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# KINETICS MODEL OF OXIDATIVE PROCESS IN BASIL SPREADS BASED ON CALCULATIVE COLOUR ANALYSIS

# KINETIČKI MODEL OKSIDATIVNIH PROCESA U NAMAZIMA NA BAZI BOSILJKA - KOLORNA ANALIZA

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## *ABSTRACT*

*Enzymatic and non-enzymatic browning of mild-heat processed spreads, based on basil and cheese was monitored as color changes, by means of multivariate color image analysis. The objective of the study was to assess the antioxidative potential of lactoferrin with combinations of ascorbic, citric, lactic acid, and NaCl on prolonged lipid oxidation in fresh basil-based spreads. The "browning"process, occuring in various foods, can be monitored by an algorithm describing the color properties of digitalised images, expressed by its color coordinates.The images were taken with a CCD camera, and afterwards transformed to frequency color distribution of color coordinate values. Different concentrations of organic acids had main influence on browning process, while lactoferrin and NaCl presence had no significant effect. The determined values of Peroxide Value (PV), Acid Value (AV) and oxidative induction time, determined by Differential Scanning Calorimetry $OIT_{DSC}$ (for both $0^{th}$ and $30^{th}$ day) were the input values for further calculation.*

***Key words:*** *food, oxidation, browning, color coordinate, SWOT analysis.*

## *REZIME*

*Enzimatsko i neenzimatsko tamnjenje („posmeđavanje") minimalno-prerađenih prehrambenih emulzija na bazi bosiljka i sira, praćeno je na osnovu promene boje digitalne slike, korišćenjem višeparametarske analize. Analiziran je antioksidativni potencijal laktoferina, proteina helatora metala na tamnjenje emulzija, u kombinaciji sa askorbinskom, limunskom i mlečnom kiselinom, kao i kuhinjskom solju, na produženje lipidne oksidacije minimalno prerađenih emulzija. Na osnovu razvijenog algoritma, ocenjivani su kolorogrami digitalizovanih slika, snimljenih kućnom digitalnom kamerom, dobijenih tokom oksidacije uzoraka. Osnovna informacija o boji dobijena je iz raspodele učestalosti boja, za RGB (crveno, zeleno i plavo) i HSV (sjaj, zasićenost i intenzitet) sisteme boja, dok je PCA analiza izvršena vrednovanjem kovarijansne matrice za osnovne, osrednjene i autoskalirane matrice početnih RGB informacija o boji i vrednovanjem sopstvenih vrednosti kovarijansne matrice i vektora sopstvenih vrednosti kovarijansne matrice za sva tri numerička modela. Rezultirajuća informacija je kolorogram, koji predstavlja jednodimenzijalni signal koji se sastoji od 4900 elemenata i opisuje osobine boja slike. Promene u materijalu, koje nastaju tokom oksidacije praćene su preko promena u raspodeli učestalosti osvetljenosti, kao i promene raspodele kolornih koordinata. Različite koncentracije organskih kiselina imaju glavni uticaj na tamnjenje emulzija, dok prisustvo laktoferina i NaCl nisu imali statistički značajan efekat.Utvrđene vrednosti peroksidne vrednosti (PV), kiselinske vrednost (AV) i oksidativni indukciono vreme, određeno tehnikom diferencijalne skenirajuće kalorimetrije $OIT_{DSC}$ (za 0-ti i 30-ti dan) bili su ulazne vrednosti za SWOT analizu.*

***Ključne reči:*** *hrana, oksidacija, tamnjenje, kolorne koordinate, SWOT analiza.*

## INTRODUCTION

The common practice in the assessment of food color is to use spectrophotometers or light sensitive cells to quantify color characteristics. The most diffused instruments evaluate only restricted areas of food samples or overall light reflectance from the entire surface of the food matrix, thus beeing not appropriate for inhomogenous food products. On the contrary, image analysis is an effective methodology, able to measure average chromatic parameters of non-homogenous surfaces. By simple programs of photo-enhancement it is possible to measure the color of digitalized images, expressing results in the usual chromatic coordinates. The changes in color, occuring due to oxidation processes of row vegetable or fruit-based foods, known as browning, can be observed by an evaluation algorithm describing the color intensity of digitalised images (*Antonelli, et al., 2004*).

