

## ANTIOXIDANT ACTIVITY OF LENTIL SEMOLINA EXTRUDATES USING CATHODE VOLTAMMETRY

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### Abstract

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Using cathode voltammetry higher antioxidant activity of lentil extrudates was proved in comparison of reference sample (without extrusion). The kinetic criterion (K) of these samples is 1.333 and 1.405, respectively in comparison of 1.111 of sample without extrusion. The analysis of the data on the antioxidant activity of the investigated samples and the extrusion conditions under which these samples were obtained showed that the combined effect of low moisture content of the lentil semolina ( $W = 18 \text{ g.kg}^{-1}$ ) and middle SCR (3:1), or high moisture content of the lentil semolina ( $W = 25 \text{ g.kg}^{-1}$ ) and low SCR (1:1), were the conditions for obtaining of samples with the highest antioxidant activity.

*Key words:* voltammogram, kinetic criterion, electroreduction, superoxide anion radical, perhydroxyl radical, phenolics

*Abridgments:* **AOA** - antioxidant activity, **BAS** - biologically active substances, **TP** – total phenolics, **PC** – phenolic compounds, **K** - kinetic criterion, **W** - moisture content, **T<sub>m</sub>** – die temperature, **n** – screw speed, **SCR** – screw compression ratio, **R<sup>2</sup>** – coefficient of determination

### Introduction

The lentil (*Lens culinaris*) is a plant of the legume family. Its nutritional and gustatory properties make it superior to all other leguminous plants. Lentils are high in proteins (20-30%), carbohydrates (50-58%), and dietary fibres (average 19.2%). Fat content is in the range of 1.0 to 1.5%. Ash content varies between 2.3 and 3.5% (Costa et al., 2006; Grela and Günter, 1995; Iqbal et al., 2006).

Legume lipids are rich in alpha-linolenic acid (Kalogeropoulos et al., 2010). Amarowicz et al. (2010) evaluated the PC of green lentil seeds using acetone extraction. Catechin and epicatechin glucosides, procyanidin

dimers, quercetin diglycoside, and *trans-p*-coumaric acid were the dominant phenolics in green lentils. Lentils are also a good source of vitamins A, E, C, and the B vitamins: B<sub>1</sub>, B<sub>2</sub>, PP, B<sub>6</sub> (Grela and Günter, 1995; Yadav et al., 2007). Kalogeropoulos et al. (2010) found that the tocopherol and squalene contents in decoctions of different legumes varied between 0.26-1.78 and 0.12-1.74 mg/100g, respectively.

Natural antioxidants, i.e. phenols, polyphenols, vitamins, and mineral substances such as zinc and selenium contained in the legumes, have a positive effect on the human health. The antioxidants possess different mechanisms of action: they remove free radicals, form chelates with the metal ions, which catalyse oxidation reactions,

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and inhibit the oxidative enzyme activity (Heimler et al., 2005). The consumption of legumes helps prevent osteoporosis (Messina, 1999), certain cancers (Lamartiniere, 2000), and reduces body lipid accumulation (Pusztai et al., 1998). The antioxidants in legumes can undergo some changes during thermal processing thereby changing the AOA of the products (Korus et al., 2007).

Extrusion is a complex technological process in which the products are subjected to the combined effect of moisture, temperature, pressure, and shear forces. It improves protein digestibility through its denaturation, rendering the molecules more accessible to the enzymes of the digestive tract (Abd El-Hady & Habiba, 2003; Milan-Carrillo et al., 2000; Onyango et al., 2005). Extrusion significantly reduces the antinutrient content of legume seeds (Abd El-Hady and Habiba, 2003; Grela et al., 2001; Nwabueze, 2007; Onyango et al., 2005). It also affects the BAS content of legumes, such as phenols, polyphenols, and vitamins determining the antioxidant properties of the new products.

Korus et al. (2007) studied the phenolic composition of flours from three bean varieties in order to evaluate the effect of extrusion process on their AOA. *Rawela* variety showed a 14% increase in the phenol content of extrudates compared to the raw beans, while the other two varieties exhibited a decrease by 19 and 21%. For all other varieties, the AOA decreased after extrusion.

