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STATISTICAL MODELING AND PREDICTION OF THE INFLUENCE OF STORED BUCKWHEAT ON THE COLOR OF THE FINAL PRODUCT

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Abstract: Incorporating plant-based ingredients into meat products faces challenges regarding raw material stability and its impact on sensory quality. This study aimed to apply multivariate statistical analysis to map the interdependency between the degradation of bioactive compounds in stored buckwheat flour and the color change of emulsion-type chicken sausages. Buckwheat flour was stored for 0, 3, 6, and 9 months at 40 °C and incorporated (3%) into sausage formulations. The relationship between flour antioxidant parameters (TPC, FRAP, individual phenolics) and sausage instrumental color was analyzed using Pearson's correlation, Principal Component Analysis (PCA), and Linear Regression. Results indicate that storage causes a drastic drop in antioxidant power (FRAP decreased 2.9-fold) and epicatechin degradation. A strong positive correlation was established between flour FRAP values and sausage redness (a*, r=0.984). PCA confirmed clear differentiation between fresh and aged samples based on oxidative status. The linear regression model predicted redness with high apparent precision (R^2=0.969), though residual analysis (RMSE=0.274) revealed non-linear deviations. In conclusion, the antioxidant power of the raw material is a critical predictor of final product color. While linear models provide high accuracy, future research should consider non-linear algorithms to fully address complex matrix interactions.

Keywords: Buckwheat flour, Emulsion sausages, Antioxidant power, Instrumental color, Multivariate analysis, Prediction

INTRODUCTION

Growing consumer awareness regarding the relationship between diet and health has led to a global increase in demand for functional food products (Grasso et al., 2014; Bigliardi and Galati, 2013). The meat industry faces significant challenges regarding consumer perception, as meat products are often associated with negative health effects due to high saturated fat and synthetic additive content (Decker and Park, 2010; Zhang et al., 2010). In response to these challenges, the reformulation of meat products through the addition of plant-based ingredients has become a leading strategy for improving their nutritional profile and oxidative stability (Jiménez-Colmenero, 2007; Petracci et al., 2013).

Among potential functional additives, buckwheat (*Fagopyrum esculentum Moench*) occupies a prominent place due to its exceptional chemical composition. Buckwheat is a gluten-free pseudocereal characterized by a high content of proteins with a balanced amino acid profile, as well as an abundance of dietary fibers and minerals (Christa and Soral-Śmietana, 2008; Wijngaard and Arendt, 2006). From a technological aspect, buckwheat phenolic compounds, primarily rutin, quercetin, and catechin, are of particular importance due to their potent antioxidant potential (Giménez-Bastida and Zieliński, 2015; Jiang et al., 2007; Holasova et al., 2002). Previous studies have confirmed that the incorporation of buckwheat flour or hulls into meat emulsions can effectively retard lipid peroxidation and improve product shelf life (Hęś et al., 2017; Belem et al., 2019; Salejda et al., 2022).

Nevertheless, the use of plant-based raw materials in the meat industry implies certain limitations, primarily related to stability during storage. It is known that degradative changes occur during the storage of grains and flour, including lipid hydrolysis and enzymatic degradation of phenolic compounds (Rakić et al., 2023; Pisinov et al., 2024; Sedej et al., 2010; Zieliński et al., 2019). Studies have shown that the content of rutin and other flavonoids decreases significantly over time due to environmental factors, directly diminishing the functional value of the raw material (Lukšič et al., 2016; Suzuki et al., 2020).

These changes in the raw material have direct implications for the sensory quality of the final product, with color being the most critical parameter. Meat color is the primary attribute by which consumers judge freshness, and myoglobin oxidation to metmyoglobin leads to the appearance of an undesirable brown color (Mancini and Hunt, 2005; Faustman and Cassens, 1990; Suman and Joseph, 2013). Although it has been established that the addition of buckwheat modifies the colorimetric parameters of sausages (Pisinov et al., 2025; Rakić et al., 2024; Yessengaziyeva et al., 2023; Sol-Hee et al., 2018), a precise quantification of the impact of the degradation of specific bioactive compounds in stored buckwheat on the color change of the final product is lacking.

Analyzing such complex interdependencies in food matrices requires the application of advanced statistical methods. Traditional linear approaches, such as Multiple Linear Regression (MLR), are often inadequate for modeling non-linear interactions between physico-chemical parameters of different dimensions (Perrot et al., 2006; Barbin et al., 2015). As shown in earlier studies on complex systems by Sekulić et al. (2019), reliable prediction of output parameters in non-linear processes requires the use of advanced multivariate techniques and models based on artificial intelligence, which generally outperform conventional regression models.

