Rapid determination of chicken meat ratios in Beef Mixtures and Beef Sausages by Near Infrared Reflectance (NIR) spectroscopy

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ABSTRACT

This study aims to determine the percentage of chicken meat in beef and chicken mixtures, which is the most common form of beef adulteration. Ground beef and beef sausages were prepared with mixtures containing chicken meat, ranging from 0.0% to 100.0% with 5.0% increments, and analyzed using a near-infrared spectroscopy device. Optimal analysis conditions were determined through the examination of a wide range of regression models. The best regression model for ground beef mixtures yielded the following results: RMSEC (Root Mean Square Error of Calibration): 2.35, RMSEV (Root Mean Square Error of Validation): 3.36, R^2C (R-Value Calibration): 0.99, R^2V (R-Value Validation): 0.98. The results for beef sausages were as follows: RMSEC: 2.56, RMSEV: 3.66, R^2C: 0.99, R^2V: 0.98. As a result, the chicken meat content in beef mixtures was detected with a margin of error of 2.05%, while the chicken meat content in beef sausages was detected with a margin of error of 2.12%.

Introduction

Meat and meat products are highly sought after by consumers worldwide due to their high bioavailability, essential amino acids, and vital nutrients such as iron and B_{12}, as well as their unique taste. However, they are often less accessible than plant-based foods with similar protein content (7). Consequently, meat and meat products may be mixed with meats from different species, making them vulnerable to adulteration (6), a problem that is not limited to developing countries but also occurs in developed countries (34). The media coverage of food adulteration has created a public opinion that food needs to be analyzed more frequently and easily (28). Infrared spectrocopy used in this study, is one method for rapidly analyzing food (33).

The present study focuses on near-infrared radiation (NIR), a segment of electromagnetic radiation with wavelengths between 800 nm and 2500 nm. In the context of food analysis, NIR has demonstrated the ability to interact with the bonds linking carbon, hydrogen, oxygen, and nitrogen atoms, where these bonds store energy similar to a spring with a mass attached to the end. Thus, NIR is a promising method for food analysis owing to its capability to provide accurate and rapid results (33).

In the field of near-infrared spectroscopy, a significant focus lies on the six types of interatomic bond vibrations that can be categorized into two groups. The first group includes symmetrical and asymmetrical stretching vibrations, while the second group comprises four bending vibrations. Specifically, two of these bending

vibrations - scissoring and rocking - occur within the same plane, while the remaining two vibrations - wagging and twisting - manifest in a different plane (1).

Infrared spectroscopy has been applied to food analysis since its inception, with the Department of Agriculture of the United States (USDA) publishing the earliest known work on the subject in 1949 (26). A significant step forward was made with the publication of the first quantitative study in 1962, which employed the technique to determine seed moisture via methanol extracts (16). The routine analysis of wheat protein using infrared spectroscopy was later adopted by the U.S. Federal Grain Inspection Service (FGIS) during the 1980s, and it has since become a widely adopted method (14).

Infrared spectroscopy has become a ubiquitous technique in the meat industry for the rapid quantification of fat, moisture, and protein contents in meat and meat products (3). In the present investigation, an adapted version of this established approach was employed to ascertain the extent of beef adulteration with chicken.

In 2013, the regulation on the inclusion of poultry meat in red meat products (e.g., salami, sausages, etc.) was revised, prohibiting any such mixtures (30). This may be attributed to the impracticality of determining the proportion of poultry meat in red meat. Although the literature reports the use of Real-time PCR to quantify this ratio (23), it has not been implemented in practice. The current study presents a modified version of a standard infrared spectroscopy method, which enables rapid detection of chicken meat - one of the most commonly used adulterants - in beef, potentially offering a practical solution to this challenge.

