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FORMULATION OF CHICKEN SAUSAGES ENRICHED WITH CLOVE (*SYZYGIUM AROMATICUM*) POWDER AND SESAME (*SESAMUM INDICUM L.*) FLOUR DURING THE FERMENTATION STAGE

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ABSTRACT: *The study evaluated the combined effect of clove and sesame flour during the fermentation stage. The impact of the incorporation of sesame flour in S1 batch (sausages with 80% chicken filet, 17% veal fat, 2% sesame flour, and 1% cloves) and S2 batch (sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves) compared to Control batch (80% chicken filet, 18% veal fat, and 2% sesame flour) on the physico-chemical, color and molecular structure during the fermentation stage. Results showed that the incorporation of clove in the chicken dry sausages significantly increased the moisture and protein contents since it passed from 14.82 on day 1 in the Control at 30.23 ± 0.90% for S2 on day 1, respectively. The results obtained also showed that clove is a source of antioxidants, since the oxidation indicator parameters decreased with the level of addition of clove. The results demonstrated the potential of using fluorescence spectroscopy for monitoring the quality of sausages, since principal component analysis and factorial discriminant analysis (applied to fluorescence spectra allowed to discriminate between samples according to the level of clove addition (97.22% of correct classification).*

KEYWORDS: Chicken sausage, Fluorescence, *Syzygium aromaticum*, *Sesamum indicum*, Fermentation.

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INTRODUCTION

Chicken sausage is one of the most consumed foodstuffs among meat products in the world. However, the product deteriorates due to lipid oxidation, microbial growth, and so on (Moawad et al., 2020). Sausages are coveted for their unique taste and are a rich source of nutrients, providing good quality animal protein, amino acids and essential fatty acids, minerals, trace elements and vitamins including B-complex (Singh et al., 2014). Different ingredients are used for producing sausages including sodium and potassium nitrite for their potential inhibitors of the growth of some pathogenic bacteria such as *Clostridium botulinum* and *Listeria monocytogenes*. However, the use of synthetic compounds is quite debatable because of their ill effects on human health (Zhang et al., 2017)**.** Therefore, some plant extracts are used as a source of antioxidants. In order to overcome this problem, charcuterie manufacturers have embarked on the use of natural preservatives with both antioxidant and antimicrobial activities such as clove powder, ginger, garlic, chitosan, oregano oil, green tea, cloudberry, beetroot, willow herb, rosemary, clove and red chili for maintaining meat quality, extending shelf-life and preventing economic loss (Kumar et al., 2014; Singh et al., 2014). These compounds are classified as food additives, generally recognized as safe for human consumption (Aminzare et al., 2016).

Spices like clove (*Eugenia caryophyllus*), a member of caryophyllaceae family, is found to be the strongest antioxidant in retarding lipid oxidation (Tajik et al., 2012). The superior antioxidant activity of clove may arise from its high content of eugenol and gallic acid (Shan et al., 2009). Clove has also been reported to exhibit inhibitory effects on many food borne pathogens such as (Singh et al., 2014). Clove powder is a natural preservative and flavoring substance that is not harmful when consumed in food products. Thus, the use of clove powder extract is attractive for keeping the quality of refrigerated chicken sausage (Moawad et al., 2020). Several other products of plant origin have been used in sausages to replace fat of animal origin. In this context, Durantona and Chéret (2013) reported that for best quality attributes in emulsion-type sausages, fat levels would be in the 30-40% range. However, increasing demand for foods having healthy properties such as meat products with reduced fat content is more and more requested by consumers. For instance, Muguerza Ansorena and Astiasara (2004) depicted that fermented dry sausages present some negative health effects because of their high fat content (25-45%) and energy (300-450 kcal/100 g). Recently, Paglarini et al. (2020) achieved a reduction in the 11-34% fat range in reformulated sausages and depicted an increase in the content of fiber and polyunsaturated fatty acids in the formulations with emulsion gels (EG) compared to standard sausages. The addition of EG in Bologna sausages increased the L* values (brightness) and reduced the a* values (redness/greenness) compared to the control sausages.

Research studies have reported that sesame (*Sesamum indicum L.*) could be used in baked goods and confectionery as a source of edible oil. It is considered in the orient to be a health food and its oil shows high stability to oxidation compared with other vegetable oils like *Thymbra spicata* oil (Bozkurt, 2007). The oxidative stability of sesame oil is due to the presence of sesamolin. sesamin. sesamel. and γ-tocopherol that presented antioxidant properties. Sesamin and sesamolin have also a beneficial effect on human beings since the former enhances hepatic detoxification, reduces the chemical-induced tumors, protects against oxidative stress, and inhibits Δ5-desaturase in polyunsaturated fatty acid biosynthesis. Therefore, the sesame flour supplementation in dry sausages could be a solution for reducing

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meat fat level and improving its nutritional profile. In our recent study (Sangaré et al., 2022), it was found that chicken sausages containing 80% chicken filet, 18% veal fat and 2% sesame flour gave similar results to the control produced with 80% chicken filet, and 20% veal fat.

Despite the advantages of cloves and sesame flour as reported above, no study has yet focused on the combined effect of sesame flour and cloves on the quality and stability of chicken dry sausages. Thus, the present study aims to investigate the combined effect of sesame flour and cloves on the physico-chemical and structural properties of chicken dry sausages during the fermentation stage.

MATERIAL AND METHODS

Preparation of cloves and sesame flour

The cloves used in this study were purchased at the Matoto market in Conakry, Guinea that were carefully sorted and ground to fine powder. The obtained powder was vacuum-packed and stored at +4 °C until use in the recipe of sausage. White sesame seeds were purchased at the Aviation Market in Conakry, Guinea. Then, a conventional roasting was carried out in a local factory. The sesame seeds were roasted using a pan at a temperature between 60°C and 70°C for 10 minutes. The roasted seeds were crushed, sieved, then vacuum-packed and stored at +4°C until used in the sausage recipe.

Production of dry fermented chicken sausages

Chicken filets (20 kg) and veal fat (5 kg) were bought in a commercial supermarket (Arras, France). Three batches were produced at the pilot scale and composed of: (i) standard (Control) batch containing 80% chicken filet, 18% veal fat and 2% sesame flour; ii) S1 made with 80% chicken filet. 18% veal fat, 2% sesame flour and 1% of cloves (iii) S2 batch produced with 80% chicken filet, 18% veal fat, 2% sesame flour, and 2% of close. The method described by Sangaré et al. (2022) was used for the production of sausages. All the physico-chemical and spectral analyses were carried out on the samples of fermented dry chicken sausages finely ground in a mixer (Retsch Grindomix GM 200) at 10.000 rpm for 1 minute.