The aim of this study is to apply a multivariate approach (PCA and correlation analysis) to map the relationship between the degradation of nutritional and antioxidant parameters of stored buckwheat flour and the instrumental color change of emulsion-type chicken sausages, thereby defining key predictors for future robust modeling.

MATERIAL AND METHODS

Experimental Design and Database Formation

The data used for multivariate analysis in this study were obtained through a controlled experiment involving raw material storage and subsequent meat product manufacturing.

Buckwheat (*Fagopyrum esculentum Moench*), variety "Novosadska", grown and harvested in the 2022 season, was subjected to an accelerated storage treatment. Grain samples were stored in thermostatic chambers at a temperature of 40 ± 2 °C and a relative humidity of 50% for four-time intervals: 0, 3, 6, and 9 months. After each interval, the grains were ground into integral flour (particle size < 1 mm) according to the procedure detailed in the study by Rakić et al. (2023).

The prepared flour was used as a functional additive in the production of emulsion-type chicken sausages (frankfurter type). Five experimental groups were produced: a control group (CON) without buckwheat addition, and four groups with 3% added buckwheat flour previously stored for 0, 3, 6, and 9 months (labeled as FB0, FB3, FB6, and FB9). The formulations were iso-protein and iso-energetic, with identical proportions of other ingredients (mechanically separated meat, ice, additives), applying standard industrial technology described by Pisinov et al. (2025).

For the purposes of this research, a unique dataset was formed where each row represents the mean measurement value for a specific time treatment, and columns represent physico-chemical parameters (variables).

Table 1. Definition of input and output variables for multivariate statistical analysis

Vector Type	Parameter Group	Parameter (Variable)	Unit	Method / Reference
INPUT (X)	Chemical Composition	Moisture	%	ISO 712
		Protein	% DM	ISO 20483
		Lipids	% DM	NMKL 160
		pH value	-	AOAC 943.02
INPUT (X)	Antioxidants	TPC (Total Phenols)	mg GAE/g	Folin-Ciocalteu
		FRAP	µmol Fe^{2+}/g	Spectrophotometry
		DPPH	µmol TE/g	Spectrophotometry
INPUT (X)	Phenolic Profile	Epicatechin	µg/g DM	HPLC-PDA
		Chlorogenic acid	µg/g DM	HPLC-PDA
		Quercetin	µg/g DM	HPLC-PDA
INPUT (X)	Flour Colour	L^*_{flour}, a^*_{flour}, b^*_{flour}	CIE system	Chromameter (CR-410)
OUTPUT (Y)	Sausage Colour	Lsausage∗, asausage∗ bsausage∗	CIE system	Chromameter (CR-400)
		C^*(Chroma), h (Hue)	-	Calculated
		ΔE (Total difference)	-	Calculated

Laboratory Analysis (Input and Output Variables)

Laboratory analyses were conducted according to standard international methods.
Input vectors (X) include quality parameters of buckwheat flour:

- Chemical composition: Moisture content (ISO 712), protein (ISO 20483), lipids (NMKL 160), total carbohydrates, and pH value,

- Antioxidant profile: Total phenolic content (TPC) determined by the Folin-Ciocalteu method, antioxidant power (FRAP), and radical scavenging activity (DPPH),
- Phenolic profile: Content of specific phenolic acids (gallic, chlorogenic, caffeic, p-coumaric, ferulic) and flavonoids (catechin, epicatechin, rutin, quercetin) was determined using the HPLC-PDA method according to the protocol defined by Pisinov et al. (2024),
- Instrumental flour color: Parameters L*(lightness), a*(redness), and b* (yellowness) were measured using a chromameter (CIE Lab* system, D-65 illuminant) in accordance with previous research on the same plant material (Rakić et al., 2024).

Output vectors (Y): Include color parameters of the final product (sausage):

- Measured values L*, a*, b*, as well as derived colorimetric parameters: chroma (C*), hue angle (h), and total color difference (ΔE) relative to the control sample.

