THE INFLUENCE OF SELECTED PHYSICAL FACTORS ON ANTIOXIDANT ACTIVITY OF QUERCETIN AND RUTIN

Tomasz Tarko, Aleksandra Duda-Chodak, Dorota Semik, Magdalena Kostrz, Ewelina Skrzyńska

Abstract: The aim of this study was to determine how physical factors (temperature, UV and visible light) influence on antioxidant activity of quercetin and rutin. In the study polyphenols treated and untreated with external factors were tested for their antioxidant activity by the ABTS method. The obtained results revealed that quercetin had higher antioxidant activity (317,2 mg Trolox/100 ml) than rutin (189,7 mg Trolox/100 ml). This fact can be explained by the presence of differences in the chemical structure of these compounds. The antioxidants activity of samples was significantly influenced by tested external factors, and the magnitude and direction of changes depended both on the polyphenol type and the kind of factor and time of exposition. Rutin was characterized by higher stability than quercetin. The increase of antioxidant activity demonstrated in some cases was probably caused by the formation of products of polyphenol degradation with higher antioxidant activity than the untreated compound.
Key words: quercetin, rutin, antioxidant activity, physical factors

INTRODUCTION

It has been proved that the content of antioxidants in foodstuffs depends on the degree of their processing that results from to the use of different technological processes. Decrease in the antioxidants content is not tantamount to a decrease of their antioxidant potential. During the processing, various reactions occur (eg. Maillard reaction) which lead to production of new compounds that may exert antioxidant activity (**Pokorny et al., 2001**). It is important to examine the effect of various physical factors on the antioxidant properties of phenolic compounds in order to more easily predict how the parameters of the production processes affect their biological value.

The aim of the study was to evaluate the influence of selected physical factors: UV radiation, visible light and temperature on the antioxidant potential of quercetin and rutin.

MATERIAL AND METHODOLOGY

The experimental material were ethanolic solutions (50 mg/100 ml) of quercetin and rutin (SIGMA). The effect of (1) temperature, (2) ultraviolet radiation and (3) visible light on the antioxidant potential of quercetin and rutin solutions was assessed.

(1) Aliquots of 5 ml polyphenol solution were placed in a water bath (100°C) for 30, 60, 90, 120, and 150 min. (2) Polyphenol solutions (5 ml) were exposed to ultraviolet light (UV-C lamp, λ = 253.7 nm) for 15, 30, 45, 60, 75 min, or visible light (3) for 24, 48, 72, 96, and 120 hours.

*Evaluation of antioxidant potential (**Tarko et al., 2009**)*

The antioxidant activity in polyphenol solutions before and after treatment was determined by using the active radical cation ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), Sigma). ABTS radical was generated by chemical reaction between 7 mM aqueous solution of diammonium salt of 2,2'-azynobis (3-ethylbenzthiazoline-6-sulfonate) and 2.45 mM potassium persulfate solution ($K_2S_2O_8$). The solution was kept overnight in the dark at ambient temperature, to terminate the reaction and to stabilize ABTS cation. During analysis, the concentrated solution of ABTS was diluted with phosphate buffer saline (PBS) at pH 7.4, to obtain solution with absorbance value of A = 0.70 ± 0.02 ($ABTS_{0.7}$) measured by a spectrophotometer (Beckman DU 650) at a wavelength of 734 nm. 100 μl of the appropriate diluted samples were added to 1 ml of $ABTS_{0.7}$ and 6 minutes after mixing the absorbance was measured. The antioxidant capacity of the samples was calculated using a standard curve performed on solutions of synthetic vitamin E (Trolox; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) and expressed in mg of Trolox/100 ml.

Statistical analysis

There were a minimum of three repetitions of the analysis and the results were shown as the arithmetic mean with standard deviation (± SD). Statistical analysis was performed using InStat v. 3.01 (GraphPad Software Inc., USA). A single-factor analysis of variance (ANOVA) with post hoc Tukey's test was applied to determine the significance of differences between means. The Kolmogorov-Smirnov test was carried out to assess the normality of distribution.

RESULTS AND DISCUSSION

The antioxidant potential of polyphenol solutions exposed to temperature of 100°C, UV and visible light as well as the initial values of this parameter (controls = polyphenols not treated) are presented in Table 1.

Antioxidant activity of quercetin (317.2 mg Trolox/100 ml) was significantly higher than that of rutin (189.1 mg Trolox/100 ml), which was associated with differences in their chemical structure as rutin is 3-O-rhamnoglucoside of quercetin. It was already proved, that glycosided are weaker antioxidants that their aglycones (**Skerget et al., 2005**).

Significant changes in the ability to free radicals scavenging by the tested polyphenols were observed after the exposition to high temperature (Tab. 1). In the case of quercetin heating caused an increase in antioxidant activity, while the ability of rutin to free radical scavenging decreased by more than 50 % after heating for 30 min, but prolonged heating caused a gradual increase of antioxidant potential reaching 53 % of initial value after 2.5 h of heating. The increase of the antioxidant properties of these compounds may result from the degradation of their original structure by high temperature as was reported by Mortira da **Costa et al. (2002).** The rate of the degradation depends on the time of exposure the polyphenols to elevated temperatures. **Deng et al. (2007)** shown that the high temperatures induce the conversion of glycosides to aglycone forms, which were characterized by higher antioxidant potential.