Physico-chemical measurements

The water activity (aw) and pH values were determined by using AquaLab equipment (Water Activity Meter. 4TE) and pH meter (3110. Germany), respectively. Fat and protein levels and thiobarbituric acid reactive substance (TBARS) values were established according to the method described by Nhouchi et al. (2019), French Standardization Association (AFNOR) (2004) and Wenjiao et al. (2014), respectively. The thiol level was determined according to the method of Berardo et al. (2015). All measurements were determined in triplicate after 1, 5, 10, and 15 days of fermentation.

Color measurement

The color measurement was carried out using Minolta Chroma Meter CR-300 (Konica Minolta Sensing Europe Roissy Charles De Gaulle. France). The color coordinates (lightness (L*),

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redness (a*) and yellowness (b*) were determined in triplicate after 1, 5, 10, and 15 days of fermentation.

Texture measurements

Textural parameters were determined using a TA-XT2i texture analyzer (Stable Micro System. London. U.K.) with a cylindrical probe (P0.25ss). The sausage was cut into 2 cm thick pieces and placed on the instrument base. The test was performed with two compression cycles. Hardness. springiness and cohesiveness were measured at 25 °C under the following conditions: 1 mm/s of test speed. 30% of compression strain and 5 s of time interval between first and second stroke. The test settings used were: pre and post-test speed: 2 mm/s. test speed: 1 mm/s, trigger force: 10 g and time gap: 5 s. All the measurements were determined in triplicate after 1, 5, 10, and 15 days of fermentation

Fluorescence measurements

Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer (Jobin Yvon, Horiba. NJ. USA). The incidence angle of the excitation radiation was set at 60° to ensure that reflected light. scattered radiation. and depolarisation phenomena were minimized. The spectrofluorometer was equipped with a thermostated cell and the temperature was controlled by a Haake A25 AC 200 temperature controller (Thermo-Scientific, France). Crushed dry fermented sausages were placed in a quartz cuvette and fluorescence spectra were recorded at 20 °C. The emission spectra of aromatic amino acids and nucleic acids (AAA+NA) (290–400), tryptophan (305-450 nm), collagen (360-650), nicotinamide adenine dinucleotide (NADH) (370-600 nm), and fluorescent Maillard reaction products (FMRP) (400-600 nm) were acquired with the excitation wavelengths set at 250, 290, 340. 360 nm, and 370 nm, respectively. The vitamin A excitation spectra (250-370 nm) were scanned after the emission wavelength set at 410 nm.

Mathematical analysis of data

To reduce the scattering effect and to compare dry sausage samples, fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1. The PCA was applied, separately, to the physicochemical and fluorescence data sets. Then, the FDA was performed on the first 5 PCs of the PCA applied to each fluorophore and physicochemical measurements accounting more than 99% of the total variance. Before applying the FDA with the leave-one-out cross-validation. 3 groups were created, namely: the Control, S1 and S2. Finally, the first 5 PCs of the PCA performed on each of the six fluorescence spectra were pooled into one matrix and the new table was analyzed by the FDA. This approach helps to improve the discrimination of the investigated dry fermented sausages. as well as to assess the ability of this technique to differentiate between the three groups. The same procedure was applied for the six fluorescence spectra and the physicochemical measurements. ANOVA and FDA were carried out with XLSTAT (2016), while the PCA and PLSR were performed using MATLAB software (Matlab, Version 6.5, Release 12, The MathWorks) and Unscramble X (V.10.4. Camo Software AS, Oslo, Norway), respectively.

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RESULTS AND DISCUSSION

Evolution of physico-chemical parameters of sausages during fermentation

The values of the physicochemical and textural parameters determined on the dry chicken sausages are mentioned in **Table 1b**. The moisture content varied from 61.07 ± 0.29% on day 1 to 20.29 ± 2.8 on day 15 in the Control batch (**Table 1b)**. From day 1 to day 15, the values ranged from 60.31 ± 0.61 to 24.51 ± 0.07% in S1 batch, and from 60.46 ± 0.87 25.51 ± 0.01% in S2 batch (**Table 1b)**. The moisture content decreased significantly in all the formulations on day 1 and 15. While on day 1 and 5, the samples belonging to S1 and S2 batches showed significantly lower levels than those of the Control batch. The S2 batch showed the lowest moisture content on day 15 attaining values of 21.36% versus 26.52 and 25.30 for S1 and Control batches, respectively. Our results are in agreement with the trend observed by Ebert et al. (2022) who observed a decrease in the moisture content in the control sausages and the hybrid sausages containing textured pumpkin seeds at 50% from 61.2 ± 0.7% to 55.1 ± 0.1% on day 1 and 21 of fermentation time. This could also explain the slightly lower aw of S1 and S2 batches.

The aw value in all three batches decreased with the fermentation time, since from day 1 to 15, these values varied from 0.98 ± 0.65 to 0.86 ± 1.09 in the Control batch, from 0.97 ± 0.78 to 0.86 ± 0.79 in S1 batch, and from 0.97 ± 0.06 0.84 ± 1.08 in S2 batch (**Table 1b**). During the fermentation stage, no significant difference ($p > O.05$) was observed between these three batches, indicating that the addition of cloves did not impact the raw values. These results are in agreement with previous work by Ebert et al. (2022) who found values of 0.909 ± 0.006 and 0.898 ± 0.004 respectively on days 1 and 21 of the fermentation. Our results disagree with those of Fernández-Diez et al. (2016) who observed a decrease in the level of raw sausages enriched with boiled quinoa during drying attaining values of 0.83 and 0.85 for control and half-fat sausages, respectively. Our results are different from those obtained by Araya-Morice et al. (2021) who found that raw values were influenced by the level of addition of substitutes, since an initial and final values of 0.96 and 0.82. 0.85. and 0.84 in the control sausages, and sausages containing a mix of extra hard cheeses, and a mix of extra hard were obtained, respectively.

As illustrated in **Table 1**, the pH values on day 1 increased significantly ($p < 0.05$) with the level of clove addition, since samples from the Control, S1 and S2 batches presented values of 5.35 ± 0.001. 5.92 ± 0.02 and 5.97 ± 0.23, respectively. On day 5, a decrease of pH values was observed reaching 4.48 ± 0.002. 4.34 ± 0.03 and 4.55 ± 0.43 in the Control, S1 and S2 batches, respectively. This tendency could be due to the activities of lactic acid bacteria, capable of transforming the sugar present into lactic acid, resulting in the acidification of the product as described by Tsun et al. (2021). On days 10 and 15, the samples of the three formulations presented similar values, because no significant difference ($p > 0.05$) was observed between the 3 formulations.

