P27: PREDICTION OF CHEMICAL COMPOSITION OF GREEK TRADITIONAL SAUSAGES BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

Tzemou M., Parasoglou V.-K., Ioannidou T., Papadopoulos V., Mitlianga P., Kasapidou E.

Technological Education Institution of Western Macedonia, Department of Agricultural Technology, Division of Agricultural Products Quality Control – Terma Kontopoulou, Florina (Greece) – ekasapidou@teiwm.gr

Abstract – The potential of Near Infrared Reflectance (NIR) spectroscopy to predict the chemical composition of traditional sausages was evaluated. Proximate composition was determined with reference methods. Calibration coefficients and standard errors were 0.92 and 1.66 (moisture), 0.90 and 0.10 (ash), 0.88 and 0.86 (protein) and 0.96 and 1.47 (fat). Cross validation coefficients and standard errors were 0.87 and 1.93 (moisture), 0.74 and 0.16 (ash), 0.75 and 1.21 (protein) and 0.92 and 1.98 (fat). NIR spectroscopy can be applied for the prediction of moisture and fat content of sausages.

Keywords: Near Infrared Reflectance spectroscopy, traditional sausage, chemical composition

1. INTRODUCTION

Traditional (village style) Greek sausages are very popular and widely consumed meat products in Greece. In the past, sausages were prepared at home shortly before Christmas whereas nowadays sausages are produced throughout the year in the adjacent premises of butcher shops and sold non-packaged in bulk [1]. Sausage composition and processing procedures tend to vary between different regions influenced by culture, history, climate and agriculture. In general, pork meat and fat are coarsely chopped and thoroughly mixed with salt, pepper and other seasonings. The sausage batter is stuffed in natural casings and the end product is kept under refrigerated storage until consumption. According to the Greek Food Legislation [2] as amended in 2011, traditional sausages are characterized as fresh (non-cooked) sausages, prepared only from lean meat and fat, salt and seasonings. Sausages are ripened and dried and, sometimes, they are smoked. Sausages do not contain added nitrites or nitrates and the fat content should not be higher than 35%. Use of poultry meat is not permitted. Sausages should be consumed only after thermal processing (frying, grilling or cooking). The quality characteristics of traditional sausages have been examined in the past [3] – [5] and the results have shown great variability in their chemical composition. Furthermore, recent research has also shown notable component variability between production batches [6]. The requirement for nutritional labelling does not apply in traditional sausages. However, the absence of this information prevents consumers from distinguishing between products with different nutritional contents during purchase [7]. NIR spectroscopy has been successfully used in the meat industry as an objective, fast, on-line, reliable, reproducible, cost effective and easy to operate environmentally friendly technique for the determination of the major constituents (moisture, fat and protein) of meat and meat products [8]. Nevertheless, there is lack of information on the application of NIR spectroscopy at individual and small scale producer level for the prediction of the chemical composition of meat products. Furthermore, the development of portable (next generation) NIR spectrophotometers that would enable the rapid online non invasive determination of product composition has become a priority in the last years [9]. Thus, the purpose of this preliminary study was to evaluate the potential of NIR spectroscopy to predict the chemical composition of traditional sausages produced in butcher shops.

2. EXPERIMENTAL

2.1. Sampling

Traditional fresh sausages (33 samples) were purchased from 14 different butcher shops located in the centre of Florina city, Greece (latitude 40.78°, longitude 21.43°) 1-2 days after the end of their

production procedure, as declared by the seller. Samples from the same butcher shop came from different production batches. Samples from the same batch were placed in oxygen permeable food bags and they were stored at 4°C until analysed. Analyses were performed in 1 kg samples, consisting of 2-4 sausages, to ensure product homogeneity due to the fact that the sausage batter often is mixed manually. Sausages were macerated in a domestic type food chopper (Moulinex®, France) taking care to produce a uniform finely ground sample. Homogenised samples were stored in plastic screw-capped containers and appropriate portions were used for proximate analysis and NIR spectroscopy.

2.2. Proximate analysis

Chemical composition analyses were performed in accordance to the recommended and standardized procedures of Official Methods of Analysis [10]. Moisture content was determined by the 950.46 method after drying of the homogenized sample in a convection chamber (ED - 115, Binder GmbH, Tuttlingen, Germany) at 102°C until a constant weight was obtained. Moisture content was calculated from pre-and post- drying weights. Ash content was determined using the 920.153 method. Samples were incinerated at 525°C for 12 h in a muffle furnace (model LM 412.07, Linn High Therm GmbH, Eschenfelden, Germany). Ash content was calculated by weight loss before and after incineration. Protein content was determined according to the method 928.08 by using nitrogen digestion (Turbotherm type TT/12M) and distillation (Vapodest type 40) apparatuses (Gerhardt Apparate GmbH & Co. KG, Germany) and converted to crude protein by multiplying the nitrogen content by 6.25. Fat content was determined according to the 991.36 method. Fat was extracted by petroleum ether using a Soxtherm/ Multistat type SE-416 macro automated system (Gerhardt Apparate GmbH & Co. KG, Germany). Fat content was calculated as the proportional difference between the weight of the sample before and after solvent extraction. All analyses were conducted in duplicate and they were completed within 1 week following sample collection.

