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DIFFERENTIATION OF FRESH AND FROZEN-THAWED POULTRY BREAST MEAT BY NEAR INFRARED SPECTROSCOPY

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Abstract

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The aim of the study is to research the feasibility of Near Infrared Spectroscopy and SIMCA classification method for discrimination of fresh and freeze-thawed chicken meat. An experiment was carried out with 4 fresh breast meats and 20 tenderloins of broiler chickens, purchased from a local meat store. All samples were measured twice - once immediately after purchase and again after frozen at minus 22°C and stored at the same temperature for 20 days, then thawed in the refrigerator at 6°C for 8 hours. NIRS measurements were performed by NIRQuest 512 spectrometer (Ocean Optics, Inc.) in the region 900-1700 nm using reflection fiber-optics probe. Differences in spectral data of fresh and frozen-thawed meat samples were found. The most significant differences were found around 938, 1018, 1310, 1374, in the region 1402-1417, around 1470 and 1584 nm. SIMCA models for discrimination of fresh and frozen-thawed meat were developed. The best models were obtained using smoothing and second-derivative transformation of spectral data, which correctly classified 100% of the samples from calibration set. The obtained recognition ratio for validation set was 94.4% for fresh meat and 96.8% for frozen-thawed meat. From the obtained results we can conclude that NIR Spectroscopy and SIMCA have a potential for discriminating fresh from frozen-thawed poultry meat.

Keywords: poultry breast meat; fresh meat; frozen-thawed meat; near-infrared spectroscopy; classification

Abbreviations: NIR – near-infrared; SIMCA – Soft Independent Modelling of Class Analogy

Introduction

In recent years the consumers have increased demand for chicken meat, which is fresh, naturally grown and without additives. Incorrect labeling, added water, added salt or sugars and other substances, and thawed chicken meat which is labeled as fresh, are among the most common frauds with chicken meat. The European Regulation 1234/2007 states that poultry meat can be labeled "fresh" when the meat has not undergone a freezing process, i.e. when stored at temperatures between -2 and + 4°C until purchase by consumers. Frozen and deep-frozen is the storage of poultry meat at -12°C and -18°C, respectively (EC Reg. 1234/2007). USDA standard for fresh meat is for meat that has never been at -4°C or lower in the processing plant or 1°C for meat during transport or in commercial stores (USDA 9 CFR 381.129, 1997).

Chicken meat consumers are expecting the meat to be fresh, properly chilled, tender, with the typical texture of fresh meat, without drip or leakage from the muscle, with same colour inside and on the surfaces, and without pathogenic micro-organisms (Leygonie et al., 2012; Benli, 2016; Boerrigter-Eenling et al., 2017). In contrast, the commercial interests of chicken meat producers and meat markets are for longer shelf-life and prolonged storage without signs of spoilage or quality losses. For these reasons, manufacturers and retailers prefer chicken carcasses and chicken meat cuts that are frozen than such in chilled storage. Unfortunately, during

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the years more and more common are the reports that frozen-thawed meat has been offered as freshly chilled (non-frozen) meat. In the case of raw meat, it is accepted as a legal requirement to be applied only one freeze cycle, and after defrosting the meat is subjected to a short (up to a few hours) refrigerated storage before culinary treatment. In stores and meat holders chicken can be storage chilled at 1-4°C or frozen at -12°C or -18°C. Freeze-thawed meat is not acceptable to be available on store shelves. Re-freezing of the thawed meat is prohibited. It is also forbidden for freeze-thawed meat to be labeled as fresh meat. Such violations are referred as food fraud and are detriment of the user and their trust in official governmental control bodies and authority organizations. The user must be able to rely on food label and when the label is for fresh meat inside the package the meat must never be frozen or frozen-thawed. Specific for the chicken meat is that after thawing it is difficult even for experts to identify changes that occur in meat at freezing and thawing. Slightly visible signs are irreversibly impaired texture, which becomes soft and loses elasticity and appears drip loss in form of muscle juice (Zhuang and Savage, 2013). In the meat industry for long storage of chicken meat it is used freezing at minus 36-40ºC in which bigger part of the water freezes and formed ice crystals are fine, thin and short and cause less muscle fiber damage. Once the ice crystals have formed with each re-freeze-thaw cycle, they are getting bigger and more and more damaging for the muscle fibers and meat tissues (Vieira, 2009). At the same time, microorganisms located on the meat surface penetrate into depth, thereby compromising the safety (Leygonie et al., 2012). In the past 10 years, the term "sub-freezing" has been used in the meat industry and commercial premises, for which it is doubt whether sub-freezing to be interpreted as frozen or chilled stages. Sub-freezing or Very-Fast-Chilling is a partial icing on the surface of the meat, where the surface freezes in the depth 3-5 millimeters but in the deeper tissue layers the temperature is up to 0-1ºC (Joseph, 1996). Sub-freezing or Very-Fast-Chilling is used for long-destination transport of meat with refrigerated containers or when stored in refrigeration chambers in the big meat holders.