Basil-based spread, similar to non heat-processed "*„pesto*", an inhomogenous Italian pasta sauce, whose main ingredients are basil, olive oil, cheese and walnuts, is identified as a benchmark to test the antioxidative properties of lactoferrin, metal-chelating protein, isolated from milk, which is an antimicrobial agent of broad spectrum, and, also, influence lipid oxidation (*Chiu, et al., 2007, Huang, et al., 1999*). Addition of natural antioxidative agents potentially influences lipid oxidation and browning (attributed to combined enzymatic and non-enzymatic oxidation processes) of minimally-processed green vegetable-based food emulsions. Pesto, obtained by the traditional recipe, from fresh basil, is limited to the very short shelf life due to oxidative procesess and microbial load (*Severini, et al., 2008, Fabiano, et al., 2000*). Oxidative browning of *„pesto"*, samples was determined by color lightness changes, applying color evaluation, by means of multivariate image analysis. Software was developed for processing of digitalized images in the form of one-dimensional signal, describing the color content. The colored images were taken with a common digital CCD camera.The basic color information was derived from frequency color distribution for red, green and blue (*R, G ,B*) (*Huang, et al., 2003, Shlens, 2009*). The changes in material due to oxidation was observed by the changes in colur coordinates frequency distribution (*R, G, B, L*).

In studying non-ezymatic browning reactions it is of main interest to define their kinetic, especially the browning production rate, expressed as reaction rate constant. In non-enzymatic browning reactions there is an initial induction period which corresponds to the stage of colored-compound formation. After this induction period the coluor of product is getting darker linearly with time (zero order kinetics $y = y_0 + k_0 \cdot t$) or exponentially (first order kinetics ( $y = \exp(-k_1 \cdot t)$ ) (*Labuza, et al. 1972, Saguy, et al.,1978, Toribio, et al., 1984, Garza, et al., 1999, Wang, et al., 2006*). It has also been reported that non-enzymatic browning of different products can follow first order kinetic, zero order kinetic or combined kinetic models (*Garza, et al., 1999, Wang, et al., 2006, Vaikousi,et al. 2008, Soliva-Fortuny, et al., 2002, Quevedo, et al., 2009*). The antioxidative effect of milk metal-binding protein lactoferrin is highly sensitive to ionic strength and pH changes (*Wakabayashi, et al., 2006, Naidu, et al., 2002, Al-Nabulsi, et al., 2007, Jacobsen, et al., 2008*). The objective of the present study was to assess the antioxidative potential of lactoferrin with combinations of ascorbic, citric, lactic acid, and NaCl on browning and prolonged lipid oxidation in fresh basil-based emulsions, similar to "*pesto*"-pasta spreads.

## MATERIAL AND METHOD

Basil-based emulsions (similar to italian *pesto* sauce), were prepared in 9 combinations of ascorbic, citric, lactic acid, NaCl and lactoferrin (Table 1). Basil was treated with hydrogen peroxide and/or short temperature shock during washing (*Lin, et al., 2002*). The product contained virgin olive oil, refined sunflower oil, cheeses, such as parmesan and kachkaval, whey, walnuts, sunflower seeds, salt and garlic, as well as organic acids (ascorbic, citric and/or lactic acids) (Sigma-Aldrich, Germany) and lactoferrin (bovine lactoferrin, 95% protein, DMV International, Veghel, The Netherlands) as regulators of acidity, antioxidative and antimicrobial agents.

### Separation of oil phase

After a certain period of storage (0 and 30$^{th}$ days), the samples of „*pesto*" spread were frozen at -70$^{o}$C for ten days. After defreezing, the content was centrifuged at 26100 g for 30 minutes and analysed for oxidative stability of separated oil phase (*Jacobsen, et al., 1998*).