In the scientific literature, only one study has been reported on determining the ratios of different species of meat added to cooked meat products using infrared spectroscopy (13). Hence, this study is one of the few investigations in this field, which aims to detect various types of animal meats added to heat-treated meat products by infrared spectroscopy. Previous literature on the detection of meat of different animal types added to beef by infrared spectroscopy (8, 11, 12, 21, 25, 29, 31, 32) demonstrates that these studies were conducted with laboratory equipment that is economically more costly than the device employed in this study. The percentage range of the prepared samples in those studies was limited, and the sample preparation process was laborious, particularly when using FTIR spectroscopy. The error margins reported in those studies were higher than those observed in this study. Therefore, the present study aims to address the above-mentioned issues related to detecting adulteration in beef using infrared spectroscopy.

Materials and Methods

Preparation of meat mixtures: Ground beef (modified atmosphere packaged, max fat content 20% [m/m], max collagen to protein ratio 15% [m/m]) and chicken breast (without skin) produced according to the Turkish Food Codex: Meat, Prepared Meat Mixtures and Meat Products Communiqué (30) were supplied from Meat and Milk Board's Ankara store. Meat mixtures were prepared from 0% chicken to 100% chicken in 5% increments. Therefore a total of 21 meat mixtures were prepared to contain 100.0%, 95.0%, 90.0%, 85.0%, 80.0%, 75.0%, 70.0%, 65.0%, 60.0%, 55.0%, 50.0%, 45.0%, 40.0%, 35.0%, 30.0%, 25.0%, 20.0%, 15.0%, 10.0%, 5.0%, and 0.0% ground beef, and chicken breast vice-versa. All mixtures were prepared with an accuracy of ±0.50g. Mixtures were homogenized using a food processor (600 W) for about two minutes. After the preparation of each mixture, the food processor was cleaned with a degreaser and dried with paper towels to avoid leaving any fat residue. Pre-analysis images of mixtures containing 100.0% beef, 50.0% beef – 50.0% chicken, and 100.0% chicken are presented in Figure 1.

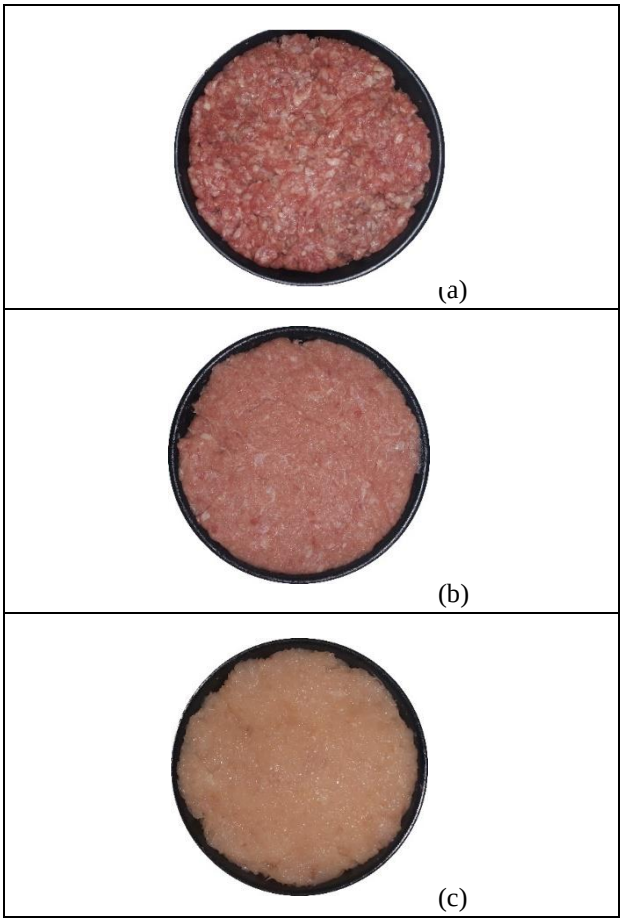

Figure 1. Pre-analyzed views of meat mixtures (a): %100.0 beef, (b): %50.0 beef, %50.0 chicken, (c): %100.0 chicken.