However, the retail price of frozen meat is lower than the price of fresh meat. A challenge to control by the governmental authorities and for consumers is the lack of a quick, inexpensive, and reliable method of recognizing fresh and freeze-thawed meat. In the current laboratory practice of official meat control, there is a method for destructive determination of the enzymatic activity of muscle juice from the meat, which method is based on the activity of mitochondrial β-hydroxyacyl-CoA dehydrogenase (HADH) (Ballin and Lametsch, 2008; European Commission, 2013; Škorpilová et al., 2014; Elahi et al., 2016; Boerrigter-Eenling et al., 2017). The method is only laboratory-applicable and with very expensive test kits and reagents, which are used after activation for a small number of meat samples. The other disadvantage of the method is that it is necessary to be carried out quickly and with well-trained and experienced laboratory staff.

Considerable interest for development of instrumental techniques for objective, faster, non-destructive and less expensive assessments of meat quality exists. Near infrared (NIR) spectroscopy has been used as a method to predict the quality of different foods and agricultural products due to the speed of analysis, minimal sample preparation and low cost. NIR spectroscopy has been applied to the quantitative determination of major constituents in meat and meat products and as authentication tool in order to prevent frauds and to detect handling aspects such as freezing, thawing etc. The lack of consumables and easy measurement conditions determines NIR methods as cheap and easy to apply. The ability to repeatedly measure the same sample and repeat at any time is also a convenience for user level input and even use in the consumer's kitchen. Many examples in recent years have seen the application of NIR directly through smartphones software and applications that enable the user to measure food in the store before buying it. Successful application of NIR in meat science was found for determination of fat, water content, protein content, acidity, drip loss, pH value, color, cooking loss and Warner-Bratzler sheer force of the product (Togersen et al., 2003; De Marchi et al., 2011; Barbin et al., 2015; Xie et al., 2015; Alamprese et al., 2016; Wu et al., 2016). Such an extended NIR application gives the lead of the methods useful in the self-control of the food we want to buy, cook and consume.

The purpose of the present study is to determine the suitability of a NIR method for rapid, cheap and non-destructive detection of fresh and freeze-thawed chicken meat and to propose a classification model for application in the routine chicken meat control.

Material and methods

Experimental design

An experiment was carried out with 4 fresh carcasses of broiler chickens collected from the poultry slaughterhouse on the day of their slaughter and 20 tenderloin purchased from a local meat store. The carcasses and the meat were transported to the laboratory within one hour at a temperature of 4-6°C. From the chicken carcasses in laboratory conditions left and right breast muscle (Musculus pectoralis major) and left and right tenderloin (Musculus pectoralis minor) were removed and trimmed from the fat and connective tissues. All the samples were fresh meat that had not been frozen.

Test samples were prepared as each of the muscles was cut into 8 transversal slides (pieces with 1.5-2 cm tick and 55-60 g weight). The slides were placed in sterile disposable petri dishes (90 mm in diameter) which were petrifilm sealed to prevent evaporation of water (moisture) during storage.

All samples (n = 24) fresh, chilled meat were frozen on the day of their laboratory preparation. Samples were frozen at minus 22°C in the freezer and stored at the same temperature for 20 days, then thawed in the refrigerator at 6°C for 8 hours. All samples were measured twice - once immediately after purchase and again after freezing and thawing.

NIR Spectral Measurements

NIR measurements were performed by NIRQuest 512 spectrometer (Ocean Optics, Inc.) in the region 900-1700 nm using reflection fiber-optics probe without destruction or any kind of treatment of the samples. Four or five measurements at different part of the samples were made to minimize any possible effects of structural variation in the samples.

A commercial program Pirouette Version 4.5 (Infometrix, Inc., Woodinville, WA, USA) was used for performing of spectral data processing.

