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Research Article

# Determination of the Quality of Dry Sausages by Targeted and Untargeted Techniques

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## Abstract

The objective of this work is to study the potential of mid infrared spectroscopy (MIR) for the determination of the quality and the prediction of the level of oxidation Peroxide Indices (PV) and Thiobarbituric Acid Reactive Substance (TBARS) of three categories of dry sausages: Auvergne, Beef-poultry and Galbanetto. The three products showed a significant difference ( $p < 0.05$ ) for the physicochemical parameters (moisture, water activity, pH, fat, TBARS, proteins and PV). The secondary structure determined by MIR indicated a variation between the products in the percentage of  $\beta$ -turn,  $\beta$ -sheet,  $\alpha$ -helix, and random coil. The application of principal component analysis and factorial discriminant analysis to the physico-chemical, colorimetric data, and MIR spectra showed a clear differentiation between the 3 brand products. The obtained results were confirmed by the partial least squares regression since excellent prediction was observed for the PV ( $R^2 = 0.96$ ) and TBARS ( $R^2 = 0.99$ ).

**Keywords:** Sausage, MIR, Oxidation, Quality, Chemometry.

## INTRODUCTION

Nowadays, consumers are increasingly concerned about the link between diet and health in terms of the quality of foodstuffs offered by the food industry, in particular meat and meat products, which have nutritional high value due to their protein, fat, etc. content. However, the high levels of fat and salt used to process meat products are known to be associated with the development of obesity, coronary heart disease, and high blood pressure, respectively (Brewer, 2012). The world consumption of sausages was estimated to 19.58 million kg by 2019 and is expected to continue growing by 3.3 % per year until 2023 (Zhu et al., 2020). Moreover, the sausages are obtained by mincing lean and fatty animal species, to which are added spices, additives and authorized condiments. During their transformation, sausages undergo a drying process which is accompanied by a maturation (temperature varying

from 15 to 25°C, relative humidity in the 65 - 80 % range during the fermentation process) where some biochemical reactions occurred inducing the development of particular organoleptic properties of sausages (Pavli et al., 2020). Indeed, the sausages are a complex food matrix, composed mainly of water, proteins, lipids, carbohydrates and species, so microbial and chemical alterations can occur during their storage (Tirado-Gallegos et al., 2021). Its high lipid content and packaging in semi-oxygen-permeable materials allows the main deterioration to be lipid, not microbial, oxidation, which impacts its nutritional and sensory properties (Bolívar-Monsalve et al., 2019). In addition, sausages during their storage in the commercial circuit may be subject to chemical and biochemical reactions capable of negatively affecting their quality. In this context, several studies have focused on the evaluation of sausage quality during storage. For instance, (Jin et al., 2014) evaluated the

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quality properties of emulsified pork sausage containing beetroot powder during cold storage. The results obtained showed a variation in qualities depending on refrigerated storage. Several research studies reported the impact of the fermentation, drying and ingredients used in the formulation on the final quality of sausages (Hüdayi Ercoskun, 2011). For example, the partial replacement of fat by pectin and tomato paste reduced resulted in a variation in the moisture, ash, lipid, and protein contents of sausages (Jouki et al., 2021); in addition, the supplementation of 50 % collagen in the sausages allowed better results of physicochemical parameters (Sousa et al., 2017). Recently, some research studies used the physico-chemical methods to assess the quality of sausages (Araújo et al., 2019; Feng et al., 2019; Lu et al., 2014). One of the main conclusions of their studies was that these methods could be used as tools for the evaluation of the quality of sausages. However, these methods are expensive, time consuming and destructive. Thus, simple and rapid techniques are necessary to assess the quality of sausages throughout the food chain. In this context, mid infrared (MIR) spectroscopy among other spectroscopic techniques was used to assess the effect of heat treatment on the quality of sausages on the one hand, (Pavli et al., 2020). In addition, Guntarti et al., (2019) used MIR spectroscopy to authenticate grilled and steamed beef sausage products and to predict their fat content. By applying partial least square regression (PLSR) to the MIR spectra, an excellent prediction of lard level ( $R^2 = 0.99$ ) and fat content ( $R^2 = 0.99$ ) in the sausages was obtained. One of the main conclusions of their study was that MIR could be used as a tool for the detection of lard in both steamed and grilled beef sausages and to predict the fat content.

To our knowledge, few studies have combined MIR spectroscopy with physico-chemical and colorimetric methods to assess the quality of sausages. In addition, most of the previous studies that employed MIR spectroscopy were focused on one type of sausage. Furthermore, to the best of our knowledge, there are no studies on the applicability of MIR spectroscopy for the prediction of the level of lipid oxidation of dry sausages belonging to different brand products. Therefore, this research aimed to investigate the potential use of MIR spectroscopy as a rapid method for the determination of the quality and the prediction of the oxidation level Peroxide Values (PV) and Thiobarbituric Acid Reactive Substance (TBARS)) of three categories of dry sausages.

