

## Antioxidative effect of *Capsicum* oleoresins compared with pure capsaicin

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**ABSTRACT:** *Capsicum annum* L, or hot peppers are unique among the plant species, because of their ability to synthesize capsaicin. Capsaicin is an alkaloid which has been known for its analgesic, antireumatic, antiseptic, antidiabetic and few more pharmacological properties. Its antioxidative potential is also a subject of many experiments, in the last few years. The aim of this study is to examine the antioxidant potential of capsaicin and capsicum oleoresins produced from *Capsicum* sp. cultivated in R. of Macedonia.

This experiment comprises four different genotypes of *Capsicum annum* L., which were used for obtaining ethanolic oleoresins. Their antioxidant potential was measured and compared to the antioxidative potential of the pure capsaicin standards. As a method for measuring the total antioxidant capacity was used FRAP (Ferric reducing antioxidant potential) method. This is a simple photometric method for estimation of in vitro antioxidative potential which is expressed as  $\mu\text{mol/L Fe}^{2+}$ .

The results from this study are showing a very good correlation between antioxidant potential of capsaicin and capsicum oleoresins.

This confirms that antioxidative potential of hot peppers does not come only from the vitamins and phenolic compounds in them, but capsaicinoids are also included.

**KEY WORDS:** antioxidants, capsaicin, FRAP, fruit, hot peppers.

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### I. INTRODUCTION

Pepper fruits (*Capsicum annum* L.) are important vegetables used as vegetable foods, spices or dry fruits intended for isolation of capsaicin [1, 2]. Peppers are a good source of vitamins C and E [3, 4] as well as some of the carotenoids (provitamin A) as compounds with well-known antioxidant properties [5-7]. Capsaicinoids refers to a group of pungent compounds found in chilli peppers. Hot cultivars are rich in capsaicinoids, alkaloids with pharmacological properties giving the specific taste to pepper fruit [4, 8]. Interest in their biological activity is increasing. Previous studies have indicated that red pepper and capsaicinoid decrease blood cholesterol concentration, [9–11] possibly mediated by inhibition of intestinal cholesterol absorption [12]. Capsaicinoids have also been shown to be effective in weight reduction mediated by enhancing  $\beta$ -oxidation of fatty acids in vivo and increasing adrenergic activity and energy expenditure [13]. Accumulated evidence has also demonstrated that capsaicinoid has potential beneficial effect on the human cardiovascular system [14, 15, 16]. It has also been reported that capsaicinoid possesses antitumor activity [17, 18]. Capsaicinoids have been shown to possess antioxidant activity with an ability to prevent excessive formation of ROS (reactive oxygen species) [19, 20]

There are few reports on the antioxidant activity of capsaicin [21, 22]. The antioxidant activities of this compound may be the result of the presence of the groups in the phenolic ring (a methoxy group in ortho position to OH) of capsaicinoids and ferulic acid ester, which influenced the antioxidant properties. This is in agreement with Henderson et al. [21], who showed that the amide group present in capsaicin does not play a major role in its antioxidant activity under free radical oxidation conditions, that the antioxidant behavior for capsaicin was due primarily to the phenolic moiety in the molecule, and that the main product of capsaicin oxidation is its dimersdicapsaicin. A comparison of the results of antioxidant activities of pure capsaicin and *Capsicum* extracts obtained in the present studies showed that the antioxidant activity of oleoresins is dependent of the concentration of capsaicin.

The aim of the present work was to complete the knowledge on *Capsicum* alkaloids. We have determined the antioxidant activity of the capsaicinoid fraction isolated from hot peppers and the antioxidant activity of the ethanolic oleoresins. The primary objective of this study was to determine which genotype has higher antioxidant potential and how it correlates with antioxidant potential of the pure capsaicin.

