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CHANGES IN PHENOLIC CONTENT IN GROUND RED PEPPER (*CAPSICUM ANNUUM* L.) DURING STORAGE

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ABSTRACT

Dynamics of changes in phenolic content in ground red pepper spice (*Capsicum annuum* L.) was monitored during the period of 10/ 2016–01/2018. Phenolic compounds were determined: gallic acid, catechine, vanillic acid, caffeic acid, coumaric acid, ferulic acid, rutin, resveratrol, cinnamic acid. In order to carry out a relevant statistical evaluation, individual measurements were interpolated to obtain a weekly time series. The fastest weekly reduction in content occurs for cinnamic acid (0.9%), coumaric acid (0.6%), resveratrol (0.54%), catechine (0.53%), quercetin (0.47%), vanillic acid (0.47%), ferulic acid (0.39%), gallic acid (0.34%), and rutin (0.29%). For the total content of phenolic substances, weekly decrease of 0.4% was statistically significant (at significance level of 0.001). The losses of phenolic substances during storage are possible to be minimized partially by keeping in cold, dry, and dark storage areas, in airtight containers for wholesale and retail packages.

Keywords: storage, sweet pepper spice, phenolic substances

INTRODUCTION

Spices represent a very diverse group of natural substances characterized by their distinctive taste, aroma, and, very often, also healing effects. In gastronomy, the spices are used to intensify the outside attractiveness of food, colour and aroma thereof. More than 200 plant species belonging to more than 30 families are used as spices. Plants coming from the tropical regions of Asia, Africa and America mostly provide the most important spice species **(Kadlec *et al.*, 2009)**. One of their important features is the ability to eliminate the unpleasant or specific odour of initial raw materials. They play an essential role in flavouring and enhancing the taste of finished meals.

The seasoning agents are, in particular, various essential oils, bitter substances, phenolic substances, and organic acids, formed by fermentation of fresh and dry parts of plants. One of the most important spices used in gastronomy is the red pepper.

The red pepper is a ground spice made from red dried fruits of larger and sweeter varieties of *Capsicum annuum* (L) with changing taste. Bell pepper fruits are vegetables consumed worldwide as raw or as spices. The bell peppers are regarded to be a source of nutrients that can prevent various types of disease, such as ascorbic acid, carotenoids, and phenolic compounds, with known antioxidant properties and potential health benefits **(Hervert-Hernandez *et al.*, 2010; Kim *et al.*, 2010)**. Hot cultivars are rich in capsaicinoids, responsible for a specific hot taste **(Liu and Nair, 2010)**. The presence of capsaicinoids in hot peppers has a favourable effect on the stability of carotenoids during high-temperature drying. The capsaicinoids are synthesized and accumulated in epidermal placental tissue **(Topuz *et al.*, 2011)**. Capsaicin production increases with ripeness until the maximum is reached and, then, it decreases due to rapid reversal and degradation up to 60% via acting of oxidative enzymes **(Yaldiz *et al.*, 2010)**. **Reyes-Escogido *et al.* (2011)** report that the content of capsaicin and dihydrocapsaicin decreases in the case of cellular disturbance of fruits, which is probably due to the effect of oxidation at higher temperatures.

The amount of bioactive compounds depends on the pepper variety, the ripening stage, and the growth conditions. Studies carried out have shown a significant increase in ascorbic acid and carotenoid content during ripening in hot peppers **(Guil-Guerrero *et al.*, 2006; Iqbal *et al.*, 2013,2015)**. The sweet pepper contains many other active ingredients such as volatile oils, capsaicinoids, carotenoids, vitamins, proteins, fibre, and minerals (**Padayatty *et al.*, 2003; Materska and Perucka, 2005; Bosland and Votava, 2012)**. All the compounds exhibit antioxidant activity and other biological activities, such as a potential action against certain types of cancer; they stimulate immune system; thanks to them, we can prevent cardiovascular diseases; and they contribute to delaying the aging process **(Podsedek, 2007; Chuah *et al.*, 2008)**.

According to **Velíšek (1999)**, some phenolic substances exhibit significant biological effects and, therefore, rank among natural antioxidants, natural toxic substances of food or also among plant defense agents; over 8,000 species are known. In addition, the phenolic substances are partly responsible for the sensory and nutritional properties of spices. From the phenolic substances, phenolic acids (derivatives of benzoic acid and cinnamic acid) and flavonoids representing 30–60% of the total phenolic compound content are most commonly found in food.

