THE USE OF UV-VIS SPECTROPHOTOMETRY FOR DETERMINING THE MALONDIALDEHYDE AS A QUALITY MARKER IN PORK TRACEABILITY

Valentina STICI[1], Tatiana COSTIN[1], Anatol RACUL[1]

e-mail: anatol.racul@gmail.com

Abstract

The aim of the paper is to define the biochemical approach in assessing the quality of pork traceability using the UV-VIS spectrophotometric method to determine the concentration of malondialdehyde. The mechanism of the proposed chemical reaction consists in the formation of the red chromogen, where λ=532 nm, between the thiobarbituric acid and malondialdehyde in the glacial acetic acid medium. The proposed concept is related to food safety, consists in using the biochemical method of evaluating the activity of peroxidase in the agri-food products, and represents the basis of the medical-veterinary approach of the accuracy in evaluating the substances that result in the process of pork alteration. The near-infrared spectroscopy (NIR) and the techniques of pH measurement served as methodological support in quality assessment. The result of the experimental evaluation confirms that the concentration of red chromogen is an genuine marker of the pork quality.

Key words: pork, peroxidase, thiobarbituric acid, malondialdehyde, near-infrared spectroscopy

The quality assurance system in the technological chain (animal breeding, slaughtering, processing and consumption of pork) involves the HACCP approach in the livestock farming sector. Food safety and the quality of agricultural products represent essential conditions that a producer must offer to consumers in the Republic of Moldova. The basic principle of HACCP is to assess the risks and determine the basic components in the evolution of the biotechnological process by determining the critical control points in the traceability of pork. Conceptually, the principle of evaluating product quality in the livestock sector is approved by defining these biochemical processes that generate the alteration of the raw material at the level of all its structures (Cheng, J., 2016).

Meat and meat products represent a substantial part of the consumed agri-food products and pork quality is an adequate indicator of the country's technological and industrial development level. In fact, the main goal of industry and researchers is to understand the mechanisms of lipid oxidation and to identify the most effective methods of controlling this process (Amaral A.B. *et al*, 2018). The technological stages are of crucial importance in pork traceability as they influence the degradation processes of unsaturated fatty acids and other perishable compounds. In the evaluation of the technological process in terms of ensuring food safety, an important role is given to the oxidative processes which, under the influence of microbiological decomposition, affect lipids, proteins, vitamins and pigments (Alamprese C. *et al*, 2013).

Simultaneously with the above-mentioned oxidative processes, the aldehydes, which are the predominant derivatives of this process, interact with proteins, causing changes that generate a decrease in the nutritional and organoleptic properties of the raw material (Chan D.E. *et al*, 2002). The consequences of these processes represent critical points in pork traceability and can cause substantial decline of the market value.

MATERIAL AND METHOD

The research was carried out in the period 2020-2021 within the cross-border project „Promoting sustainable production and implementing good practices in cattle farms in the cross-border area Romania, Republic of Moldova and Ukraine" MIS Code ETC 1549 using in experiment meat samples purchased from a local pig farmer. Experimental evaluation of the raw material quality was performed using the laboratory equipment purchased for the „Cattle Production Monitoring Center": VWR UV-6300PC spectrophotometer, WTW pH 7110 meter, NIR MultiScan 3000 analyzer, WTW thermostat OF-01E, Orbita CLU-1 centrifuge, Komovski pump and WTW analytical balance.

