CH bonds in methyl and methylene. For the positive side of PC_2 the intensities are more important bands corresponding to stretching vibrations for methyl/methylene and carbonyl in triglycerides. In the negative part of PC_2, the variables related to the bands corresponding to the vibrations for the double bonds are important (both WNs and intensities). The eigenvalues greater than 1 (inflection) suggested a number of five significant PCs for these classifications (92.37% of explained variance) (Figures 7-9). If the two types of variables (WNs and intensities of FTIR bands) are separated, the discrimination of lipid fraction samples from sheep meat and those from chia seed oil is maintained, especially when using WNs as independent variables. However, there are no significant improvements in terms of sample discrimination.

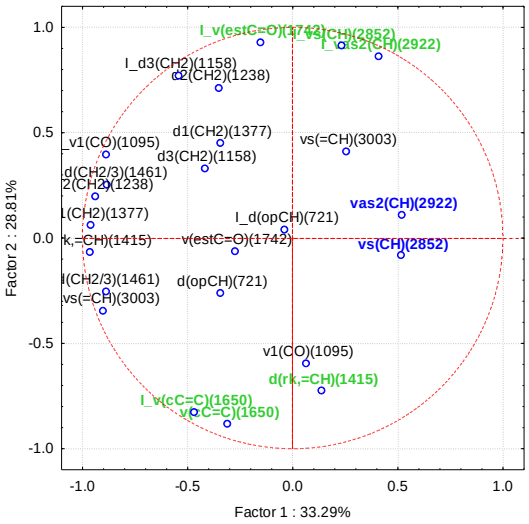

Figure 7. PC_2 *versus* PC_1 loadings plot from the PCA analysis of animal and vegetable lipid fractions using both FTIR wavenumers and intensities as input variables

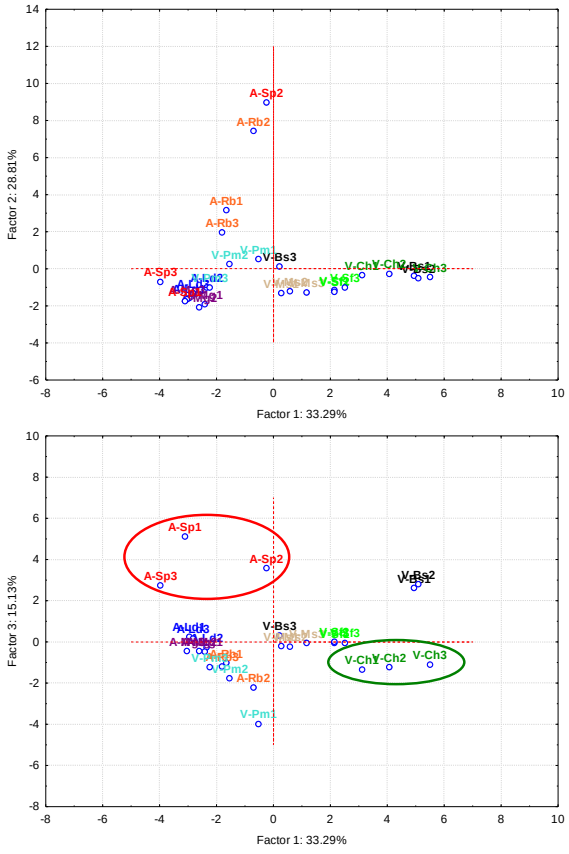

Figure 8. PC_2 *versus* PC_1 (top) and PC_3 *versus* PC_1 scores plots (bottom) from the PCA analysis of the animal and vegetable lipid fractions using both FTIR wavenumers and intensities as input variables