## MATERIALS AND METHODS

### Sampling

Thirty ( $n = 30$ ) sausage samples were purchased in the markets of Conakry, Republic of Guinea. It concerns three

types of dry sausages including one type of Beef-poultry sausage ( $n = 10$ ) and two other types made with pork meat (Auvergne ( $n = 10$ ), and Galbanetto ( $n = 10$ )). These samples were first frozen at  $-18\text{ }^\circ\text{C}$  for 24 hours, then placed in an icebox and sent to the laboratory in France (Arras) where they were carefully packed and stored at  $-20\text{ }^\circ\text{C}$  until analyses. All the analyses were made in triplicate.

### Physico-chemical analysis

Each dry sausage was thawed for 12 h at  $4\text{ }^\circ\text{C}$  and ground in a mixer (Retsch Grindomix GM 200) at 10,000 rpm for 1 minute.

The moisture content was determined according to the AOAC (1990) method and the water activity was measured using a water activity analyzer (Awalometer Aqualab). The pH was determined by using pH meter 3110 (Germany). The fat and protein contents were established by using the method described by (Nhouchi et al., 2019) and (AFNOR, 2004), respectively.

Regarding the oxidation measurements, the PV and TBARS were determined according to the method described by (Zakaria & Sarbon, 2018).

### Colour measurements

To assess dry sausages colour, the Minolta Chroma Meter version CR-300 (Konica Minolta Sensing Europe, Roissy Charles De Gaulle, France) was used. The dry sausage slices were placed in the Petri dishes for measuring  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness). The total colour difference ( $\Delta E^*$ ) between the sausage samples was calculated as follows:

$$\Delta E^* = \sqrt{[(L^*)]^2 + [(a^*)]^2 + [(b^*)]^2}$$

### Mid infrared spectroscopy measurements

The MIR spectra were recorded at room temperature ( $20\text{ }^\circ\text{C}$ ) between 4000 and  $700\text{ cm}^{-1}$  using a resolution of  $4\text{ cm}^{-1}$  on a Fourier transform spectrometer IRTracer-100 (Shimadzu, Duisburg, Germany) which was mounted with an Attenuated Total Reflection (ATR) accessory equipped with a grip (Pike Technologies, Inc. Madison, United States). The ATR cell was made of a horizontal ZnSe crystal which presented an incidence angle of  $45^\circ$  and a total reflection of 10. Gentle pressure was applied to the handle to allow good contact between the crystal and the sausage sample. For each sausage sample, three spectra were recorded by using 40 scans for each sample. Before each measurement, the spectrum of the ZnSe crystal was recorded and used as background. Between different sausage samples, the crystal was carefully cleaned using ethanol and ultra-pure water.

All data were carried out using a Lab Solution Software and raw absorbance spectra were cut between 1700 and

1600 cm<sup>-1</sup> for analysing the Amide I region. The second derivative (Number of Points 11) was calculated on baseline correction, normalisation (with position 1700), and smoothing (Parameter Number 7). The integrated areas of each peak was calculated and related to the secondary structure of  $\alpha$ -helix (1654–1662 cm<sup>-1</sup>),  $\beta$ -sheet (1611–1640 cm<sup>-1</sup>),  $\beta$ -turn (1660–1690 cm<sup>-1</sup>), and random coil (1640–1650 cm<sup>-1</sup>) according to the method applied by (Sow et al., 2019).

### Mathematical analysis of data

To compare the three sausage categories, ANOVA was applied to each variable of the physicochemical and colorimetric parameters. With regard to the MIR spectra, and in order to reduce the scattering effects and to compare the sausage samples, the spectra were normalised by reducing the area under each spectrum to a value of 1. Then, the principal component analysis (PCA) and factorial discriminant analysis (FDA) were applied. The FDA was performed on the first five principal components (PCs) resulting from the PCA applied to MIR spectra and physicochemical and colorimetric measurements. Before applying the FDA, three groups were created corresponding to Auvergne, Beef-poultry and Galbanetto dry sausages.

The PLSR was applied on the normalised MIR spectra to predict the primary and secondary oxidation products (PV and TBARS). The first group designated as the calibration set is composed of 81 spectra, representing 9/10 of the samples, while the validation model was composed of 1/10 of the population (9 samples). The determination coefficient (R<sup>2</sup>) indicates the percentage of the variance in the variable Y which is represented by the variable X. A value of R<sup>2</sup> between 0.50 and 0.65 indicates that more than 50 % of the variance of Y is taken into account by the variance X, so that the discrimination between high and low concentrations can be made. An R<sup>2</sup> value between 0.66 and 0.81 indicates rough quantitative predictions, while an R<sup>2</sup> value between 0.82 and 0.90 indicates a good prediction.

Calibration models with an R<sup>2</sup> value greater than 0.91 are considered excellent.

In addition, the robustness of the model was also studied by determining the square of the correlation ratio of the Standard Deviation (SD) to the square Root of The Squared Error of Prediction (RMSEP), called the Ratio of the Prediction to the Deviation (RPD). This determines the factor by which the accuracy of the prediction has been increased over using the average composition for all samples. This ratio must be greater than 2 for a good calibration. An RPD ratio of less than 1.5 indicates poor predictions and the model cannot be used for new predictions (Karoui R., Mouazen A., M., Dufour É., Laurent Pillonel L., Picque D., Bosset J-O., 2006).