*Table 1. Treatments of basil-based "pesto" spreads. Nine formulations were obtained by modulating levels of ascorbic acid, citric acid, lactic acid, NaCl and lactoferrin.*

| *No.* | *Treatment* |
|---|---|
| 1. | 0.5g·kg$^{-1}$ ascorbic acid, 0.4% NaCl |
| 2. | 0.5 g·kg$^{-1}$ ascorbic acid, 0.4% NaCl, 80 mg·kg$^{-1}$ lactoferrin, |
| 3. | 0.5 g·kg$^{-1}$ ascorbic acid, 0.4% NaCl, 400 mg·kg$^{-1}$ lactoferrin |
| 4. | 0.5 g·kg$^{-1}$ ascorbic acid, 0.4% NaCl, 2000 mg·kg$^{-1}$ lactoferrin |
| 5. | 0.5 g·kg$^{-1}$ ascorbic acid, 3% NaCl |
| 6. | 1 g·kg$^{-1}$ ascorbic acid, 1.5 g·kg$^{-1}$ citric acid, 0.4% NaCl |
| 7 | 1 g·kg$^{-1}$ ascorbic acid, 1.5 g·kg$^{-1}$ citric acid, 0.4% NaCl, 400 mg·kg$^{-1}$ lactoferrin |
| 8. | 1 g·kg$^{-1}$ ascorbic acid, 1.5 g·kg$^{-1}$ citric acid, 0.4% NaCl, 3.6% lactic acid |
| 9. | 1 g·kg$^{-1}$ ascorbic acid, 1.5 g·kg$^{-1}$ citric acid, 0.4% NaCl, 3.6% lactic acid 400 mg·kg$^{-1}$ lactoferrin |

### Oxidative stability analysis

The development of primary products of lipid oxidation was followed by peroxide value (*PV*), (ISO 3960:1998). Free fatty acids were analyzed by acid value (*AV*), (ISO 660:1996), in the separated oil phase of the sample. Oxidative stability of „*pesto*" oil phase, was analysed according to the method of differential scanning calorimetry (*DSC*), at isothermal conditions in oxygen current on "Tzero" technology calorimeter, (*Velasco, et al., 2004*), DSC Q1000, TA instruments, (Delaware, USA), and expressed as oxidation induction time ($OIT_{DSC}$). „*Pesto*" oil phase was isothermally oxidized in oxygen atmosphere (purge flow 50 ml·min$^{-1}$) at, 105 $^{o}$C by DSC, using oxidative induction time (*OIT*) standard method (ASTM E 1858-08). All experiments were done in triplicate.

### Colour analysis

Colour images of basil-based "*pesto*" spreads were captured by a Canon PowerShot A550 CCD camera, which is a common digital camera for home use. Each sample was smeared to an approximately constant 3-5 mm thickness and to an area sufficient to cover completely the image scene, in order to avoid the presence of background in the image. Then, it was placed on a white paper napkin set on a flat white painted surface, 15 cm below the digital camera. Paper napkins were used in order to absorb the excess of oil and therefore to avoid undesired reflection effects. With this setup, it was possible to capture images with negligible shadows and without specular reflections. There were 9 different pesto formulations to be observed, and the images of each different treatments of *„pesto"* were taken periodically 22 times during the period of approximately 320 minutes.

The first step in the elaboration of every single digital *RGB* image with size {*r*, *c*, *R*, *G*, *B*} (where *r* is the number of pixel row, *c* is the number of pixel column and *R, G* and *B* channels) consists of unfolding it to a {(*r* x *c*), (*R*, *G*, *B*)} two-dimensional matrix of the *R, G* and *B* values (variables) for the *r* x *c* pixels (objects), i. e., (*r* x *c*) x 3 dimension. Since the imported files are 24 bit color images, each one of the three *R, G* and *B* "slices" of the 3D array are (24/3) 8 bit greyscale images, and therefore the three *R, G* and *B* variables can assume all the integer values in the range 0-255. After this first step these coordinates were normalized, i. e., divided by 255, in order to be represented as values varying from 0 – 1.

The peek values for *R*, *G*, *B* and *L* distribution vectors was used to represent data recorded during the experiment, and these data is represented in relative form, i. e., values were divided by maximum, initial, values.

### Kinetic model

The experimental values of relative lightnes $\left(L_{rel}\right)$ of *„pesto"* formulations were ajusted to the zero order ( $y = y_0 + k_0 \cdot t$ ) and first order ( $y = \exp(-k_1 \cdot t)$ kinetic models (*Garza, et al., 1999*). The experimental lightness $\left(L_{rel}\right)$ value data of pesto sauce spreads obtained were fitted to the 2 commonly used regression models by using non-linear least squares regression solved by a Levenberg–Marguardt numerical method. The fitting function used for determination of model constancies ($y_0$, $k_0$, $k_1$) were exponential curve (for *„pesto"* spreads 1-5) and linear model (for pesto spreads 6-9). The multiple combinations of different parameters that gave the highest $R^2$ were finally included in the logarithmic model.

