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TBARS and Microbial Growth Predicative Models of Pork Sausage Stored at Different Temperatures

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Abstract

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Kinetic models were developed for quality changes of emulsified pork sausages to predict the TBARS (2-thiobarbituric acid reactive substance) and TVC (total viable counts) changes at different temperatures during storage. Kinetic models of TBARS changes with respect to the temperature during storage were developed based on Arrhenius equation. The regression coefficients ($R^2 > 0.86$) indicated the acceptability of the zero-order reaction and Arrhenius model for predicting TBARS changes. The activation energy (E_a) of TBARS was 109 kJ/mol and the corresponding rate constant (k_0) was 2.6979×10^{18}. External model validation was performed for the sausage stored at 12°C. A high correlation between the observed and modelled TBARS values as well as low RMSE level were obtained. The Baranyi model was fitted to the growth curves. The polynomial model predicted more accurately the influence of temperature on the growth rate, reaching the high adjusted determination coefficient (0.98). Therefore, the established models could effectively predict TBARS content and TVC growth in the emulsified pork sausages.

Keywords: lipid oxidation; microbiological quality; Arrhenius equation; square root model; TBARS

Emulsified pork sausages are very popular and often purchased by consumers. This is caused by the relatively low price and versatility of the culinary use. This type of sausage is very willingly eaten by children, therefore it should be of good quality. Furthermore, it should be safe for human health. Emulsified pork sausages are characterised by a high fat content. Fat is an important constituent of the processed pork sausage products due to its affecting tenderness and juiciness. However, fat content is associated with a higher degree of lipid oxidation during storage, so consumers' acceptance of pork sausages declines due to the development of rancidity caused by lipid oxidation and/or microbial growth (Bradley *et al.* 2011). Lipid peroxidation is one of the primary causes of quality deterioration of meat and meat products, generating compounds that may be detrimental to human health. Therefore, oxidative rancidity is the main cause of pork sausage quality decline. Processed products, which are minced, mixed, and heated, are very susceptible to lipid oxidation and off-flavour development. Mincing and heating accelerate lipid peroxidation and volatile production in meat by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments. High temperature causes reduction of activation energy for lipid peroxidation and decomposes preformed hydroperoxides to free radicals that further stimulate the autoxidation process and off-flavour development (Min & Ahn 2005).

The thiobarbituric acid (TBARS) test was proposed over 40 years ago and is now one of the most extensively used methods to detect oxidative deterioration of fat-containing foods. During lipid oxidation, malonaldehyde (MA), a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of polyunsaturated fatty acids. It is usually used as an indicator of the lipid oxidation process, both for the early appearance in the course of oxidation and for the sensitivity of the analytical method. It should be noted that the oxidation of pure fatty acids is fairly well understood; new approaches to the examination and control of

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oxidative stability in the more complex food systems like particular meat products are still needed.

Microbiological safety of the product during its storage is the most important from the consumer and food manufacturer points of view. A very useful tool for the researchers and food companies is ComeBase platform. That platform is an online collection of data that show how food processing and storage conditions affect the growth of food poisoning bacteria, by describing how different bacteria are affected by the changes in pH, temperature, water content, and other factors in a variety of different foods. The growth of bacteria depends on the external factors (parameters of the environment) and internal characteristics of food products. Temperature is the most important environmental parameter governing microbial growth (Juneja *et al.* 2009). The determination of the temperature influence on the behaviour of bacteria is one of the tools that enable us to control the growth of bacteria in final products. Therefore, in addition to the oxidative changes, the microbiological changes were also examined during the storage of the product. The knowledge of the microbial and lipids oxidative changes during pork sausages storage would be a very helpful to determine optimal storage conditions and shelf life.

Therefore, the objective of this research was to determine predicative models for TBARS changes and microbial growth in emulsified pork sausage during storage.

MATERIAL AND METHODS

Sample preparation. Sausages were purchased directly from the local manufacturer. All sausages came from the same batch and were packed in their usual commercial packages. Sausages were analysed for their moisture, protein and fat contents according to the procedures from the Polish Standards Collection Meat and Meat Products (PN-ISO 1442:2000, PN-75/A-04018:2000, and PN-ISO 1444:2000), respectively. Also sodium chloride as well sodium nitrite levels, were determined using the Polish Standards Collection (PN-73/A-82112 and PN-74/A-82114). The sausages were divided into 5 groups and stored at 3, 6, 9, 12, and 18°C with air access. Three sausage samples were taken randomly every 3 days for analysis.