It has been shown that UV irradiation of rutin solutions did not affect their ability to free radicals scavenging. Regardless of the irradiation time the antioxidant activity of ethanolic solutions of rutin did not change significantly. This confirms the theory that the rutin is a relatively stable compound (**Murakami et al., 2004**). Completely different trends were observed for solutions of quercetin. It was shown that the exposure to 15 minutes of UV radiation affects the antioxidant potential of quercetin solution and caused its increase by appr. 65 % compared to the control. It could be probably related with degradation of quercetin and obtaining it derivatives with a higher antioxidant potential in comparison to the initial value. **Zvezdanović et al. (2012)** demonstrated that UV irradiation of the quercetin solution induced the cleavage of the ring C and the quercetin degradation. When the irradiation time was elongated the progressive reduction of antioxidant activity of quercetin could be observed.

Table. 1 Influence of various factors on antioxidant potential of querctin and rutin solutions

Factor	Time [h]	Antioxidant activity (mg Trolox/100 ml)	
		Quercetin	Rutin
Control		317.2 ± 2.95 ^{b}	189.7 ± 5.45 ^{c}
Temperature 100 °C	0.5	346.6 ± 31.38 ^{a,b}	76.9 ± 5.52 ^{a}
	1.0	346.9 ± 2.55 ^{a,b}	78.2 ± 11.17 ^{a}
	1.5	328.5 ± 0.00 ^{a,b}	79.4 ± 9.23 ^{a}
	2.0	351.7 ± 56.21 ^{a,b}	83.3 ± 1.98 ^{a}
	2.5	373.7 ± 3.09 ^{a}	106.9 ± 5.59 ^{b}
Control		317.2 ± 2.95 ^{b}	189.7 ± 5.45 ^{a}
UV	0.25	521.4 ± 54.41 ^{a}	185.1 ± 5.48 ^{a}
	0.5	457.9 ± 29.18 ^{a}	184.4 ± 1.18 ^{a}
	0.75	419.2 ± 101.26 ^{a,b}	183.4 ± 4.09 ^{a}
	1.0	403.8 ± 42.68 ^{a,b}	184.8 ± 3.43 ^{a}
	1.25	442.9 ± 117.38 ^{a,b}	187.7 ± 1.20 ^{a}
Control		317.2 ± 2.95 ^{a}	189.7 ± 5.45 ^{a}
Visible light	24	389.6 ± 19.41 ^{a}	186.8 ± 0.68 ^{b}
	48	325.0 ± 41.13 ^{a}	184.0 ± 13.31 ^{b}
	72	320.9 ± 49.24 ^{a}	177.1 ± 16.15 ^{b}
	96	320.7 ± 12.48 ^{a}	168.6 ± 8.60 ^{b}
	120	339.1 ± 18.38 ^{a}	166.6 ± 2.52 ^{b}

a,b,c – means obtained for particular factor marked with the same letter superscript in a column are not significantly different at $p<0.05$

Exposure of the samples on the visible light caused the smallest changes in antioxidant potential when compared to the previously described physical factors. Already after 24 hours of exposition an increase of the antioxidant activity of quercetin solution by 23 % in relation to the control sample was observed. **Sisa et al. (2010)** reported that a short exposure of plant to visible light caused the light absorption by phenolic compounds, and could even lead to their biosynthesis in plant tissue. However, the analysis showed that elongated exposure of quercetin to the visible light led to a decline in this antioxidant activity, probably due to degradation of the compound. In the case of rutin continuous decrease of ability to free radicals scavenging was observed, but, as in the case of UV radiation rate of change was small.

CONCLUSION

The exposure of polyphenols on physical factors, such as temperature, UV and visible light, significantly influenced their antioxidant activity. Heating the polyphenols at 100 °C causes the degradation of these compounds which may result both in a reduction (eg., rutin) and an increase (eg., quercetin) of their antioxidant properties. Ultraviolet and visible light induced decomposition of quercetin and the products obtained had a higher antioxidant activity than the polyphenol not subjected to external factors. In the case of rutin there a significant decrease in the antioxidant potential was demonstrated after the exposure to the light. The presence of the sugar moiety in the rutin molecule implies its greater stability and resistance to external factors.

REFERENCES

Deng, S., West, B. J., Jensen ,C. J. 2008. Simultaneous characterization and quantitation of flavonol glycosides and aglycones in noni leaves using a validated HPLC-UV/MS method. *Food Chemistry*, 2008, 111, 526-529.

Moreira da Costa, E., Filho, J. M. B., Gomes do Nascimento, T., Macedo, R. O. 2002. Thermal characterization of the quercetin and rutin flavonoids. *Thermochimica Acta*, 2002, 392–393, 79-84.
Murakami, A., Yamaguchi, T., Takamura, H., Matoba, T. 2004. Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. *Journal of Food Science*, 2004, 69, FCT7-FCT10.
Pokorny, J., Janishlieva, N., Gordon, M. 2001. Antioxidants in food. Practical applications. Abington Hall. Abington. Cambridge, CRC Press 2001.
Sisa, M., Bonnet, S. L., Ferreira, D., Van der Westhuizen, J. H. Photochemistry of Flavonoids. *Molecules*, 2010, 15, 5196-5245.
Skerget, M., Kotnik, P., Hadolin, M., Rizner, H. A., Simonic, M., Knez, Z. 2005. Phenols, proanthocyanidyns, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry*, 2005, 98, 191-198.
Tarko, T., Duda-Chodak, A., Sroka, P., Satora, P., Michalik, J. Transformations of phenolic compounds in an in vitro model simulating the human alimentary tract. *Food Technology and Biotechnology*, 2009, 47, 456-463.
Zvezdanović, J. B., Marković, D. Z., Cvetković, D. J., Stanojević J. S. UV-induced change in quercetin antioxidant activity toward benzophenone initiated lipid peroxidation, *Journal of the Serbian Chemical Society*, 2012, 77(11), 1571-1588.

Contact adress: Tomasz Tarko, Department of Fermentation Technology and Technical Microbiology, ul. Balicka 122, 30-149 Krakow, POLAND, email: t.tarko@ur.krakow.pl