### SWOT analysis

Furthermore, the SWOT analysis was applied, and the lipid oxidation criteria were: primary products of lipid oxidation, analyzed by peroxide value (*PV*), (ISO 3960:1998), free fatty

acid content, analyzed by acid value (*AV*), (ISO 660:1996) and total oxidative stability, determined by oxidation-induction time ($OIT_{DSC}$), according to the method of differential scanning calorimetry (DSC) in the separated oil phase of emulsions (*Velasco, et al., 2004*).

The SWOT diagram was evaluated on lipid oxidation criteria, according to the four factors of decision-making: alternatives, criteria, performance, and weight (*Saaty, et al., 1990*).. Alternatives refer to data (objects) to be compared with (e.g., emulsions on $0^{th}$ and $30^{th}$ day). Criteria refer to the key factors of value assessment (e.g. the factors that represent the state of the objects to be compared, *AV*, *PV* and $OIT_{DSC}$. Performance structure refers to the values of the key factors representing their importance. The weights of key factors are evaluated according to previous investigation or by some analytical method (analytic hierarchy method – AHP, principal component analysis – PCA (*Saaty, et al., 1990*).

## RESULTS AND DISCUSSION

*„Pesto"*, obtained by the traditional recipe, from fresh basil, is limited to the very short shelf life. For this reason, pasteurisation or sterilisation and/or lowering of pH and addition of antimicrbial and antioxidative agents are needed for industrial production, but these treatments lead to color, taste and flavour degradation. Changes in color, occuring due to oxidation processes (oxidation of phenols, degradation of chlorophyll and non-enzymatic browning, due to the reaction of oxidated lipids with proteins), can be observed in *„pesto"*sauce by evaluation algorithm describing the color changes during oxidation process, obtained through colorgram analysis (*Antonelli, et al., 2004*). The relative color coordinates (red, green and blue), Figure 2., in all samples with 0.5 $g \cdot kg^{-1}$ of ascorbic acid containing lactoferrin concentrations 80, 400 and 2000 $mg \cdot kg^{-1}$ (formulations 1, 2, 3, 4 and 5 respectively), decreased more quickly during the time, i.e. the browning was more intensive than in the control treatment 1.