The protein values of sausages belonging to the 3 batches are shown in **Table 1b**. The protein values were 14.82 ± 0.03, 16.58 ± 0.04, 21.18 ± 0.21, and 30.18 ± 0.12 for the Control, 15.99 ± 0.09, 18.96 ± 0.65, 22.18 ± 0.23 and 30.21 ± 0.01 for S1, and 15.89 ± 0.67. 19.22 ± 0.43. 22.12 ±1.00 and 30.23 ± 0.90% for S2 on day 1, 5, 10 and 15, respectively. On day 5, samples belonging to the Control batch presented lower significant values compared to S1 and S2

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batches ($p < 0.05$). The increase in protein level during fermentation, for a considered batch, could also be attributed to the decrease of moisture content during fermentation, in agreement with the findings of Paule, (2005) On days 10 and 15, no difference was observed between the samples of the three formulations indicating that the addition of clove did not influence the protein level. The protein levels obtained on day 15 are similar to the findings of Ruiz-Capillas, Triki, Herrero, and Rodriguez-Salas (2012) who observed protein content of 25.64 ± 0.13%, 30.37 ± 0.81% and 32.52 ± 0.44% in control, reduced fat, and low fat dry fermented sausage, respectively on day 1, 10 and 15. The results obtained showed that the addition of cloves significantly impacted the b* value. The fat contents contained in the samples of the three batches are presented in **Table 1b**. On days 1, 5, 10 and 15, the fat content in the Control (15.65 ± 1.03, 30.50 ± 0.01, 29.81 ± 0.05, and 27.10 ± 0.01%), S1 (15.23 ± 0.10, 28.00 ± 0.09, 29.81 ± 0.01, 27.17 ± 0.04 %), and S2 (15.27 ± 0.07, 28.03 ± 0.76, 29.22 ± 0.08, and 26.89 ± 0.65%) batches significantly increased. Our results are in line with those of Fernández-Diez et al. (2016) who found a fat content of 63.3, 47.2 and 35.2% in control (no fat replacement; 30% of pork back-fat), a quinoa half-fat (50% of fat replacement; 15% of pork back-fat), and a quinoa low-fat (85% of fat replacement; 4.5% of pork back-fat) sausage, respectively. In short, the results showed that the addition of cloves did not influence the fat content of the Control. S1 and S2 batches.

As illustrated in **Table 1b**, The TBARS values found on day 1, 5, 10 and 15 were respectively 0.03 ± 0.03, 0.07 ± 0.01, 0.07 ± 0.01, and 0.15 ± 0.33 mg MDA/kg of sausage in Control batch; 0.03 ± 1.09. 0.04 ± 0.32. 0.04 ± 0.03. and 0.03 ± 0.001mg MDA/kg of sausage in S1 batch, and 0.03 ± 0.001. 0.04 ± 0.01. 0.03 ± 0.01. and 0.02 ± 0.02 mg MDA/kg of sausage in the S2 batch. These values were affected significantly ($p < 0.05$) by the fermentation stage and the addition of cloves. In addition, the obtained results showed that the TBARS values gradually decreased throughout the fermentation stage. As shown in **Table 1b**, no significant difference ($p > 0.05$) was observed between the samples of the three batches on day 1. On the other hand, these variations could be attributed to the decomposition of the formed TBARS to volatile compounds throughout the fermentation stage. Furthermore, from day 5 to 15, the samples from the batches containing the clove showed similar and significantly lower values than the samples of the Control batch. The low level of TBARS in the S1 and S2 batches could be attributed to the clove that is considered as an important source of antioxidants. Our results are in agreement with the work of Zhang et al. (2017) who reported that the addition of clove extract to sausages significantly delayed the increase of TBARS values for 21 days. On the other hand, the high level of TBARS in the Control batch would be due to the fact that during the fermentation of the sausages, the sugars could be broken down into aldehydes, which could react with the TBA reagent and lead to an increase in the TBARS values (Zhang et al., 2013). These results indicate that the addition of clove had a negative effect on retarding lipid oxidation in sausages.

The levels of thiol content found in the Control, S1 and S2 batches decreased significantly during the fermentation stage, since these values were 2.58 ± 0.007, 2.88 ± 0.21, 2.78 ±0 1.09, and 2.45 ± 1.21 nmol mg/ protein in the Control batch, 3.03 ± 0.001, 3.06 ± 0.43, 3.00 ± 0.72, and 3.00 ± 1.04 nmol mg/ protein in S1 batch, and 0.04 ± 0.909, 3.79 ± 0.21, 3.01 ± 0.01, and 3.00 ± 0.45 nmol mg/ protein in S2 batch **(Table 1b)**. No significant difference was observed between the samples of the S1 and S2 batches ($p > 0.05$) compared to the samples belonging to the Control batch; in agreement with the findings of Zhang et al., (2013) who found that temperature promoted protein oxidation in Chinese-style sausages and that the addition of

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Salvia officinalis decreased the loss of thiol groups. These results indicate that the addition of clove had a positive effect on retarding protein oxidation in sausages.

Regarding the color measurements (**Table 1b**), it appeared that the incorporation of clove induced a decrease of L* values; indeed, L* values on day 1 for Control, S1 and S2 batches were 42.97 ± 0.45. 40.59 ± 1.09, 41.86 ± 0.32 and 49.38 ± 0.34, respectively. On day 15, these values were 36.83 ± 2.00, 39.85 ± 1.07, and 40.07 ± 0.01, respectively. During the 15 days of fermentation, no significant difference ($p > 0.05$) was observed between the S1 and S2 batches compared to the Control batch. This difference could be due to the addition of clove, since clove contains purple pigments as described by Nuñez de Gonzalez et al. (2009); and/or ii). Another explanation for this variation would be due to the fact that the polyphenolic compounds present in cloves could oxidize to quinones and then to darker compounds in sausages, this is in agreement with the observations of Zhang et al. (2017) who observed a decrease in the value of L* with the addition of clove extract.

The samples belonging to the Control batch showed higher a* values ($p > 0.05$) than S1 and S2 **(Table 1b)**. Throughout the fermentation, the a* value for the Control batch decreased significantly compared to the S1 and S2 batches. From these results, it appeared that cloves could have a preventive effect on the discoloration of sausage samples during the fermentation stage. These results are in line with the investigation of Kong et al. (2010) who noted that pork patties with clove extract significantly decreased the a* value compared to the control batch and those of Zhang et al. (2017) who also found that clove extract considerably affected the value of a* in sausages.

Regarding b* value, the Control batch exhibited values that ranged from 37.48 ± 1.98 to 27.50 ± 0.54 during the fermentation stage, while S1 and S2 batches exhibited values that varied from 33.53 ± 1.104 to 25.70 ± 2.09, and from 33.44 ± 1.12 to 24.90 ± 1.07, respectively (**Table 1b**). The S1 and S2 batches showed significant differences ($p > 0.05$) compared to the Control batch on.