ANOVA and FDA were carried out with XLSTAT, 2016. The PCA and PLSR were performed using MATLAB (Matlab, Version 6.5, Release 12, The MathWorks) and Unscramble X (V.10.4, Camo Software AS, Oslo, Norway) software, respectively.

## RESULTS AND DISCUSSION

### Physico-chemical measurements

The results of the physico-chemical composition of dry sausages are shown in (Table 1). The moisture content of Beef-poultry sausages was significantly higher ( $48.61 \pm 0.31$  %) than Auvergne and Galbanetto dry sausages presenting levels of  $31.67 \pm 0.27$  % and  $18.44 \pm 0.44$  %, respectively. These differences could be attributed, on the one hand, to the manufacturing process, and on the other hand, to the nature of the used raw materials (beef, poultry or pork). Our values are lower than those observed by: i) (Araújo et al., 2019)) who found moisture contents of 68.39, 62.81 and 67.81 % for standard sausage, sausage containing 50% fat replacement with hydrolysed collagen powder and sausage with 50% fat replacement with chicken leg collagen in gel, and ii) (El-Nashi et al., 2015), who noted moisture content of 61.89, 60.65, 60.02, and 52.82 % for beef sausages

**Table 1.** Physico-Chemical Parameters Determined On Auvergne, Beef-Poultry, and Galbanetto Dry Sausages.

Parameters	Dry sausages category			
	Auvergne	Beef-poultry	Galbanetto	
Moisture (%)	31.67±0.31 <sup>A</sup>	48.61±0.27 <sup>B</sup>	18.44±0.44 <sup>C</sup>	
Water activity	0.88±0.002 <sup>A</sup>	0.93±0.001 <sup>B</sup>	0.75±0.002 <sup>C</sup>	
pH	5.93±0.007 <sup>A</sup>	5.31±0.077 <sup>B</sup>	5.86±0.014 <sup>C</sup>	
Fat (%)	31.15±0.08 <sup>A</sup>	17.14±0.95 <sup>B</sup>	36.38±0.57 <sup>C</sup>	
Proteins (%)	29.84±0.02 <sup>A</sup>	26.50±0.06 <sup>B</sup>	30.41±0.07 <sup>C</sup>	
colour	L*	49.11±1.67 <sup>A</sup>	41.86±1.24 <sup>B</sup>	41.70±1.57 <sup>B</sup>
	a*	10.92±0.89 <sup>A</sup>	14.13±0.67 <sup>B</sup>	15.82±0.77 <sup>C</sup>
	b*	7.25±0.83 <sup>A</sup>	9.67±0.55 <sup>B</sup>	7.43±1.03 <sup>C</sup>
	$\Delta E$	50.82±1.13 <sup>A</sup>	45.23±1.12 <sup>B</sup>	45.21±1.12 <sup>C</sup>

Mean values and standard deviations from three replicates are presented.

Different capital letters (A, B, C) represent the statistical difference between the three types of dry sausages (P <0.05)

samples containing 0%, 1 %, 2% and 3 % of pomegranate peels powder, respectively.

As shown in Table 1, the water activity of Auvergne, Beef-poultry, and Galbanetto dry sausages was  $0.88 \pm 0.002$ ,  $0.93 \pm 0.001$ , and  $0.75 \pm 0.002$ , respectively, in agreement with the findings of (Gunter Heinz, 2007) who pointed out that fermented dry sausages exhibited water activity ranging between 0.70 and 0.96 and those of (Zhang et al., 2021) who noted water activity values ranging from 0.83 to 0.89 for dry fermented sausages.

As illustrated in Table 1, the pH values of Auvergne, Beef-poultry, and Galbanetto dry sausages was  $5.93 \pm 0.007$ ,  $5.31 \pm 0.077$ , and  $5.86 \pm 0.014$ , respectively. A significant difference ( $p < 0.05$ ) was observed between the three types of dry sausages. Our results are: i) similar to those of (Monteiro et al., 2017) who depicted pH values between 5.88 and 5.97 for raw and cooked sausages prepared with 2.5, 5, 7.5, and 10 % of canola oil; ii) superior to the pH values obtained by (Mikami et al., 2020) who noted pH values of  $4.80 \pm 0.02$  and  $4.58 \pm 0.05$  for fermented dry sausages; and iii) inferior to the findings of (Zhang et al., 2021) who depicted pH values ranging from 6.27 to 6.60 for fermented dry sausages. These differences in the pH values could be attributed to the differences in the recipes and the microbiological quality of meat particularly the number of total coliforms, which cause degradation of proteins to amino acids resulting in the formation of ammonia and consequently an increase in pH values.