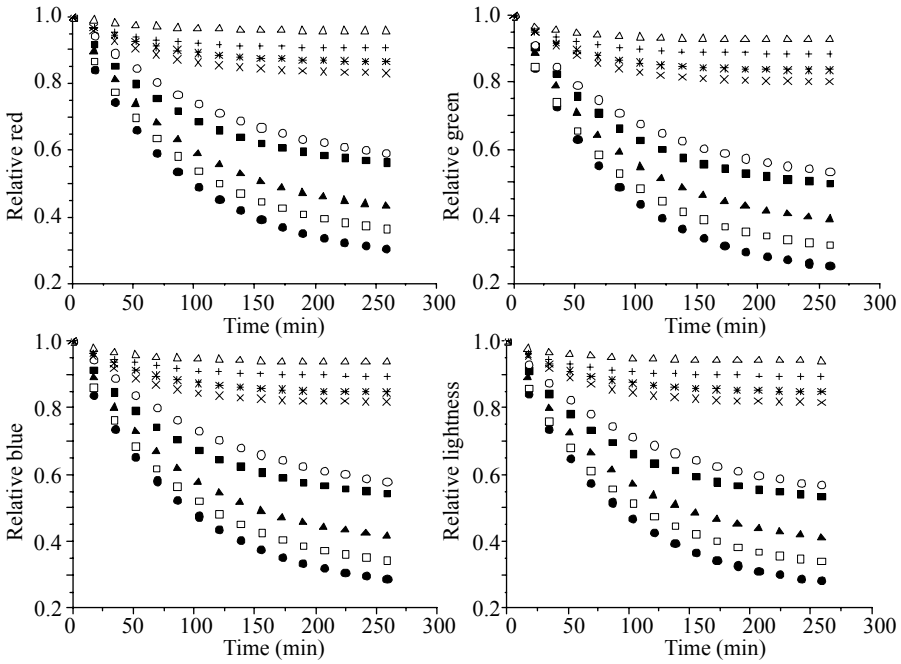


*Fig. 1. Relative changes of most frequent values of colour coordinates (red, green and blue, as well as lightness) of 9 treatments (Table 1., 1 -■, 2 -●„ 3 -▲, 4 - □, 5 - ○, 6 - △, 7 - +, 8 - ×, 9 - ✳) of basil-based („pesto",) spreads during oxidation at room temperature*

*Table 2. The first order browning rate kinetic constants, calculated by the value of relative lightness, obtained from the slope of the regression lines, obtained after plotting function of time (for pesto spreads 1, 2, 3, 4, 5):* $y = \exp(-k_1 \cdot t)$

| *No.* | $k_1$ | $\chi^2$ | $R^2$ | RMSE | P |
|---|---|---|---|---|---|
| 1. | 0.003 ± 0.001 | 0.004 | 0.962 | 0.048 | 0.009 |
| 2. | 0.005 ± 0.002 | 0.004 | 0.975 | 0.052 | 0.012 |
| 3. | 0.004 ± 0.001 | 0.004 | 0.966 | 0.056 | 0.013 |
| 4. | 0.005 ± 0.001 | 0.004 | 0.967 | 0.058 | 0.014 |
| 5. | 0.003 ± 0.001 | 0.003 | 0.955 | 0.049 | 0.008 |

*Table 3. The zero order browning rate kinetic constants, calculated from the slope of the regression lines, obtained after plotting function of time (for pesto spreads 6,7,8,9):* $y = y_0 + k_0 \cdot t$

| *No* | $y_0$ | $k_0$ | $\chi^2$ | $R^2$ | RMSE | P |
|---|---|---|---|---|---|---|
| 6. | 1 | 0.004± 0.001 | 1e-5 | 0.99 | 0.001 | 0.002 |
| 7. | 1 | 0.004± 0.001 | 1e-5 | 0.99 | 0.001 | 0.001 |
| 8. | 1 | 0.005± 0.001 | 1e-5 | 0.99 | 0.002 | 0.003 |
| 9. | 1 | 0.006± 0.001 | 1e-5 | 0.999 | 0.002 | 0.002 |

Samples containing lactic acid (3.6%) in combination with ascorbic acid (1 $g \cdot kg^{-1}$) and citric acid (1.5 $g \cdot kg^{-1}$), treatments 8 and 9, and samples containing ascorbic acid (1 $g \cdot kg^{-1}$) and citric acid (1.5 $g \cdot kg^{-1}$), treatments 6 and 7, proved to be least prone to oxidation.

In our study, it have been found, according to the fitting model, that the pesto formulations 1-5 are getting darker (browning effect) while exposed to air at room temperature, according to exponential law: $y = \exp(-k_1 \cdot t)$; first order kinetics, and pesto formulations 6-9 according to zero order kinetic $y = y_0 + k_0 \cdot t$, where y is the value of property for any time, $y_o$ is the initial value of this property, t is time, $k_o$ is the zero order kinetic constant and $k_1$ is the first order kinetic constant (*Labuza, et al. 1972, Saguy, et al.,1978, Toribio, et al., 1984, Garza, et al., 1999, Wang, et al., 2006*). Evaluated browning rate constants ($k_1$, $k_o$) and correlation coefficients for these curves ($R^2$), root mean square error (RMSE), mean relative percent error (P) and χ-square ($\chi^2$) are presented in Table 2**,** and Table 3.

The first order model kinetic constant values ($k_1$) (Tab 2) where much higher in all cases than those of the zero order model ($k_0$), suggesting that browning process, in all samples containing 0.5 $g \cdot kg^{-1}$ of ascorbic acid (formulations 1, 2, 3, 4 and 5 respectively) was faster than in samples with 1$g \cdot kg^{-1}$ of ascorbic acid added (formulations 6, 7, 8 and 9), what is atributed to antioxidant and browning reduction properities of ascorbic acid, citric acid and lactic acid (*Lopez-Nicolas, et al., 2007, Son, et al., 2001, González-Aguilar, et al., 2004, Rico, et al., 2007*). The effect of lactoferrin, on kinetic constant values, as it was shown on Tables 2 and 3, was negligible.