Evolution of textural parameters of sausages during fermentation

The textural properties of the sausages with the nail during the fermentation stage are shown in **Table 1b**. At the start of the fermentation (day 1), textural properties of Control, S1 and S2 batches showed no significant difference ($p > 0.05$). The hardness in Control batch varied from 1 314.75 ± 194 g to 18167.437 ± 1329 g, from 1 313.75 ± 190 to 16500.2635 ± 1233 g for S1 batch and from 1 313.49 ± 109 to 17112.4975 ± 141 g for S2 batch.

Regarding the hardness, no significant difference was observed between the three formulations on day 1. While on day 5, 10 and 15, the samples containing the clove (S1 and S2 batches) were significantly higher than the Control batch. Based on the fermentation stage (day 1, 5, 10, 15). it appeared that the hardness values increased during the fermentation stage (**Table 1b**). These results show that the addition of clove impacted the hardness value.

The springiness values are presented in **Table 1b**. These results showed that the Control and S1 batches presented similar values on day 1 compared to the S2 batch which presented a significant difference ($p > 0.0$). The springiness values during the fermentation stage varied from 0.60 ± 0.01 to 0.575 ± 0.00 in the Control batch, 0.60 ± 0.003 to 0.49 ± 0.024 in the S1 batch, and 0.98 ± 0.001 to 0.644 ±0.01 in the S2 batch. These results showed that the level of

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clove as well as the fermentation stage induced a variation in springiness values. It also appears from our results that the samples containing a low level of fat presented a high value of springiness. This trend is similar to previous studies of Surasani et al. (2020) who found that sausages incorporated with 2.5% g pangas protein isolates exhibited higher values of springiness than those produced foiswith 5% pangas protein isolates.

The cohesiveness values were affected by the addition of clove throughout the fermentation stage (day 1, 5, 10 and 15) (**Table 1b**). The obtained values varied from 0.27 ± 0.001 to 0.43 ± 0.002 in the Control batch, 0.27 ± 0.012 to 0.43 ± 0.012 in the S1 batch, and from 0.19 ± 0.012 to 0.44 ± 0.00 in the S2 batch. On day 1, 10 and 15, the Control and S1 batches presented similar values ($p > 0.05$) compared to the S2 batch. It was only on day 5 that batches S1 and S2 showed significantly higher values. The obtained results showed that the addition of the cloves and the duration of fermentation influenced the cohesiveness value since an increase was observed. The similar trends were found by Abbasi et al. (2019) who reported that the addition of gums in sausage recipes up to 0.5% increased cohesiveness whereas an inverse trend was observed for sausages containing 1% gums. An inverse trend was observed by Ozturk-Kerimoglu et al. (2022) who found no significant difference between cohesiveness of sausages containing microparticulate.

The information in the physicochemical, colorimetric and textural data tables was extracted by applying principal component analysis (PCA) (**Figure 1**). The map defined by PC1 and PC2 (62 and 15.8% of the total variance. respectively) clearly differentiated sausage samples according to their fermentation stage and a less manner their recipes. The FDA with leave-one-out cross-validation was performed on the 5 PCs of the PCA performed on the physico-chemical data tables. Overall, correct classification of 97.22% was obtained (**Table 2c)**.

Evolution of fluorescence spectra during dry sausage fermentation

The meat products contain many intrinsic fluorophores, such as aromatic amino acids and nucleic acids (AAA+NA), tryptophan, collagen, nicotinamide adenine dinucleotide (NADH), and fluorescent Maillard reaction products (FMRP). The presence of these intrinsic fluorophores in these products has favored the use of fluorescence spectroscopy techniques as a rapid and non-destructive method for monitoring their quality (Andersen & Wold, 2003).

Fluorescence spectra acquired on fermented dry sausages after excitation and emission set at different wavelengths

The fluorescence spectra acquired after excitation set at 250 nm exhibited maximum intensities located between 397 and 400 nm, regardless of the batch type (**Figure 2a**). A drastic change in the shape of the fluorescence spectra of the three batches was observed during the fermentation stage. These variations could be attributed to the proteolysis that induced change in the protein-protein. protein-lipid, and/or protein-water interactions. The PCA applied to the spectra acquired after excitation fixed at 250 nm allowed to differentiate the samples according to the fermentation time and the level of clove addition (data not shown). This trend was confirmed by the FDA applied to the first 5 PCs of the PCA, since an overall rate of 97.22% was obtained **(Table 1c).** Indeed, all the samples were correctly classified (100%), except the Control batch for which 66.66% correct classification were obtained.

Regarding the excitation spectra fixed at 370 nm (Figure 2b)

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Regarding the excitation spectra fixed at 370 nm (Figure 2b), it appeared that the Control and S1 and S2 batches on day 1 and Control batch on day 15 exhibited a maximum intensity located ~567 nm. On the other hand, on day 15, the intensities have drastically decreased. The variation in the intensity of the S1 and S2 batches compared to the Control batch could be attributed to the clove. The PCA similarity map allowed a differentiation of the sausage samples based on the recipes and the fermentation time (data not shown). By applying the FDA to the AAA+NA and collagen spectra, an overall rate of 97.22% was obtained (Table 1c). It is important to specify that the samples of the Control batch on day 5 had a rate of 66.66% of correct classification. All other samples were correctly classified (100% correct classification) (**Table 1c**). As shown in **Figure 2c** the fluorescence spectra acquired after excitation fixed at 340 nm on the dried sausages showed a maximum intensity located at 565 nm for all batches. Then, it appeared that the shape of the NADH emission spectra correlated with both the recipe and the fermentation stage.

The PCA applied to the emission spectra allowed clear separation of samples according to their formulation (data not shown). This trend was confirmed by the FDA since an overall rate of 80.55% of correct classification was obtained (**Table 1c**). All samples of S2 batch were correctly classified (100%). The fluorescence spectra showed a maximum ~568 nm at 1, 5, 10 and 15 days for all the formulations (**Figure 2d**.

Regarding the peak observed ~568 nm that could be attributed to the FMRP originating from the reaction between free amino groups and carbonyl compound as depicted by Karoui et al., (2021). The Control batch seemed to be more affected by the Maillard reaction than the S1 and S2 batches. This could be due to the presence of antioxidants in the two latter batches containing clove.

The PCA applied to the emission spectra showed a discrimination between the three recipes depending on the fermentation stage (data not shown). This trend was confirmed by the FDA applied to the FMRP spectra that allowed some discrimination of the sausage samples according to the recipe and the fermentation stage. Indeed, a correct classification rate amounting to 50% was observed. It should be specified that the S1 and S2 batches at day 1, 10 and 15, and the Control batch at day 5 have not been classified **(Table 1c).**

Figure 2e showed the normalized emission spectra acquired after the excitation wavelength set at 290 nm. The Control, S1 and S2 batches showed maximum intensity located at ~380 and 395 nm. Although the appearance of all the spectra was the same, a shift was observed depending on the recipe and the duration of fermentation. The change that occurred in the tryptophan fluorescence spectra could be due to the modifications in the protein-protein, protein-lipid, and/or protein-water interactions (Viljanen et al.. 2005). Another explanation could arise from the oxidative reactions such as the loss of functional groups due to the degradation of amino acid residues, the modification of side chains, the aggregation and/or the polymerization of the protein. which could decrease the tryptophan fluorescence quantum yield.