SIMCA (Soft Independent Modeling of Class Analogy) method was performed to classify samples. SIMCA is based on the assumption that the samples that lie closer to each other in measurement space are likely to belong to the same class. In SIMCA, each training set class is described by its own model using principal components analysis (PCA). Principal components are separately calculated for the objects of each class. The method is useful for classifying high-dimensional observations because it incorporates properties of PCA for dimension reduction and provides additional information on the different groups such as the relevance of the different variables and measures of separation. SIMCA defines subspaces (class models) and a new object is projected in each subspace and compared to it in order to assess its distance from the class. Finally, the object assignment is obtained by comparing the distances of the object from the class models.

Class variable was assigned to each analysed samples – class "fresh-chilled meat" or "frozen-thawed", respectively. Additionally, samples were divided into calibration and validation sets. Eight samples from each class were selected randomly and used for independent validation. The rest of the samples were used as the calibration set.

SIMCA models were developed using different data pre-treatments that included smoothing, first and second derivatives. The first and second derivative and smoothing transforms are based on a Savitzky–Golay polynomial filter (Savitzky and Golay, 1964). The number of significant PCs in the model of each class was evaluated using leave-one-out cross-validation. Probability threshold was set to 0.95. Probability threshold is a value used to determine whether a sample belongs to a certain class or not. Finally, obtained SIMCA models were used to classify samples from the independent test set.

For estimation of SIMCA models and spectral information essential for classification, we used the following three parameters: the number of incorrectly classified samples from calibration and test sets of samples and class distance (*CD*). *CD* describes the distance from the center of the classes. In SIMCA, *CD* is defined as the ratio of the sum of the residual standard deviations for all variables within one class when fitted to the other class as compared to when fitted to their own class. *CD* is used to measure the distance (dissimilarity) between two classes: $CD < 1$ indicates that the two classes overlap, $1 < CD < 3$ indicates partial separation of the classes and $CD > 3$ indicates good separation of the classes. These *CD* values represent a useful metric for characterizing the overall prediction ability and success rate of the SIMCA models for discriminating the respective class pairs.

Additionally, the so called aquagrams were calculated, using specific water vibrations, connected with free water, specific water configuration such as dimers, trimmers, solvation shells etc., and named water matrix co-ordinates at 1344, 1364, 1372, 1382, 1398, 1410, 1438, 1444, 1464, 1474, 1492 and 1518 nm, which cover the most distinctive species of water structure. Aquagram is a radar chart which displays normalized absorbance values at several water bands on the axis originating from the center of the graph. Water matrix coordinates were used for axes. The values for aquagram Aq_λ are calculated using the following equation;

$$Aq_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda},$$

where $\mathbf{A_\lambda}$ **is** absorbance after multiplicative scatter correction (MSC), $\boldsymbol{\mu_\lambda}$ **is the** mean value of all spectra, and $\boldsymbol{\sigma_\lambda}$ is standard deviation of all spectra at wavelength $\boldsymbol{\lambda}$, respectively.

Results and Discussions

Analysis of meat spectra

In order to specify the exact positions of spectra maxima for general spectral interpretation, second-derivative transformation of the meat spectra was performed. In the second-derivative spectra, absorption maxima were converted to minima. These sharply reduce the apparent spectral bandwidth, allowing for resolution of overlapping peaks and largely eliminating baseline differences between spectra.

The average second derivative absorbance spectra of measured samples of each class are presented in Figure 1.

The shapes of the NIR spectra are similar, but differences in spectral data of fresh-chilled meat and frozen-thawed meat at particular wavelengths were found. The biggest variation in spectral data were observed around 938, 1018, 1310, 1374, in the region 1402-1417, around 1470 and 1584 nm. The absorption at these wavelength regions might be assigned mainly to first overtone of O–H stretch around 1400 nm, C-H bands at 938 and 1374 nm and N–H in amino and amide groups at