According to their chemical structure, the phenolic substances are divided into three basic groups – non-flavonoid, flavonoid, and others. In addition to the distribution of phenolic compounds according to their chemical structure, these substances can be classified into several other groups according to their primary properties, including phenolic acids, flavonoids, tannins, lignans, and stilbenes **(Parades-Lopez *et al.*, 2010; Heleno *et al.*, 2015)**. The phenolic acids are present in food of plant origin predominantly in a bound form. The phenolic acids belong to the group of phenolic substances, structure of which is derived from the benzoic acid derivatives (e.g. gallic acid, salicylic acid, vanillic acid, hydroxybenzoic acid) and cinnamic acid derivatives (e.g. coumaric acid, ferulic acid, and caffeic acid). Probably, the most predominant form of hydroxycinnamic acid is chlorogenic acid composed of caffeic acid and quinic acid **(Duthie *et al.*, 2000; Robbins, 2003; Mattila *et al.*, 2006; Mattila and Hellström, 2007)**. The phenolic acids are deposited predominantly in surface layers of fruits and vegetables.

A number of studies describe the effects of storage and packaging materials on the long-term stability of polyphenols in food and beverages, warning that they are very sensitive to light, oxygen, and heat **(Kim *et al.*, 2011; Munin and Edwards-Levy, 2011)**. Several previous studies focused on the stability of capsaicinoids and antioxidants in pepper stored at different temperatures and reported substantial fluctuations during storage. Consequently, the concentration of capsaicinoids in the pepper was significantly reduced during prolonged storage under ambient storage conditions and the maximum reduction was observed for the dihydrocapsaicin **(Topuz and Ozdemir, 2004)**.

According to **Daood *et al.* (1996,2006)** the proportion of ascorbic acid in ground pepper significantly decreased by 35% compared to the original value after four months of storage, while carotenoids and flavonoids showed greater stability according to **(Kalt, 2005)**. **Perez-Galvez *et al.*, (2009)** monitored the proportion

of carotenoids in pepper powder and found that 83% of the original content was kept in a product stored in plastic bags at 4°C for 12 months.
The aim of the work was to monitor changes in the phenolic substance content in sweet pepper spice during storage in the period of 10/2016–01/2018.

MATERIAL AND METHODS

Samples of commercially supplied ground sweet pepper from Raps - CZ, s.r.o. were used in our experiment. In each time interval, 8 pepper samples were used for analysis. The pepper samples weighing 1.0±0.0001 g were mixed with 100 mL of an extraction mixture consisting of methanol, distilled water, and acetic acid (30:69:1). Then, the mixture was shaken in a water bath at 70°C for 50 min the mixture was cooled to 18°C and filtered into vials through a 0.5 µm PTFE Advantec filter. The phenolic substance content was determined using UHPLC Dionex Ultimate 3000 liquid chromatograph equipped with a UV/VIS detector, Phenomenex Kinetex C18 150×4.6 mm column in a mobile phase consisting of A (water:acetic acid – 99:1) and B (water:acetonitrile:acetic acid – 67:32:1). The mobile phase flow was 1 mL.min^{-1} in a gradient mode (A–90%, B–10%) at 0 min and (A–30%, B–70%) at 40 min followed by column equilibration to initial conditions – 5 min. The detection was performed at 275 nm. Within the experiment, the phenolic compounds were determined: gallic acid, catechine, vanillic acid, caffeic acid, coumaric acid, ferulic acid, rutin, resveratrol, cinnamic acid, and quercetin.

Statistical Analysis and Methods

A cubic spline was used to obtain a sufficiently large time series. Formally, a spline function consists of polynomial pieces, the third-order polynomials for cubic spline, on subintervals joined together with certain continuity conditions. For more information, see (**Gerald, 2003; Kaya, 2014**). A growth is analysed for the time series. The simplest approach in this regard is to obtain the geometric mean of the series. One of the disadvantage the approach is that it focuses only on the first and last observations in series, i.e. it ignores the information in the ongoing observations and any trend of growth that could develop over the period. This problem can be partially solved by using the ordinary least squares (OLS) method for estimating the unknown parameters in a regression model.
The simplest is linear regression model

$$y_t = \alpha + \beta x_t,$$

where the coefficient β gives us directly the change in dependent variable, y_t, for a one-unit change in independent variable, x_t (time in our case) and α is constant. Thus, this model is not suitable for comparing growth rates when comparing the growth rate of different dependent variables (with different value ranges). On the other hand, log-linear model

$$\ln y_t = \alpha + \beta x_t,$$

it converts the regression coefficient into relative values, i.e. it represents the percentage change of the dependent variable with absolute change of time (shift by one time).
In order to demonstrate a statistically significant difference in coefficients of two regression models, β_i, β_j, $i \neq j$, the null hypothesis that the coefficients are the same is tested. For the test statistic holds, if the homogeneity of error variances (between groups) cannot be assumed and we have at least 25 observations (within each series), the test statistic has a normal distribution and is determined as

$$z = \frac{\beta_i - \beta_j}{s_{\beta_i - \beta_j}}, i \neq j,$$

where $s_{\beta_i - \beta_j}$ is the standard error of the difference between the two coefficients. For detailed information see **(Kleinbaum and Kupper, 1978)**.