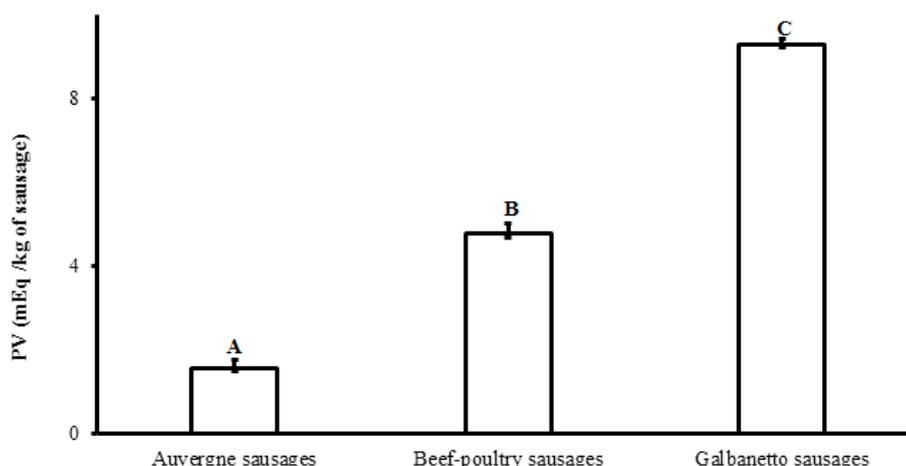
The fat contents found for Auvergne, Beef-poultry, and Galbanetto sausages were  $31.15 \pm 0.08$  %,  $17.14 \pm 0.65$  %, and  $36.38 \pm 0.57$  %, respectively (Table 1). Again, a significant difference ( $p < 0.05$ ) was observed between the three brand products of sausages, in agreement with the findings of Juliana et al. (2021) who depicted levels in

lipid varying in the of 32.25 to 44.53 % range for sausages made with chicken skin and / or abdominal fat. The high fat content observed for Auvergne, and Galbanetto sausages could be ascribed to the nature of the raw materials, since these dry sausages were made with pork meat rich in lipid (36.38%), contrary to Beef-poultry sausages that contained lipid at a level of 17.14%.

The protein values found for Auvergne, Beef-poultry, and Galbanetto sausages (Table 1) were  $29.84 \pm 0.02$ ,  $26.50 \pm 0.06$  and  $30.41 \pm 0.07$  %, respectively. This variation in the level of protein between the three dry sausages could be related to the quantity and quality of the ingredient used during the technological process. Such a hypothesis was in line with the findings of Carvalho et al. (2020) who also found a variation in the protein content ranging from 18.27 to 18.73 % in sausages prepared with vegetable oil and starch.

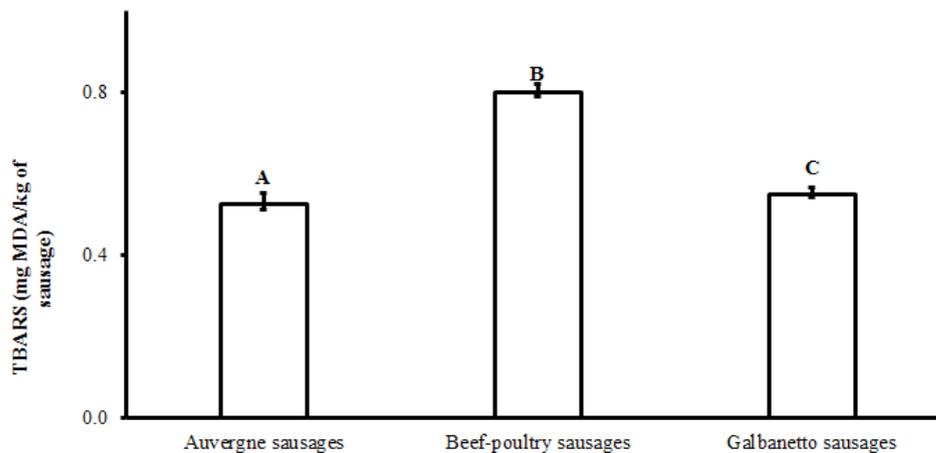
In the present study, PV was used to assess the primary products of lipid oxidation in the three types of dry sausages. The highest level of the PV was found in Auvergne sausage ( $9.27$  mEq / kg of sausage), followed by Beef-poultry sausage ( $4.79$  mEq / kg of sausage) and Galbanetto sausages ( $1.58$  mEq / kg of fat) (**Figure 1**). A significant difference ( $p < 0.05$ ) was observed between the three brand products ( $p < 0.05$ ). These variations could be due to the nature of fat and antioxidants used in the recipes. The results obtained in the present study for PV are higher than those reported by Panea & Ripoll, (2021) who found PV ranging from 0.23 to 3.44 mEq O<sub>2</sub> / kg and Zhao et al., (2020) who depicted PV of 0.06 mEq / kg and 0.08 mEq / kg in the sausages containing 2 % and 4 % NaCl, respectively.