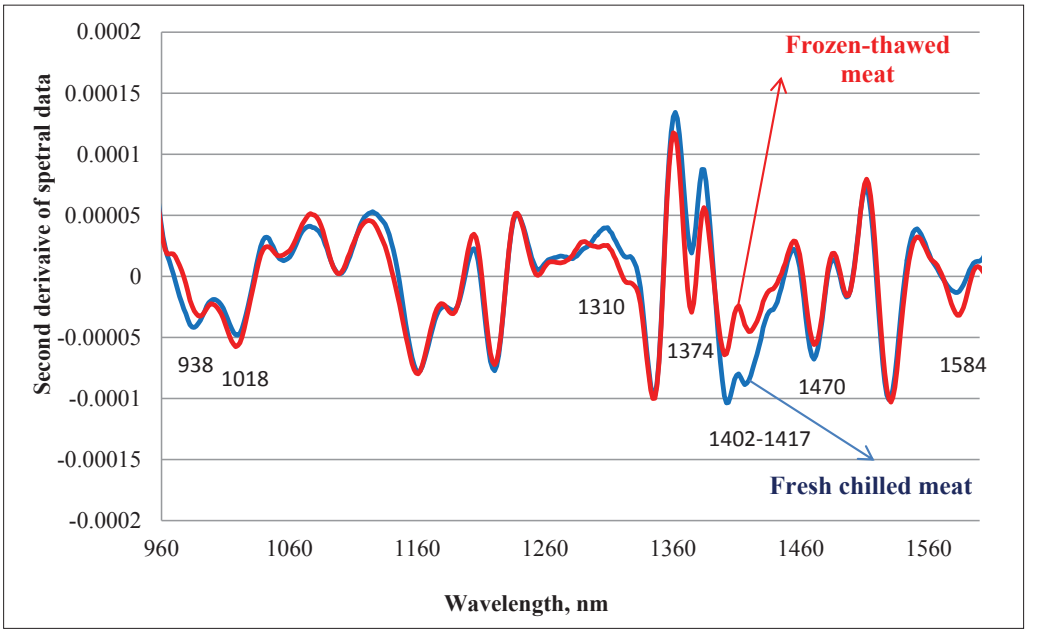

Fig. 1. Average second derivative absorbance spectra of measured fresh chilled and frozen-thawed meat samples

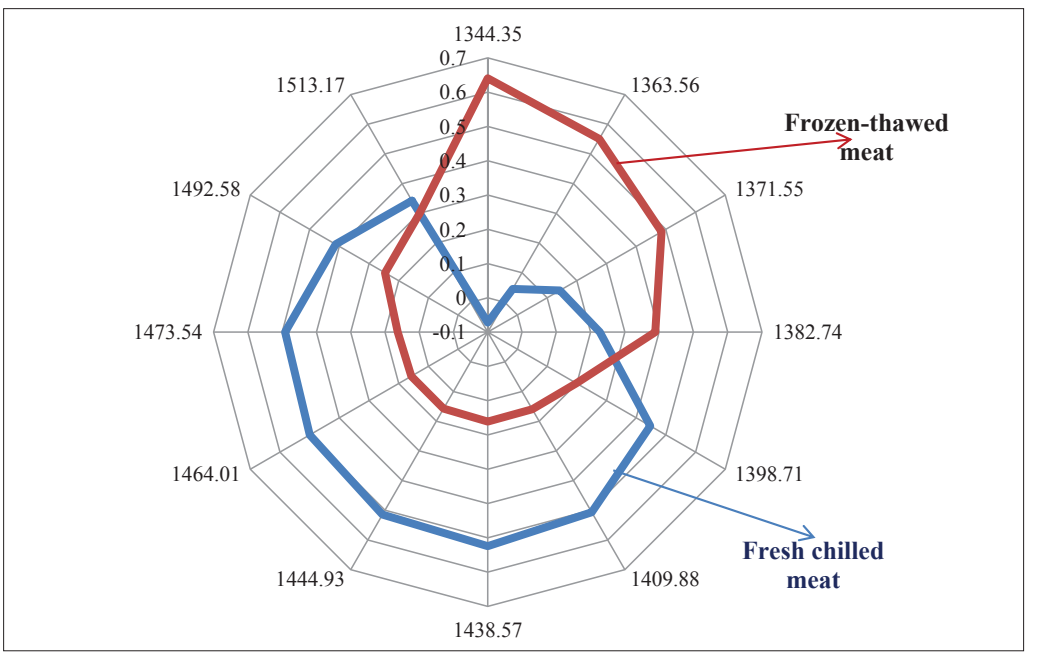

Fig. 2. Aquagram of fresh chilled and frozen-thawed meat

1018 and 1430 nm (Workman and Weyer, 2008). Bowker et al., 2014 found similar spectral region (1380, 1404, 1474, 1492 and 1510 nm) to be important for discrimination of broiler breast raw and freeze-dried fillets according to water-holding capacity. Barlocco et al. (2006) and Prieto et al. (2009) reported the wavelengths most related to the prediction of tenderness to be 1400 nm, connected with water absorption, and region 1300-1400 nm - C-H molecular bonds.

