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NON-TARGETED ANALYSIS OF VOCS BY HS-SPME-GC/MS COUPLED WITH CHEMOMETRICS AS A POTENTIAL TOOL FOR AUTHENTICATION OF WHITE KOŁUDA OAT GOOSE*

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Abstract

This study tested the possibility of using non-targeted analysis of volatile organic compounds by headspace solid-phase microextraction–gas chromatography–mass spectrometry coupled with chemometrics as a potential tool for differentiating leg meat of oat- and wheat-fed (*ad libitum*) White Kołuda geese. Thirty-six classification models were obtained for which the correct classification rate and classification accuracy for oat-fed and wheat-fed geese were calculated based on a seven-fold cross-validation. Generally, the most advantageous method of the sample preparation was the high-temperature heat treatment version, whereas the highest correct classification rate was obtained when the chemometric analysis was carried out on the female, then male, and finally male + female variant of group comparisons (P<0.01). Furthermore, log-transformation appeared to be a slightly better data preprocessing technique in comparison to systematic ratio normalization. The obtained classification models can potentially differentiate the meat of oat-fattened from wheat-fattened White Kołuda geese.

Key words: White Kołuda goose, food authentication, volatile organic compounds, chemometrics, Fisher ratio, systematic ratio normalization

Nowadays, Poland, next to Hungary, is one of the leading producers of goose meat in Europe (Buzała et al., 2014; Biesiada-Drzazga, 2014). The production of goose is based on the genotype of the White Kołuda goose, which was developed as a result of breeding work carried out over several dozen years at the National Research Institute of Animal Production at the Experimental Station in Kołuda Wielka. The White Kołuda goose, suitably fed on oats, obtained a word and figurative trademark in 2001, registered in the Patent Office of the Republic of Poland. This is so-called the White Kołuda oat goose. The geese are farmed in natural conditions so as to maintain high welfare standards – they are reared semi-intensively, with easy access to pasture, and fattened usually up to 16 or 17 weeks of age, and are given only water and fattened with a whole oat grain (*ad libitum*) at the end of the rearing period. Such fattening results in good quality meat, rich in numerous aroma compounds (Gąsior et al., 2021 b) and with excellent sensory properties (Buzała et al., 2014). However, for the goose to be called White Kołuda oat goose, the fattening must include chicks from White Kołuda goose farms, which should be fattened only with oat grain in the last feeding period. Meanwhile, doubts are being raised by the increasingly frequent replacement of oat grains with other grains, (including wheat grain) which is taking place among a growing number of goose producers. Therefore, there was a need to verify the possibility of confirming the authenticity of the oat White Kołuda goose in comparison with a goose fed with other grains (e.g. wheat).

The influence of various factors on the volatile organic compounds (VOCs) profile makes their analysis a potential tool for assessing the authenticity of a product. Elmore et al. (2000), Nuernberg et al. (2005) and Scollan et al. (2006) showed that the composition and amount of VOCs depend, among others, on the method of animal feeding. Other factors, such as the smoking of meat (Merlo et al., 2021; Kosowska et al., 2018; Nowicka et al., 2017), degree of maturity (Stetzer et al., 2008), pH (Cerny and Briffod, 2007), the species of the animal (Calkins and Hodgen, 2007) and even weight at slaughter (Insausti et al., 2005) also influence the volatile compounds composition in meat. It is possible to find many works in the literature presenting the possibilities of applying various techniques for analysis of volatile compounds in food samples. Authors use both one-dimensional (Wang et al., 2021; Monahan et al., 2018; Majcher et al., 2015; Lv et al., 2015, 2014) and two-dimensional gas chromatography–mass spectrometry (Giri et al., 2015; Cordero et al., 2010, 2008). However, the one-dimensional gas chromatography combined with solid-phase microextraction (SPME) technique for VOCs

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isolation and concentration in SPME fiber meets the conditions of an inexpensive and relatively easy method to implement in a laboratory. Thus, this is the method usually used, both for identification, using a series of standard n-alkanes for calculating retention indices (Gąsior et al., 2021 a; García-Béjar et al., 2020; Liu et al., 2019; Nowicka et al., 2017; Ba et al., 2010; Soncin et al., 2007) and for semi-quantitation (Bennato et al., 2021; Gąsior et al., 2021 b; García-Béjar et al., 2020; Liu et al., 2019) or quantitation of volatile compounds (Liu et al., 2019). Perhaps this method could also be potentially used as a fingerprinting method (Majcher et al., 2015; Kiefl et al., 2012; Cordero et al., 2010) to confirm the authenticity of the White Kołuda oat goose. Authentication is a broad topic that requires the implementation of chemometric techniques for processing the obtained results, including the Fisher ratio method (Prebihalo et al., 2020), principal component analysis (PCA, Majcher et al., 2015; Lv et al., 2015, 2014), linear discriminant analysis (LDA, Majcher et al., 2015), or systematic ratio normalization (SRN, Lehallier et al., 2012). The chemometric approach can be used to differentiate food products (Majcher et al., 2015, 2010; Lv et al., 2015; Narváez-Rivas et al., 2011; Stanimirova et al., 2010).