[1] Agrarian University of Moldova, Chișinău, Republic of Moldova

1. *Reagents and chemicals.* Thiobarbituric acid (TBA) 99% (Makrohim-Ukraine); 96% tetrabutylammonium malondialdehyde (MDA) salt (Sigma-Aldrich-Germany); 99.5% methyl alcohol (Makrohim-Ukraine); 99.8% glacial acetic acid (Makrohim-Ukraine) and double-distilled water.
2. *Preparation of the standard malondialdehyde solution*. In order to achieve the calibration curve (nomogram), it was prepared the 1mM primary MDA solution in glacial acetic acid. For this purpose, an amount of 31.35 mg MDA was dissolved in 100 mL of solvent. Subsequently, from the primary solution, different concentrations related to the calibration curve with the values 0.025 ÷ 0.33 mM MDA were prepared according to Table 1. The range of MDA concentration values shows a 10-fold upward trend and thus the experimental values of the extinction index give the possibility to perform interpolation.
3. *The basic reagent TBA in chromogen formation*. The standard solution of thiobarbituric acid was of 4.0 mM. For this purpose, 57.66 mg of TBA were dissolved in 100 ml of glacial acetic acid. Fresh TBA solution was prepared for each experimental day.
4. *Sample preparation and analytical procedure of chromogen evaluation.* Fresh pork in a quantity of 1gr is disintegrated in 10 mL of solvent. As a solvent served a mixture of 50% of distilled water with 50% of glacial acetic acid. Methyl alcohol at a concentration of 0.01% was used to prevent the oxidative processes in the environment. The samples were passed through Schott filters under the pressure of 0.3 MP created by the Kmovski pump. The filtrate was centrifuged at 4000 rpm and subsequently used for spectrophotometric analysis. The chromogen evaluation procedure involves the creation of a homogeneous mixture of 1ml of disintegrated solution with 1ml of TBA solution and subsequently stabilized at 95°C for 60 minutes. As a result of the oxidative reaction of peroxidase, the red chromogen is formed and the measurement of the absorbance indicator at λ=532 nm is performed using the UV-VIS VWR6300 spectrophotometer. The fresh meat samples were placed in the thermostat at a stabilized temperature of 25^0C for a period of 24 hours. The chromogen concentration calculation formula (TBARS) assumes that the reference concentration of thiobarbituric acid is of 4 mM and the stoichiometry of the biochemical reaction is 2:1.

$$C(TBARS)=(Ac*V)/W$$

where Ac is the absorbance indicator and it is evaluated according to the regression analysis in the calibration curve, W is the weight of the pork sample evaluated in the experiment and V is the multiplicative factor depending on the dilution in ml of the measurable extract (Alam Z.F. *et al*., 2016). Defining the calibration curve requires performing five repetitions for each reference point according to the requirements of the Student distribution with 95% accuracy. The evaluation of the absorbance indicator of the control sample was performed with the repetitiveness n = 5 replacing the standard solution with the sample of acetic acid or water. The analytical methods of chromogen evaluation are in accordance with the requirements of the International Conference on Research Techniques and the calculation accuracy in the experiments is 95% according to the Student.

RESULTS AND DISCUSSIONS

For an accurate evaluation of the quality indicator in pork traceability it is necessary to apply a complex methodology in which the biochemical factors are evaluated with a significant weight (greater than 30%) in the obtained result. An important factor that denotes the damage degree of pork at biochemical level is the concentration of MDA, which results from the oxidation processes of peroxidase. Unsaturated fatty acids and free oxygen represent the basic components in the biochemical reaction of lipid oxidation. Lipids can be oxidatively damaged in three main directions which include complex self-oxidation reactions, fermentatively catalyzed oxidative processes and photo-oxidation (Chan J., 2016).

In the presented mechanisms, self-oxidation is based on the chain of free radicals that attack the unsaturated bonds in fatty acids and it can be mentioned that this represents the basic procedure in biochemical degradation of meat (Ripoll G. *et al*., 2008). The fermentative and photo-oxidative mechanisms differ insignificantly from the exposed biochemical reaction, but they have a significant contribution in the formation of peroxides in the initial phase of pork degradation. Because of this, free radicals obviously explain most of the changes in meat degradation and can be the fundamental hypothesis of the biochemical processes of oxidation-reduction of the Krebs cycle in mitochondria.

The basic objective of the research was to oxidation of unsaturated lipids by determining the intermediate agents that include the formation of radicals in the process of substrates degradation by peroxidase in pork. This is the basic hypothesis put forward in the paper that attributes to malondialdehyde the quality of self-oxidation intermediate by means of hydrogen peroxide and serves as a quality marker in the traceability of the raw material. Traditionally, meat and meat products are exposed to light in the UV-VIS range in marketing networks in order to be attractive to consumers. This creates photo-oxidative biochemical premises, which are more intense according to the degree of free radical formation than the self-oxidative processes.

Table 1

Dependence of the extinction index on the MDA concentration and the parameters of related linear regression

N	MDA concentration mM	Absorbance	Standard deviation	Confidence interval		Standard skewness	Standard kurtosis
				Lower limit	Upper limit		
1	0.330	0.287	0.0031	0.283	0.291	0.656	-0.221
2	0.250	0.241	0.0020	0.238	0.243	0.408	-0.275
3	0.200	0.194	0.0034	0.190	0.199	-0.408	-1.279
4	0.166	0.161	0.0016	0.159	0.163	-0.297	-1.299
5	0.125	0.101	0.0109	0.087	0.114	0.396	-1.264
6	0.076	0.060	0.0035	0.056	0.064	0.504	-1.242
7	0.025	0.022	0.0014	0.020	0.023	-0.091	-0.197