The differences were biggest around 1400 nm – the region of first overtone of O-H bond of water. To visualize in detail the changes in water absorbance pattern a chart termed "Aquagram" is used. The aquagram, calculated using spectral data the same meat samples, measured initially fresh, and after that frozen-thawed meat (Figure 2). Aquagram pattern of meat sample after freezing is changed significantly. Aquagram values of frozen sample decreased in the region from 1398 to 1518 nm and increased in the region 1344-1382 nm. Changes in an aquagram showed decreasing the bounded water in frozen-thawed meat compared to fresh meat (Tsenkova, 2009). Similar aquagram pattern were found for the rest of the samples.

Lean muscle contains approximately 75% water. Water in the meat existed in form of bound, entrapped and free water. Bound water is the water that exists in the vicinity of non-aqueous constituents (like proteins) and has reduced mobility. Entrapped water is another fraction of water that can be found in muscles. It can be easily converted to ice during freezing. Free water is defined as the water whose flow from the tissue is unimpeded. During freezing and thawing of meat, ice crystal growth causes biochemical and physical changes. The latter result in the disruption of cellular organelles and release of their contents into the meat drip juice. These changes lead to changes in water content and proportion of free and bounded water, change of pH, water-holding capacity, protein denaturation, texture and tenderness of the meat (Vieira et al., 2009). At the micro-level, changes lead to oxidative processes and oxidation of lipids and proteins in destroyed cells (Xia et al., 2009).

The differences in near-infrared spectra of fresh and frozen-thawed meat show that variations in the spectra reflect the changes that occur in the meat when freezing and thawing and could be used for creation of model for discrimination of poultry meat.

SIMCA classification of meat samples

From the unprocessed spectral raw data it is not possible to classify the meat samples as fresh or as frozen-thawed. Detailed data processing is required for development a classification model based on freezing quality changes in chicken meat samples.

A total of 113 spectra were obtained from fresh meat samples and 102 spectra of frozen-thawed samples. Samples were divided into calibration and validation sets. Eight samples from each class were selected randomly and used for independent validation (36 spectra of fresh samples and 31 spectra of fresh-thawed samples). The rest 16 samples (77 spectra of fresh samples and 70 spectra of fresh-thawed samples) were used as the calibration set.

SIMCA models for discrimination of fresh and frozen-thawed meat were developed. The SIMCA models were compared in terms of class distance values and number of misclassified samples. The best models were obtained using smoothing and second-derivative transformation of spectral data, which correctly classified 100% of the samples from calibration set (Figure 3). These SIMCA models included 9 principal components, which explained 98.87% of variations in the spectral data of fresh meat and 98.75% of variation in the spectra of frozen-thawed samples. The value of parameter „Interclass distance", which describes the distance from the center of the classes, was 3.79. Large class distances imply well-separated classes. A distance of more than 3 is an indication of good SIMCA class separation and that the models are really different. An obtained value of interclass distance indicates good separation of the classes.

The results of predicting of class values of samples from independent validation set were presented at Table 1 and Figure 4. The most of the meat spectral measurement allowed correct discrimination between classes. Three measurements were not classified into any of the modeled classes. The obtained recognition ratio was 94.4% for class of fresh meat and 96.8% for class frozen-thawed meat.

Results from our study were consistent with those of Wang and Peng (2014), Pu et al. (2015) and Huang et al. (2016). Wang and Peng (2014) reported accuracy of the discrimination of fresh and frozen-thawed pork of 96.67% for calibration set and 100% for validation set, using spectral range from 385 to 935 nm with Fisher discriminant method and Bayes discriminant method. Pu et al. (2015) investigated possibilities for classifying fresh and frozen-thawed meats by integrating critical spectral and image features extracted from hyperspectral images in the region of 400-1000 nm. Probabilistic neural network models for classification of fresh and frozen-thawed pork meats were established and the highest classification rate of 93.14% and 90.91% for calibration and validation sets, respectively, were obtained. Fresh, chilled, frozen and repeatedly frozen pork meats in spectral region 760-2500 nm were investigated by Huang et al. (2016). Self-organizing competitive neural network model was used for establishing model for classification. The second differential pre-treatment of spectral data exert-

ed the optimum effect. The obtained recognition ratio was from 93.3 to 100%.