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Preparation of mixed sausages: The same meats and the same proportions used in the preparation of mixed meats were also used in the preparation of mixed sausages. Sausages were prepared according to the formula of: 20.0% ice, 5.0% starch (cornstarch), 3.5% salt (NaCl), 3.0% spice mixture (red sweet pepper, red chili pepper, thyme, black pepper, white pepper, paprika, garlic powder, onion powder, fennel, basil, sage, mustard, cumin, coriander, ginger), 0.25% polyphosphate (P_2O_5), 0.10% ascorbic acid (E300), 0.035% sodium nitrite (E250), [all proportions are m/m, modified from (24)]. The sausage mixes were emulsified and then filled into synthetic sausage cases. The sausages were then dried and pre-cooked in hot air at 60.0°C for 15 minutes. Then they were boil-cooked at 80.0°C until their core temperature reached 72.0°C. Their cooking finished as their core temperature was kept at 72.0°C for 15 minutes in the oven. Cooked sausages were then showered with cold water (approx. 15.0°C-17.0°C). When their core temperature dropped to room temperature, they were transferred to the cold room which was set to +4.0°C. They were kept in a cold chain until their analysis. The sausages were analyzed under the same conditions as the meat mixtures. Cross-sections of sausages made from 100.0% bef, 50.0% beef – 50.0% chicken meat, and 100.0% chicken are shown in Figure 2.

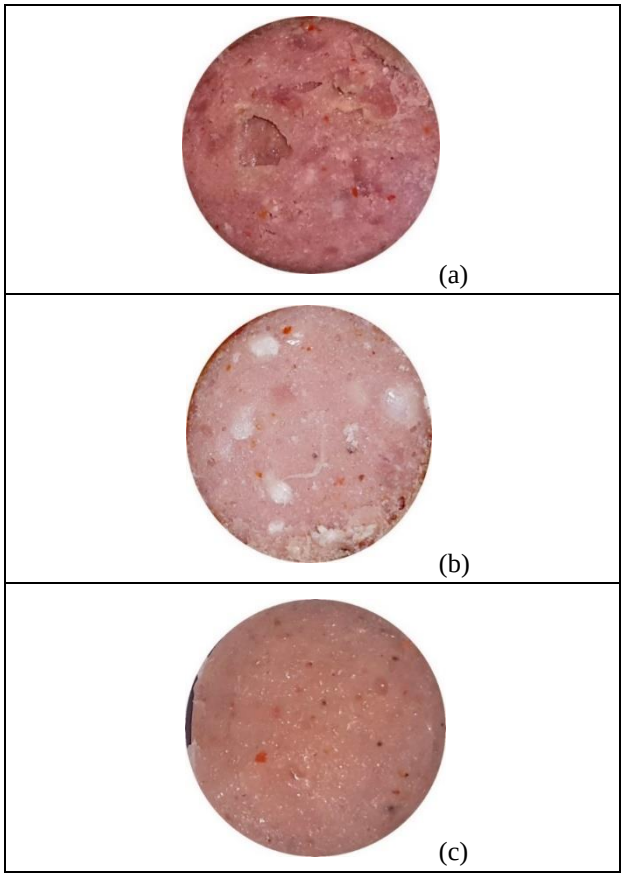

Figure 2. Cross-sections of mixed sausages (a):100.0 beef, (b): %50.0 beef, %50.0 chicken, (c)%100.0 chicken.

Obtaining near-infrared spectrums: Near-infrared reflectance (NIRS) spectra were obtained via NIR Spectrometer (Perten DA 7250, Perkin Elmer, United States of America; wavelength range: 950 nm – 1650 nm; wavelength sensitivity: better than 0.05 nm). The device has two different sample containers (500 ml and 150 ml), and both of them rotate 360° around their x-axis during the analysis. The analysis of the mixtures was performed twice in both large and small containers and the spectrums were collected at a resolution of 0.5 nm, 1 nm, 2 nm, and 5 nm. The analyses have been performed in so different ways to determine the optimal analysis conditions.