Therefore, in this study we propose a quantitative non-targeted analysis of VOCs by headspace solid-phase microextraction–gas chromatography–mass spectrometry, i.e., HS-SPME-GC/MS (Majcher et al., 2015, Cordero et al., 2010; Kiefl et al., 2012) coupled with chemometrics as a potential tool for differentiating meat of the oat- and wheat-fed White Kołuda geese. We also study three different factors influencing the discriminatory power of classification models which might help in selecting the optimal chemometric patterns to distinguish the animal groups investigated.

Material and methods

Animal experiment

The research included two identical experiments carried out for two consecutive years. The experimental material consisted of 160 birds (geese and ganders) of the White Kołuda breed reared for 16 weeks in accordance with the fattening technology developed at the Experimental Station of the National Research Institute of Animal Production in Kołuda Wielka. Until the age of 13 weeks, the birds were fed the same way. At that time, they received feed mixtures KB-1 (geese fed at 1–4 weeks of age), KB-2 (geese fed at 5–9 weeks of age), KB-3 (geese fed at 10–13 weeks of age), mowed green forage and had access to pasture. The feed mixtures (11.26, 11.24 and 11.0, MJ/kg, for KB-1, KB-2 and KB-3, respectively) comprised maize, wheat, barley (65–68%), wheat bran, soybean meal and sunflower meal (29–31%), as well as supplements of minerals, vitamins and amino acids (3–4%). At the beginning of the 14th week of life, the geese were randomly assigned to two feeding groups, the first of which were fattened up to 16 weeks of age with whole grain oats and the second with whole grain wheat. In this last period, the birds were given only these grains and water (both *ad libitum*). During the rearing period, the body weight of each goose was monitored and 80 birds were selected for slaughter (on average, males 7.1 kg, SD = 0.55 and females 6.2 kg, SD = 0.38) so that the coefficient of variation for this parameter did not exceed 15%. As a result, 20 males and 20 females fed on oats, and 20 males and 20 females fed on wheat were used for the chemical research. After slaughter and removal of feathers, the goose carcasses were chilled (+5°C) for 24 hours, and then the leg muscles were sampled for VOCs analysis. The analyses were conducted in the given year of the experiment.

Meat samples

A leg muscle (approximately 300 g) was taken from each bird and cut into smaller pieces (approximately 2 × 2 cm). Next, the samples were vacuum-packed into polyamide/polyethylene bags, and stored at –80°C for no more than 2 months. The still unthawed meat was blended at a low temperature using liquid nitrogen, and next, a 10 g sample was transferred into a 20 ml headspace vial sealed with an aluminum crimp cap and PTFE/silicon septum. The prior portioning of the sample into small pieces and using the liquid nitrogen was very relevant since it allowed the material to be kept frozen until the analysis started (Gąsior et al., 2021 a).

Versions of meat heat treatment

Three versions of the samples were prepared for the tests: meat without heat treatment (raw meat), low-temperature heat treatment (30°C, 24 h), high-temperature heat treatment (170°C, 35 min). Thermal treatment was carried out in sealed SPME vials followed by immediate chromatographic analysis (Ba et al., 2010), which ensured the repeatability of the process in the all analyzed samples.

Analysis of VOCs

Volatiles were isolated by headspace solid-phase microextraction using 50/30μm DVB/CAR/PDMS fiber (Supelco, Merck group, Poznań, Poland) and analyzed by a GCMS-QP 2010 Plus gas chromatograph mass spectrometer (Shimadzu, Duisburg, Germany). A semi-polar column (Gąsior et al., 2021 a; Węsierska et al., 2021; Wang et al., 2021; Rivas-Cañedo et al., 2013; Vasta et al., 2010; Moon and Li-Chan, 2004) – Zebron ZB-5MSi 30 m × 0.25 mm, 0.25 μm column (Phenomenex; Shim-Pol, Izabelin, Poland) – was used for volatiles separation. The sample was equilibrated at 50°C for 30 min and next injected by SPME needle. The fiber exposition time was 15 min at 50°C and then VOCs were desorbed in the splitless port at 240°C for 2 min. The injection was performed using the AOC-5000 SPME autosampler Combi Pal System (Shimadzu, Duisburg, Germany). The GC-MS operating conditions were as follows: helium flow

(He, purity, 99.999; Linde Gaz Polska, Kraków, Poland) 1 ml min^{-1}, oven temperature 37°C (10 min), then increased to 132°C (4°C/min) and 240°C (8°C/min). The total time of the analysis was 60 min. The quadrupole electron ionization (70 eV) mass spectrometer was operated in full scan mode in a range of 35–450 m/z (416 ions). The temperature of the ion source was 250°C. The autosampler rack was cooled to about +3°C and the samples in the rack were placed in a staggered manner to lower analytical trend effects disturbing the results due to aging of the chromatographic system (Calik et al., 2017).