Similar results, showing possibilities for discrimination of fresh and frozen-thawed fish were reported. Fasolato et al. (2012) reported that percentage of correctly classified samples obtained with Vis-NIR spectroscopy was ≥ 96.7%, whereas that for NIR was ≥ 90.0%.

In the present study, the main point is not for a high correlation with a specific chicken meat trait, but is intended to detect the ability to apply a NIR method for direct recognizing of non-frozen and frozen-thawed meat on the base of total quality changes in the meat after thawing. The variation in spectral information of meat samples in the near-infrared range of 900 to 1700 nm are connected with changes caused by the freezing of water in ice crystals and chemical changes in the meat components and could be used for discrimination of fresh and frozen-thawed meat without use of value or a specific indicator as a reference. Our results showed a successful classification of the samples - 94.4% for class of fresh meat and 96.8% for class frozen-thawed meat. The most informative wavelength connected with quality changes in the meat after freeze-thawing were discussed.

Conclusions

Differences in absorption spectra of fresh and frozen-thawed poultry breast meat in the region from 900 to 1700 nm existed, which could be explained with the changes that occur in the meat when freezing and thawing and could be used for creation of a model for discrimination of poultry meat. The most significant differences were found around 938, 1018, 1310, 1374, in the region 1402-1417, around 1470 and 1584 nm.

SIMCA models were successfully developed based on NIR spectra for discrimination of fresh and frozen-thawed poultry breast meat. NIR spectroscopy combined with multivariate methods of classification could serve as a rapid alert system against fraud in meat products, food safety and control procedures may thus be considerably enhanced. NIR spectroscopy should be used as alternatives to time, cost and personnel intensive wet chemical standard analytical methods.

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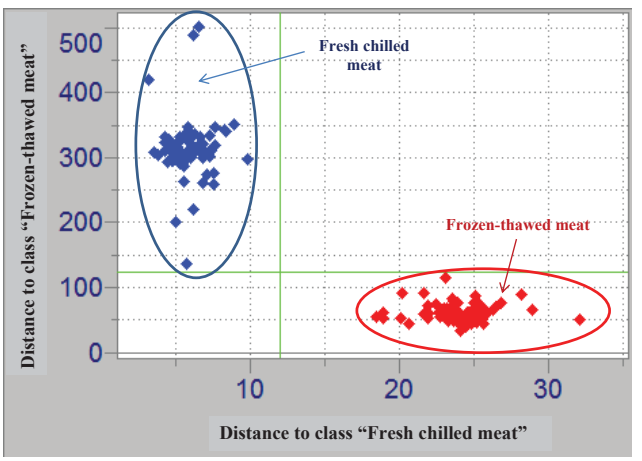

Fig. 3. Calibration data set in Plots for visualisation of distance between class Fresh-chilled meat and class Frozen-thawed meat

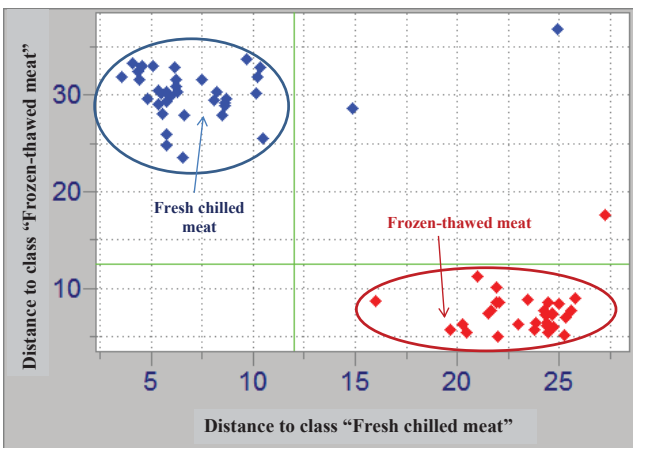

Fig. 4. Validation data set in Plots for visualisation of distance between class Fresh-chilled meat and class Frozen-thawed meat

Table 1
Results of validation data set in SIMCA prediction of meat in class Fresh-chilled meat and class Frozen-thawed meat

	Determines as fresh chilled meat	Determines as frozen-thawed meat	No match	% Correct determination
Fresh chilled meat	34	0	2	94.4
Frozen-thawed meat	0	30	1	96.8

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