TBARS values are indicators of the secondary oxidation products of sausages (Wenjiao et al., 2014). The TBARS values found for Auvergne, Beef-poultry, and Galbanetto



**Figure 1:** Evolution of Peroxide Values (PV) of Auvergne, Beef-poultry, and Galbanetto dry sausages.

Different capital letters (A, B, C) represent the statistical difference between the three types of dry sausages ( $P < 0.05$ )



**Figure 2:** Evolution of Thio Barbituric Acid Reactive Substance (TBARS) values of Auvergne, Beef-poultry, and Galbanetto dry sausages. Different capital letters (A, B, C) represent the statistical difference between the three types of dry sausages ( $P < 0.05$ )

sausages were  $0.80 \pm 0.03$ ,  $0.55 \pm 0.02$  and  $0.53 \pm 0.00$  mg MDA / kg of sausage, respectively (**Figure 2**). Statistically, these three categories of dry sausages are different ( $p < 0.05$ ). The obtained TBARS values are higher than those observed by Wenjiao et al., (2014) who found 0.22 mg MDA / kg for pork sausages stored at 10 and 20 °C. The differences between TBARS values of the 3 dry sausages could be attributed to the fat constituents, the muscle nature as well as the storage time. Based on TBARS values, the 3 types of dry sausage are acceptable for human consumption since the limit of acceptability of sausage is fixed to 1 mg MDA/kg of sausage (Wang et al., 2018). The TBARS values of Auvergne sausages are similar to those depicted by Liu et al. (2019) who found values in the 0.71-0.8 range mg MDA / kg for dry Harbin sausages.

### Colour measurements

Colour is one of the main characteristics that affect the quality of consumer perception of sausages. The values found for the lightness ( $L^*$ ) of Auvergne, Beef-poultry, and Galbanetto dry sausages were  $49.11 \pm 1.67$ ,  $41.86 \pm 1.24$  and  $41.70 \pm 1.57$ , respectively (Table 1). Statistically, no significant difference was observed between Beef-poultry, and Galbanetto dry sausages ( $p < 0.05$ ), but Auvergne dry sausages were different from the two other dry sausages ( $p > 0.05$ ). This difference could be due to the technological process that was found to present an impact on the  $L^*$  values as depicted by Yang et al. (2015).

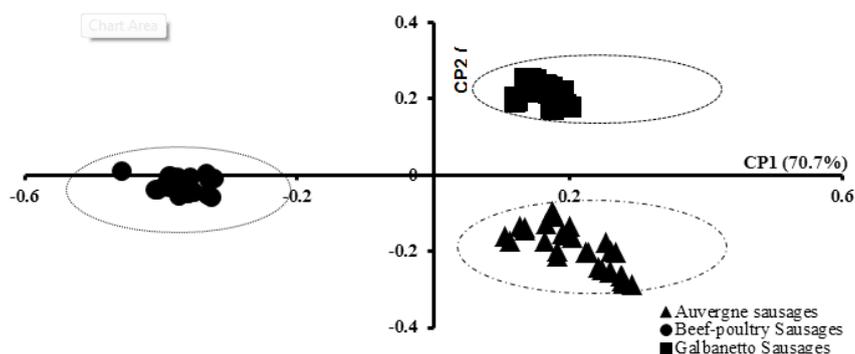
Regarding  $a^*$  parameter (Table 1), the Auvergne, Beef-poultry, and Galbanetto dry sausages had values of  $10.92 \pm 0.89$ ,  $14.13 \pm 0.67$  and  $15.82 \pm 0.77$ , respectively. A significant difference was observed between the 3 brand products ( $p < 0.05$ ). The lowest value was obtained for Auvergne dry sausages which could be attributed to a low level of lipid oxidation; this result is in agreement with

the values of primary and secondary oxidation products since the highest values of PV and TBARS were found for Auvergne dry sausages. Our results are also in agreement with the findings of Feng & Makino, (2020) who reported that the decrease in the  $a^*$  values may be ascribed to lipid oxidation.

The  $b^*$  values for Auvergne, Beef-poultry, and Galbanetto dry sausages was  $7.25 \pm 0.83$ ,  $9.67 \pm 0.55$ , and  $7.43 \pm 1.03$ , respectively (Table 1). Again, Beef-poultry and Galbanetto dry sausages showed no significant difference ( $p > 0.05$ ), but Auvergne sausages were statistically different from the two latter ( $p < 0.05$ ). It could be concluded that Auvergne sausages were yellower than the two other sausages. These differences could be due to the recipe and the technological process. This observation is confirmed by the  $\Delta E^*$  values, since no difference was observed between Beef-poultry and Galbanetto dry sausages ( $p > 0.05$ ), while Auvergne dry sausages were different from the two others ( $p < 0.05$ ). These variations could be attributed to the composition of the raw material, and/or storage conditions as depicted by Eisinaité et al. (2020).

The PCA was performed jointly on the physico-chemical and colour parameters (**Figure 3**). The map defined by the PC1 and PC2 representing 70.07 and 22.7 % of the total variance, respectively differentiated clearly between the 3 groups of dry sausages. Indeed, dry sausages made with Beef-poultry presented negative scores according to the PC1, while the other samples exhibited positive values. The PC2 differentiated between Galbanetto dry sausages having positive scores from Auvergne dry sausages presenting negative values.