## Prolonged oxidative stability

Oxidative stability of separated oil phase of basil-based spreads was followed regarding peroxide value (*PV*), acid value (*AV*) and according to the method of differential scanning calorimetry (DSC) oxidation induction time ($OIT_{DSC}$). Results obtained are presented in Table 4.

*Table 4. Peroxide value, (PV), (mmol $O_2 \cdot kg^{-1}$), acid value, (AV) and oxidation induction time ($OIT_{DSC}$) (min.), of basil-based spreads during 30 day of storage at 4°C, (determined in separated oil phase) - the effect of 9 combinations of ascorbic, citric, lactic acid, NaCl and lactoferrin (Table 1).*

| *No.* | *PV* 0 day | *PV* 30 day | *AV* 0 day | *AV* 30 day | $OIT_{DSC}$ 0 day | $OIT_{DSC}$ 30 day |
|---|---|---|---|---|---|---|
| 1 | 1.10 | 1.61 | 1.03 | 1.37 | 177.84 | 248.15 |
| 2 | 0.93 | 0.89 | 0.95 | 1.38 | 118.66 | 226.18 |
| 3 | 0.98 | 0.36 | 0.98 | 1.42 | 134.45 | 225.58 |
| 4 | 0.82 | 1.21 | 1.01 | 1.34 | 143.20 | 220.51 |
| 5 | 1.08 | 0.47 | 1.04 | 1.47 | 147.94 | 293.08 |
| 6 | 2.23 | 0.89 | 0.67 | 0.90 | 291.32 | 382.98 |
| 7 | 1.85 | 0.69 | 0.69 | 1.07 | 236.86 | 307.54 |
| 8 | 1.78 | 1.20 | 0.67 | 0.73 | 326.71 | 328.69 |
| 9 | 1.72 | 1.22 | 0.81 | 0.87 | 317.18 | 321.72 |

Ascorbic and combination of ascorbic and citric acid, lowered primary lipid oxidation products (peroxide value, *PV*) after 30 days of storage at 4°C, (treatments 6 and 7). It was noticed previously that the increase of ascorbic acid concentration decreases the *PV* in fish oil enriched mayonnaise during storage (*Jacobsen, et al., 2001*). The effect of lactoferrin on PV, was observed on day 30, as decrease in *PV* related to the control (treatment 1), at concentrations of 80 and 400 $mg \cdot kg^{-1}$ (treatments 2 and 3). The absence of decrease in peroxide value, at 2000 $mg \cdot kg^{-1}$ of lactoferrin (treatment 4) could be explained by the prooxidative effect of high concentrations lactoferrin.[3] The lowering of pH, by adding lactic acid, (treatments 8 and 9), enhance, however, peroxide values after 30 days, compared to the treatments 6 and 7. The increase of acid value was generally observed between the 0th and 30th day and could be mostly attributed to the influence of lactobacteria, originating from cheese.

## SWOT analysis

The determined values of lipid oxidation criteria: *PV*, *AV* and $OIT_{DSC}$ (on 0th and 30th day) for 9 types of basil based emulsions were the input values for SWOT calculation **(**Table 2**).** The input values were normalized both for quantified and qualified performance, for objective performance scoring (e.g., 1–5 points, or 0.0 – 1.0 score). The aim of normalization is to unify the scales of the key factors (it could be also done by autoscaling). The used normalization method is realized by using fuzzy set, and calculated as benefit-criteria normalization (the higher the better for $OIT_{DSC}$), or cost-criteria normalization (the lower the better for *PV* and *AV*). In this article, A matrix is the correlation matrix, and the calculation of key factor weights is done as suggested by Saaty, et al., 1990. After calculation of the 0th and 30th day weight scores of the comparing objects, and the normalization of key factors, the score value was calculated for each data object. The mean was accepted as the benchmarking value. The scores of the compared basil-based emulsions were added together and the benchmarking value substracted. The final value was the coordinate value of the compared samples in the SWOT analysis matrix. In order to show the comparison on the four-quadrant coordinate system, the ordinate is prescribed to stand for the 30th day of basil-based emulsion, while the abscissa is prescribed to stand for the 0th day. The final benchmark values of lipid oxidation parameters are, also, calculated using weight factors for 0th and 30th day and their score values. The values of weight factors are also calculated using PCA analysis. The score values and SWOT coordinates obtained by evaluation are multiplied by 4 and added to 1 (in order to present the results in range 1 – 5 points, which is far more understandable, then the calculation range 0 – 1).