Delgado-Licon et al. (2009) studied the effect of extrusion on the bioactive compounds and the antioxidant capacity of bean / corn mixtures (in a 60:40 proportion) extruded at different moisture contents and temperatures (150°C, 160°C, 170°C, 180°C, and 190°C). The mixture with moisture 16.3% extruded at temperature 142°C had the highest polyphenol content and AOA. Balunkeswar et al. (2011) reported an increase in the TP, flavonoids, and AOA of extruded potato and pea flour mixtures.

The effect of extrusion on the stability of the B vitamins has been studied mainly for cereals. The water-soluble vitamins of the B group are coenzymes of exceptional importance for the cell function. Their AOA protects cells against the toxic oxidative stress. Mitochondria are exposed to risk in the event of any B vitamin deficiency (Depeint et al., 2006). Vitamin  $B_1$  (*thiamine*) is most extensively studied for stability during ex-

trusion, followed by vitamin  $B_2$  (*riboflavin*) and vitamin  $C$  (*ascorbic acid*). There have been fewer studies on the other vitamins of the B group or vitamin  $E$  (Athar et al., 2006; Beetner et al., 1974; Björck and Asp, 1983; Cha et al., 2003; Gadiant and Fenster, 1994; Ibanoglu et al., 1997; Killeit, 1994).

Athar et al. (2006) found that vitamins  $B_2$  and  $B_3$  (*niacin*) were the most stable ones during extrusion of cereals under different conditions, whereas vitamin  $B_1$  was the least stable. Their preservation rate varied between 44 and 62%.

The studies of Killeit and Wiedmann (1984) proved that the increase in the initial water content by addition of 3-11% water improved the stability of  $B_1$ ,  $B_6$  and  $B_9$  (*folic acid*). Wheat flour extrusion resulted in  $B_1$  reduction from 88.5 to 57.5% when the temperature product increased from 131 to 176°C (Guzman-Tello and Chef-tel, 1987).

There is no data in the literature for optimum conditions of lentil semolina extrusion with regard to preservation of the BAS. No data are available on the AOA for lentil semolina extrudates.

The aim of the present study was to evaluate the AOA of lentil extrudates obtained under different extrusion conditions with varying moisture content of lentil semolina, die temperature, screw speed, and screw compression ratio.

## Materials and Methods

### *Lentil semolina*

Representative sample of commercial lentil cultivar, namely *Ilina*, was obtained from Dobroudja Agricultural Institute, General Toshevo, Bulgaria. The variety was created through multiple individual selections on basic selection characters in a hybrid population of X84L1 cross between *Naslada* x *Laird* (Canada) varieties.

Lentil seeds were ground using a hammer mill and passed through standard sieves. Prepared particle size of lentil semolina was about 0.001 m in diameter. Lentil semolina was mixed with distilled water to be obtained the desired moisture contents (18, 25 g.kg<sup>-1</sup>). The wet materials were placed and kept in sealed plastic bags for 12 h in a refrigerator at 5°C. The samples were tempered for 2 h at room temperature prior to extrusion.

### Extrusion

Lentil semolina was extruded in a laboratory single screw extruder (BRABENDER 20 DN, Germany) with 0.019 m screw diameter and 0.005 m die diameter. The feed screw speed was fixed at  $1.17 \text{ s}^{-1}$ . The temperature of the feed zone and that of the metering zone were 150 and  $160^\circ\text{C}$ , respectively. The  $T_m$ ,  $n$ , and SCR varied (Table 1).

### Total phenolics

The TP in the samples were evaluated according to the method of Kerina et al. (1995). The TP were read off a pre-constructed calibration straight line of  $0.01 \text{ g (100 cm}^3)^{-1}$  gallic acid.

### Antioxidant activity

AOA was determined by cathode voltammetry using solutions obtained from the extrudates specified above.

An extrudate of the selected samples was ground then 0.002 kg of it were measured and transferred to a centrifuge tube. After the addition of  $50 \text{ cm}^3$  distilled water, the sample was tempered in water bath to  $30^\circ\text{C}$  for 30 min under continuous stirring. Centrifugation was applied for 20 min at  $50 \text{ s}^{-1}$ . The supernatant was separated for determination of AOA.