RESULTS AND DISCUSSION

The analysis results for ground sweet pepper root show that there were no significant changes in dry matter content during storage. Dry matter of the samples ranged from 88.40±0.98 to 88.60±1.08%. Moisture of the pepper spice is specified and should not exceed 11% (i.e. at least 89% of dry matter).
The dynamics of changes in phenolic substances during storage was carried out between 2016 and 2018, with samples being collected and analysed on 10/10/2016, 10/04/2017, and 08/01/2018.
Table 1 shows the proportion of aromatic substances in the dry matter.

Table 1 Representation of acids and aromatic substances in dry matter (µg·g^{-1})

code	substances	10/2016			4/2017			1/2018		
I	gallic acid	79.89	±	0.82	70.99	±	1.42	64.19	±	1.73
II	catechin	423.91	±	3.73	361.28	±	2.85	301.56	±	2.66
III	vanilic acid	33.18	±	0.42	31.23	±	0.28	24.34	±	0.66
IV	coffee acid	28.54	±	0.62	26.97	±	0.89	21.85	±	0.33
V	coumaric acid	11.69	±	0.66	10.37	±	0.27	10.37	±	0.31
VI	ferulic acid	12.16	±	0.22	11.56	±	0.09	9.37	±	0.80
VII	rutin	181.97	±	2.60	176.25	±	2.99	150.73	±	2.22
VIII	resveratrol	4.23	±	0.08	4.01	±	0.70	2.92	±	0.18
IX	cinnamic acid	2.00	±	0.07	1.84	±	0.06	1.10	±	0.02
X	quercetin	11.69	±	0.42	10.95	±	0.45	8.57	±	0.54
Sum		789.25	±	9.64	705.45	±	10.08	595.01	±	9.44

Weekly time series with decreasing tendency were obtained after interpolation. The cubic interpolation spline was chosen as the interpolation technique. A total of 65 values are available for each series. Figure 1 shows the development of interpolated time series of individual acids with 95% confidence intervals.
The graphs in Figure 1 show a nonlinear downward trend. The rate of decline is estimated using a log-linear regression model. Table 2 shows the estimate of regression slope coefficients. The coefficients (their negative value) represent the percentage decrease in single acid content per week. The values in parenthesis represent the standard errors of the coefficient. In this case, the regression slope coefficients are statistically significant at 1% level of significance.

Table 2 The estimate of regression slope coefficients in percentage terms (the values in parenthesis represent the standard errors of the coefficients).

Acid	I	II	III	IV	V
coefficients	-0.34 (0.007)	-0.53 (0.005)	-0.47 (0.014)	-0.41 (0.011)	-0.60 (0.008)

Acid	VI	VII	VIII	IX	X
coefficients	-0.39 (0.011)	-0.29 (0.009)	-0.54 (0.020)	-0.90 (0.035)	-0.47 (0.013)

Caption: I – X = code acids and aromatic substances (See Table 1)

The results indicate that the most rapid reduction in cinnamic acid (0.9% per week) occurs. In contrast, the slowest in routine (0.29% per week). When the sum of all the phenolic compounds is analysed, there is a statistically significant negative linear trend. Overall, the amount of phenolic compounds is reduced by 0.4% per week.
Our results are consistent with the findings of other authors who also experience a decrease in total phenolic content during storage, or storage factors with a direct impact on the content and antioxidant activity of selected phenolic compounds; see **(Khali and Selselet-Attou, 2007; Medina-Juárez *et al.*, 2012)**. Analogously, **Daood *et al.* (1996,2006)** report that phenolic compounds gradually decreased about 24% and 14% respectively, according to storage temperature during the storage period five months.
Further, at a 1% level of significance, it was tested whether there were statistically significant differences between the decline rates. Specifically, we tested hypotheses:

$$H_0: \beta_i = \beta_j, i \neq j, i, j = I, II, \ldots, X$$
$$H_A: \beta_i \neq \beta_j, i \neq j, i, j = I, II, \ldots, X$$