Chemometrics

Classification models distinguishing the meat of goose groups fed with oats (males and females – 40 samples) vs. wheat (males and females – 40 samples) were developed after pooling the data from both animal experiments on the basis of the results of VOCs analysis using the HS-SPME-GC/MS method. Each chromatogram was divided into 71 sections. There were 416 m/z ions (35–450) in the mass spectrum of each section. The logarithms of the 29536 intensities of these ions (416 ions × 71 sections, for each sample) were the variables used for creating the classification models based on 2 techniques of data preprocessing prior to chemometrics (LOG REL and LOG ABS based on relative (REL) and absolute (ABS) intensity of spectral data, respectively). For creating the models based on the next 2 SRN techniques, i.e., SRN REL and SRN ABS for relative and absolute data, respectively, each of the 71 chromatogram sections was divided into 3 subsections (in the ranges: 35–88 m/z, 89–181 m/z, 182–450 m/z). In each subsection, the ion intensities were averaged. After such a modification, 3 × 71 = 213 raw variables were obtained, which were then subjected to SRN transformation into corresponding log-ratios (Lehallier et al., 2012). As a result, 22578 new variables (p × [p-1]/2, p = 213) were obtained for each sample, which were then used for chemometrics. The transformation was carried out in Microsoft Excel using the standard functions of this program. Next, the most differentiating variables were preselected from the variables derived from the 4 data preprocessing techniques (LOG REL, LOG ABS, SRN REL, SRN ABS) using the Fisher ratio method (Prebihalo et al., 2020; Magagna et al., 2017; Brokl et al., 2014). The list of the preselected variables, as mean values of 2 replicates with the highest Fisher ratio values was then submitted for PCA-LDA. The values amounting to more than 11.3 significantly exceeded the critical values to minimize the multiple comparisons problem (Parsons et al., 2016). These critical values were of 4.41 (for the number of degrees of freedom = 18, male to male and female to female comparisons – for both cases 10 mean values vs. 10 mean values, Figures 1, 2 and 3) and 4.09 (for the number of degrees of freedom = 38, male + female to male + female comparisons – 20 mean values vs. 20 mean values, Figure 4). In PCA, the number of variables was reduced to the principal components by Cattell's scree test in order to avoid classification model overfitting. After the PCA-LDA was performed, the classification accuracy was calculated on the basis of seven-fold cross-validation. The Fisher ratio values were computed adopting statistical functions in Microsoft Excel. The PCA-LDA analysis was executed using Statgraphics Centurion XVI equipped with a multivariate statistics package (Statpoint Technologies, Inc.; Gambit, Kraków, Poland).

Three accuracy measurement parameters of the classification models were calculated: correct classification rate (CCR), classification accuracy for oat-fed (ACCo) and for wheat-fed (ACCw) geese. CCR expresses the percentage of total correctly classified samples and is calculated from formula (1), while ACCo and ACCw are calculated from formula (2), in which T1, T2, T3 and T4 are the numbers of correctly classified oat-fed males, oat-fed females, wheat-fed males and wheat-fed females, respectively; N is the number of all samples; Tx is the number of correctly classified samples to the x^{th} group; and Nx is the number of samples in the x^{th} group.

$$CCR = \frac{T1 + T2 + T3 + T4}{N} \times 100\% \quad (1)$$

$$ACC_x = \frac{T_x}{N_x} \times 100\% \quad (2)$$

ART-ART-c and ANOVA statistics

A three-factor experiment was applied. There were 3 levels for the first factor (versions of meat heat treatment): raw meat, low-temperature heat treatment (30°C, 24 h), high-temperature heat treatment (170°C, 35 min); and also 3 levels for the second factor (variants of oat vs. wheat comparisons): male, female, and male + female; and 4 levels for the third factor (techniques of data preprocessing prior to Fisher ratio-PCA-LDA): LOG REL, LOG ABS, SRN REL, SRN ABS.