In a second step, FDA with leave-one-out cross-validation was performed on the 5 PCs of the PCA performed on the physico-chemical and colorimetric data tables. A complete



**Figure 3:** Principal component analysis similarity maps of the physico-chemical and colour data tables of Auvergne, Beef-poultry, and Galbanetto dry sausages presented by Principal Components 1 (PC1) and 2 (PC2).

**Table 2:** Classification Table of Factorial Discriminant Analysis (FDA) With Leave-One-Out Cross-Validation Of Physico-Chemical And Colour And Mid Infrared (MIR) Data Sets.

Predicted / Observed	Auvergne sausages	Beef-poultry sausages	Galbanetto sausages	Total	% correct classification
<b>Physico-chemical and colorimetric measurements</b>					
Auvergne sausages	30	0	0	30	100 %
Beef-poultry sausages	0	30	0	30	100.00%
Galbanetto sausages	0	0	30	30	100.00%
<b>Total</b>	30	30	30	90	100 %
<b>Mid infrared measurements</b>					
Auvergne sausages	24	0	6	30	80.00%
Beef-poultry sausages	0	30	0	30	100.00%
Galbanetto sausages	0	0	30	30	100.00%
<b>Total</b>	24	30	36	90	93.33%

(100 %) of correct classification was obtained for the three groups (**Table 2**) indicating that these techniques could be used jointly as tools for the evaluation of the quality of dry sausages.

### Mid infrared spectroscopy measurements

(**Figure 4**) exhibits MIR spectra scanned between 4000-700  $\text{cm}^{-1}$  on the 3 types of dry sausages. This region provides information about molecular bonds with fundamental valence vibrations of functional groups (Karoui et al., 2006). As shown in Figure 4, the spectra presented a similar shape. A strong peak in the region located between 3205 and 3485  $\text{cm}^{-1}$  was noted and could be due to O–H and N–H stretch vibration (Ganesan et al., 2019). The bands observed in the 3005-2855  $\text{cm}^{-1}$  spectral region are very similar to those depicted by (Karoui et al. 2011) indicating that these bands could be due to lipids.

The peak observed at 2920  $\text{cm}^{-1}$  and 2855  $\text{cm}^{-1}$  could be due to the C–H saturated aliphatic stretch (Pavli et al., 2020) and asymmetric and symmetrical stretching methylene ( $\text{>CH}_2\text{>}$ ) group (Sari & Any Guntarti, 2018), respectively. The peak at 1745  $\text{cm}^{-1}$  could be attributed to the carbonyl groups ( $\text{C=O}$ ), while that observed at 1654  $\text{cm}^{-1}$  could be

due to the cis stretch C=C (Sari & Any Guntarti, 2018), and/or ) H–O–H stretch of the water (Polshin et al., 2011).

The Amide I band (1600-1700  $\text{cm}^{-1}$ ) is recognised as the most useful spectral region used to determinate the secondary structure of proteins that changed considerably depending on the type of sausages. Different peaks could be assigned to different secondary structures, including  $\alpha$ -helix (1654–1662  $\text{cm}^{-1}$ ),  $\beta$ -sheet (1611–1640  $\text{cm}^{-1}$ ),  $\beta$ -turn (1660–1690  $\text{cm}^{-1}$ ), and random coil (1640–1650  $\text{cm}^{-1}$ ) as depicted by Sow et al. (2019). The quantitative analysis and the proportion of the secondary structure are calculated and presented in (**Table 3**). The total ratio of more organised secondary structures ( $\alpha$ -helix and  $\beta$ -sheet) in Beef-poultry and Galbanetto dry sausages (15.29–15.22 %) was significantly lower ( $P < 0.05$ ) compared with Auvergne dry sausages (15.73 %). The low level of  $\alpha$ -helix in these samples could be ascribed to the hydroxyl groups of procyanidins which disrupted the hydrogen bonds of the protein and caused the unwinding of  $\alpha$ -helix ((Song et al., 2022). While, the highest levels of  $\alpha$ -helix and Random coil were obtained for Auvergne dry sausages exhibiting 15.73 and 14.66 %, respectively. The  $\beta$ -sheet structure varied from 31.79 to 41.57% (%), higher than the results observed by Zhao et al. (2019) who found  $\beta$ -sheet in the levels between 21.93–26.10 %.