Moisture, protein, fat, collagen, ash, salt, and pH analyses: All these results were obtained using the device's (Perten DA 7250) own built-in calibration models with two significant numbers (10, 17, 27).

Statistical analysis of infrared spectrums: The obtained infrared spectra was analyzed using The Unscrambler® X program (Version 10.4, 32 bits). Spectra were examined with and without several pre-processing methods. The pre-processing methods to be applied and the calibration models to be established were chosen from (4, 15, 19, 21, 22). Normalization (Area Normalization), Saviztky – Golay Derivative (First Derivative), Gaussian Filter (Standart Deviation 2), and Multiplicative Scatter Correction methods were used for pre-processing. Then, partial least squares regression (PLSR) models were constructed from pre-processed and non-pre-processed spectra. When constructing regression models, predicted values were verified with full cross-validation.

A total of 180 statistical models were constructed for each infrared radiation resolution (0.5 nm, 1 nm, 2 nm, and 5 nm), whether the spectra were pre-processed or not, for the sample container used (large, small, or both), and for the sample's analysis count (once, twice or using both). All generated models were examined for RMSEC (Root Mean Square Error of Calibration), RMSEV (Root Mean Square Error of Validation), Slope (calibration), Slope (validation), R^2 (calibration), and R^2 (validation) values. In addition, the average error values of the estimates of the 10 models with the highest R^2 (validation) values were calculated according to the following formula:

$$Average\ margin\ of\ error\ (\%) = \frac{\sum_{i=1}^{n}|x|}{n}$$

Where:

$|x|$ = absolute value of the error rate of each predicted value (%)

n = total number of analyses.

All models with similar characteristics were then compared and the best-performing model with the lowest average margin of error was determined. It was found that

the best-performing model is achieved by scanning once using the large sample container, using the spectra with a resolution of 5 nm, and pre-processing the spectra with the normalization method. Therefore, the design of this model was chosen as optimal conditions, and the parameters in this model were used in the analysis of mixed sausages.

Results

Composition analysis of meat mixtures with varied proportions of beef and chicken: The moisture, protein, fat, collagen, ash, salt ratios, and pHs of meat mixtures are given in Table 1. Table 1 shows that as the proportion of chicken meat in the meat mixtures increased; the amount of moisture, protein, and collagen-free protein also increases, but on the other hand, the percentage of fat decreases. A graphical representation of the moisture, fat, protein, and collagen-free protein values of meat mixtures along with the chicken meat ratios is presented in Figure 3 (a, b, c, d).

Table 2 (MM) shows that chicken meats added to beef meats could be detected with an average of 2.05% margin of error. In addition, the values showing the statistical success of the model are RMSEC: 2.35, RMSEV: 3.36, Slope (calibration): 0.99, Slope (validation): 0.96, R^2 (calibration): 0.99, R^2 (validation): 0.98.

Among the wavelengths of infrared radiation used in the creation of the model, the wavelengths that contributed the most to the regression model were found to be 950 nm, 1140 nm (±20 nm), 1210 nm (±25 nm), and 1310 nm (±35 nm).

Composition analysis of mixed sausages with varied proportions of beef and chicken: The analysis of the mixed sausages was carried out according to the parameters in the optimal model (spectrum obtained at 5 nm resolution, analyzed only once in the large analysis tray, and pre-processed with normalization).

Moisture, protein, fat, collagen-free protein, ash, salt ratio,s, and pH values of mixed sausages are presented in Table 3. As shown in Table 3, as the chicken ratio increased in the mixed sausages, the moisture, protein, and collagen-free protein ratios increased; the fat ratio decreased. Graphical representations of these ratios are presented in Figure 3 (e, f, g, h).