The results of validation of classification models were developed using a non-parametric equivalent of 3-way ANOVA, Aligned Rank Transform (ART), for indicating significant impacts from the main factors (Wobbrock et al., 2011; Higgins et al., 1990), and Aligned Rank Transform Contrasts (ART-c) for analysis of interaction by multifactor contrast tests (Elkin et al., 2021). These tests were applied since some groups of input data did not meet the assumptions of a normal distribution (visualization by Q-Q plots, Shapiro-Wilk test). Moreover, these powerful statistical tests can be applied on data that should not be drawn from a Cauchy distribution (i.e., has Cauchy-distributed residuals) and has no extreme outliers (Elkin et al., 2021). This was also the case with this study. To prove this, the Shapiro-Wilk test and an outlier plot with sigma limits were used. The common ANOVA

was used after the ART and ART-c procedures. The ART and ART-c described by Wobbrock et al. (2011) and Elkin et al. (2021) were applied using the Windows version algorithms (Wobbrock et al., 2006). The Shapiro-Wilk test, visualization by Q-Q plots, outlier plot with sigma limits, and ANOVA were performed using Statgraphics Centurion XVI equipped with a multivariate statistics package (Statpoint Technologies, Inc.; Gambit, Kraków, Poland).

Results

Fisher ratio-PCA-LDA scatter plot examples (mean values of 2 replicates) showing meat grouping of oat- and wheat-fed females for the LOG REL data preprocessing technique are shown in Figure 1 (raw meat, with an abnormally high mean CCR value, n = 7) and in Figure 2 (high-temperature heat treatment version, with an abnormally low mean CCR value, n = 7). A Fisher ratio-PCA-LDA scatter plot for oat- and wheat-fed males in the high-temperature heat treatment version and the SRN ABS technique (mean values of 2 replicates) is presented in Figure 3 as the example with the highest accuracy mean values (n = 7) of the all three validation parameters CCR, ACCo and ACCw. In turn, a Fisher ratio-PCA-LDA scatter plot example (mean values of 2 replicates, male + female variant, raw meat version and the SRN ABS technique) with an accuracy measurement parameter with the lowest mean value across the entire experiment (ACCo, n = 7) is shown in Figure 4.

Table 1 presents the values of the CCR, ACCo and ACCw parameters after cross-validation, taking into account three factors: 3 versions of meat heat treatment, 3 variants of oat vs. wheat comparisons, and 4 techniques of data preprocessing prior to Fisher ratio-PCA-LDA. Table 2 shows the mean values of the validation parameters after the ART-ART-c algorithms and the ANOVA statistics.

Table 1. Validation parameters of 36 Fisher ratio-PCA-LDA classification models for each parameter: CCR, ACCo, ACCw, based on VOCs analysis of White Kołuda goose meat by HS-SPME–GC/MS, after 7-fold cross-validation. 3 versions of meat heat treatment: raw meat, low-temperature heat treatment (30°C, 24 h), high-temperature heat treatment (170°C, 35 min); 3 variants of oat vs. wheat comparisons: male, female, and male + female; 4 techniques of data preprocessing prior to Fisher ratio-PCA-LDA: LOG-REL, LOG-ABS, SRN-REL, SRN-ABS

Raw meat									
Techniques of data preprocessing	Male			Female			Male + female		
	CCR*	ACCo	ACCw	CCR	ACCo	ACCw	CCR	ACCo	ACCw
LOG REL**	93.2	93.2	93.2	**100.0**^{i1***}	**100.0**^{i3}	100.0	94.5	95.4	93.6
LOG ABS	86.4	81.4	91.4	100.0	100.0	100.0	83.3	75.2	91.4
SRN REL	85.5	72.9	98.2	94.8	100.0	89.6	85.4	76.6	94.1
SRN ABS	88.8	79.3	98.2	91.4	91.4	91.4	82.7	71.4	93.9
Low-temperature heat treatment (30°C, 24 h)									
Techniques of data preprocessing	Male			Female			Male + female		
	CCR	ACCo	ACCw	CCR	ACCo	ACCw	CCR	ACCo	ACCw
LOG REL	99.1	100.0	98.2	90.5	81.1	100.0	83.3	80.4	86.3
LOG ABS	95.7	100.0	91.4	96.6	100.0	93.2	90.1	95.5	84.6
SRN REL	89.8	91.4	88.2	100.0	100.0	100.0	84.6	86.3	82.9
SRN ABS	86.3	91.4	81.1	96.6	93.2	100.0	85.8	87.9	83.8
High-temperature heat treatment (170°C, 35 min)									
Techniques of data preprocessing	Male			Female			Male + female		
	CCR	ACCo	ACCw	CCR	ACCo	ACCw	CCR	ACCo	ACCw
LOG REL	**100.0**^{i5}	**100.0**^{i6}	100.0	**84.6**^{i2}	**76.1**^{i4}	93.2	88.3	82.7	93.9
LOG ABS	89.6	89.6	89.6	100.0	100.0	100.0	88.8	83.8	93.9
SRN REL	97.5	100.0	95.0	100.0	100.0	100.0	87.6	89.6	85.5
SRN ABS	100.0	100.0	100.0	**96.6**^{i7}	100.0	93.2	88.5	89.8	87.1

*CCR: correct classification rate; ACCo: classification accuracy for oat-fed geese; ACCw: classification accuracy for wheat-fed geese.
**LOG REL, LOG ABS, SRN REL, SRN ABS: data preprocessing techniques, see Materials and methods for details.
***Examples of the observed interactions (in **bold**):
– raw meat vs. high temperature heat treatment ($^{i1\text{-}i2}$ and $^{i3\text{-}i4}$);
– female vs. male ($^{i2\text{-}i5}$ and $^{i4\text{-}i6}$);
– LOG REL vs. SRN ABS ($^{i2\text{-}i7}$).

