# Authentication of Wild Boar Meat in Meatball Formulation Using Differential Scanning Calorimetry and Chemometrics

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Research Article

# Authentication of Wild Boar Meat in Meatball Formulation Using Differential Scanning Calorimetry and Chemometrics

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ABSTRACT

*Bakso* or meatball is one of the Indonesian favorite foods, commonly made from beef. This food is quite popular among Indonesian societies. Due to the high price of beef, unethical producers may adulterate beef with wild boar meat (WBM). In this study, the potential use of differential scanning calorimetry (DSC) combined with multivariate calibration was used to verify adulteration of WBM in meatball formulation. Oil extracted from WBM is characterized by significantly different cooling and heating DSC thermal profiles. The change of characteristic exothermic and endothermic event in oil with increasing crystallization, melting enthalpy and developing both process over a narrower temperature range is investigated. In this research, we developed DSC and multivariate calibration of Partial Least Square (PLS) calibration to analyze WBM in beef meatball. Meanwhile, the chemometrics of Principle Componen Analysis (PCA) is used to classify WBM and beef in the meatball. The validation model using crystalization profiles yield the coefficient of determination ($R^2$) of 0.999 for the correlation between actual value of WBM (x-axis) and DSC predicted value (y-axis) with equation of y= 0.9999 x + 0.0027, root mean square error of cross validation (RMSECV) of 0.380%, and root mean square error of prediction (RMSEP) of 0.203%. PCA is successfully used for classification of WBM in beef meatball. DSC in combination with PLS and PCA can be an alternative technique for analysis of WBM in meatball.

**Key words:** Differential scanning calorimetry, Wild bear meat, Crystallization profile, Melting profile, Partial least square.

## 1. Introduction

Meatball is processed meat which can be classified as restructured meat. Beef, pork, fish, or chicken is meat commonly used for meatball products. The most popular one used in meatball and widely met in Indonesian market is beef (Kurniawati *et al.*, 2013). The correct labelling of meat used in the meatball is an obligation for the sake of consumers' protection and religious reason regarding the consumption of banned components (Aparicio *et al.*, 2013; Doosti *et al.*, 2014). As consequence, interest in meat authenticity has increased recent years.

Many consumers are concerned about the meat used and accurate labeling in the product they eat (Reid *et al.*, 2006; Ballin, 2010). Some countries make a regulation to assure that the food products available in the market are halal and safe (Ali *et al.*, 2012). Therefore, the detection of meat product including meatball is important for costumer protection and for the verification of non-halal products (Rohman and Che Man, 2012). The identification of wild bear meat (WBM)

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and beef is necessary for two reasons. First, economical reasons, because WBM has the lower price value that beef, and second, to assure the halalness of food because WBM is considered as nonhalal components (Regenstein *et al*., 2003). In some countries such as Indonesia, Malaysia and Middle East countries, halal certified food is compulsory for all kinds of food (Nakyinsige *et al*., 2012). Indeed, it is important to create a reliable verification system to determine whether a food is halal or not. As a consequence, some analytical methods have been developed, proposed and used for identification and quantification of non-halal component in the food products (Mursyidi, 2013).

Some analytical methods are developed for identification of WBM, mostly using polymerase chain reaction (Meyer *et al*., 1995; Samaraweera *et al*., 2011; Abd. Mutalib *et al*., 2012). However, PCR methods are complex and needs complex instrument, therefore, some simple methods such as Fourier transform infrared (FTIR) spectroscopy (Guntarti *et al*., 2015) and differential scanning calorimetry (DSC) is potential to be used for authentication of WBM. DSC is one of thermal analysis methods that is the most widely used for analysis of oils and fats, especially for authentication studies of oil as a quality control. Besides, thermal analysis has long been used in material science and testing, particularly in the field of polymer (Angiuli *et al*., 2009). DSC give the information about melting and crystallization phenomena of oils that is directly influenced by their physicochemical properties such as fatty acid, triglyceride (TAG) composition and chemical structure (Kumar *et al*., 2014). Several studied have evaluated DSC application to detection of adulteration of edible oils and fats, such as detection of animal fat in canola oil (Tan and Man, 2000), soybean, sunflower and canola oils in olive oil (Marikkar *et al*., 2000), refined hazelnut oil in extra virgin olive oil/EVOO (Jafari *et al*., 2009), but as far as result from literature searching, DSC has not been applied to analyse WBM in meat ball formulation.