The PCA performed on the normalized spectra allowed some discrimination of samples throughout the fermentation stage (data not shown)**.** An overall correct classification of 100% was obtained following the application of the FDA to the first 5 PCs of the PCA (**Table 1c**). All samples were correctly classified (100%). The excitation spectra of the vitamin A scanned

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on fermented dry sausages between 252 and 390 nm with emission wavelength set at 410 nm are shown in **Figure 2f.** These excitation spectra exhibited a maximum located ~288 nm and another one located ~ 322 nm for the Control and S1 batches. These observations are in agreement with previous findings of Karoui et al. (2006) reporting that the maximum fluorescence intensity of vitamin A excitation spectra scanned on different varieties of soft cheese after emission at 410 nm were located at ~322 and 305 nm. The fluorescence intensity at 322 nm increased with the fermentation stage which could be explained by the increase of the viscosity of triglycerides. This could be due to the crystallization of triglyceride during the fermentation stage, in agreement with previous findings of Charlotte, Møller, Andersen and Mortensen (2008). Additionally, the intensity of the sample spectra was drastically affected by time and clove incorporation.

The PCA applied to the normalized spectra allowed clear discrimination of samples according to the recipes and the fermentation stage (data not shown). This trend was confirmed by the FDA since an overall correct classification of 97.22% was obtained (**Table 1c**). All the samples were correctly classified (100% correct classification) during the fermentation period, except the Control batch on day 5 (66.66% correct classification).

CONCLUSION

The results obtained in the present study showed that clove can be used as a natural source of antioxidant, since a negative correlation between the level of clove and that of oxidation of batches S1 and S2 compared to Control batch. The principal component analysis (PCA) applied to the physico-chemical and textural data allowed discrimination between the samples according to the stage of fermentation and the level of clove addition. This trend was confirmed by the FDA, which achieved an overall correct classification rate of 97.22%. Regarding the fluorescence spectra, PCA also allowed a clear discrimination of the samples according to the duration of fermentation and the level of clove addition. The FDA confirmed this trend, since an overall average of 87.03% was obtained for all the fluorophores.

FUTURE RESEARCH

From the findings of this research, further research could be carried out to:

- monitor the quality of sausages during storage in order to extend the duration of monitoring their quality,

- use other analytical methods (gas chromatography coupled with mass spectroscopy, nuclear magnetic resonance, etc.) to identify the antioxidant molecules present in sausages.

- use other, more robust chemometric tools for the scientific exploitation of data, and

Notwithstanding, the findings of this research indicated that clove could be combined with sesame flour and considered as a source of antioxidants in sausages during the fermentation stage.

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Table1: (**a**) list and levels of additives and ingredients used in the manufacture of fermented chicken dry sausages; (**b**) physico-chemical and colorimetric parameters determined on dry fermented chicken sausages during the fermentation stage, and (**a**) Classification table of factorial discriminant analysis (FDA) with leave-one-out cross-validation of fermented chicken dry sausages (Control, S1 and S2 batches) for physicochemical and fluorescence spectra data sets

Table 1a:

Ingredients	Sausages with different formulations		
	Control	S1	S2
Salt (g)	121.88	121.88	121.88
Corn starch (g)	27.00	25.70	24.40
Garlic powder (g)	81.25	81.25	81.25
Black pepper (g)	24.37	24.37	24.37
Sucrose (g)	40.62	40.62	40.62
Chili (g)	24.37	24.37	24.37
Sweet paprika (g)	81.25	81.25	81.25
Traditional maturing ferment 302	4.06	4.06	4.06

C: standards sausages with 80% chicken filet. and 20% veal fat;

S1: sausages with 80% chicken filet. 18% veal fat and 2 % sesame flour;

S2: sausages with 80% chicken filet. 16% veal fat and 4% sesame flour.

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Table 1b:

Fermentation (day)	Control	S1	S2
	Moisture (%)		
1	61.07 ± 0.29[a]	60.01 ± 0.61[b]	60.46 ± 0.87[c]
5	42.07 ± 0.74[a]	43.74 ± 0.37[b]	42.67 ± 0.02[b]
10	27.81 ± 0.20[a]	25.31 ± 0.06[b]	25.23 ± 0.04[b]
15	20.29 ± 2.8[a]	24.51 ± 0.07[b]	25.51 ± 0.01[c]
	Protein (%)		
1	14.82 ± 0.03[a]	15.99 ± 0.09[b]	15.89 ± 0.67[b]
5	16.58 ± 0.04[a]	18.96 ± 0.65[b]	19.22 ± 0.43[C]
10	21.18 ± 0.21[a]	22.18 ± 0.23[a]	22.12 ±1.00[a]
15	30.18 ± 0.12[a]	30.21 ± 0.01[a]	30.23 ± 0.90[a]
	Fat (%)		
1	15.65 ± 1.03[a]	15.23 ± 0.10[a]	15.27 ± 0.07[a]
5	30.50 ± 0.01[a]	28.00 ± 0.09[b]	28.03 ± 0.76[b]
10	29.81 ± 0.05[a]	29.81 ± 0.01[a]	29.22 ± 0.08[a]
	27.10 ± 0.01[a]	27.17 ± 0.04[a]	26.89 ± 0.65[a]
	Aw		
1	0.98 ± 0.65 [a]	0.97 ± 0.78 [a]	0.97 ± 0.06 [a]
5	0.94 ± 0.32 [a]	0.94 ± 0.21 [a]	0.94 ± 1.00 [a]
10	0.89 ± 0.12 [a]	0.89 ± 1.08 [a]	0.88 ± 0.89 [a]
15	0.86 ± 1.09 [a]	0.86 ± 0.79 [a]	0.84 ± 1.08 [a]
	pH		
1	5.35 ± 0.001[a]	5.92 ± 0.02 [b]	5.97 ± 0.23[c]
5	4.48 ± 0.002 [a]	4.34 ± 0.03 [a]	4.55 ± 0.43 [a]
10	5.36 ±0.10 [a]	5.28 ± 0.12 [a]	5.28 ± 0.003 [a]
15	5.32 ± 0.001 [a]	5.34 ± 0.23 [a]	5.55 ± 0.90 [a]
	Thiol		
1	2.58 ± 0.007 [a]	3.03 ± 0.001 [b]	3.04 ± 0.909 [b]