Table 2. ART-ART-c and ANOVA for validation parameters of Fisher ratio-PCA-LDA classification models: 3 versions of meat heat treatment: raw meat, low-temperature heat treatment (30°C, 24 h), high-temperature heat treatment (170°C, 35 min); 3 variants of oat vs. wheat comparisons: male, female, and male + female; 4 techniques of data preprocessing prior to Fisher ratio-PCA-LDA: LOG-REL, LOG-ABS, SRN-REL, SRN-ABS, based on VOCs analysis of White Kołuda goose meat by HS-SPME–GC/MS

Validation parameters	Versions of meat heat treatment			Variants of oat vs. wheat comparisons			Models of data preprocessing prior to PCA-LDA				P-value
	Raw meat	Low-temperature heat treatment (30°C, 24 h)	High-temperature heat treatment (170°C, 35 min)	Male	Female	Male + female	LOG ABS**	LOG REL	SRN ABS	SRN REL	
CCR*	90.5 A	91.5 A	93.5 B	92.7 B	95.9 C	86.9 A	92.3 AB	92.6 B	90.7 A	91.7 AB	<< 0.01
ACCo	86.4 A	92.3 B	92.6 B	91.6 B	95.1 C	84.5 A	91.7 B	89.9 AB	89.4 A	90.8 AB	<< 0.01–0.0014
ACCw	94.6 B	90.8 A	94.3 B	93.7 B	96.7 C	89.3 A	92.9 A	95.4 B	92.1 A	92.6 A	<< 0.01
Interaction for CCR, ACCo and ACCw	Versions of meat heat treatment x variants of oat vs. wheat comparisons x techniques of data preprocessing prior to Fisher ratio-PCA-LDA										<< 0.01

*CCR: correct classification rate; ACCo: classification accuracy for oat-fed geese; ACCw: classification accuracy for wheat-fed geese.
**LOG REL, LOG ABS, SRN REL, SRN ABS: data preprocessing techniques, for details see Materials and methods.
Values in rows with different letters: A, B, C, differ at P<0.01.

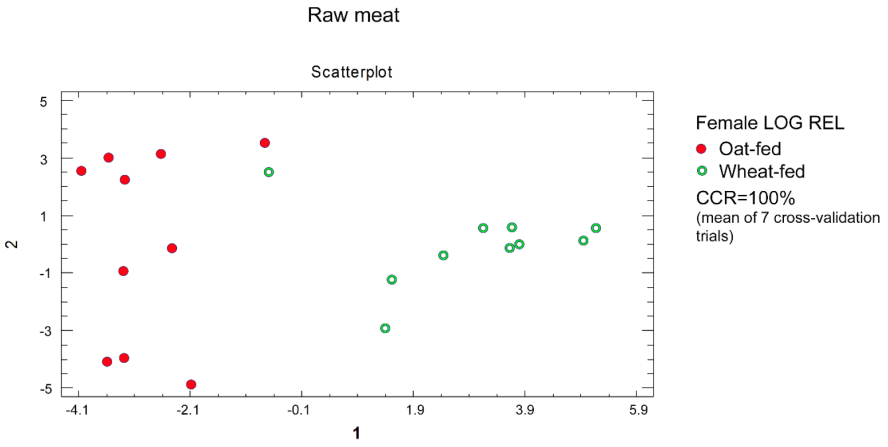


Figure 1. Example of interaction (P<0.01) – Fisher ratio-PCA-LDA scatter plot (mean values of 2 replicates) showing meat grouping of oat- and wheat-fed females in version of raw meat and LOG REL technique, with abnormally high mean CCR value (n = 7), based on VOCs analysis of White Kołuda goose meat by HS-SPME-GC/MS