Average error rates of predictions of beef ratios in mixed sausages are presented in Table 2 (MS). Table 2 (MS) shows that the estimation rate of beef in mixed sausages was found to be seven per ten-thousand percent worse than the estimated rate of meat mixtures'.

Indicators of the success of the statistical model in predicting the meat content of mixed sausages are found as RMSEC: 2,56, RMSEV: 3.66, Slope (calibration): 0.99, Slope (validation): 0.97, R^2 (calibration): 0.99, R^2 (validation): 0.98. While creating the regression model, the wavelengths that contributed the most to the model were found to be 1160 nm (±30 nm), 1210 nm (±15 nm), and 1390 nm (±10nm).

Table 1. Meat mixtures' moisture, protein, fat, collagen, ash, salt ratios and pH values.

Beef Ratio %	Chicken Ratio %	Moisture %	Protein %	Fat %	Collagen %	Ash %	Salt %	pH
100.0	0.0	63.6	18.6	16.4	1.75	1.32	0.35	5.59
95.0	5.0	65.0	19.0	15.1	2.50	1.19	0.28	5.56
90.0	10.0	65.3	19.0	14.7	2.38	1.04	0.32	5.54
85.0	15.0	65.7	19.2	14.0	1.96	1.09	0.45	5.52
80.0	20.0	65.5	19.3	14.3	2.28	1.48	0.31	5.7
75.0	25.0	65.9	19.2	13.9	2.51	1.19	0.07	5.56
70.0	30.0	67.1	19.1	12.0	2.16	1.97	0.32	5.63
65.0	35.0	66.2	19.1	13.0	2.16	2.23	0.29	5.69
60.0	40.0	67.8	19.6	10.7	1.83	1.1	0.30	5.57
55.0	45.0	67.2	19.4	11.7	1.66	1.17	0.29	5.61
50.0	50.0	68.6	19.2	10.6	1.68	1.84	0.19	5.73
45.0	55.0	70.1	19.1	9.26	1.72	1.22	0.35	5.62
40.0	60.0	72.2	19.4	8.02	1.34	1.06	0.22	5.77
35.0	65.0	70.9	18.9	8.46	-0.25	1.21	0.50	5.58
30.0	70.0	74.1	19.2	6.15	1.37	1.39	0.37	5.77
25.0	75.0	73.6	19.0	5.18	0.75	1.45	0.45	5.77
20.0	80.0	73.5	19.5	4.40	1.01	1.33	0.40	5.79
15.0	85.0	74.0	20.6	4.07	0.16	1.39	0.45	5.55
10.0	90.0	75.1	20.5	3.03	-0.04	1.51	0.81	5.49
5.0	95.0	74.4	20.8	3.18	0.46	1.41	0.85	5.55
0.0	100.0	76.3	20.6	1.06	-0.72	1.85	1.16	5.45

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Table 2. Average error margin of the regression model for meat mixtures (MM) and mixed sausages (MS).

Beef Ratio (%)	Predicted (MM) (%)	Difference (MM) (%)	Predicted (MS) (%)	Difference (MS) (%)
100.0	99.6	0.33	98.1	1.86
95.0	89.5	5.42	94.7	0.29
90.0	92.3	2.35	85.0	4.98
85.0	84.3	0.67	85.8	0.85
80.0	76.7	3.22	78.8	1.16
75.0	75.8	0.88	76.1	1.13
70.0	69.9	0.02	72.6	2.63
65.0	63.6	1.33	68.1	3.13
60.0	58.6	1.36	62.4	2.44
55.0	58.8	3.89	50.8	4.21
50.0	52.3	2.30	52.4	2.39
45.0	48.8	3.88	49.2	4.17
40.0	39.6	0.39	42.1	2.15
35.0	37.2	2.28	36.6	1.58
30.0	27.6	2.36	25.9	4.05
25.0	23.2	1.73	24.5	0.52
20.0	17.1	2.85	18.8	1.21
15.0	12.7	2.25	13.6	1.42
10.0	6.72	3.27	12.9	2.87
5.0	3.85	1.14	5.69	0.69
0.0	1.15	1.15	0.89	0.89
Average Error (%) :		**2.05 (MM)**	Average Error (%) :	**2.12 (MS)**

Table 3. Mixed sausages' moisture, protein, fat, collagen-free protein, ash, salt ratios and pH values.