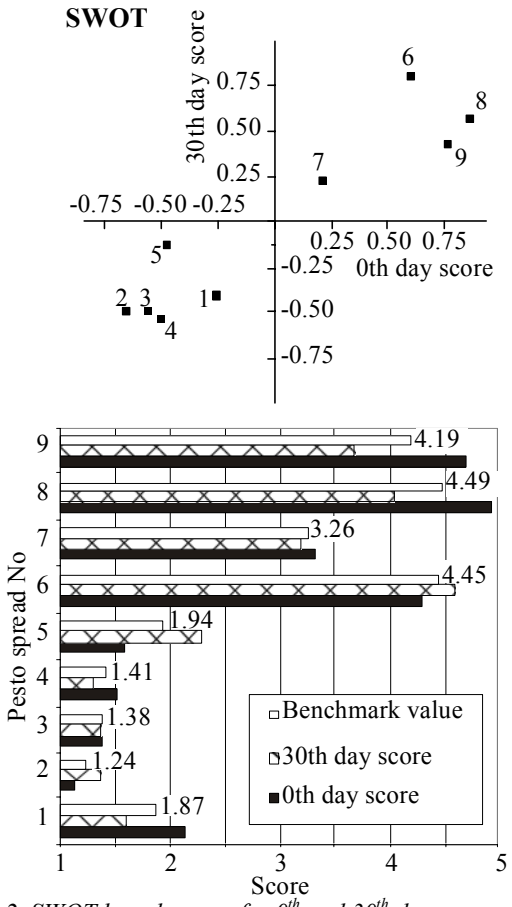


*Figure 2. SWOT based scores for 0th and 30th day, represented in SWOT diagram and benchmark value of lipid oxidation parameters (PV, AV and $OIT_{DSC}$) of basil-based emulsions 1 - 9.*

0th day score vector shows results that fit very well to those acquired by color image analysis of basil-based emulsion, corresponding to oxidative browning. Both analysis showed that the score of basil-based emulsions 2, 3 and 4 (containing lactoferrin and low acid concentration, 0.5 $g \cdot kg^{-1}$ ascorbic acid, pH 5.2) oxidize much faster then other emulsions, and gain the worst scores, compared to the control (emulsion 1). The best antioxidative effects were obtained with combination of ascorbic, citric and lactic acid at pH 4.4 (emulsion 8 and 9). There are variations in results obtained by image (benchmark value) and SWOT analysis, especially in emulsion type 6, which seems to be more like emulsions 8 and 9, as seen from SWOT analysis, while it is much closer to emulsion 7 according to benchmark value, Figure 3.

## CONCLUSION

Colour analysis of minimally-processed fresh basil-based food emulsions (*„pesto"* spreads), by means of multivariate image analysis, was applied on color lightness changes corresponding to oxidative browning of the product treated with antioxidative agents:, metal chelating protein, lactoferrin and/or ascorbic,

citric or lactic acid. The basic color information was derived from frequency color distribution for *RGB* color system.The changes in material due to oxidation was observed by the changes in color coordinates frequency distribution (*R, G, B, L*). The first order model kinetic constant values ($k_1$) where much higher in all cases than those of the zero order model ($k_0$ ), suggesting that browning process, in all samples containing 0.5 $g \cdot kg^{-1}$ of ascorbic acid was faster than in samples with $1g \cdot kg^{-1}$ of ascorbic acid added, what is atributed to anti oxidant and browning reduction properities of ascorbic acide, citric acid and lactic acid. The effect of lactoferrin, on kinetic constant values was negligible. Ascorbic, citric and lactic acid had shown significant impact on prolonging lipid oxidation in *„pesto"* spreads, according to SWOT analysis, while lactoferrin had negative impact, especially at high pH (5.2). $0^{th}$ day score vector revealed results that fit very well to those acquired by color image analysis, corresponding to oxidative browning, of same products.