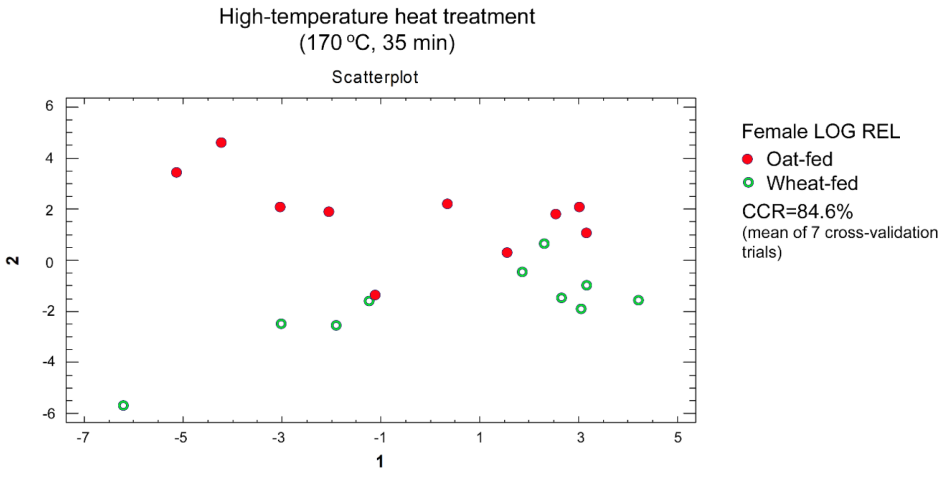


Figure 2. Example of interaction (P<0.01) – Fisher ratio-PCA-LDA scatter plot (mean values of 2 replicates) showing meat grouping of oat- and wheat-fed females in version of high-temperature heat treatment and LOG REL technique, with abnormally low mean CCR value (n = 7), based on VOCs analysis of White Kołuda goose meat by HS-SPME-GC/MS

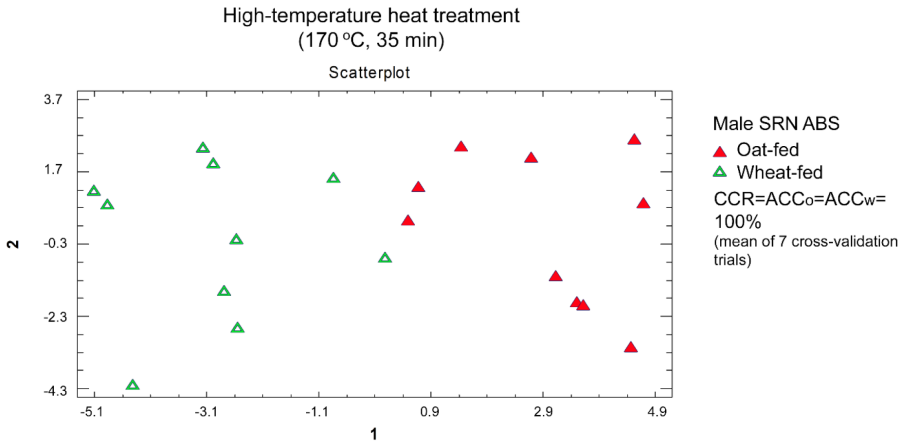


Figure 3. Example with the highest accuracy mean values (n = 7) for all three validation parameters – Fisher ratio-PCA-LDA scatter plot (mean values of 2 replicates) showing meat grouping of oat- and wheat-fed males in version of high-temperature heat treatment and SRN ABS technique, based on VOCs analysis of White Kołuda goose meat by HS-SPME-GC/MS

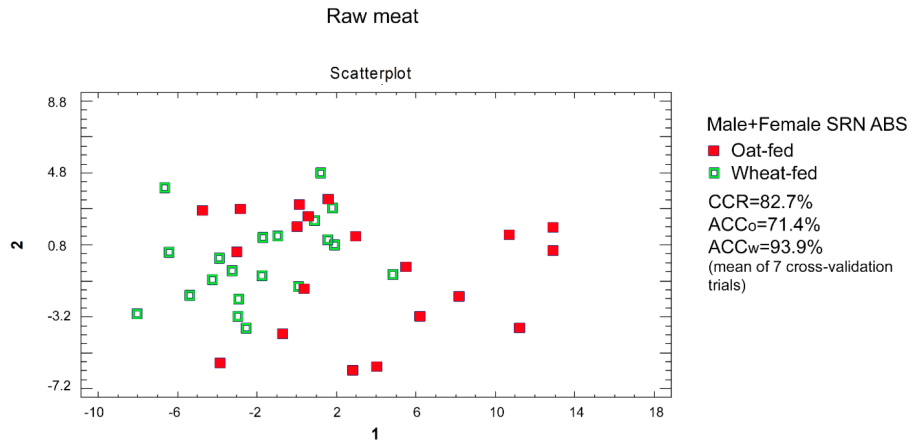


Figure 4. Example with the lowest mean ACCo value (n = 7) – Fisher ratio-PCA-LDA scatter plot (mean values of 2 replicates) showing meat grouping of oat- and wheat-fed geese (male + female variant) in version of raw meat and SRN ABS technique, based on VOCs analysis of White Kołuda goose meat by HS-SPME-GC/MS

Discussion

PCA and the basic statistical description of the validation parameters

In the present study, the first two and three principal components accounted for 54.5–84.8 and 68.6–92.7, respectively (data not shown in Figures and Tables). Values of the 3 parameters measuring the accuracy varied from 71.4% to 100%. The calculated values were similar to those in other studies, and what is more, the classification models in some selected combinations (versions of meat heat treatment/variants of comparisons/techniques of data preprocessing) were characterized by high performance.