Beef Ratio %	Chicken Ratio %	Moisture %	Protein %	Fat %	Collage-Free Protein %	Ash %	Salt %	pH
100	0	57	18.82	18.74	14.19	2.88	2.13	5.86
95	5	58.26	18.47	18.78	14.34	2.7	2.18	5.84
90	10	59.92	18.86	17.43	14.75	2.96	2.14	5.91
85	15	58.28	19.28	18.03	14.59	2.85	2.19	5.86
80	20	58.06	19.96	17.37	15.12	3.06	2.09	5.88
75	25	60.27	21.58	15.38	16.26	3.16	2.1	5.93
70	30	59.92	21.22	15.51	16.73	3.04	2.16	5.9
65	35	61.43	20.88	13.06	15.92	2.74	2.03	5.94
60	40	61.3	21.12	13.58	17.05	3.13	2.02	5.92
55	45	61.13	20.24	12.15	17.43	2.99	2.23	5.9
50	50	60.13	21.57	11.67	20.06	3.21	2.3	5.9
45	55	59.11	21.77	10.37	18.79	3.09	2.22	5.94
40	60	60.09	21.33	9.01	19.9	2.99	2.25	5.98
35	65	59.48	21.87	8.62	20.59	3.07	2.03	5.95
30	70	59.94	23.53	7.54	21.36	3.21	2.15	5.96
25	75	61.63	24.4	6.79	21.76	3.3	2.21	6
20	80	62.42	24.03	5.95	22.3	3.36	2.3	6.01
15	85	64.02	25.04	4.61	22.88	3.77	2.16	6.07
10	90	64.98	24.3	4.23	22.34	3.16	2.13	6.07
5	95	64.46	24.96	3.41	23.82	3.66	2.33	6.05
0	100	65.35	24.48	2.23	23.96	3.4	2.08	6.06