Statistical Processing and Modeling

Given the different units of measurement and ranges of input parameter values, all data were normalized (Z-score normalization) prior to processing to eliminate the influence of different dimensions on multivariate analysis results. All statistical analyses were conducted using the software package IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

The data processing procedure included the following steps:

- **Correlation Analysis**. Interdependency between buckwheat degradation variables and sausage color changes was examined using Pearson's correlation coefficient (r). Statistical significance of correlations was tested at the level of $p < 0.05$. Results are presented in the form of a correlation matrix (heatmap) for easier visualization of relationship strength and direction,
- **Principal Component Analysis (PCA).** As a robust chemometric method for dimensionality reduction and pattern detection in food systems (Granato et al., 2014), PCA was applied to the correlation matrix. The number of significant principal components was determined based on the Kaiser criterion (eigenvalue > 1) and the "Scree plot",
- **Regression Modeling**. To quantify predictive potential, Simple Linear Regression was used.

A detailed schematic representation of the entire statistical analysis flow, from data preparation to model validation, is illustrated in Figure 1.

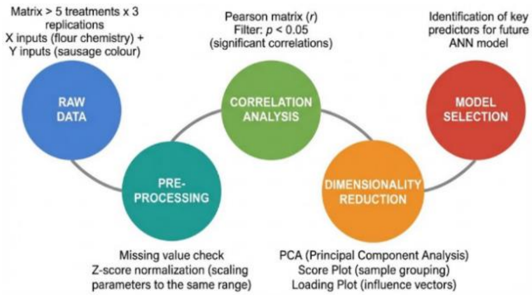

Figure 1. Schematic representation of experimental design and statistical data processing flow

Criteria for Evaluating Prediction Success

To quantify the strength of the relationship between input parameters (flour quality) and output variables (sausage color), and to estimate the predictive potential of the defined statistical models, standard statistical error indicators were used, according to methodology previously applied in complex non-linear systems (Sekulić et al., 2019).

Model success was evaluated by calculating the Coefficient of Determination (R^2), which shows the percentage of variance explained by the model, as well as error metrics, Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) (Barbin et al., 2015).

These parameters were calculated according to the following equations:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_{obs,i} - y_{pred,i})^2}{\sum_{i=1}^{n}(y_{obs,i} - \overline{y_{obs,\iota}})^2} \quad (1)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{pred,i} - y_{obs,i})^2} \quad (2)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|O_i - P_i| \quad (3)$$

Where:
n - total number of samples in the dataset,
$y_{pred,i}$ - predicted value of the output parameter (based on the regression model),
$y_{obs,i}$ - experimentally measured (observed) value,
$y_{obs,i}$ - mean value of all experimental measurements.

According to criteria accepted in predictive microbiology and food technology, higher R^2 values (tending towards 1), as well as lower RMSE and MAE values (tending towards 0), indicate greater model precision and more reliable prediction capability for the final product quality (Granato et al., 2014).

RESULTS AND DISCCUSION

Overview of Matrix Degradation and Parameter Variability

Before conducting multivariate analysis, descriptive statistics were applied to examine trends in input and output vectors and confirm the variability necessary for modeling. A summary of key chemical parameters of the raw material and colorimetric properties of the final product, expressed as mean values, is given in Table 2.

Raw material analysis confirmed that the storage process at 40 °C significantly alters the chemical profile of buckwheat flour ($p < 0.05$). A drastic drop in antioxidant power was observed, with the FRAP value decreasing by approximately 2.9 times (from 63.45 to 22.20 μmol Fe^{2+}/g DM) over 9 months. This trend was accompanied by a specific redistribution of phenolic compounds: while potent antioxidants like epicatechin significantly degraded, quercetin content paradoxically increased, likely due to release from glycosidic bonds or hydrolytic processes during storage.

Table 2. Changes in selected chemical parameters of buckwheat flour and sausage color parameters during the storage period (mean values only)

Storage Time	**Flour FRAP** (μmol Fe^{2+}/g CM)	**Epicatechin** (μg/g DM)	**Quercetin** (μg/g DM)	**Sausage a*** (Redness)	**Sausage ΔE** (Total difference)
0 months (FB0)	63.45	95.7	9.8	18.57	1.53
3 months (FB3)	28.68	78.5	12.5	15.86	3.53
6 months (FB6)	25.32	77	16.6	15.09	4.29
9 months (FB9)	22.2	59.8	47.1	14.5	5.08

As seen from these data, chemical changes in the raw material resulted in measurable variations in the final product. Chicken sausages produced with aged flour showed a progressive loss of characteristic redness, where the a* value dropped from 18.57 (FB0) to 14.2 (FB9). Simultaneously, the total color difference (ΔE) reached a value of 5.08, clearly exceeding the threshold for consumer sensory detection. This dataset, with clearly expressed but opposing trends (decrease in FRAP and epicatechin versus increase in quercetin and ΔE), represents a valid basis for further correlation modeling.