In other studies, comprising PCA statistics for differentiation of animal food samples based on VOCs analysis, the first two principal components accounted for 63.6% (Polish smoked ewes' cheeses, Majcher et al., 2015), 88.4% (Rhode Island Red (R-11) capons and cockerels, Calik et al., 2017), and 45.48% (Polish smoked meat "półgęsek" from White Kołuda goose, Nowicka et al., 2017) of the total variance. On the other hand, 77.99% of the total variability was explained by the first three principal components in a study by Berezińska et al. (2007) on blue-veined cheeses. In turn, the rate of correctly classified samples ranged between 58.3% (PDO oscypek cheese, Majcher et al., 2015), and 100% (Rhode Island Red (R-11) capons and cockerels, Calik et al., 2017; PDO oscypek cheese, Majcher et al., 2015; Iberian pigs' subcutaneous fat, Narváez-Rivas et al., 2011).

As shown in Table 1, there were 36 total classification models for the each validation parameter (3 × 3 × 4 × 3, 108 models in total). The average values of CCR, ACCo and ACCw from 7 replicates were calculated (i.e. based on a seven-fold cross-validation). These average values for measuring the accuracy of all parameters were

greater than 70. The lowest values (70–80%) were obtained for the ACCo (Table 1, a Fisher ratio-PCA-LDA scatter plot example is shown in Figure 4). The number of these values was 6 for combinations – versions of meat treatment/variants of comparisons, as follows: raw meat/male + female (3 values), raw meat/male (2 values), and high-temperature heat treatment/female (1 value). It can be assumed that the remaining other parameters – the values of which exceeded 80% – can be considered acceptable (Stanimirova et al., 2010). On the other hand, there were 29 mean values of the parameters measuring the accuracy which were equal to 100% (7 for CCR, 13 for ACCo, and 9 for ACCw). In the case of 7 classification models, the accuracy value was 100% for all 3 parameters. These were: 2 models – for raw meat, 1 model – for low-temperature heat treatment; and 4 models – for high-temperature heat treatment (a Fisher ratio-PCA-LDA scatter plot example is shown in Figure 3). More validation parameters with values of 100% were obtained for the analysis of the meat of females (20) than males (9). Worse results were obtained for the male + female variant of oat vs. wheat comparison – there were no parameters with a value of 100%. However, here too most of the values were above 80%, and only 3 were below this (for ACCo, raw meat version, Table 1).

Versions of meat heat treatment

Generally, the most advantageous method of sample preparation was the high-temperature heat treatment version (Table 2). The relevant CCR value was 2 p.p. and 3 p.p. higher ($P<0.01$) than for the versions of low-temperature heat treatment and of raw meat (Table 2). Similar, but not identical, was the case with ACCo and ACCw parameters. For the high-temperature heat treatment version, the ACCo value was statistically significantly higher than for raw meat, and for ACCw value than for meat subjected to low-temperature heat treatment (for both $P<0.01$).

It seems that thermal treatment contributes to a better classification of the groups of the geese. This is related to the greater number of compounds formed during the roasting of the meat, as the chromatograms in this study showed that the amount of VOCs increased significantly during the thermal treatment (data not presented). The reason for this is that volatile compounds precursors in meat are predominantly free amino acids, reducing sugars, lipids (especially fatty acids), vitamins, nucleotides and peptides (Ba et al., 2012), and that the main chemical reactions – oxidation and degradation of lipids, Maillard reactions, and reactions between the products of these processes (Resconi et al., 2013) – occurring especially intensively at elevated temperatures, lead to the formation of the VOCs. However, care should be taken to ensure that roasting takes place in controlled and as stable conditions as possible, as was the case with this study – i.e., heat treatment in a sealed SPME vial (Ba et al., 2010), and cooling the samples and alternating translocation of the samples on the rack before the chromatographic analysis (Calik at al., 2017).

Although, generally, heat treatment favored better classification of the goose groups, one can also find the highest values (100%) of the accuracy measurement parameters within the raw meat version. This is confirmed by the interactions between the versions of raw meat and high-temperature heat treatment ($P<0.01$, Table 2). The CCR is an example which has the highest value of 100% (oat vs. wheat comparison) for raw meat/female variant/LOG REL, and lower value for high-temperature heat treatment version/female variant/LOG REL (Table 1 – interactions $^{i1\text{-}i2}$, Figures 1 and 2). Similarly, ACCo value was higher for raw meat/female variant/LOG REL than for high-temperature heat treatment version/female variant/LOG REL (Table 1 – interactions $^{i3\text{-}i4}$).