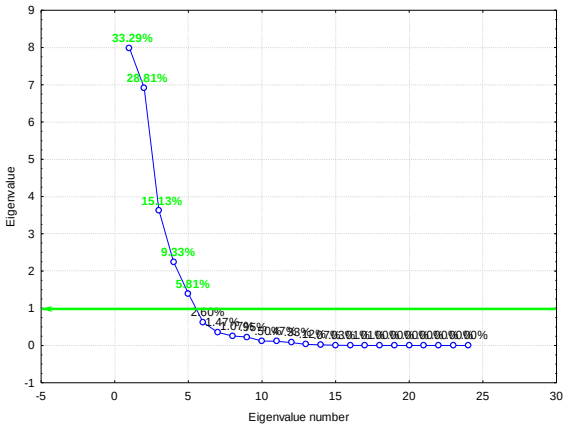

Figure 9. Eigenvalues for all principal components from the PCA analysis of the animal and vegetable lipid fractions using both FTIR wavenumers and intensities as input variables; variables in green explain 92.37% of the variance of the data

An attempt was made to apply the combined ATR-FTIR-PCA technique only by using the codes related to the type of animal ("A") or plant ("V") source for the studied samples. Following this combined analysis, the classification of plant samples according to PC_1 and animal samples according to PC_2 is better observed. Even the PC_3 *vs.* PC_1 scores plot allows some clustering of the two types of samples (Figure 10). If the ATR-FTIR-PCA analysis is limited only to the WNs of the characteristic bands, respectively only to their intensities, the classification is no longer so obvious, although the classification remains quite good in this last case. This fact demonstrates the greater importance of the intensities of the characteristic FTIR bands and less of the WNs of these bands in the discrimination of animal and vegetable lipid samples.

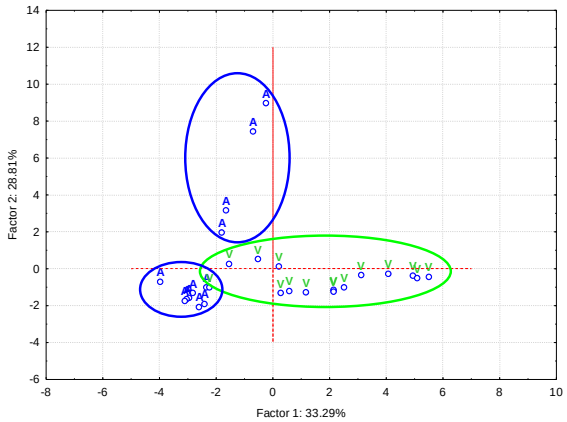

Figure 10. PC_2 *versus* PC_1 scores plots from the PCA analysis of the animal ("A") and vegetable ("V") lipid samples using FTIR wavenumers and intensities as input variables

4. Conclusion

The studies were carried out regarding the separation and preliminary analysis of lipid fractions from animal sources (meat) and vegetable oils, respectively the evaluation of the similarity/dissimilarity of animal and vegetable lipid fractions. Four types of lipid fractions were separated from the meat of some autochthonous animal species, as well as five types of non-conventional and classical vegetable oils. They were analyzed by ATR-FTIR for the identification of specific bands that allow the quick, easy and non-destructive discrimination of the samples using the combined ATR-FTIR-PCA technique. ATR-FTIR analyzes revealed the presence of hydrophobic chain components, through the bands corresponding to the stretching vibrations of CH bonds, but also of esters (especially triglycerides) through the existence of the intense band for the carbonyl group in esters. An important aspect is the presence and intensity of bands corresponding to stretching and bending vibrations for groups involving double bonds in *cis* configuration. The intensity and even the WN corresponding to the stretching vibration of the =CH bond from animal lipid fractions or vegetable oils allowed the discrimination of the two types of samples (animal and vegetable) by applying the combined ATR-FTIR-PCA technique. The animal samples and vegetable oils can be easily classified according to the lipid profile by infrared spectroscopy if the differences between the concentrations of saturated and unsaturated fatty components are significant, suggesting the use of these combined techniques to evaluate the food product quality and to detect possible adulterations in some cases of animal products that have higher costs.

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