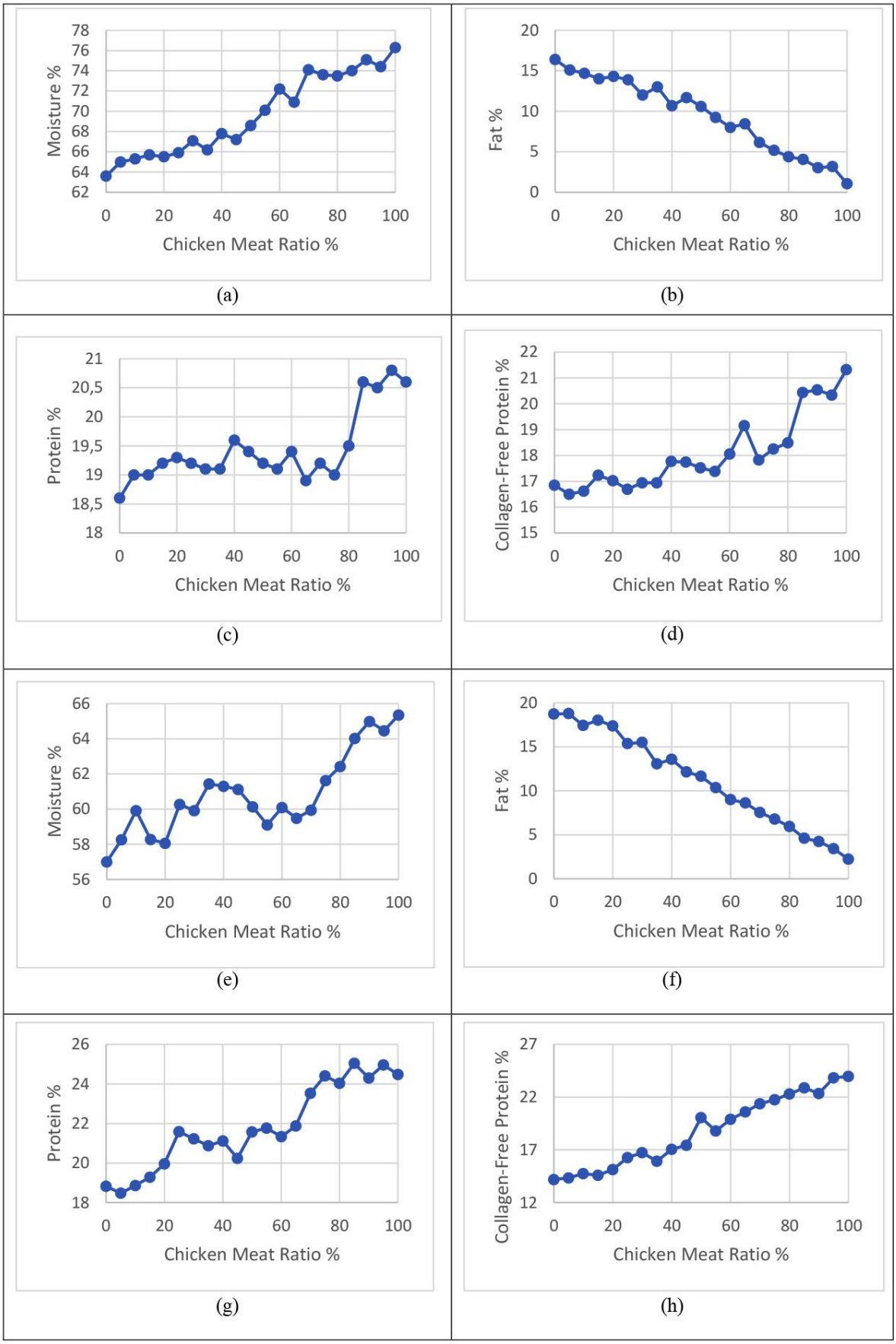

Figure 3. Graphical representations of moisture, fat, protein and collagen-free protein ratios of meat mixtures (a, b, c, d) and mixed sausages (e, f, g, h).

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Discussion and Conclusion

This study demonstrates that near-infrared spectroscopy can detect chicken added to ground beef and chicken added to beef sausages with a precision of 2.05% and 2.12%, respectively. The results indicate that the higher error rate of mixed sausages, with a discrepancy of 0.07%, is due to the presence of diverse spices and additives incorporated in the sausage formulation and the alterations that occur during heat processing. The infrared spectra of spices and additives differ from those of beef and chicken, resulting in the development of various compounds with distinctive infrared spectra during heat treatment.

Ding and Xu (13) have previously demonstrated the ability to detect adulterated beef hamburgers using infrared radiation in the 400–2500 nm region, with a resolution of 2 nm. Their study involved the preparation of adulterated hamburgers by substituting beef with minced pork and mutton at 5%, 10%, and 25% (m/m) levels, and collecting infrared spectrums from both raw and cooked samples. The R^2 values obtained in their study for mutton and pork were relatively low, at 0.87 (raw), 0.79 (cooked), 0.84 (raw), and 0.74 (cooked), respectively. In our study, we were able to achieve higher R^2 values (0.98) for both adulterated ground beef and adulterated beef sausages. The differences in infrared radiation range, meat types, sample sizes, and adulteration rates employed may account for the differences in R^2 values between the two studies.

In a previous study by Restaino et al. (31), beef and pork patés were analyzed using infrared radiation in the 1100-2500 nm region with a resolution of 2 nm in reflectance mode. While the individual beef and pork paté samples were accurately classified at 100%, binary mixtures received only 72% correct classification. Despite paté being one of the most homogenized meat products, the low classification rate was attributed to the statistical model used, namely Stepwise Linear Discriminant Analysis (SLDA). In contrast, our study employed the PLSR model and was able to achieve 100% classification accuracy for all mixtures. This suggests that the PLSR model provides superior results compared to SLDA.