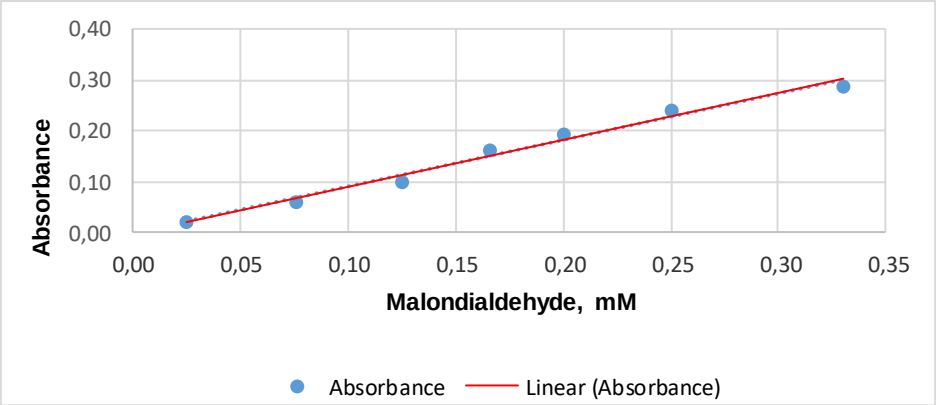

Figure 1 T**he calibration curve of MDA concentration evaluated spectrophotometrically with linear trend**

Table 1

NIR multifactorial analysis related to the method of sample storage and the determination of the significant difference in confidence intervals

				Stnd.	*Lower*	*Upper*
Multifactorial analysis		*Count*	*Mean*	*Error*	*Limit*	*Limit*
General average		18	55.3	0,045	55.2	55.4
Storage method (NIR factors)						
fat	refrigertor	3	13.5	0.102	13.3	13.8
fat	thermostat	3	14.5	0.102	14.3	14.7
moisture	refrigertor	3	66.1	0.102	65.9	66.3
moisture	thermostat	3	65.7	0.102	65.5	65.9
collagen	refrigertor	3	86.2	0.102	85.9	86.4
collagen	thermostat	3	85.6	0.102	85.4	85.8

Usually, the photo-oxidative mechanism is the initiator of lipid oxidation processes. During this process, hydroperoxides are formed in the presence of hemoglobin as a photo light sensitizer in the corresponding range. In conclusion, it can be stated that the main factors that influence the oxidation of lipids in meat are the content of fats and especially the unsaturated fatty acids that represent the substrate of oxidative processes (Candek - Potokar, M. *et al*., 2006).

The experimental part of the paper aims to evaluate the correlation between MDA concentration, pork acidity and biochemical factors included in NIR spectroscopy along with the degradation degree of the raw material. Therefore,

the techniques for assessing the MDA concentration in the analyzed samples represent a major objective in developing the methodology in the field of food safety. It is proposed to use UV-VIS spectrophotometry for determining the MDA in control solutions (100% acetic acid) or in the pork extract using as a solvent the mixture of 50% distilled water and 50% of glacial acetic acid. An important step in the proposed experimental methodology represents the definition of the calibration curve using the 1 mM MDA of control

solution with the respective dilution to the aldehyde concentration in the analyzed samples. The range of MDA concentrations refers to a 10-fold difference of the ratio between the lower limit and the upper limit of the elaborated nomogram. UV-VIS spectrophotometric analysis assumes that the chromogen absorbance indicator determines the amount of malondialdehyde in solution (Cozzolino D., *et al*., 2003). *Figure 1* shows the calibration curve of the MDA concentration in the range of 0.025 ÷ 0.33 mM with the calculation accuracy according to the Student distribution of 95%. Each reference point in the evaluation is repetitive (n=5) and denotes a satisfactory quality of the measurement technique using the UV 6300 spectrophotometer. The linear regression equation for determining the MDA concentration is the following

$y = 0{,}923*x - 0{,}0024$

where:

y - absorbance indicator,

x - MDA concentration in mM.

Table 1 presents the accuracy of the calculation in the spectral analysis nomogram in obtaining the linear dependence between the extinction index and the MDA concentration. The Student coefficient for the line slope in the linear regression t=43.3 represents a precision clearly higher than the level of 95%. The argument in favor of the regression model used to evaluate the parameters of the calibration curve is the Fisher criterion of dispersion analysis F=1880 and the determination coefficient R^2=0,98. The results obtained to define the MDA nomogram are acceptable for the control solution and thus the standard deviation of the values estimated within the proposed methodology represents a coefficient of variation below 10% according to Table 1.