Current studies about DSC lead to the combination of DSC with chemometrics techniques. Chemometrics is the application of statistical and mathematical techniques for chemical data, including DSC profiles. The use of chemometrics to evaluate the quality of edible oils is extensively studied by correlating thermal parameters to major and minor components or concentrations of adulterant (Chiavaro *et al*., 2008). The use of combination of DSC-Partial Least Square (PLS) to construct a predictive model for fatty acid composition in 63 samples of oil (olive oil, hazelnuts, sunflower and canola) has been studied (Cerretani *et al*., 2011). The results are quite satisfactory with high coefficient determination ($R^2$) and low root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). Using literature review, DSC applications for detection adulteration of WBM in meatball formulation in combination with multivariate analysis has not been reported yet. The aim of this study was to use of DSC in combination with partial least square (PLS) regression for quantitative analysis and principal component analysis (PCA) for classification of WBM in beef meatball.

## 2. Materials and Methods

### 2.1. Materials

Wild boar meat (*Sus barbantus*) is obtained from wild boar hunter in Kalimantan, Indonesia, while beef was purchased from several local markets in Yogyakarta Indonesia. In order to anticipate the variation among meat composition, the wild boar and beef used are mixture from different farmer's land and local market. All the collected samples were transported under ice-chilled condition (4°C) and were stored at -20°C for further processing and for the preparation of meatball formulation.

### 2.2. Preparation of meatball samples

Meatball was prepared according to Purnomo and Rahardiyan (2008). It is made by emulsifying 90% of beef and or wild boar meat, then added with 10% of starch and mixed vigorously with salt and certain ingredient (garlich powder, cumin powder, chopped onion and black pepper). The meat and all other ingredients were blended by vigorous mixing and the emulsified homogenous meat mixture was shaped into ball. The meatball is then cooked in boiling water (100°C) for 10-20 min.

### 2.3. Preparation of calibration and validation standard

During the preparation of calibration samples, a set of standards consisting of WBM and beef was prepared by mixing of both meat at concentration ranges of 0, 10, 15, 20, 35, 50, 65, 80, 85 and 100% (wt/wt) of WBM in beef. To check the accuracy and precision of a calibration model, some other samples known as validation samples were also prepared for making a validation model. The validation sample refer to meatball samples prepared independently in the laboratory with known amount of WBM composition. Meatball was further subjected to lipid extraction according to the traditional Soxhlet method using hexane as an extraction solvent (AOAC, 1995). The hexane containing lipid fraction was evaporated under a vacuum rotary evaporator at 60°C and dried with anhydrous sodium sulfate. The lipid fraction obtained was subjected to DSC measurements.

### 2.4. Thermal analysis by DSC

Thermal analysis was carried out on a Mettler Toledo differential scanning calorimeter DSC-60 Plus (Shimadzu, Jepang) equipped with a thermal analysis data station (TA60WS). Nitrogen (99.99% purity) was used as the purge gas at a rate of 20 mL minG1. The DSC instrument was calibrated with indium (m.p. 157.99EC) Hf = 28.62 J gG1). Approximately of 9.0-12.5 mg (15 µL) of oil samples (extracted from WBM and beef) was placed in a standard DSC aluminum pan and then hermetically sealed. As the reference, an empty and hermetically sealed DSC aluminum pan was used. The samples were subjected to the following temperature program: The

sam[10]was held at -100°C isotherm and held for 2 min. The sample was th[1] heated from -100 to 50°C at the same rate. The DSC parameters of melting and crystallization curve were determined to characterize each sample. The DSC parameters consisting of the onset temperature (Ton, °C), the offset temperature (Tof, °C) (points where the extrapolated leading edge of the endotherm/exotherm intersects with the baseline), the range (range temperature between Ton and Tof), enthalpy, and the various temperature transitions (peak temperatures between Ton and Tof) were determined.