ANALIZATOR AOA-1 ("Polyant", Tomsk Polytechnic University, Russia) was used for the AOA determination. The measurement was performed according to the cathode voltammetry method in a solution of the samples tested. The process included plotting a voltammogram in the absence of an antioxidant (the current in the background electrolyte was measured) and plotting a voltammogram in the presence of an antioxidant.

**Table 1**  
**Extrusion conditions**

Sample №	Moisture, W, $\text{g.kg}^{-1}$	Die temperature, $T_m, ^\circ\text{C}$	Screw speed, $n, \text{s}^{-1}$	Screw compression ratio, SCR
1	18	160	3.0	3:1
2	25	160	3.0	3:1
3	25	136	3.0	3:1
4	25	160	3.8	3:1
5	25	160	3.0	1:1
6	25	160	3.0	5:1

A phosphate buffer solution with pH 6.86 in which  $0.1 \text{ M NaClO}_4$  was dissolved as an indifferent electrolyte was used as background electrolyte. The reference electrode was filled with supersaturated KCl solution. When voltage was applied to the reference electrode, the electroreduction of molecular oxygen resulted in the formation of oxygen radicals as the superoxide anion radical ( $\text{O}_2^-$ ) and perhydroxyl radical ( $\text{HO}_2^\cdot$ ), electric current flowed (measured in  $\mu\text{A}$ ) and the apparatus plotted the voltammogram of the background electrolyte ( $I_0$ ). In the presence of antioxidants neutralising the oxygen radicals, the current was reduced and a new voltammogram (I) was recorded which was below the background curve and dependent on the antioxidant concentrations.

The software included with the apparatus enabled the determination of K. It indicated the quantity of active oxygen radicals, which reacted with the antioxidants (or the overall antioxidant content) within 1 min, i.e. its dimension was  $\mu\text{mol (l.cm}^3)^{-1}$ . It was determined according to the formula:

$$K = C_{\text{O}_2} \cdot (1 - I / I_0) / t, \mu\text{mol (l.cm}^3)^{-1} \quad (1)$$

where:

I – the current from the electrically reduced oxygen in the presence of antioxidants in the solution,  $\mu\text{A}$ ;

$I_0$  – the current from the electrically reduced oxygen in the absence of antioxidants in the solution,  $\mu\text{A}$ ;

$C_{\text{O}_2}$  – initial oxygen concentration in the solution,  $\mu\text{mol/l}$ ;

t – duration of the reaction between the antioxidant and the active oxygen radicals, min.