## II. MATERIALS AND METHODS

**2.1 Plant Material.** Fruits of three hot pepper cultivars, (genotypes Vezena, Feferona, Bombona), and one mild genotype Sivrija, (as a control), were taken from the field experiment conducted in the years 2012-2013. Fruits were harvested at the stage of full ripeness (red) [22, 23]. After the fruits have been washed and the seeds removed, fresh pepper fruits were cut and dried at a room temperature, in a dark and dry place for about two weeks. They were dried to constant weight, and the percent of water in them was counted. Dried fruits of the peppers were grounded and kept in an exsiccator.

**2.2 Method of extraction.** For the extraction of oleoresin we have used the maceration method [24]. Process of extraction with vacuum filtration was made using 0,2g of grounded peppers in 25 ml of extraction solvent. According to the literature a few organic polar and non polar solvents can be used for capsaicin extraction. We have tried to use acetone and ethanol, but acetone has shown as an inappropriate solvent for spectrophotometric measurements on wavelength of 280nm. Therefore, in the focus of this experiment were taken only the ethanolic extracts. Maceration process was performed in volumetric flasks for 5 hours, on temperature of 50°C. Separation of extracts from the powder was conducted with vacuum filtration using a Buchner funnel and a water vacuum pump. Final extracts for this experiment were diluted 2:25 with the same solvent.

**2.3 Method for quantification of capsaicin.** Concentrations of capsaicin in the standard solutions and in ethanolic extracts were measured by one of the cheapest method for quantification of capsaicin, the UV/VIS spectrometric method [25, 26]. Measurements of the concentration of capsaicin in the extracts were performed through their absorbencies measured on UV/VIS spectrometer model Cary 100, 9.0 wavelength of 280nm.

**2.4 Method for measuring the antioxidant potential of capsaicin.** As a method for measuring the total antioxidant capacity (TAC) of the pure capsaicin or *Capsicum* extracts was used the *in vitro* spectroscopic method, FRAP (Ferric Reducing Antioxidant Power) assay [27]. This method is based on the reduction of the  $Fe^{3+}$  ions to  $Fe^{2+}$  ions, when they are captured in TPTZ (2,4,6-Tri(2-pyridinyl)-1,3,5-triazine), under the influence of the antioxidant (reductance) in the system. The reaction in this assay is running under acidic conditions.  $FeSO_4$  has been used as a standard solution in this measuring, so the results are expressed as  $\mu\text{mol/L } Fe^{2+}$ . Detection of the final coloring of the samples on which this assay was applied was measured on wavelength of 595nm.

## III. RESULTS AND DISCUSSION

The capsaicin content in the ethanolic oleoresins was determined on the basis of standard solutions of capsaicin (Sigma-Aldrich, Schnelldorf, Germany) [28]. The results for concentration of capsaicin in the samples were calculated using the linearity curve and linearity equation  $y = 9.484x + 0.016$ , obtained from the standard solutions of capsaicin Fig. 1.

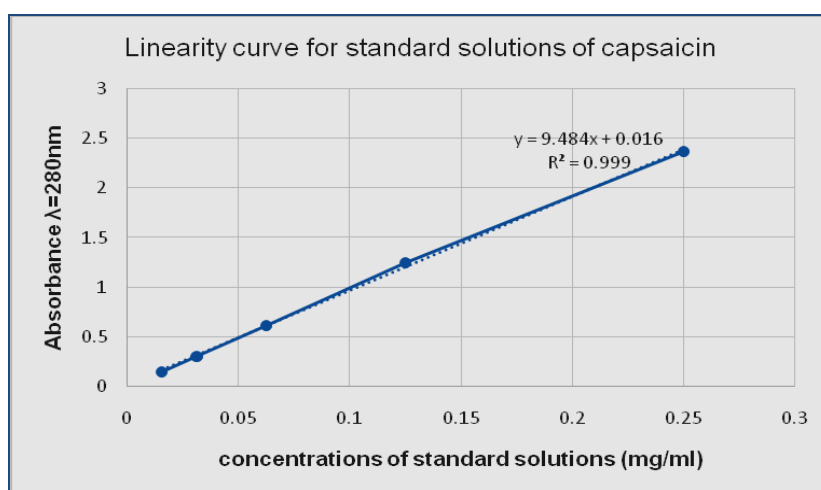


Figure1. Linearity curve for standard solutions of capsaicin

Table 1 is presenting the results of capsaicin concentration ( $\lambda=280\text{nm}$ ) and TAC values of the standard solutions of capsaicin, measured by spectrophotometer on wavelength of 595nm.