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Fermentation (day)	Control	S1	S2
5	2.88 ± 0.21[a]	3.06 ± 0.43[b]	3.79 ± 0.21[b]
10	2.78 ±0 1.09[a]	3.00 ± 0.72[b]	3.01 ± 0.01[b]
15	2.45 ± 1.21[a]	3.00 ± 1.04[b]	3.00 ± 0.45[b]
	TBARS		
1	0.03 ± 0.03[a]	0.03 ± 1.09[a]	0.03 ± 0.001[a]
5	0.07 ± 0.01[a]	0.04 ± 0.32[b]	0.04 ± 0.01[b]
10	0.07 ± 0.01[a]	0.04 ± 0.03[b]	0.03 ± 0.01[b]
15	0.15 ± 0.33[a]	0.03 ± 0.001[b]	0.02 ± 0.02[b]
	L*		
1	42.97 ± 0.45[a]	40.59 ± 1.09[b]	41.86 ± 0.32[b]
5	38.14 ± 0.21[a]	41.28 ± 0.21[b]	40.87 ± 0.25[b]
10	38.14 ± 0.56[a]	41.28 ± 0.42[b]	41.42 ± 1.90[b]
15	36.83 ± 2.00[a]	39.85 ± 1.07[b]	40.07 ± 0.01[b]
	a*		
1	16.00 ± 0.12[a]	13.82 ± 1.04[b]	13.29 ± 1.08[b]
5	14.68 ± 0.25[a]	13.56 ± 0.60[b]	12.51 ±0.00[b]
10	13.68 ± 1.23[a]	12.56 ± 1.52[b]	12.66 ±0.01[b]
15	13.11 ± 0.43[a]	12.00 ± 1.21[a]	12.03 ± 0.21[a]
	b*		
1	37.48 ± 1.98[a]	33.53 ±1.104[b]	33.44 ± 1.12[b]
5	31.78 ± 0.09[a]	30.42 ± 0.21[b]	29.62 ± 1.09[c]
10	30.62 ± 0.43[a]	29.42 ± 1.53[b]	29.28 ± 1.21[b]
15	27.50 ± 0.54[a]	25.70 ± 2.09[b]	24.90 ± 1.07[b]
	Hardness		
1	1 314.75 ± 194[a]	1 313.75 ± 190[a]	1 323.49 ± 109[a]

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Fermentation (day)	Control	S1	S2
5	1 913.49 ± 153[a]	13205.625 ± 401[b]	13750.4085 ± 46[b]
10	12870.47 ± 255[a]	14010.083 ± 891[b]	14602.016 ± 1729[b]
15	18167.437 ± 1329[a]	16500.2635 ± 1233[b]	17112.4975 ± 141[c]
	Springiness		
1	0.60 ± 0.01[a]	0.60 ± 0.003[a]	0.98 ± 0.001[b]
5	0.60 ± 0.02[a]	0.66 ± 0.003[b]	0.88± 0.002[c]
10	0.624 ± 0.03[a]	0.55 ± 0.020[b]	0.59 ± 0.021[c]
15	0.575 ± 0.00[a]	0.49 ± 0.024[b]	0.644 ±0.01[c]
	Cohesiveness		
1	0.27 ± 0.001[a]	0.27 ± 0.012[a]	0.19 ± 0.012[b]
5	0.30 ± 0.002[a]	0.39 ± 0.012[b]	0.39 ± 0.012[b]
10	0.44 ± 0.010[a]	0.44 ± 0.012[a]	0.45 ± 0.001[b]
15	0.43 ± 0.002[a]	0.43 ± 0.012[a]	0.44 ± 0.001[b]

Means ± standard deviations

Different capital letters (A. B. C) represent statistical difference between the formulations ($p < 0.05$)

Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

Table 1c:

Predicted/ Observation	Control (1 day)	S1 (1 day)	S2 (1 day)	Control (5 days)	S1 (5 days)	S2 (5 days)	Control (10 days)	S1 (10 days)	S2 (10 days)	Control (15 days)	S1 (15 days)	S2 (15 days)	Total	% correct classification
	Physico-chemical measurements													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	3	0	0	0	0	0	0	0	0	3	100.00%

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S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00%
S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	1	0	0	0	0	0	0	0	2	0	0	3	66.67%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	4	3	3	3	3	3	3	3	2	3	3	36	97.22%
	Fluorescence emission spectra acquired after excitation at 250 nm (AAA+AN)													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	2	0	0	0	0	0	0	1	0	3	66.66%
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00%
S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	3	3	2	3	3	3	3	3	3	4	3	36	97.22%
	Fluorescence emission spectra acquired after excitation at 370 nm (Collagen)													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	2	0	1	0	0	0	0	0	0	3	66.66%
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00%
S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	3	3	2	3	4	3	3	3	3	3	3	36	97.22%

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	Fluorescence emission spectra of nicotinamide adenine dinucleotide (NADH) acquired after excitation at 340 nm													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	2	0	0	1	0	0	0	0	0	3	66.66%
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	0.00%
S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	0.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	3	3	2	3	3	4	3	3	3	3	3	36	80.55%
	Fluorescence emission spectra acquired after excitation at 360 nm (fluorescent Maillard Reaction Products (FMRP))													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	0	3	0	0	0	0	0	0	0	0	3	0.00%
Control (5 days)	0	0	3	0	0	0	0	0	0	0	0	0	3	0.00%
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	0.00%
S1 (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	0.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	0.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	0.00%
Total	3	3	3	3	3	3	3	3	3	3	3	3	36	50.00%
	Fluorescence emission spectra acquired after excitation at 290 nm (Tryptophan)													
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	3	0	0	0	0	0	0	0	0	3	100.00%
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00%

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S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	3	3	3	3	3	3	3	3	3	3	3	36	100.00%
Fluorescence emission spectra acquired after emission at 410 nm (Vitamin A)														
Control (1 day)	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00%
S1 (1 day)	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00%
S2 (1 day)	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00%
Control (5 days)	0	0	0	2	0	0	0	1	0	0	0	0	3	66.66
S1 (5 days)	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00%
S2 (5 days)	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00%
Control (10 days)	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00%
S1 (10 days)	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00%
S2 (10 days)	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00%
Control (15 days)	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00%
S1 (15 days)	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00%
S2 (15 days)	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00%
Total	3	3	3	2	3	3	3	4	3	3	3	3	36	97.22%

Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

LIST OF FIGURES

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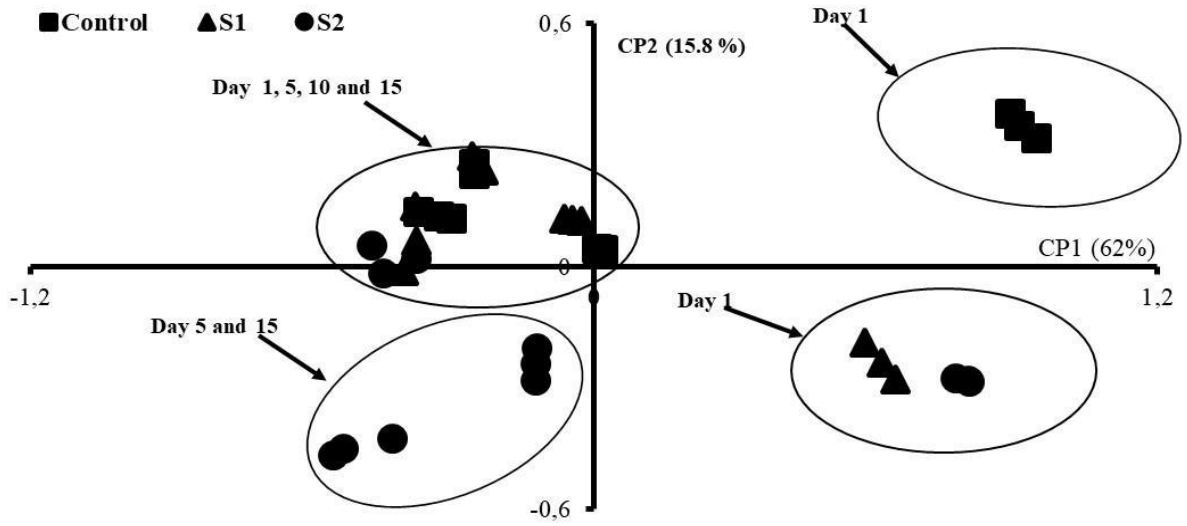
Figure 1:

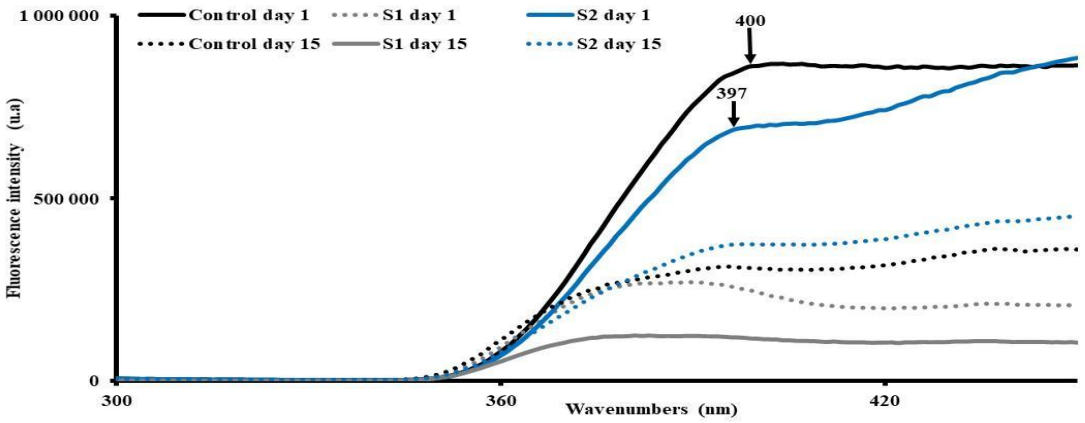


Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

Figure 2a:



Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

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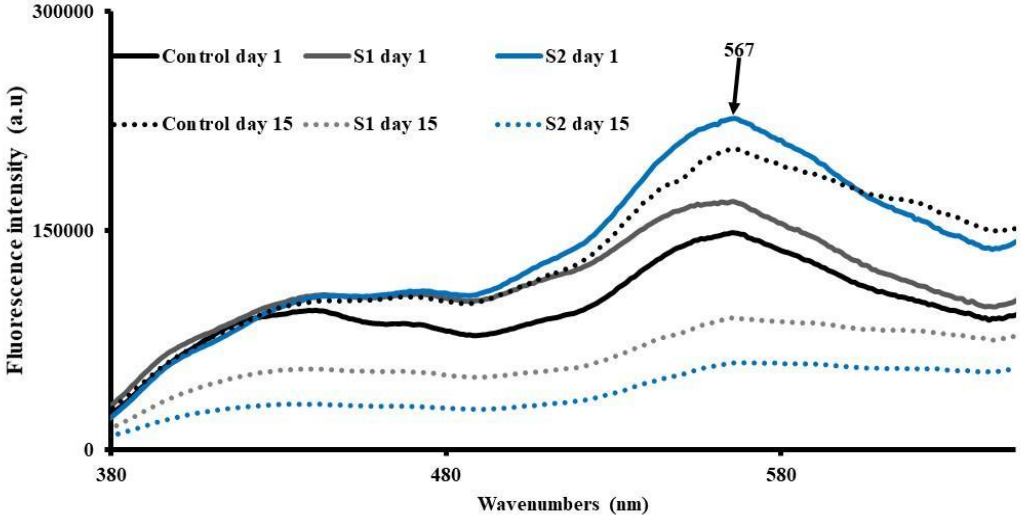
Figure 2b:

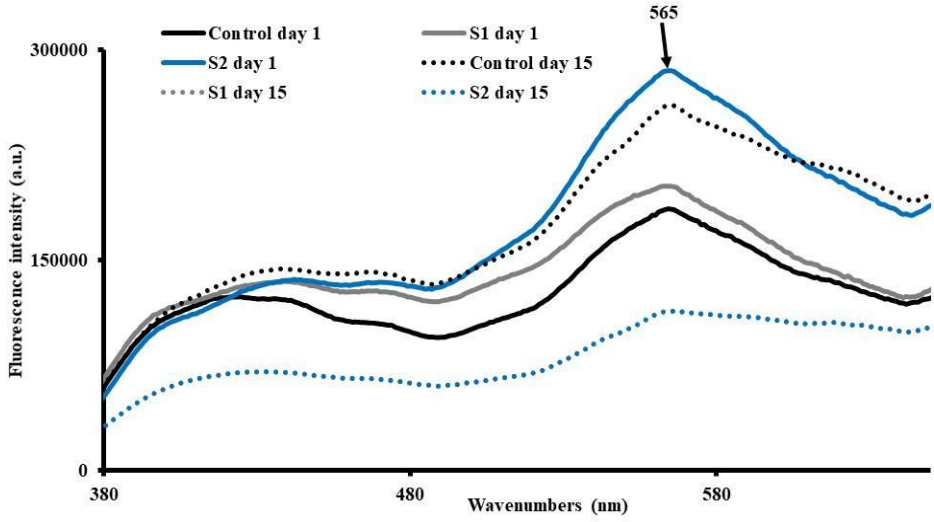


Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

Figure 2c:



Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

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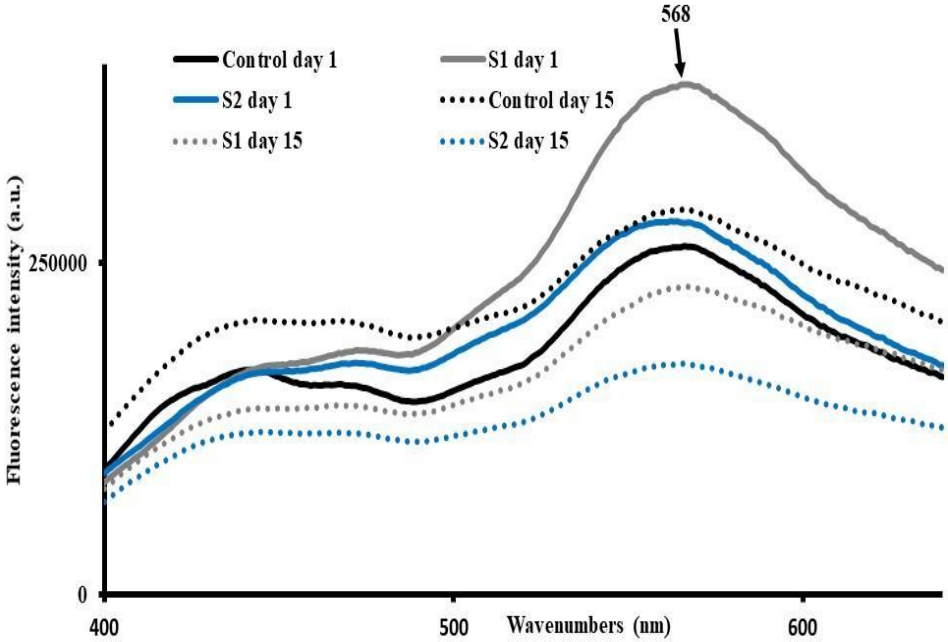
Figure 2d:

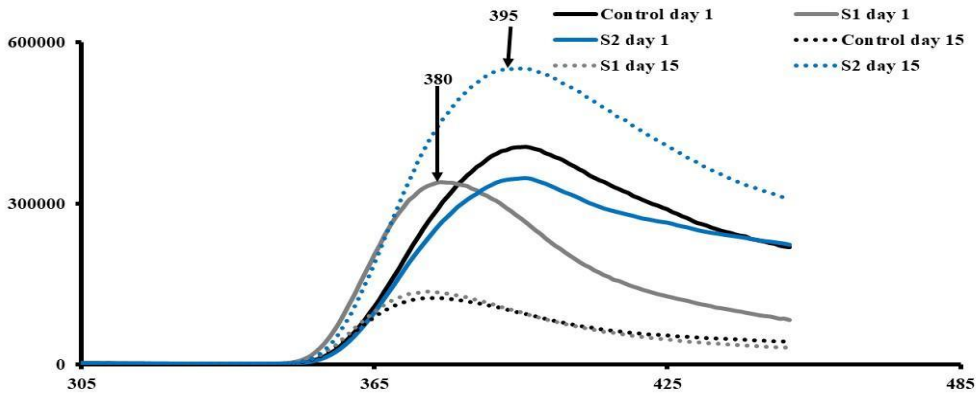


Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

Figure 2e:



Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

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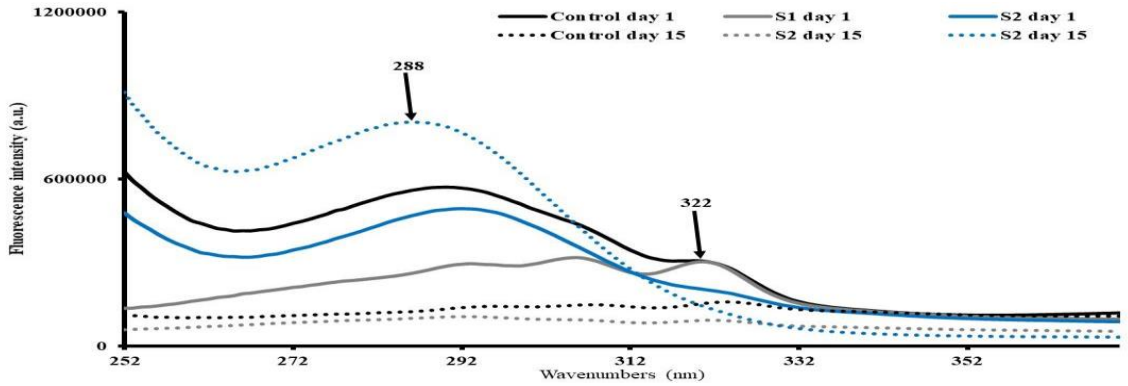
Figure 2f:

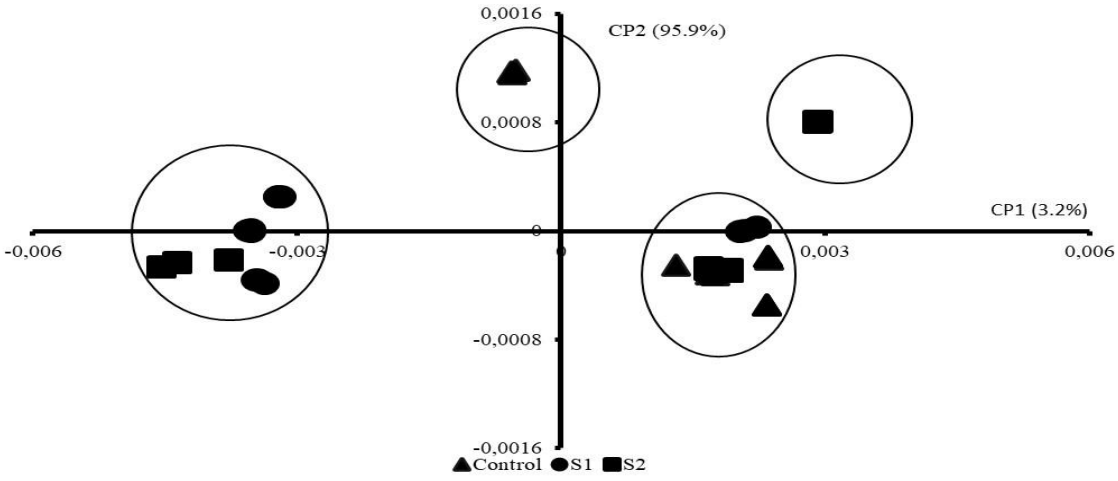


Control: sausages with 80% chicken filet, 18% veal fat and 2% sesame flour;

S1: sausages with 80% chicken filet, 17% veal fat, 2 % sesame flour, and 1% cloves;

S2: sausages with 80% chicken filet, 16% veal fat and 2% sesame flour, and 2% cloves

Figure 2g:



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