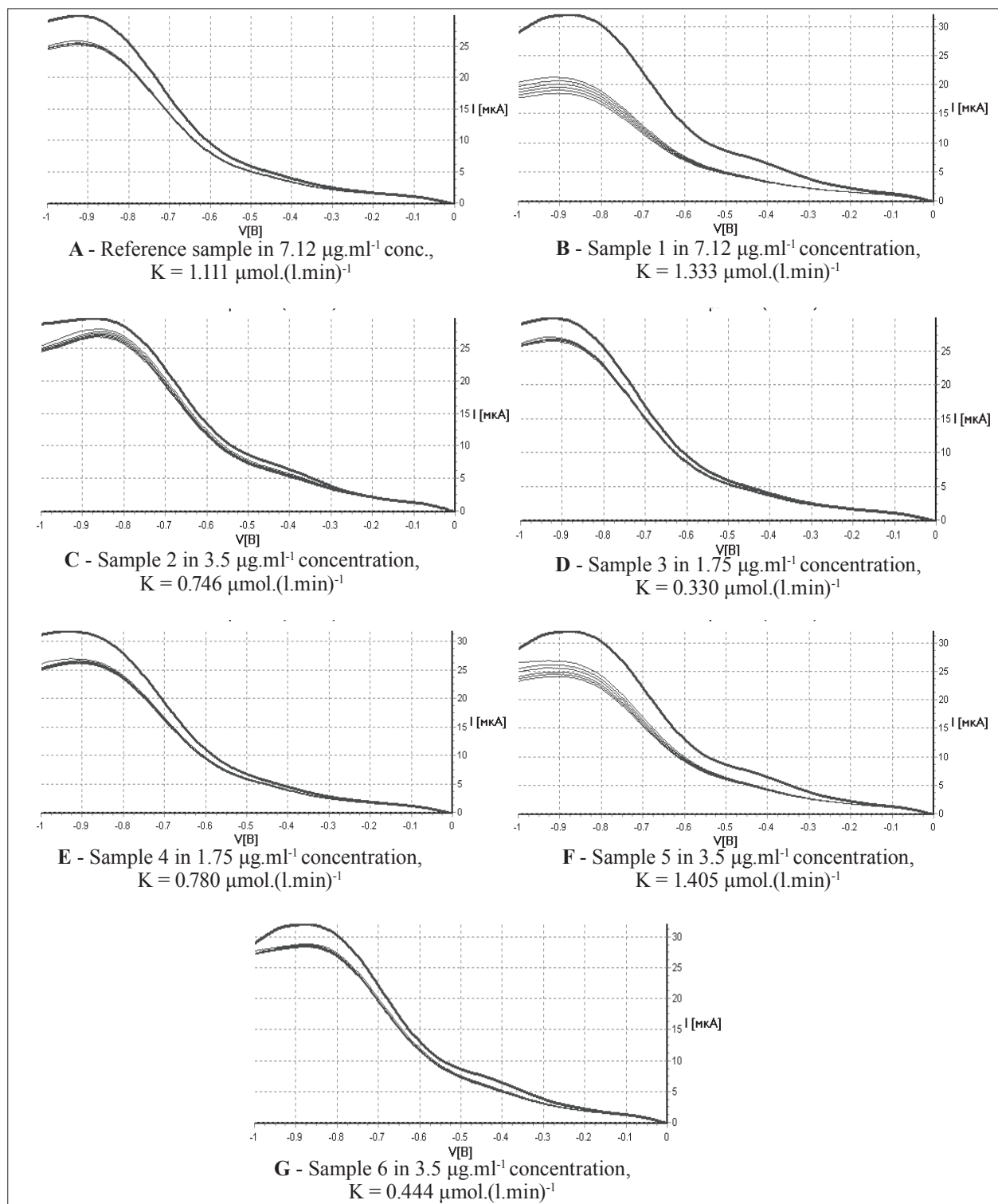
Based on the analysis results, the dependence of function  $(1 - I/I_0)$  on time t was graphically displayed.

The values for each experimental point were obtained after 6 measurements for AOA.

The mathematical processing of the experimental results was performed according to the least squares method (Excel program).

## Results

AOA is most frequently determined using chemical methods. The results of these methods however are hardly comparable due to the lack of a unified measurement unit.



**Fig. 1.** Voltammogram of oxygen electroreduction in the absence (thick upper curve) and in the presence (lower curve) of: **A** – reference sample; **B** – Sample 1; **C** – Sample 2; **D** – Sample 3; **E** – Sample 4; **F** – Sample 5; **G** – Sample 6

In this paper, an electrochemical method was used whereby the determination of AOA could be tracked in its dynamics. Another advantage of cathode voltammetry was the simultaneous neutralisation of both the superoxide anion radical ( $O_2^{\cdot-}$ ) and the perhydroxyl radical ( $HO_2^{\cdot}$ ).

AOA is known to depend on the antioxidant concentration in the sample. To establish this dependency, the extrudate solutions studied were added to the background electrolyte in 5 concentrations: 0.07, 1.75, 3.5, 5.25 and 7.12  $\mu\text{g (cm}^3\text{)}^{-1}$ .

Figure 1 (A, B, C, D, E, F, G) presents the voltammograms of the extruded samples and the reference sample (non-extruded lentils) at extraction concentrations for which K had the highest value.