Table 1 Capsaicin concentration and TAC of the standard solutions of capsaicin

Standard solutions	Concentration of capsaicin (mg/ml)	Absorbance in FRAP assay	TAC (Fe <sup>2+</sup> μmol/L)
St. solution 1	0,25	1,175	1395
St. solution 2	0,125	0,726	763
St. solution 3	0,062	0,457	383
St. solution 4	0,031	0,332	207
St. solution 5	0,016	0,265	113

The coefficients of correlation (R values) given in Fig.2 and Fig.3 are showing a very good correlation between the content of capsaicin and its total antioxidative potential. As we can see, Fig.2 is showing the results obtained from the standard solutions of capsaicin.

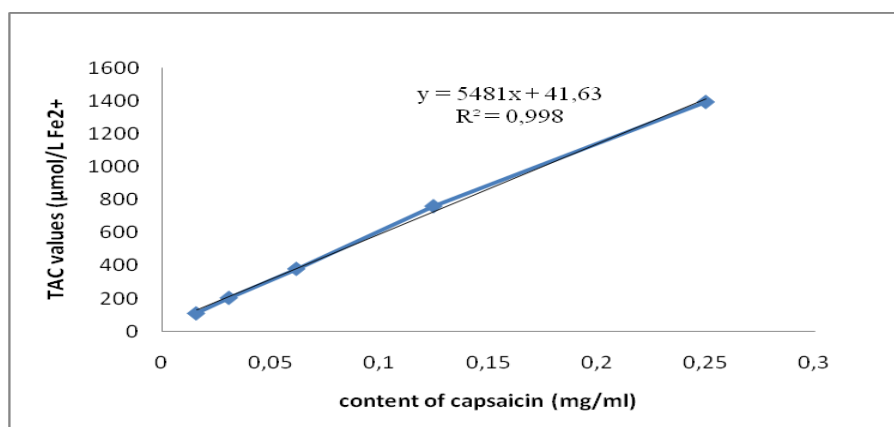


Figure 2 Correlation between capsaicin content and TACs in standard solutions of capsaicin

Table 2 is giving the results of Capsaicin concentration and TAC in the ethanolic oleoresins. Consequently on the next figure, Fig. 3 is presented the correlation between capsaicin content in oleoresins and their TAC values. But, only the hot peppers oleoresins are taken in consideration because as expected for the control there are very low antioxidant potential that cannot be measured by this method.

Table 2 Capsaicin concentration and TAC of the ethanolic oleoresins

Extracts	Concentration of capsaicin (mg/ml)	Absorbance in FRAP assay	TAC (Fe <sup>2+</sup> μmol/L)
Vezena	0,014	0,227	59
Feferona	0,019	0,228	61
Bombona	0,052	0,249	90
Control (Sivrija)	0,018	0,198	18

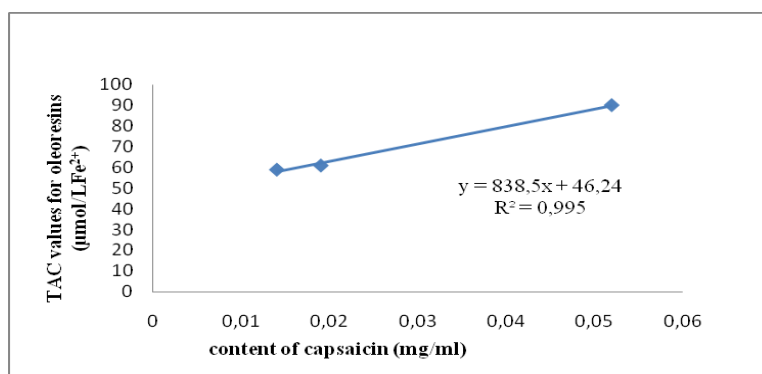


Figure 2 Correlation between capsaicin content and TACs in oleoresins obtained from hot peppers genotypes