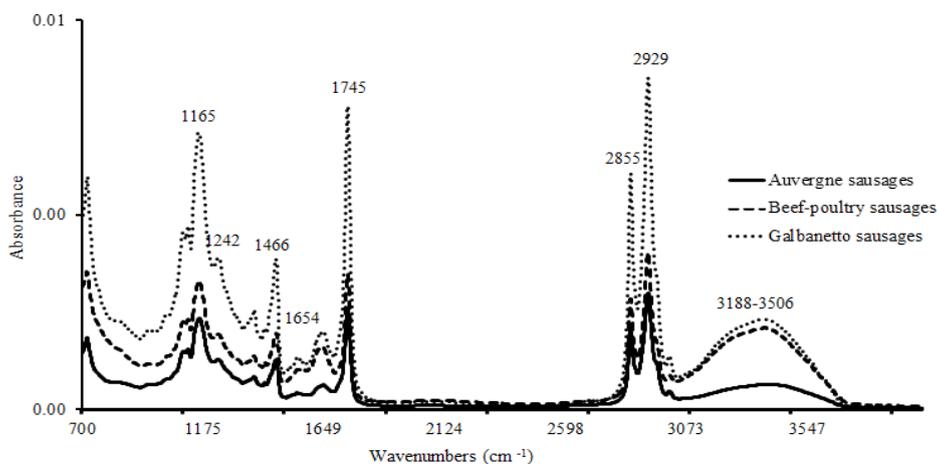


Figure 4: Raw mid-infrared spectra.

Table 3: Secondary Structure Determined From Mid-Infrared Spectra Of Dry Sausages Belonging To Three Brand Products: Auvergne, Beef-Poultry, And Galbanetto.

Parameters	Dry sausages category		
	Auvergne	Beef-poultry	Galbanetto
$\alpha$ -helix (%)	15.73 $\pm$ 0.005 <sup>A</sup>	15.29 $\pm$ 0.001 <sup>B</sup>	15.22 $\pm$ 0.001 <sup>B</sup>
$\beta$ -sheet (%)	36.24 $\pm$ 0.028 <sup>A</sup>	41.57 $\pm$ 0.004 <sup>B</sup>	31.79 $\pm$ 0.03 <sup>C</sup>
$\beta$ -turn (%)	33.39 $\pm$ 0.026 <sup>A</sup>	29.05 $\pm$ 0.004 <sup>B</sup>	40.18 $\pm$ 0.02 <sup>C</sup>
Random coil (%)	14.66 $\pm$ 0.009 <sup>A</sup>	14.10 $\pm$ 0.0007 <sup>B</sup>	12.82 $\pm$ 0.01 <sup>C</sup>

Mean values and standard deviations from three replicates are presented.

Different capital letters (A, B, C) represent the statistical difference between the three types of dry sausages ( $P < 0.05$ )

Table 4: Cross-Validation Results of The Peroxide Values (PV) And Thiobarbituric Acid Reactive Substance (TBARS) Values Using Partial Least Squares Regression (PLSR) Of The Calibration and Validation Models.

Data	Calibration			Prediction		
	R <sup>2</sup>	RPD	RMSEC	R <sup>2</sup>	RPD	RMSEP
PV	0.80	3.43	1.37	0.96	6.62	0.76
TBARS	0.98	6.45	0.001	0.99	6.83	0.004

PV: Peroxide Value

TBARS: Thio Barbituric Acid Reactive Substances

RMSEP: Root Mean Square Error of Prediction.

RMSEC: Root Mean Square Error of Calibration

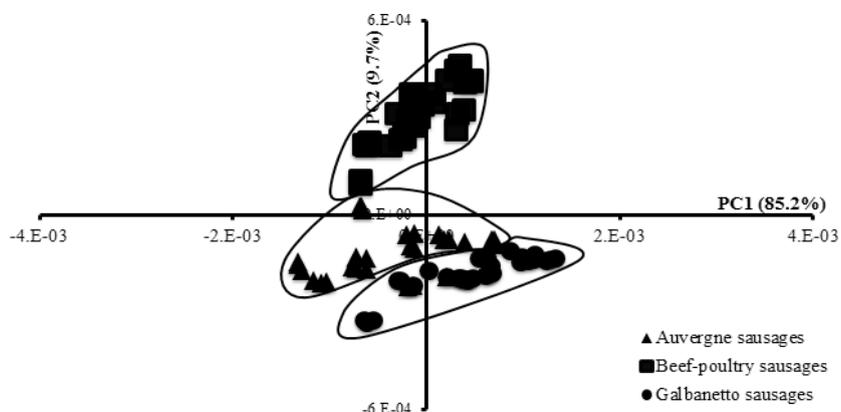
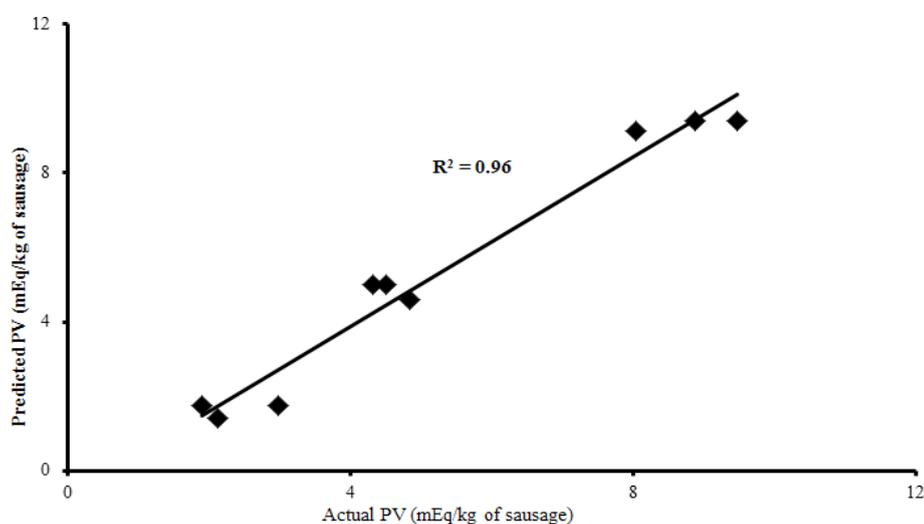