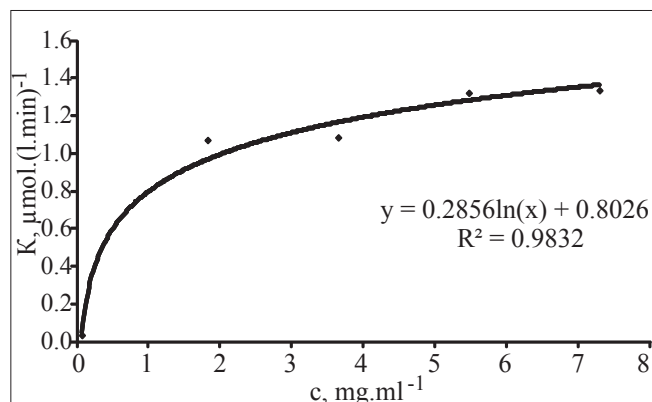
All samples studied showed current reduction in relation to the current of the background electrolyte. The greater the current reduction followed the higher the AOA due to the greater antioxidant content. This correlated with a higher K value per 1 min. The voltammograms also made it possible to determine the action mechanism of the antioxidants contained in individual extracts. The change in voltammograms in the presence of antioxidants is characteristic for the group of PC, vitamins A, E, C, and the microelements having antioxidant effect, i.e. zinc and selenium.

The dependence between the AOA (shown by K) and the concentration of antioxidant substances in the samples with most significant antioxidant capacity, i.e. Sample 1 and Sample 5, and the reference sample, have been shown in Figures 2, 3, and 4 respectively.

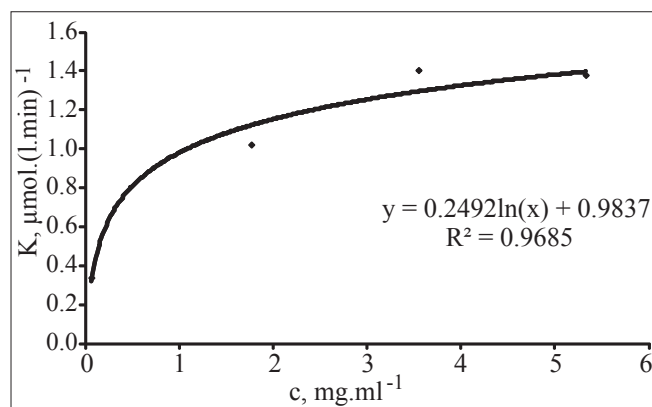
The suggested model described with significant accuracy the effect of the concentration of antioxidant substances on K (logarithmic dependence). The  $R^2$  was high both in the test samples and in the reference sample. Fischer's coefficient ( $F_{\text{stat}}$ ) was between 61.56 and 175.06, and the standard error varied between 0.01 and 0.11, which indicated minimum deviation from the model.

The logarithmic dependence between K and the concentration of antioxidant substances in the samples provided the grounds for a comparative profile of the different extracts and the reference sample in relation to the obtained maximum value of K (Figure 5). Sample 1 and Sample 5 showed higher AOA than the reference,

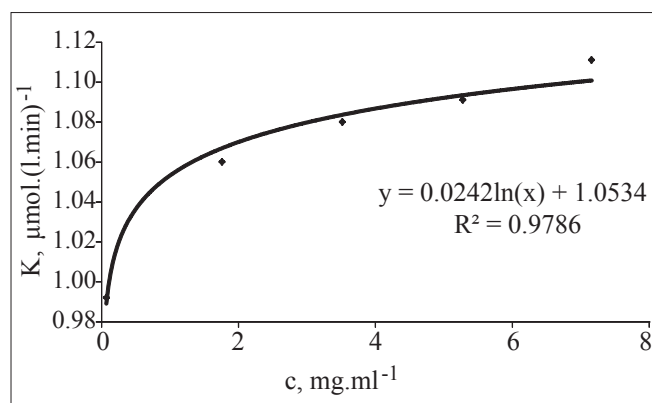
whereas Samples 2, 3, 4, and 6 had weaker antioxidant capacity than the reference sample.