It can be mentioned that the confidence interval of the extinction indicator in the proposed method of assessing meat quality has an accuracy within the range of 95% and indicates a correct determination of MDA concentration according to the spectra shown in Figure 2. A fundamental support in arguing the spectrophotometric approach in MDA determination is represented by the indicators of normal distribution (Gauss) of the curves presented in Figure 2 Standard kurtosis and Standard skewness.

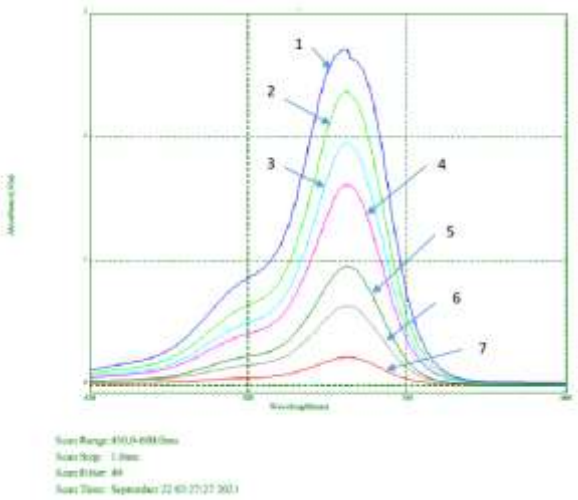

Figure 2. **Chromogen absorption spectra in the range of 450÷600 nm attesting the absence of adjacent triglycerides in the basic peroxidase reaction**

According to *Figure 2*, the correlation between the absorption spectra of the MDA-TBA standard is presented with the amount of intermediate triglycerides of peroxidase, which had to appear as wavelengths with significant intensity apart from λ=532 nm (Abbasali Z. *et al*., 2019). The spectrophotometric determination of the MDA concentration represents the basic criterion in the evaluation of the oxidative processes of unsaturated fatty acids.

The value of the chromogen absorbance indicator with the wavelength λ=532 nm obtained as a result of the reaction with TBA is an indisputable marker of the pork quality. The determination of this aldehyde in meat is of major importance due to the fact that in minor amounts of MDA a specific odor of product degradation with high protein content is produced. The specialized literature admits the amount of MDA of 2-2.5 mg/kg as an acceptable limit in which there is no rancidity in pork and its by-products (Merás I. *et al.*, 2020). In this paper, the MDA concentration resulting from the oxidative reactions of peroxidase on unsaturated fatty acids is proposed as a marker of pork quality. The methodological approach of evaluating this factor simultaneously with the determination of acidity (pH) and biochemical factors in NIR spectroscopy is the basic objective of the research (Bertolín J.R. *et al*., 2019).

The comparative analysis of the difference between refrigerated meat and meat kept in a thermostat at 25^0C for 24 hours is shown in Figure 3. The average value of the MDA concentration absorbance indicator for the meat sample kept in the thermostat is 25.1% higher than the samples kept in the refrigerator. The standard deviation of the 5 spectrophotometric measurements in each

variant is sufficient to assert that the confidence intervals do not overlap with 95% accuracy. The variation coefficient of the samples represents 2.96% for the meat kept in the thermostat and 7.49% for the one kept in the refrigerator. The ANOVA dispersion analysis based on primary data represents a strong argument to affirm that the MDA concentration depends on the storage temperature of the meat. At the same time, the acidity of the samples was determined and as a result of the repetitive evaluation, it was obtained the pH values of 5.82 for the meat kept in the thermostat and the pH=5.63 of the sample from the refrigerator. The increase of the sample acidity in the thermostat confirms the evolution of the destructive biochemical processes, demonstrating that the oxidation of lipids is favored by the increase of the temperature.

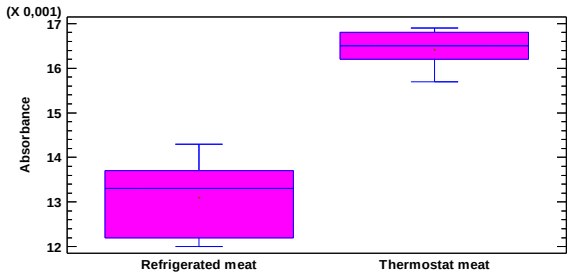

Figure 3. **Evaluating the significant difference in the storage of samples with the confidence interval of the MDA concentration**