Oxidation. The extent of lipid oxidation was monitored by the formation of thiobarbituric acid-reactive substances (TBARS). The TBARS indeces were determined in triplicate samples by the extraction method of Sørensen and Jørgensen (1996). The results expressed as mg malondialdehyde/kg meat were calculated from the standard curve of TEP (1,1,3,3-tetraethyoxypropane) standards.

Microbiological analysis. Total viable aerobic bacteria counts (TVC) in the emulsified pork sausage as microbial spoilage indicator were determined. The samples (10 g) were homogenised with 90 ml of sterile peptone water (1 g/l) using an Ultra-Turrax T25 homogeniser (IKA, Königswinter, Germany). Serial decimal dilutions were made and plated onto Standard Plate Count Agar (CM 463; Oxoid, Basingstoke, UK). The incubation was run at 30°C for 72 hours. Bacterial counts were enumerated and expressed as $\log_{10}$ CFU/g.

Statistical analysis. All analysis were run in triplicate. Data were analysed using the general linear model (GLM). All analysis were made using STATISTICA v.10.0 package. The significance level was set at 5%.

RESULTS AND DISCUSSION

Chemical composition. The examined sausages contained about 14% (± 0.35) protein, 15% (± 0.76) fat and 65% (± 1.64) water. Sodium chloride helps to maintain the texture, it improves the taste and increases the products shelf life. Its level in the emulsified sausage was about 1.5%. Another additives which is commonly used as chemical preservative is sodium nitrite. In the analysed sausage samples, the nitrite value did not exceed 35 (± 1.72) mg/kg.

TBARS content of emulsified pork sausage during different storage temperatures. TBARS index was used to evaluate the degree of lipid oxidation during storage. The presence of TBARS reactive substances is caused by the second stage of auto-oxidation, in which peroxides are oxidised to aldehydes and ketones. Lipid oxidation was affected by the time (intrinsic factor) and temperature (environmental factor) ($P < 0.001$) with 0.66 and 0.70 (SE = 0.05) beta coefficients respectively. The higher is the beta coefficient, the stronger is the relationship observed between the independent (time and temperature) and depended variables is observed. Almost 75% of the variance was explained by the independent variables. The changes in TBARS of emulsified pork sausages are shown in Figure 1. TBARS showed a constant increase till the end of storage. The initial content of TBARS was 1.06 mg malondialdehyde (MDA)/kg, which is higher than 0.22 mg MDA/kg reported by Wenjiao *et al.* (2014) for dried sausage but close to the

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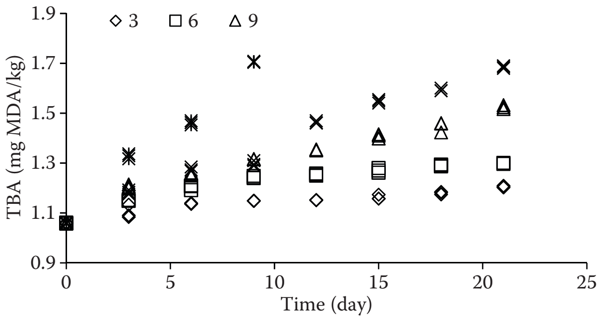

Figure 1. Variation of TBARS index value

0.9 mg MDA/kg reported by Jayasingh and Cornforth (2003) for ground and cooked pork meat. These results proved that lipid oxidation products are accumulated during processing (homogenising and heating). Additionally, the initial TBARS index may be affected by the fat composition, type of muscles (Cobos *et al.* 2003) and seasoning (Shah *et al.* 2014) as well as the production and packaging processes of each type of products (Hernández *et al.* 1999; Veberg *et al.* 2006).

***Establishment and validation of TBARS predicative* model**. All sausage samples were kept under controlled conditions and taken for analysis in appropriate time intervals to allow efficient kinetic analysis of quality deterioration. To validate the applicability of the models, TBARS index values of the sausage samples stored at 3, 6, 9, and 18°C were used to establish the predicative model. The samples stored at 12°C were treated as controls, to verify the application techniques as well as demonstrate the validity of the approach. The values of the TBARS were plotted vs. time for all temperatures studied and the apparent order of TBARS changes was determined based on the least square statistical fit. The highest regression coefficients values were obtained for TBARS value vs. time relationship (Table 1). Therefore, the zero-order reaction model was applied.