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## REFERENCES

Al-Nabulsi A. A., Holley R. A.: Effercts on Echerichia coli O157:H7 and meat starter cultures of bovine lactoferrin in broth and microencapsulated lactoferrin in dry sausage batters. *Int. J. Food Microb.,* 2007, 113(1), 84-91.

Antonelli A., Cocchi M., Fava G., Franchini G. C., Manzini D., Ulrici A.: Automated evaluation of food color by means of multivariate image analysis coupled to a wavelet-based classification algorithm. Anal. Chim. Acta., 2004, 515, 3-13.

Chiu C.H., Kuo C.C.: Antioxidative and antimicrobial properties of lactoferrin in hot-boned ground pork during storage. *J. Food Process. Preserv.,* 2007, 31, 157-166.

Fabiano B., Perego P., Pastorino R., Del Borghi M. The extension of the shelf-life of "*pesto*" sauce by a combination of modified atmosphere packaging and refrigeration. Int. J. Food Sci. Techn. 2000, 35, 293-303.

Garza S., A. Ibarz, J. Pagan, J. Giner, Non-enzymatic browning in peach puree during heating, Food Res. Int., 1999, 32, 335-343.

Huang S-W, Satue-Gracia T., Frankel E. N., German B.: Effect of lactoferrin on oxidative stability of corn oil emulsions and liposomes. *J. Agric. Food Chem*., 1999, 47: 1356-1361.

Jacobsen C., M. B. Let, N. S. Nielsen, A. S. Meyer Antioxidant strategies for preventing oxidative flavour deterioriation of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation.Trends Food Sci. Technol., 2008, *19*, 76-93.

Labuza T.P., CRC Crit. Rev. in Food Technol., 1972, 3, 217-223., CRC Crit. Rev. in Food Technol., 1972, *3*, 217-223.

Lin C.M., Moon S. S., Doyle M. P., McWatters K. H.: Inactivation of *Echerichia coli* O157:H7, *Salomonella enterica* Serotype enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide and mild heat. J. Food Protection, 2002, 65: 1215-1220.

Naidu A. S.: Activated lactoferrin – a new aproach to meat safety. *Food Technol.,* 2002, 56: 40-45.

Quevedo R., O. Díaz, B. Ronceros, F. Pedreschi, J. M. Aguilera, Description of the kinetic enzymatic browning in banana (Musa cavendish) slices using non-uniform color information from digital images, Food Res. Int. , 2009, *42*, 1309–1314.

Saaty T.L., L.G. Vargas, Models, Methods, Concepts & Applications of the Analytic Hierarchy Process, Kluwer Academic Publishers, Boston, 1990.

Saguy J., I.J. Kopelman, S. Mizrahi, Extent of nonenzymatic browning in grapefruit juice during thermal and concentration processes: Kinetics and prediction, .J. Food Processing & Preservation. 2:175-184. 1979, *2*, 175-184.

Severini, R. Corbo, A. Derossi, A. Bevilaqua, Giulliani, R., Use of humectants for the stabilization of "*pesto*" sauce, Int. J. Food Sci Technol., 2008, *43*(6), 1041-46.

Shimazaki K. Lactoferrin: A marvelous protein in milk,.Anim. Sci J, 2000, 71, 329–347.

Soliva-Fortuny R. C., P. Elez-Martınez, M. Sebastian-Caldero, O. Martın-Belloso, Kinetics of polyphenol oxidase activity inhibition and browning of avocado purée preserved by combined methods, J. Food Eng. 2002, *55*, 131–137.

Toribio J.L., J.E. Lozano, J. Food Sci., Nonenzymatic browning in apple juice concentrate during storage 1984, *49*, 889-892.

Vaikousi H., K. Koutsoumanis, C. G. Biliaderis , Kinetic modelling of ... under isothermal and dynamic heating conditions, Food Chem., 2008, 107, 785–796.

Velasco J., Andersen M. L., Skibsted L.H.: Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. Food Chem., 2004, 85: 623-625.

Wakabayashi H., Yamauchi K., Takase M.: Lactoferrin research, technology and application. *Int. Dairy J.,* 2006, 16: 1241-1251

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