### 2.5. Statistical analysis [6]

All thermal analyses were carried out in duplicate and the results were expressed as the mean value ± SD (standard deviation). All statistical analyses were performed using Minitab software (versi[4]n 16, Minitab. USA). Data were statistically analyzed by one-way Anova analysis of variance and Tukey's multiple comparison test with family error rate of 5%. Multivariate regression of DSC thermal data were evaluated with PLS. Quantification models that offering the highest values of coefficient of determination ($R^2$) and the lowest values of root mean square error of calibration (RMSEC) were selected for developing PLS. The calibration models were further used to predict the concentration lev[7]of oil adulterants in samples. The values of $R^2$ and root mean square error of prediction (RMSEP) were used for prediction criteria.

[13]

## 3. Results and Discussion

### 3.1. The profile crystallization of DSC of analysis

The DSC crystallization profile is obtained for meat boar and their admixtures (10-85% wild boar meat in meatball formulation). The measured parameters during DSC measurements are the beginning of crystal formations (onset, Ton), the end of crystallization (offset, Tof), the amount of energy that lost from samples during crystallization (enthalpy) and the range temperature between Ton and Tof (range). Table 1 summarizes the DSC parameters that characterize the crystallization profile of evaluated samples. The crystallisation thermograms of wild boar meat 100% and their admixtures are shown in Figure 1.

**Table 1. DSC parameters obtained from crystallization thermograms of wild boar meat in meat ball formulation and their admixtures**

| | DSC Parameters | | | |
|---|---|---|---|---|
| Sample | Ton (°C) | Enthalpy ($Jg^{-1}$) | Tof (°C) | Range (°C) |
| WB 0 | 25.63±0.4738 | 57.52±3.3915 | -28.09±3.2526 | 53.71±2.786 |
| WB 10 | 11.93±0.6718 | 17.87±0.8485 | -53.28±8.3576 | 64.71±7.6933 |
| WB 15 | 15.18±9.2630 | 14.7±4.1436 | -53.28±3.7578 | 68.46±14.1845 |
| WB 50 | 5.57±1.8667 | 49.14±8.5843 | -47.76±0.0141 | 53.33±1.8667 |
| WB 60 | 2.96±0.7778 | 12.2±7.2124 | -46.8±14.3825 | 49.76±15.1604 |
| WB 65 | 6.63±2.2627 | 43.53±4.7800 | -44.81±6.8589 | 51.44±9.1216 |
| WB 85 | 3.59±0.3798 | 33.5±4.5820 | -39.5±1.8667 | 43.09±1.2869 |
| WB 100 | 13.48±5.2609 | 28.88±6.6609 | -47.42±9.6732 | 60.9±14.9340 |

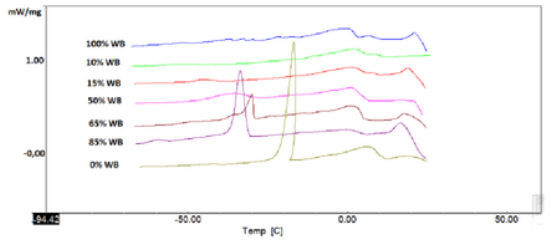


Figure 1. Representative DSC crystallization thermograms of meat boar meat and their admixtures. Peak height (Mw mgG1) expressed the crystallization thermograms at different percentages of WBM added. WB: meat boar meat.