Variants of oat vs. wheat comparisons

The analysis of variance showed that the highest correct classification rates – when comparing oat vs. wheat geese – were obtained when the chemometric analysis was carried out on female, then male, and finally male + female data ($P<0.01$, Table 2). In the latter case, all mean CCR values were below 100% (Table 1). The average predictive performance (as CCR) of the male + female variant of the classification models was lower by 9 p.p. and 5.8 p.p. than of the female, and male variants, respectively (Table 2). The ACCo and ACCw performed similarly. The values of CCR, ACCo and ACCw differed significantly for each variant ($P<0.01$, Table 2).

There were also strong interactions ($P<0.01$, Table 2) within the variants of oat vs. wheat comparisons. The female variant was, generally, the best. However, it is also possible to find here cases with low values of the accuracy measurement parameters. As an example – for high-temperature heat treatment version and LOG REL data preprocessing technique – CCR was lower for the female than for the male variant (Table 1 – interactions $^{i2\text{-}i5}$), and similarly for the ACCo parameter (Table 1 – interactions $^{i4\text{-}i6}$).

Techniques of data preprocessing prior to Fisher ratio-PCA-LDA

There are many alternative chemometric techniques, the choice of which is not obvious and often depends on the nature of the original data. Therefore, it is advisable to build alternative classification models and select the most favorable one in the process of validation (Dreižienė et al., 2015).

In the present study, log-transformation appeared to be a slightly better technique in comparison to SRN, although for CCR a statistically significant difference was shown only between LOG REL and SRN ABS technique (Table 2, difference 1.9 p.p., $P<0.01$). In the case of the ACCw parameter, LOG REL proved to be a better data preprocessing technique comparatively to the others (difference comparatively to SRN ABS, SRN REL and

LOG ABS – 3.3 p.p., 2.8 p.p. and 2.5 p.p., respectively, P<0.01). In turn, in the case of the ACCo parameter, a significant difference was noted between LOG ABS and SRN ABS (2.3 p.p., P<0.01). The observations made in this study differ from those in the work of Lehallier et al. (2012), in which SRN transformation on 2 data sets (samples of different oils and samples of different lamb meat) yielded a considerable improvement in discriminatory power. Perhaps this inconsistency can be explained by the influence of many different factors, in particular differences in data preprocessing. Namely, the Fisher ratio (between-groups variance divided by within-groups variance) for preselection of variables before the proper multivariate statistics was used in our study, in contrast to the studies by Lehallier et al. (2012). Furthermore, in both studies, SRN was compared with different statistical techniques; in this study these were log-transformed data, while in the latter study they were pre-SRN data based on comprehensive combinatory standard correction (CCSC). Another influencing aspect which was also probably important was that the additional division of the sections into subsections on the chromatogram and averaging the data in them (for SRN) were performed in our study, in order to obtain a reasonable reduced number of variables, compatible with the capabilities of the software used, further applied for chemometric analysis. Thus, this could also explain the above-mentioned inconsistency. It is worth adding that techniques based on SRN transformation are more complex than those based on log-transformed data.

As was the case with previous factors, strong interactions (P<0.01, Table 2) were also observed within the techniques of data preprocessing prior to Fisher ratio-PCA-LDA. For example, for the high-temperature heat treatment version and female variant a CCR value which was abnormally low for LOG REL and high for SRN ABS was obtained (Table 1 – interactions $^{i2\text{-}i7}$).

Conclusions

Fisher ratio-PCA-LDA classification models based on VOCs analysis have the potential to distinguish meat of oat-fattened White Kołuda geese from wheat-fattened White Kołuda geese. They showed good discriminatory power.

The most favorable results of the discriminant analysis were obtained for the variant based on comparing birds of each sex separately (females are preferred).

Generally, the best accuracy measurement parameters were obtained for the classification models based on analysis in meat after high-temperature heat treatment or after low-temperature heat treatment. The values of these parameters were the highest or one of the highest.

When choosing a classification model for a given application, it is suggested to construct a model based on log-transformed data, which is simpler than constructing a model based on more complex SRN-transformed data. Only when the LOG-REL or LOG-ABS models are not satisfactory is it worth applying SRN.

Although some options within the tested factors (versions of meat heat treatment, variants of comparisons, and data preprocessing techniques) favored better classification of the goose groups, nevertheless classification models with potentially high discriminatory power could also be found for these weaker cases.

The authors hope that this work will promote traditional food and sustainable agricultural production systems while maintaining high animal welfare standards, as well as the development of food product quality control ensuring food safety from farm to fork.

Competing interests

The authors have no conflicts of interest to declare. They declare that they carried out their duties impartially and honestly.

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