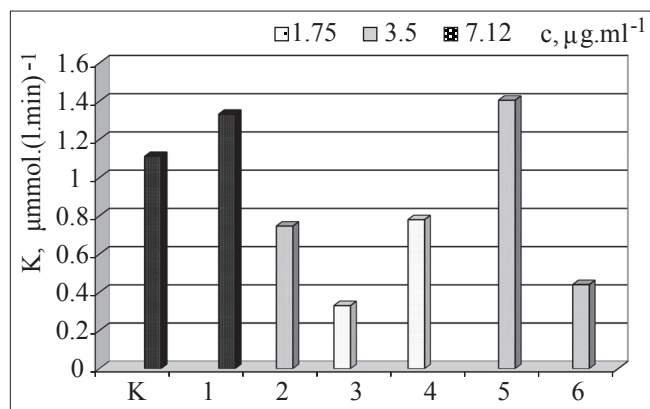
**Fig. 2. Dependence between K and the concentration of antioxidant substances in Sample 1**



**Fig. 3. Dependence between K and the concentration of antioxidant substances in Sample 5**



**Fig. 4. Dependence between K and the concentration of antioxidant substances in the reference sample**



**Fig. 5. Comparative profile of the samples in relation to the obtained maximum values of K**

## Discussion

The antioxidant capacity studies of samples obtained through extrusion under different conditions confirmed the presence of antioxidant substances. Their action mechanism, according to the shift in the voltammogram from the background voltammogram recorded in their presence, referred them to the group of phenolic and flavonoid compounds, vitamins A, B, E, C, metals. Apparently, the extrusion conditions in Sample 1 ( $W = 18 \text{ g.kg}^{-1}$ ,  $T_m = 160^\circ\text{C}$ ,  $n = 3 \text{ s}^{-1}$ , SCR 3:1) and Sample 5 ( $W = 25 \text{ g.kg}^{-1}$ ,  $T_m = 160^\circ\text{C}$ ,  $n = 3 \text{ s}^{-1}$ , SCR 1:1) were most favourable for the extraction of antioxidant substances in these extrudates. In the other extracts, the AOA was lower than that of the reference sample.

The studies for TP made using the Folin–Ciocalteu reagent showed that PC had the highest concentration in the reference sample and decreased in the samples almost twofold after extrusion (the data have not been shown). Korus et al. (2007) also reported a reduction in the PC and AOA after extrusion. The studies of Balunkeswar et al. (2011), however, informed of an increase in the TP, flavonoids and AOA in extruded products of potato and pea flour mixtures. The authors determined the TP in extrudates using the Folin–Ciocalteu reagent, after twofold extraction with 80% acetone, followed by water extraction of the extrudates. Perhaps this kind of treatment permits a fuller extraction of PC, which are the main carriers of the biological activity of extruded products. That is, the reduced concentration of PC in

the extruded products in our studies could be attributed to their incomplete extraction due to the application of water extraction only. The increased AOA of Sample 1 and Sample 5 in comparison with the reference sample (non-extruded lentils) established by use of a new and highly sensitive method, was the strongest evidence this study could provide of the presence of BAS in them.

It is also possible that the extrusion conditions in Sample 1 and Sample 5 favoured the extraction of B vitamins and the microelements zinc and selenium, and, consequently, these samples showed higher antioxidant capacity than the reference sample or the other extrudates.

## Conclusions

The analysis of the data on the AOA of the investigated samples and the extrusion conditions under which these samples were obtained showed that the combined effect of low moisture content of the lentil semolina ( $W = 18 \text{ g.kg}^{-1}$ ) and middle SCR (3:1), or high moisture content of the lentil semolina ( $W = 25 \text{ g.kg}^{-1}$ ) and low SCR (1:1), were the conditions for obtaining Sample 1 and Sample 5, i.e. the samples with the highest AOA. This indicated that, all other conditions being equal, the most significant factor was the combined effect of  $W$  and SCR parameters.

Further studies are needed which ought to include the manner of preparation of extracts from extrudates with a view to a fuller separation of the PC, the main AOA carriers. This would lead to a more accurate assessment of the changes in antioxidants during extrusion. Additional research for the presence of other antioxidant substances in the extrudates would contribute both to the evaluation of the biological activity of new products and to the search for better extrusion conditions.

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