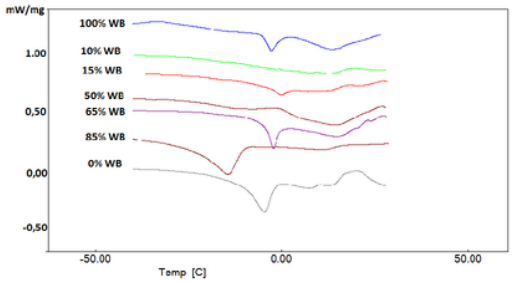


Figure 2. Representative DSC melting thermograms of meat boar meat and their admixtures. Peak height (Mw mgG1) indicated the melting thermograms at different percentages of WMB added. WB: meat boar meat.

### 3.2. The profile melting of DSC of analysis

Melting profile of oils and fats were not easily interpretable like crystallization profile due to the phenomenon of polymorphism of TAG as a major content of oils which depends on thermal history of samples. Melting thermogram of wild boar meat samples exhibited one major endothermal as shown in Figure 2 that were similar to those previously reported by Man *et al.* (1999).

Results of peak melting (endothermic) of wild boar 100% and 0% indicate big difference, enabling to be used for authentication of WBM in meatball containing beef. Temperature (onset, Ton) 100% with more meat boar appear on -7.17°C, while Tof appear at 36.69°C. But temperature wild boar 0% revealed Ton -15.14°C and Tof of 20.2°C. This difference can be seen in the results of a wider range of meatball containing 100% WBM. The melting profiles of oils extracted from wild boar were [16]ther complicated by multiple endothermic transitions as shown in Table 2.

**Table 2. DSC parameters obtained from melting thermograms of wild boar 100%, beef (0%) and their admixtures**

| | DSC Parameters | | | |
|---|---|---|---|---|
| Sample (%) | Ton (°C) | Enthalpy ($Jg^{-1}$) | Tof (°C) | Range (°C) |
| WB 0 | -15.14±1.3717 | -26.13±4.3416 | 20.2±7.0286 | 35.29±8.4711 |
| WB 10 | -1.93±1.0465 | -6.78±4.7517 | 27.82±5.5720 | 25.75±6.6185 |
| WB 15 | -4.97±6.7740 | -24.3±7.8065 | 22.5±0.0282 | 27.46±6.7599 |
| WB 50 | -14.1±3.6628 | -53.1±7.1983 | 36.56±5.0487 | 50.66±8.7115 |
| WB 60 | -17.19±6.1942 | -19.17±2.786 | 30.16±7.8630 | 47.35±1.6687 |
| WB 65 | -5.04±0.3676 | -28.55±6.9579 | 47.22±5.2750 | 52.26±5.6427 |
| WB 85 | -23.57±0.0848 | -37.43±3.4931 | 43.9±0.7353 | 67.47±0.6505 |
| WB 100 | -7.17±1.5981 | -43.48±0.7636 | 36.69±8.3862 | 43.86±6.7882 |

### 3.3. Quantitative of wild boar meat in meatball formulation

In order to build the quantitative calibration model for adulteration study, the mixtures of wild boar meat as adulterants were prepared in a range of 0-100% wt./wt. All DSC parameters for both the crystalization and melting profiles including onset, offset, enthalpy and range of 8 samples (i.e., Lipid fraction obtained from wild boar 100%, beef 100%, and its binary mixtures) were subjected to Partial Least Square (PLS) regression. In PLS calibration, variables that show a high correlations with the response variables are chosen and given extra weight for predictions (Rohman and Man, 2011).

Table 3 compiled the statistical results of authentication study of beef meatball from WBM. The $R^2$ and RMSECV obtained during cross validation using leave one technique were used for the internal validity criteria. RMSECV value obtained from crystalization is 0.380%. In addition, during internal validation using independent samples with known concentration of WBM, $R^2$ value for the correlation between actual value of WBM and DSC predicted value (y-axis) is > 0.99 that describes the goodness of fit of the predicted concentrations and actual values. The difference between actual and predicted value is calculated and can be expressed with predicted residual errors sum of squares (PRESS). The PRESS and RMSEP values obtained are 0.2468% and 0.203%, respectively. The smaller the PRESS and RMSEP values, the lower errors of the developed model. While using melting parameters, the RMSECV, PRESS, and RMSEP values obtained are 0.005%, 42.967% and 2.676%, respectively.