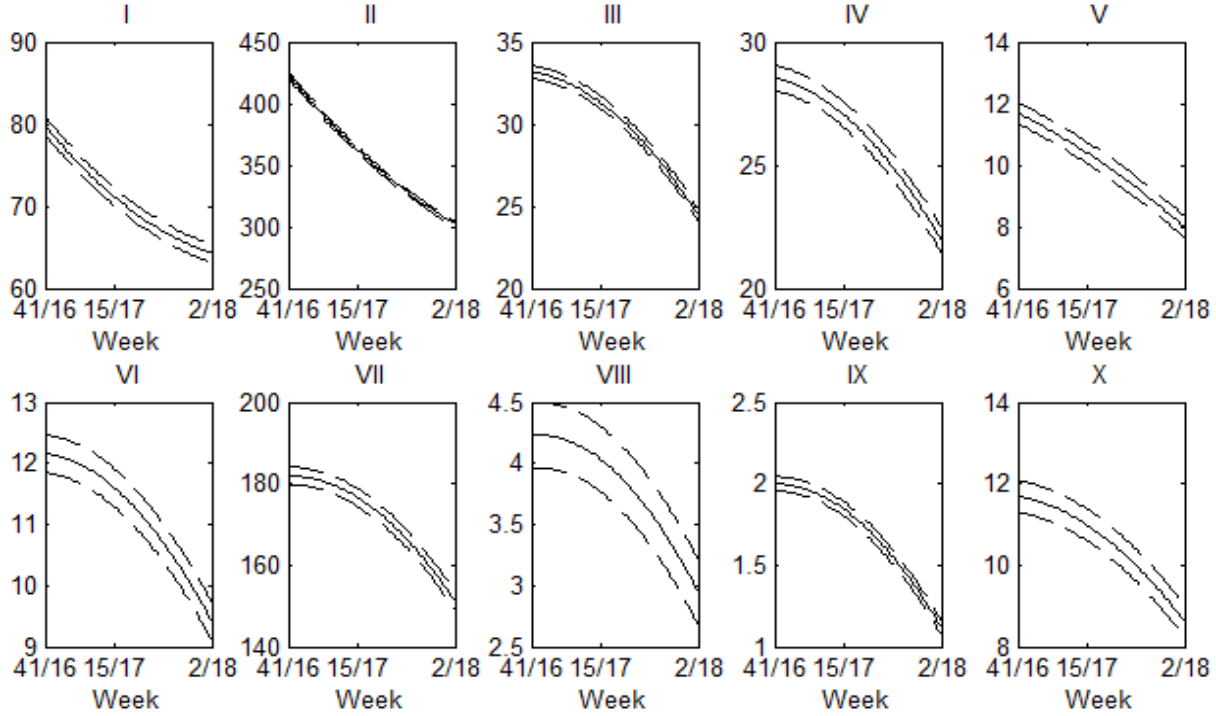

Figure 1 Development of I-X acids with 95% confidence intervals (See Table 1)

Table 3 presents the results in rejection or non-rejection of the null hypothesis and acceptance (A) or non-acceptance (N) of the alternative hypothesis.
The results show that there are statistically significant differences between the rates of decrease in individual acids. The exception is the relationship between feluric acid and caffeic acid, where the difference between 0.39% and 0.41% is not statistically significant. Similarly, the rate of decline in resveratrol is not statistically significantly different from that of catechine and vanillic acid.

Table 3 The results of hypotheses testing

	I	II	III	IV	V	VI	VII	VIII	IX
II	A								
III	A	A							
IV	A	A	A						
V	A	A	A						
VI	A	A	A	N	A				
VII	A	A	A	A	A	A			
VIII	A	N	N	A	A	A	A		
IX	A	A	A	A	A	A	A	A	
X	A	A	N	A	A	A	A	A	A

Caption: I – X = code acids and aromatic substances (See Table 1);
A= acceptance; N= non-acceptance

CONCLUSION

The results of the monitoring show that there is a significant decrease in phenolic substances in the spice of ground red pepper during prolonged period of storage. Storage under controlled optimal conditions allows for the overall quality of spice to be preserved in terms of sensory properties, such as hotness, colour, and antioxidant content, and, thus, reducing the impact of natural reduction in phenolic substance content. More gradual losses were observed for gallic acid, ferulic acid, caffeic acid, vanillic acid, rutin, and quercetin (0.40% per week). On the contrary, fast reduction occurs for cinnamic acid, coumaric acid, catechine, rutin, and resveratrol (0.64% per week). The fastest loss during our monitoring was reported for cinnamic acid (0.9% per week). The lowest decrease was observed for rutin (0.29% per week). In total, the phenolic substance content decreased significantly by 24.6%. In order to reduce the loss of phenolic substances during storage, it is important to store the spices in dry, dark, and cold areas, possibly under inert atmosphere. If the optimum storage conditions are met, the phenolic substance losses are possible to be minimized partially. For spice storage, sealable packaging is suitable to be used, which can help to slow down negative changes, or more precisely, decrease in content of biologically active substances contained in the red pepper within the warehouse as well as from the perspective of the retail consumer.

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