Correlation Analysis of Interdependencies (Pearson Matrix)

To quantify the strength and direction of the relationship between chemical changes in flour and the sensory quality of the sausage, Pearson's correlation matrix (r) was calculated. The results of cross-referencing key raw material parameters and the final product are displayed in Table 3, visualized using a color scale ("heatmap") to facilitate pattern identification.

Table 3. Pearson correlation matrix (r) between flour quality parameters (inputs) and sausage color parameters (outputs)

	Sausage L^* (Lightness)	Sausage a^* (Redness)	Sausage b^* (Yellowness)	Sausage ΔE (Total change)
TPC (Total Phenols)	0.824	**0.874**	0.855	-0.869
FRAP (Antioxidant power)	0.851	**0.984**	0.912	-0.978
Epicatechin	0.795	**0.930**	0.880	-0.925
Quercetin	-0.810	**-0.895**	-0.860	**0.910**

Note: Values are displayed on a "Heatmap" color scale: intense red ("hot") indicates a strong positive relationship (values closer to +1), while intense blue ("cold") indicates a strong negative relationship (values closer to -1).

Analysis of the "heatmap" immediately reveals key zones of influence. A zone of distinctively "hot" (red) correlation dominates between the antioxidant parameters of the raw material and the redness parameter of the final product. The strongest positive correlation in the entire matrix was observed between the antioxidant power of the flour (FRAP) and the redness of the sausage (a^*), with a coefficient of $r = 0.984$. This implies an almost functional dependence: the preservation of the flour's reducing ability directly dictates the intensity of the red color in the product. The high statistical reliability of this link confirms the mechanism by which buckwheat phenols protect myoglobin from oxidation.

On the other hand, the total color change column (ΔE) shows strong "cold" (blue) correlations with antioxidants, particularly with the FRAP value ($r = -0.978$). The negative sign indicates an inverse proportion: the lower the antioxidant protection of the flour, the greater (worse) the color change of the sausage. An interesting exception is quercetin, whose row in the table shows an inverse color arrangement compared to other antioxidants. It shows a strong negative relationship with redness ($r = -0.895$) and a positive relationship with ΔE ($r = 0.910$). This confirms that quercetin accumulation during storage can be viewed as a reliable chemical indicator (marker) of aging that indicates the degradation of colorimetric properties.

Principal Component Analysis (PCA)

Before interpreting PCA components, the suitability of the dataset for factorization was checked. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.72, which is above the recommended threshold of 0.5. Additionally, Bartlett's test of sphericity showed statistical significance ($p < 0.001$), justifying the application of PCA analysis to this dataset.

Multivariate analysis enabled dimensionality reduction and detection of mutual relationships between variables, with the first principal component (PC1) clearly defined as the "oxidative status axis". Geometric arrangement of vectors in Figure 2 reveals key structural laws in the data, primarily observing a distinct grouping of variables FRAP, TPC, Epicatechin, and redness (a*) in the right part of the graph (positive PC1 axis). The proximity and directionality of these vectors in the same direction confirm that high antioxidant power of the raw material directly supports the preservation of redness.

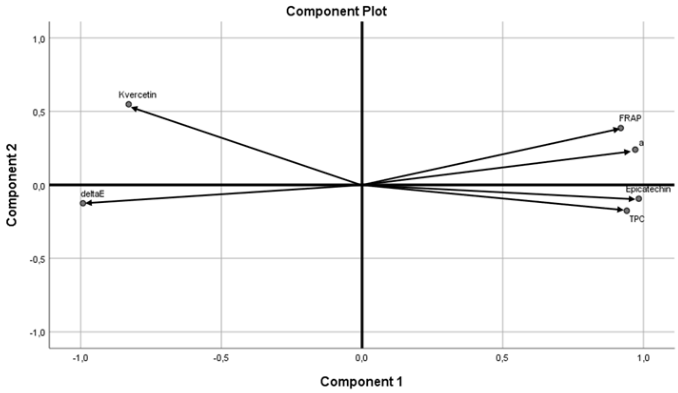

Figure 2. Component Plot in Rotated Space obtained by SPSS analysis, showing vector relationships and grouping of examined variables

In contrast, the total color change vector (ΔE) is isolated on the opposite, left side of the graph, visually demonstrating a strong inverse correlation with antioxidants. Additionally, the Quercetin vector occupies a specific position in the upper left quadrant, separated from the primary group of antioxidants, indicating its role as a specific marker of matrix degradation that behaves differently during the storage process.