For classification between meatball WBM 100%, beef meatball 100% and commercial samples (S-1, S-2 and S-3), principal component analysis (PCA) is used. PCA is one of the unsupervised pattern recognition techniques used for classification among samples evaluated. Figure 1 exhibited PCA score plot of evaluated samples. PCA is successfully used for classification of WBM meatball, beef meatball and samples. This results deduced that the commercial samples do not contain WBM in its formulation, as indicated by enough separation of commercial samples from training set of 100% WMB meatball.

**Table 3. Multivariate statistical summary from DSC-PLSR calibration for crystallization and melting thermograms of wild boar oil, and their admixtures Kriteria Validasi**

| | | Validaty criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Internal validation | | | External Validation | | | | |
| Calibration models | Factor | R(adj) | PRESS | R Pred. | RMSEC | RMSECV | PRESS | R Pred. | RMSEP |
| *Crystallization* | | | | | | | | | |
| PLS | 3 | 0.9999 | 10.30 | 0.9975 | 0.4855 | 1.8096 | 14.5851 | 0.9999 | 1.7168 |
| *Melting* | | | | | | | | | |
| PLS | 3 | 0.9998 | 43.026 | 0.9892 | 0.9339 | 1.8308 | 14.8946 | 0.9998 | 1.7259 |

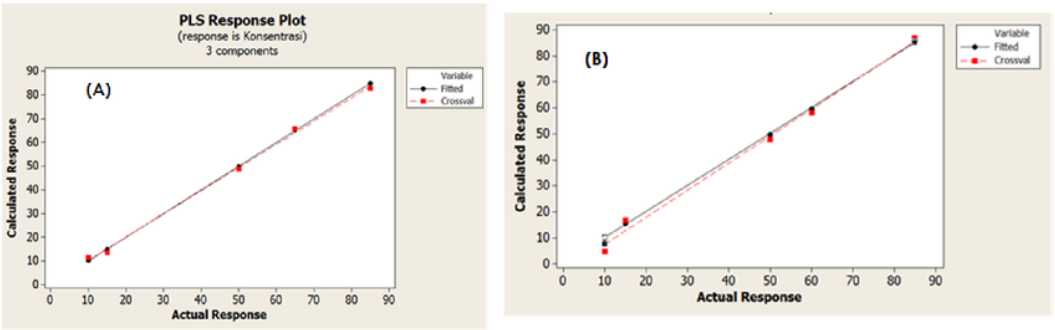


Figure 3. Scatterplot of actual vs. predicted values of wild boar oil as adulterant in wild boar met ball formulation in the internal validation (cross validation) using Partial Least Square Regression (PLSR). (A) Analysis using crystallization profile, (b) validation using melting profile.

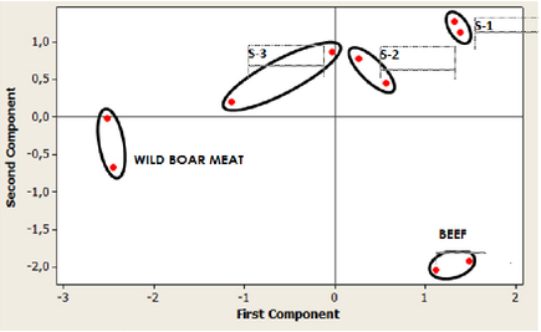


Figure 4. Classification of meatball containing 100% wild boar meat, 100% beef and commercial samples (S-1, S-2, and S-3).

## 4. Conclusion

The results in this study suggest that DSC analysis can be a useful tools for detecting adulteration of wild boar meat in meatball formulation. The DSC combined with multivariate calibration of partial least square represent a rapid, environmentally friendly for wild boar meat authentication without sample pretreatments and the use of hazardous solvent. The results are satisfied for determination concentrations of adulterant with a good correlation coefficient, low RMSECV and RMSEP in both crystallization and melting, either in calibration or validation model.

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