Zero-order kinetic model:

$$TBA = TBA_0 + k \times t \quad (1)$$

where: TBARS – value of TBARS indicators; $TBARS_0$ – initial TBARS value; k – rate constant (day^{-1}) at a given temperature

The values of k were obtained from the slope of regression of TBARS value versus time. As concerns the effect of temperature on the reaction rates, the higher the temperature is, the larger the absolute value of reaction rates. Reaction constants k are shown in Table 2. The data were then applied to the Arrhenius equation (Boekel 2008):

$$k = k_0 \times (-E_a a/(R \times T)) \quad (2)$$

where: k_0 – pre-exponential factor (or frequency factor); E_a – activation energy (J/mol); T – absolute temperature (K); R – gas constant (8.3144 J (mol/K); k_0, E_a – all experience constants related to the nature of the response systems

The modified Logistic Arrhenius equation was given by the equation:

$$\log k = \log k_0 - E_a/RT \quad (3)$$

In the calculated rate constants under different temperatures, the slope of the regression line ($-E_a/R$) was obtained through the use of $\log k$ on the thermodynamic temperature of the reciprocal (T^{-1}) plot. Activation energy (E_a) was obtained through the slope of the regression line, and k_0 was obtained through the interception of the regression line. The temperature dependence of the rates on TBARS changes is described adequately by Arrhenius kinetics in the whole temperature range studied (Figure 2). The results from Figure 2 show that the slope of the regression line was –13119.39 (SE = 791), so the values of E_a and rate constant (k_0) for the emulsified pork sausages in TBARS were 109 kJ/mol (SE = 16.54) and 2.6979×10^{18} (SE = 6.58×10^3), respectively, and the corresponding R^2 in TBARS was 0.993. The equation of the TBARS predictive model is:

$$TBA = ([TBA]_0 + 2.6979 \times 10^{18} \times \exp(-13\,119.39/RT)) \times t \quad (4)$$

where: TBARS – predicative values of the TBARS value of the emulsified pork sausages stored for a particular time; $TBARS_0$ – initial values of TBARS index value of the sausages

In order to model validate, the comparison of the predicted and measured TBARS values of the emulsified pork sausages stored at 12°C was evaluated (Fig-

Table 1. Estimation of the order of TBARS index by examining the R^2 coefficient (n = 28)

Temperature (K)	Zero-order reaction (TBA vs. time)	First-order reaction (Log TBA vs. time)	Second-order reaction (1/TBA vs. time)
276.15	0.91	0.90	0.89
279.15	0.86	0.84	0.82
282.15	0.96	0.94	0.96
291.15	0.99	0.97	0.91

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Table 2. Zero-order kinetic parameters for TBARS index of emulsified pork sausage stored at different temperatures

Temperature (K)	Regression coefficients			
	slope (mg MDA/g/day)	confidence interval	constant (mg MDA/g)	confidence interval
276.15	0.00591	(0.00493; 0.00689)	1.08145	(1.06924; 1.09368)
279.15	0.01028	(0.00843; 0.01213)	1.11289	(1.08973; 1.13605)
282.15	0.01967	(0.01788; 0.02145)	1.11464	(1.09219; 1.13709)
291.15	0.06906	(0.06302; 0.07509)	1.07782	(1.04395; 1.11168)

MDA – malondialdehyde

ure 3). The results show that the established TBARS predicative model gives a very good prediction of the TBARS content. This confirms the high correlation (r = 0.99) between the observed and modelled TBARS values as well as a low RMSE level (Figure 3). Therefore, TBARS values can be quickly and accurately predicted in emulsified pork sausages stored between 3 and 18°C. Wenjiao *et al.* (2014) created with success TBARS predicative model for dried pork sausage stored at different temperatures. The influence of temperature on TBARS changes was modelled using Arrhenius equation. This relationship is widely applied in meat quality prediction. Ishiwatari *et al.* (2013) predicted the umami decomposition residues in beef during cooking. Bolumar *et al.* (2012) modelled the effect of temperature on the formation of free radicals during high pressure processing. Hence, the established TBARS content predicative model can lead to effective management systems for lipid oxidation, which can be a very helpful tool to optimise the quality of pork sausage products.