The adulteration of beef with horse meat was studied by Boyacı et al. (8) using Raman spectroscopy, a variant of infrared spectroscopy. In this study, Raman spectra were obtained using a laser and infrared radiation with 200-2000 cm^{-1} wavenumber at a $2cm^{-1}$ resolution. Adulterated samples were prepared by adding horse meat to beef at ratios of 0%, 25%, 50%, 75%, and 100% by weight, and all adulterated samples were accurately classified. However, the analysis of samples using Raman spectroscopy requires long and laborious preparation steps, such as extracting the fat with hexane and centrifugation. In contrast, in our study, sample preparation only involved homogenization in a food processor for approximately two minutes, with an analysis time of approximately six seconds using NIRS. Therefore, we concluded that the NIRS setting we used in our study is more easily applicable than Raman spectroscopy for detecting food adulteration.

The study conducted by Nolasco-Perez et al. (25) aimed to compare the effectiveness of portable NIR and NIR + hyperspectral imaging (NIR-HSI) systems in detecting beef adulterated with chicken. Adulterated samples were prepared by varying the chicken content from 0% to 50% in 2% increments (w/w). The portable NIR system showed R^2 values of 0.93 (calibration) and 0.7 (validation), while the NIR-HSI system showed R^2 values of 0.98 (calibration) and 0.94 (validation). Our study, using the NIR system, produced better results with R^2 values of 0.99 (calibration) and 0.98 (validation). We attribute this to the sample size and range of adulteration rates used in our study, which outperformed the more advanced NIR-HSI system.

This study is distinctive from previous researches mentioned above, because it utilizes a cost-effective infrared spectroscopy instrument and a simple sample preparation technique. The prepared samples used in this study are 500g, which provides a more precise representation of complex meat samples. Moreover, the margin of error in this study is considerably low.

The regression model used in this study revealed that the infrared radiation with a wavelength of 950 nm was particularly significant in distinguishing mixed beef and chicken meats. This wavelength is known to interact mainly with O-H bonds, which are influenced by the water ratios in the meats. Moreover, infrared radiation with wavelengths of 1140 nm, 1210 nm, 1310 nm, and 1160 nm, 1201 nm, 1390 nm, were found to be important in identifying chicken meat in beef mixtures and mixed sausages, respectively. These wavelengths are known to interact primarily with C-H bonds, which are influenced by the proportions of fat, chromophores, and hydrocarbons in the different meats (2, 5, 9, 18, 20, 35).

In this study, the efficacy of NIRS in detecting chicken meat in ground beef and beef sausages was investigated, and accuracy rates of 2.05% and 2.12%, respectively, were achieved. This suggests that NIRS could be a viable candidate for a rapid and straightforward method of detecting adulteration in these meat products, as well as in other beef products. Moreover, as the cost of food continues to rise, there is a growing demand for affordable and high-quality protein alternatives, which may include blends of beef and chicken meat. To ensure the quality and ratio of such blends, NIRS could potentially provide an effective means of analysis.

For NIRS to gain acceptance as a reliable analysis method for determining the ratio of meat mixtures, further

research is required using whole chicken carcasses and mechanically separated meats (MSM). Moreover, in order to use NIRS in detecting the ratio of beef products such as beef sausages, salami, etc., calibration models incorporating common ingredients of such processed meats, including milk proteins, soy proteins, starch, vegetable oils, food additives, and dyes, need to be developed.

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Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

BT and ÖK designed the experiments. BT prepared the samples under the supervision of ÖK and carried out experiments. Both authors interpreted the results. BT took the lead in writing the manuscript. Both authors provided critical feedback and helped shape the research, analysis and manuscript.

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