At the end of this study, a comparison between the antioxidant potential of the extracts and the total antioxidant potential of standard solutions of pure capsaicin are shown here in Fig. 4 (Antioxidant potential of capsicum oleoresins vs. capsaicin standard solutions). These measurements were made using water as negative and ascorbic acid as positive control, against ethanol as sample blank.

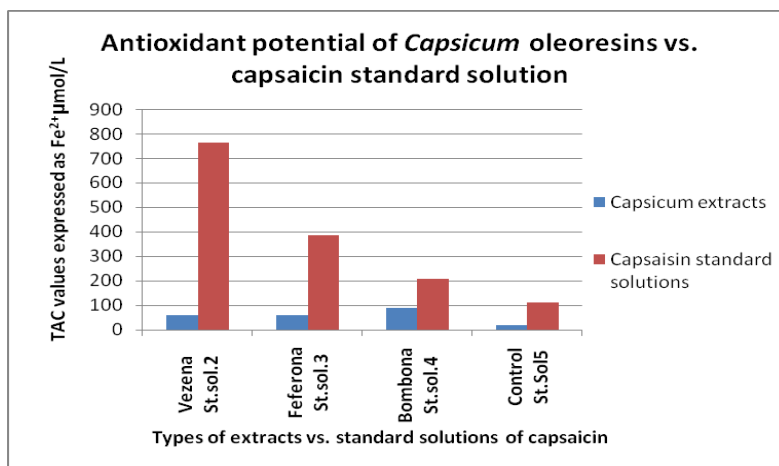


Figure 4 Antioxidant potential of Capsicum oleoresins vs. capsaicin standard solutions

Results are showing good correlation between capsaicin concentration in the extracts and their antioxidant potential. As we can see the genotype Bombona has higher concentration of capsaicin, so we can see, also, higher antioxidant potential, from the examined genotypes of hot peppers. The total antioxidant capacity of the oleoresins obtained from genotypes Vezena and Feferona, (capsaicin concentrations are 0,014 and 0,019 mg/ml) are lower than TAC values for pure capsaicin solution with concentration of 0,016 mg/ml. The control, as a mild genotype, has obviously lower antioxidant potential, than hot pepper genotypes. Compared to the TAC values of pure capsaicin the total antioxidant potential of oleoresins is little lower but it has a good correlation with their capsaicin concentration.

#### IV. CONCLUSION

As a conclusion: From the previous findings in this study and in the literature, there is a likelihood that pepper fruits may provide the types of nutritional and health benefits associated with the consumption of fresh pepper fruits in general[29-31]. From the results obtained by FRAP (ferric reducing antioxidant power) method, oleoresin obtained from hot peppers genotype that has higher concentration of capsaicin exhibits higher total antioxidant potential than other genotypes, especially from the mild control. This can mean that hot peppers can be used as a potential source of antioxidants because they are rich in capsaicin. This in vitro method for measuring the antioxidant potential is not a specific test for showing the in vivo effects of the antioxidant, because of the known ADME (absorption, distribution, metabolism, elimination) effects on the capsaicin in the human organism. Although the bioactive forms of the capsaicin might not be the one found in the plants, the pepper fruits are still widely used as a source of antioxidants. Because of their often use as a spice and as a food protector further studies into the activity of this compound of peppers are needed to evaluate their potential antioxidative effect and their health benefits for the human organism.

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