Bacterial growth model development in emulsified pork sausage during different storage temperatures. Bacterial (TVC) growth curves were generated by fitting the obtained data with the Baranyi and Roberts model (Baranyi & Roberts 1994).

$$y(t) = y_0 + \mu_{max} F(t) - \log(1 + e^{(\mu_{max} F(t))} - 1)/e^{(y_{max} - y_0)} \quad (5)$$

$$F(t) = t + 1/\theta \ln(e^t + e^{(h_0)} - e^{(\theta t - h_0)}) \quad (6)$$

where: $y(t)$ – cell concentration at time t (log CFU/g); y_0 – initial cell concentration (log CFU/g); y_{max} – maximum cell concentration (log CFU/g); μ_{max} – maximum specific growth rate (day^{-1}); θ – rate of increase of the limiting substrate; h_0 – dimensionless parameter quantifying the initial physiological state of the cells

From that, the lag time λ can be calculated as h_0/μ_{max}. Fitting of h_0 primary models to the observed data was performed using Excel add in $h_0$application DMFit. The goodness of fit of the fitted primary models was evaluated, including the calculation of: the sum of squares due to error (SSE), adjusted R^2, and RMSE (Figure 4). It has been noted that the model applied describes the observed growth with satisfactory accuracy. At all temperatures analysed, the adjusted regression coefficients reached the values above 97%. Therefore, the growth rate generated by this model can be used for the secondary modelling purposes. Secondary modelling was analysed according to two formulas. First, the square root model described by Ratkowsky *et al.* (1982):

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (7)$$

where: μ_{max} – maximum growth rate (day^{-1}); b – coefficient determined during the modelling process; T – temperature (°C); T_{min} – determined minimum temperature for the growth of microorganisms (°C)

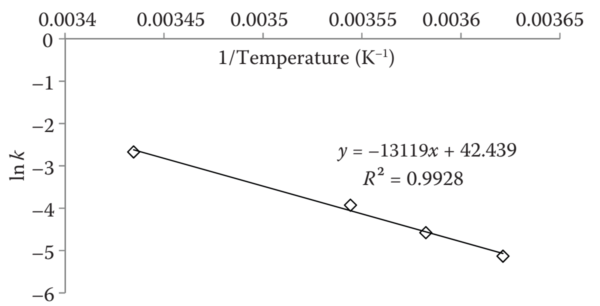

Figure 2. Modelling temperature dependency of changes rate of TBARS for Arrhenius equation

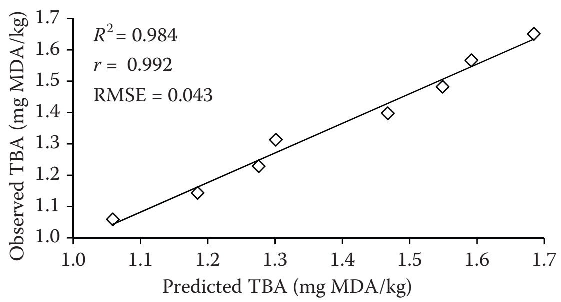

Figure 3. Validation of generated TBA model for emulsified pork sausage stored at 12°C

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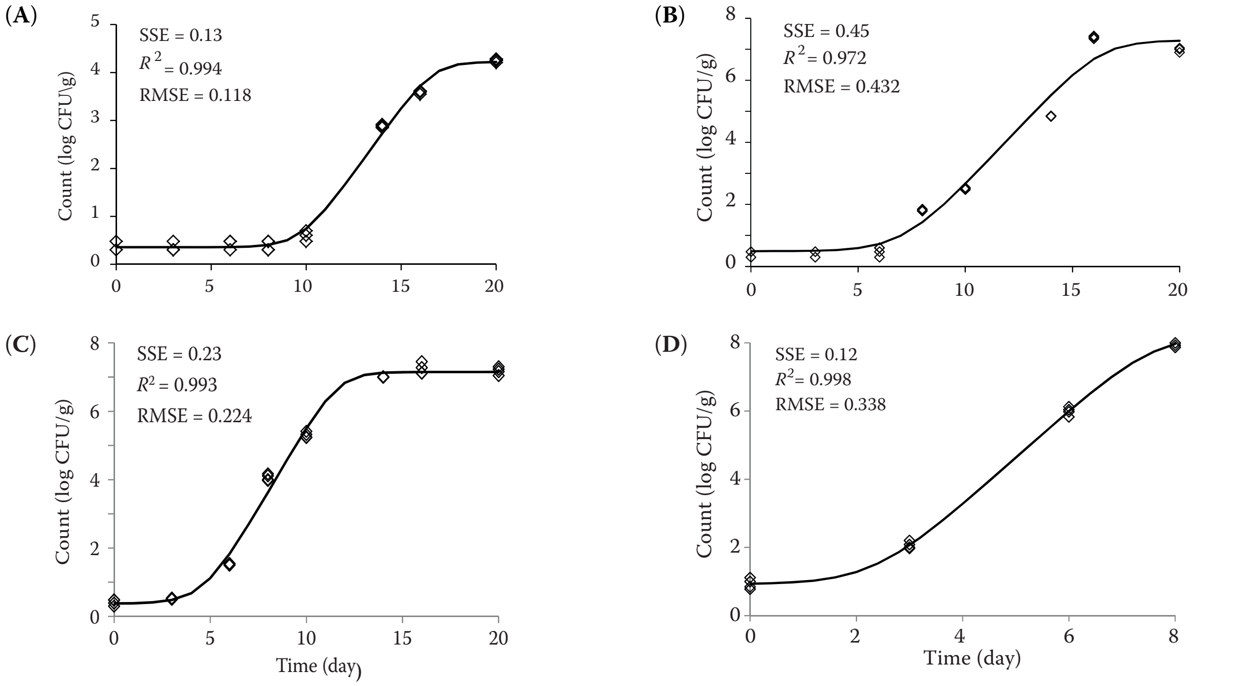