In near-infrared spectroscopy, the biochemical factors identified in the meat samples are fat, moisture and collagen. The evaluation of the above mentioned NIR factors, which attests the alteration degree of pork depending on the storage method (thermostat or refrigerator) for 120 hours are presented in *Table 2*. The evaluated fat shows a 7% increase for the samples kept in the thermostat compared to the option of keeping it in the refrigerator. The calculation accuracy according to the Student criterion of 95% shows that the confidence intervals do not overlap and it can be stated that the NIR approach for determining the significant difference in the pork degradation degree is demonstrated. The moisture factor in the NIR assessment also shows a significant difference for the samples kept in the thermostat compared to those kept in the refrigerator. The confidence intervals of the arithmetic mean depending on the storage method with 95% accuracy do not overlap. The evaluation of the collagen factor also shows a significant difference with the accuracy of 95% based on the confidence intervals of the arithmetic means. The multifactor analysis model presented in Table 2 by the total standard deviation σ=0.102 shows that according to Fisher's criterion the NIR approach in the evaluation of pork degradation factors is true with an accuracy of 95%. At the same time, it was evaluated pork acidity for these samples and consequently, the 7% increase in the pH value for the thermostat storage method proves an advanced degradation. The determination of the absorbance indicator of the MDA concentration depending on the method of sample storage shows an increase of 34.6% of the aldehyde for the meat sample kept in the thermostat. The minimum values of the standard deviation confirm that the confidence intervals do not overlap for 95% accuracy (Grazioli C. *et al*., 2021).

CONCLUSIONS

As a result of researching pork traceability using specific methods of assessing the degradation degree, a complex of measures was proposed that can serve as a quality marker. The concept defined in the field of food safety in terms of biochemical methodology for assessing peroxidase activity in agri-food products, represents the scientific novelty of the research and requires experimental confirmation in the practice of product quality control. An obvious advantage of the proposed experimental research represents the definition of the pork disintegration technique using as a solvent the glacial acetic acid in order to avoid the formation of products coloured by the barbituric acid. This method is precise, sensitive and with a high reproducibility for the quantitative determination of the chromogen and falls within the 95% evaluation accuracy according to the Student distribution. The complex approach of MDA concentration as a marker in pork traceability may be useful later also for biochemical research of other animal products.

REFERENCES

Abbasali Z., Armin F., 2019 - *Extraction and preconcentration of trace malondialdehyde from lipid-rich foods using ion pair–based solvent bar liquid-phase microextraction*. Food analytical methods,12(7): 1625-1634.

Alam Zeb, F. Ullah, 2016 - *A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances (TBARS) in fried fast foods. Journal of analytical methods in chemistry*. Article ID 9412767, p.5.

Alamprese, C., Casale, M., Sinelli, N., Lanteri, S., & Casiraghi, E., 2013 - *Detection of minced beef adulteration with turkey meat by UV–vis, NIR and MIR spectroscopy. LWT*. Food science and technology, 53(1), 225–232.

Amaral, A.B.; da Silva, M.V.; da Lannes, 2018 - *S.C.S. Lipid oxidation in meat: Mechanisms and protective factors—a review*. Food sci. Technol, 38: 1–15.

Bertolín J.R., M. Joy, M. Blanco, 2019 - *Malondialdehyde determination in raw and processed meat products by UPLC-DAD and UPLC-FLD*. Food Chemistry, 298.

Candek-Potokar, M., Prevolnik, M., & Skrlep, M., 2006 - *Ability of near infrared spectroscopy to predict pork technological traits*. Journal of near infrared spectroscopy, 14(4): 269–277.

Chan, D.E., Walker, P.N., & Mills, E.W., 2002 - *Prediction of pork quality characteristics using visible and near-infrared spectroscopy*. American society of agricultural engineers, 45(5): 1519–1525.

Cheng, J., 2016. *Lipid oxidation in meat*.J. Nutr. Food Sci,6: 1–3.

Cozzolino, D., Barlocco, N., Vadell, A., Ballesteros, F., & Gallieta, G., 2003: *The use of visible and near-infrared reflectance spectroscopy to predict colour on both intact and homogenised pork muscle. LWT*. Food science and technology, 36(2): 195–202.

Grazioli C., Faura G., Dossi N., Toniolo R., Tubaro F., Terzi F., Bontempelli G., 2021 - *A colorimetric paper-based smart label soaked with a deep-eutectic solvent for the detection of malondialdehyde*. Sensors and Actuators, 329.

Merás I., Cesar P., Jesús P. Elisabet M. T., Anunciación E.M.,2020 - *Optimization of the thiobarbituric acid-malonaldehyde reaction in non-aqueous medium. Direct analysis of malonaldehyde in oil samples by HPLC with fluorimetric detection*.159.

Ripoll G., Albertí P., Panea B., Olleta J.L. and Sañudo C., 2008 - *Near-infrared reflectance spectroscopy for predicting chemical, instrumental and sensory quality of beef*. Meat Science, 80(3), 697–702.