Regression Model Evaluation and Limitations

Based on the established high correlations, a Linear Regression procedure (SPSS Linear Regression) was applied to form a predictive model for sausage redness (a*) based on flour FRAP value. The statistical reliability of the model was confirmed by Fisher's F-test (ANOVA), which showed high regression significance ($F = 62.53$, $p = 0.016$), thereby rejecting the null hypothesis of no linear relationship.
The obtained model equation is:

$$a^*_{pred} = 0.092 * FRAP + 12.787 \tag{4}$$

Quantitative indicators of this model's success are summarized in Table 4.

Table 4. Parameters and performance of the developed linear regression model

Input Variable (x)	Output Variable (y)	Model Equation	R^2	RMSE	MAE
FRAP (μmol Fe^{2+}/g)	Sausage a* (CIE redness)	y=0.092x+12.787	0.969	0.274	0.215

The graphical fit of experimental data with predicted values is shown in Figure 4. Visual inspection of the diagram confirms a high degree of linearity, where experimental points (markers) closely follow the regression line, especially in zones of fresh (FB0) and long-term stored raw material (FB9).

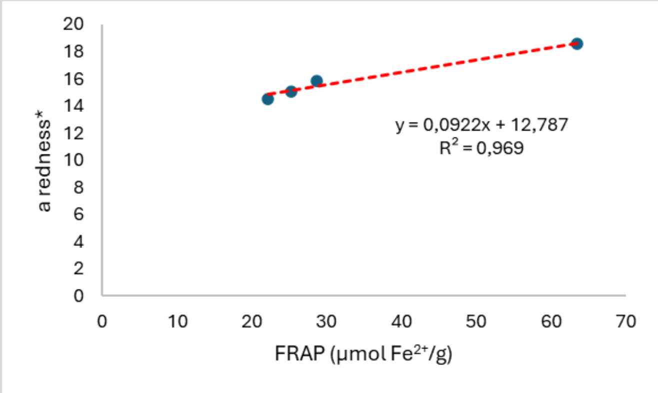

Figure 3. Linear regression model of the dependence of parameter a* on FRAP value

In contrast, a more detailed residual analysis reveals limitations of this approach. Although R^2 is high, the presence of detected error (RMSE = 0.274) suggests that there are deviations that cannot be ignored in the context of precise standardization. Linear models are limited when describing complex biological systems where opposing mechanisms act simultaneously. These results suggest a clear room for improving prediction accuracy by applying more sophisticated, non-linear artificial intelligence algorithms (ANN) in future research.

CONCLUSION

This study applied multivariate statistical analysis for the first time to map the interdependency between the degradation of bioactive compounds in stored buckwheat flour and the color change of emulsion-type chicken sausages.

Based on the results obtained, the following conclusions are drawn:

- Matrix Degradation: The buckwheat storage process leads to significant chemical changes ($p < 0.05$), with the most critical being the loss of antioxidant power (FRAP value decrease by 2.9 times) and degradation of specific phenols such as epicatechin. These changes in the raw material directly correlate with the loss of characteristic redness in the final product,
- Key Predictors: Correlation and PCA analysis identified the FRAP value of flour as the most reliable single marker ($r = 0.984$) for predicting the colorimetric quality of the sausage. This confirms that preserving the reducing ability of the additive is a key mechanism for preventing meat pigment oxidation,
- Model Reliability: The developed linear regression model showed high precision ($R^2 = 0.969$) and statistical significance ($F = 62.53$, $p = 0.016$), providing the industry with a simple and fast tool for estimating expected product color based on input raw material analysis, prior to the start of the production process,
- Guidelines for Future Research: Despite the high coefficient of determination, the detected residual error (RMSE = 0.274) and mean absolute error (MAE = 0.215) indicate the limitations of the linear approach in describing complex interactions within the food matrix.

Final conclusion of this research is that statistical modeling can successfully link raw material and product quality, but complete elimination of error requires the application of more sophisticated algorithms. The next phase of research will focus on developing models based on Artificial Neural Networks (ANN), aiming to further improve prediction precision for robust standardization of functional meat products.

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