Figure 4. Primary modelling results describing the growth of TVC in emulsified pork sausage (3 replicates) at (A) 3°C, (B) 6°C, (C) 9°C, and (D) 18°C (Solid line = Baranyi model)

Second, the polynomial model (Eq. 8):

$$\log y = a_1 + a_2x_2 + a_3x_1^2 \quad (8)$$

where: y – response of microorganisms (e.g., specific growth rate (day^{-1}); a_1, a_2, a_3 – adjustment factors; x_1 – temperature (°C)

The generated models analyse only 1 independent variable, namely the storage temperature. Figure 5 illustrates the graphical version of the elaborated models with the parameters of the generated mathematical equations. The polynomial model better describes the effect of temperature on the bacterial growth better than the square root model, reaching the values of the adjusted R^2 = 0.9829 and 0.924, respectively. The last stage of the work was to validate the secondary models obtained. Comparisons of the predicted and measured bacterial growth rates in the emulsified pork sausages stored at 12°C were made. The observed maximum growth rate was 1.129 whereas those modelled using the polynomial and square root models were 1.123 and 1.057, respectively. The predicative ability of the polynomial model is slightly better than that of the square root model. The elaborated mathematical model can be successfully used to asses the bacterial growth probability in the emulsified pork sausage, however, caution is needed when applying it to other sausage types. To improve the quality of the predicative models in the food industry, the generated models should be orientated toward a specific product or a group of similar products.

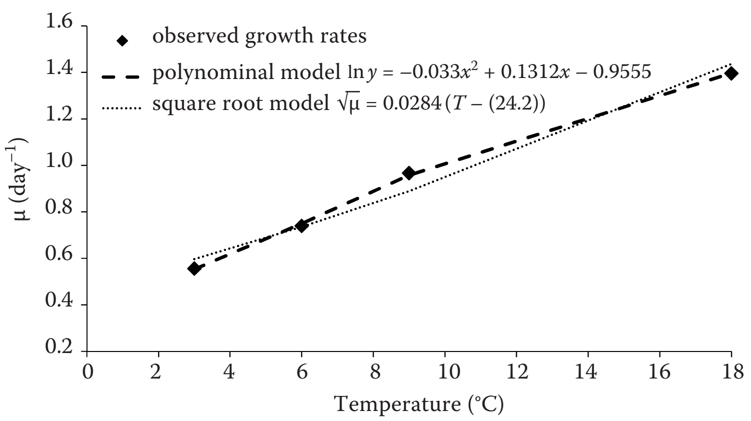

Figure 5. Secondary modelling results describing the effect of temperature on bacteria growth in emulsified pork sausage according to polynominal and square root models

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CONCLUSION

The results show that TBARS value of the emulsified pork sausages increased during storage and this increase was closely connected with temperature. The amount of MDA increased as the storage temperature increased. TBARS value is constantly correlated with the storage time and temperature. The TBARS predicative model gives a realistic prediction of the TBARS level in the emulsified pork sausage. Therefore, this model is effective for TBARS predictions in the emulsified pork sausages. This study also reports on the development and evaluation of the models for TVC growth in the emulsified pork sausage. The results showed the superiority of the polynomial model in TVC growth rate modelling in this particular meat product. This research demonstrates the usefulness of such models in predicting the quality of meat products. Predicative models could provide an excellent tool in the estimation of the effects of the storage conditions on the quality of the final product. Modelling and further validation of the generated models emphasised the issue of usability and applicability of predicative models in the food processing industry by elaborating models targeted at a specific product.

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