2.3. NIR spectroscopy – Spectra acquisition

Homogenised sausage samples were subjected to NIR spectroscopy using a Spectra Star 2400 – D (Unity Scientific, Milford, MA, USA) spectrophotometer. Samples were placed in a rotating circular sample cup, set in the stepped mode at 24 scans, and scanned at 2 nm intervals over the 1200-2400 nm spectral range and automatically recorded as log (1/Transmittance). Samples were prepared in duplicate and two spectra were collected per single sample.

2.4. Data analysis

Construction of the calibration models was performed by the UCal (3.0.0.23) software that supports optimized Partial Least Squares (PLS) chemometric models. Calibration models were built using the calibration dataset and optimized by internal cross-validation (leave-one out). Before calibration, proximate analysis (raw) data was "cleaned" by removing errors. The calibration data set was screened for anomalous spectra and outliers were removed to optimize the accuracy of the calibration. Standard error of calibration (SEC), regression coefficient of calibration (R^2), standard error of cross validation (SECV) and regression coefficient of cross validation (r^2) were calculated for each chemical parameter.

3. RESULTS AND DISCUSSION

The ability of NIR Spectroscopy to predict the chemical composition of traditional sausages is presented in Table 1. Regression coefficients of calibration (R^2) were higher than 0.80 in all examined parameters confirming the good ability of NIR spectroscopy to estimate the chemical composition of meat and meat products [11]. The highest regression coefficient of calibration (R^2) was reported for fat content and the lowest for protein content. This is in agreement with the studies of Ortiz-Somovilla et al. [7] and Gaitán-Jurado et al. [12] for pork sausages. It is important to note that ash content is not usually determined in studies evaluating the ability of NIR spectroscopy to predict the composition of meat and meat products [8], [9], [11].

Regression coefficients of cross validation (r^2) for ash and protein contents were below 0.80 indicating a degree of uncertainty in the prediction of the content of these two components. According to Shenk and Westerhous [13], good accuracy is considered for cross validation regression coefficients (r^2) values in the range of 0.7-0.89

whereas values exceeding 0.9 account as extremely accurate.

Table 1. Prediction of chemical components in Greek traditional sausages

Parameter	SEC	R^2	SECV	r^2
Moisture	1.66	0.92	1.93	0.87
Ash	0.10	0.90	0.16	0.74
Protein	0.86	0.88	1.21	0.75
Fat	1.47	0.96	1.98	0.92

SEC: standard error of calibration; R^2: regression coefficient of calibration; SECV: standard error of cross validation; r^2: regression coefficient of cross validation

Standard error of calibration (SEC) and standard error of cross validation (SECV) values were within the range of values reported in the review studies of Prieto et al. [8]-[9], Prevolnik et al. [11] and Porep et al. [14] for the application of NIR spectroscopy in meat and meat products. However, standard error of calibration (SEC) and standard error of cross validation (SECV) values were higher than the values reported in the studies of Ortiz-Somovilla et al. [7] and Gaitán-Jurado et al. [12] for pork sausages. In general, low standard error of calibration (SEC) and standard error of cross validation (SECV) values are desired [15].

Regarding the limited ability of NIR spectroscopy to predict the protein content, this is also corroborated by the results of Prieto et al. [8]-[9] and Alomar et al. [16] and it is due to the small range of values for protein content [3]-[5], causing difficulties in the detection of differences. With reference to the lack of NIR spectroscopy ability to strongly predict the ash content, this is related to the fact that near infrared radiation does not interact with pure minerals or inorganic compounds in their ionic forms and salts [8].

According to Prieto et al. [8], a large number of samples are required for the development of acceptable NIR spectroscopy calibration models because of the complex physical matrix of sausages as composed of meat and fat mixtures obtained from different anatomical regions and in some cases different animal species. Furthermore, the number of samples, required to construct a calibration model with sufficient variation range for the individual ingredients, extend between 30 to 150 [11]. The success of the NIR technique relies to a certain extent on the variability of the reference data set [8]. In the present study, 33 samples were used, a number which lies at the lower end of this range. It is worth mentioning that 80 dry cured pork sausage samples were used for the construction of the calibration curves in the studies of Ortiz-Somovilla et al. [7] and Gaitán-Jurado et al. [12]. Another weakness, of the present study was the fact that many samples came from the same butcher shops despite their different production batches. This limitation does not allow for large variation in sausage composition that would had enabled the development of more accurate and robust calibration models [17]. Finally, at the present study phase, the small number of examined samples did not allow to perform an external validation, using a separate and independent sample set, that would had provided a more reliable and relevant estimation of the ability of the model to predict the chemical composition of traditional sausages [14].

4. CONCLUSIONS

The results of this preliminary study show that NIR spectroscopy can be used as a reliable tool for the prediction of moisture and fat content of Greek traditional sausages. However, in order to build a fully developed prediction model, analysis of a greater number of samples, that represent a broader range of values for the content of the different components, is needed. Furthermore, additional research is required to represent for diversity in sausage composition due to local recipes and processing procedures. Finally, it is also important to note that future research should focus on the development of calibration models for the next generation of portable low cost NIR spectroscopy instruments that would help butchers to produce healthier products.

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