Figure 5: Principal component analysis similarity maps of the MIR spectra presented by Principal Components 1 (PC1) and 2 (PC2) of Auvergne, Beef-poultry, and Galbanetto dry sausages.

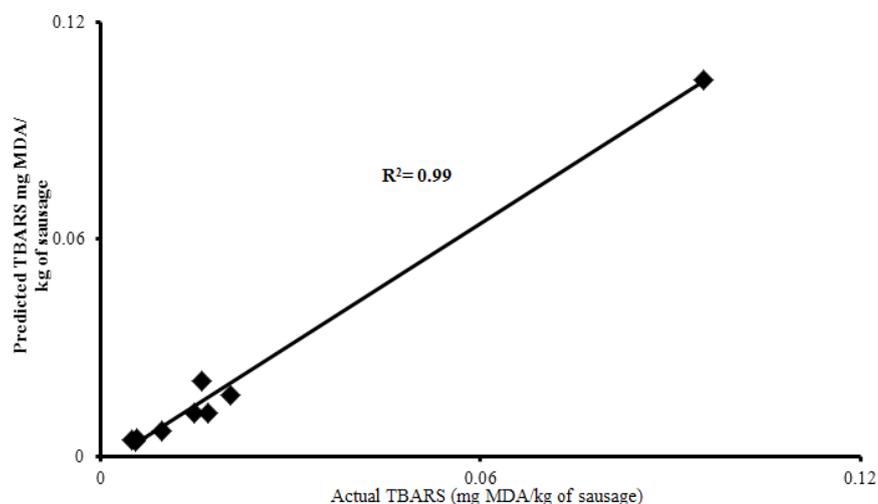
To extract information from the MIR spectra, PCA was applied to the normalised spectra. The similarity map allowed clear differentiation between Auvergne, Beef-poultry, and Galbanetto sausages (**Figure 5**). Again, Beef-poultry sausages were well differentiated from the two other dry sausages. The obtained results were confirmed by the FDA since 93.33 % of correct classification was obtained (Table 2). Beef-poultry and Galbanetto dry sausages were 100 % correctly classified. Six (6) Auvergne dry sausages out of 30 samples were classified as belonging to Galbanetto dry sausages.

#### Prediction of peroxide value and thiobarbituric acid reactive substance values from mid infrared spectra

A summary of the predictive performance of models developed for each of the chemical parameters using the 4000-700  $\text{cm}^{-1}$  is shown in (**Table 4**) and (**Figures 6, 7**). According to the  $R^2$ , the prediction of the PV ( $R^2=0.96$ , RPD = 6.62; RMSEP = 0.76) and TBARS ( $R^2=0.99$ , RPD = 6.83; RMSEP = 0.004) can be considered as excellent. Therefore, the developed model demonstrated the potential use of MIR to predict the primary and secondary oxidation products in dry sausages. Although the results found in this study should be validated using a large number of dry sausages for each category, the obtained results indicate the possibility of using MIR as a technique for rapid prediction and with excellent precision for PV and TBARS, regardless of their technological process and recipes.



**Figure 6:** Scatter plots of measured versus predicted Peroxide Values (PV) for Auvergne, Beef-poultry, and Galbanetto dry sausages with full cross validation after Partial Least Squares Regression (PLSR).



**Figure 7:** Scatter plots of measured versus predicted Thio Barbituric Acid Reactive Substances (TBARS) values for Auvergne, Beef-poultry, and Galbanetto dry sausages with full cross validation after Partial Least Squares Regression (PLSR).

## CONCLUSION

To respond to consumer concerns about the quality of dry sausages, physico-chemical, colorimetric and MIR measurements were carried out on 30 dry sausages belonging to 3 brand products (Auvergne, Beef-poultry, and Galbanetto). The potential of MIR to differentiate between the 3 brand products was demonstrated. Indeed, by applying FDA to the MIR spectra, satisfactory classification of dry sausages was obtained since 93.33% of correct classification was obtained. This trend was confirmed following the application of PLSR to the MIR spectra. The technique has demonstrated its ability to predict with excellent manner PV and TBARS values of dry sausages since R<sup>2</sup> of 0.96, and 0.98 were observed, respectively. Although further research studies are needed on a large number of dry sausages, the simplicity of the MIR technique offers rich opportunities for the characterisation of dry sausages at a very low cost. In addition, the technique has the potential to dramatically reduce the analysis time required for the determination of PV and TBARS values.

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