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By-products of vegetables from organic cultivars as antioxidant foodstuff

Gemüse-Nebenprodukte aus biologischem Anbau als antioxidative Nahrungsmittel

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Summary

Our study evaluate the influence of cropping systems (organic and conventional), polarities of solvents (aqueous water and ethanol: water) and the methods of sample preparation (dried and raw) on the ability of free radical scavenging of extracts prepared from cruciferous by-products including cauliflower, broccoli and white cabbage. Dried extracts had higher dry matter yield and a greater percentage of scavenging free radicals as compared with the raw extracts ($P < 0.05$). The amount of antioxidant substance required to reduce 50 % the initial concentration (IC_{50}) of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was variable and, following an increasing scale, it was demonstrated the greatest antioxidant capacity of the dried organic extracts was cauliflower by-product (IC_{50} = 0.33 mg/ml), followed by broccoli by-product (IC_{50} = 0.45 mg/ml) and by white cabbage by-product (IC_{50} = 0.52 mg/ml). Therefore, the organic vegetables have lower IC_{50} values as compared with the conventional crop system ($P < 0.05$). Regarding to the polarity of the solvent, the extracts solutions obtained by hydroalcoholic extraction had a higher antioxidant capacity compared to those that used aqueous water for extraction ($P < 0.05$). For hydroalcoholic extracts, the most potential antioxidant has been demonstrated by the cauliflower by-products (IC_{50} = 0.19 mg/ml), followed by broccoli by-products (IC_{50} = 0.40 mg/ml) and white cabbage by-products (IC_{50} = 0.51 mg/ml). It was concluded that (1) there is variation in antioxidant potential between species of cruciferous by-products; (2) the farming systems, the sample preparation methods and the type of solvent exert significant influence in the antioxidant property; (3) the antioxidant effect is dose-dependent.

Keywords: *Brassicaceae,* by-products, antioxidant, agricultural type, DPPH

Zusammenfassung

Die Studie untersucht den Einfluss von Anbausystemen (ökologische und konventionelle), Polaritäten von Lösungsmitteln (Wasser-Alkohol-Lösung und wässrige Lösung) und die Probenaufarbeitung (getrocknet und roh) auf die antioxidative Fähigkeit von Extrakten aus Kreuzblütler-Nebenprodukten (Blumenkohl, Brokkoli und Weißkohl). Trockenextrakte hatten einen höheren Trockenmasseertrag und somit einen größeren Anteil an freien Radikalfängern im Vergleich zu den Rohextrakten ($P < 0,05$). Die benötigte Menge an Antioxidationsmittel, um 50 % der ursprünglichen Konzentration (IC_{50}) von 1,1-Diphenyl-2-picrylhydrazyl (DPPH) zu verringern, war unterschiedlich. Die größte antioxidative Fähigkeit zeigten die getrockneten, biologisch angebauten Extrakte aus Blumenkohl-Nebenprodukten (IC_{50} = 0,33 mg/ml), gefolgt von Brokkoli-Nebenprodukten (IC_{50} = 0,45 mg/ml) und Weißkohl-Nebenprodukten (IC_{50} = 0,52 mg/ml). Demnach hatte das Bio-Gemüse die niedrigeren IC_{50}-Werte verglichen mit den konventionellen Anbausystemen ($P < 0,05$). Bezüglich der Polarität des Lösemittels zeigte sich, dass die Extrakte, die durch wässrig-alkoholische Extraktion gewonnen wurden, eine höhere antioxidative Kapazität hatten, als die Extrakte, die durch wässrige Lösungen gewonnen wurden ($P < 0,05$). Bei den Wasser-Alkohol-Extrakten, zeigten die Blumenkohl-Nebenprodukte das größte antioxidative Potential (IC_{50} = 0,19 mg / ml), gefolgt von Brokkoli-Nebenprodukten (IC_{50} = 0,40 mg / ml) und Weißkohl-Nebenprodukten (IC_{50} = 0,51 mg / ml). Die Schlussfolgerungen daraus waren, dass es zwischen den Kreuzblütler Arten Unterschiede im antioxidativen Potential gibt; die Anbausysteme, die Probenaufarbeitung und die Art des Lösungsmittels einen erheblichen Einfluss auf die antioxidative Eigenschaften ausüben; und die antioxidative Wirkung dosisabhängig ist.

Schlüsselwörter: *Brassicaceae,* Nebenprodukte, Antioxidationsmittel, Anbausysteme, DPPH

Introduction

Several studies have been shown that the antioxidant activity of fruits and vegetables helps to reduce the risk development of certain degenerative diseases associated with oxidative stress by the protective effects of phenolic compounds, flavonoids, folates, carotenoids and glucosinolates (Dekker et al., 2000). It was reported that phytochemicals which have in its structure a phenolic hydroxyl groups shows antioxidant potential by donation of electrons from the OH groups to the free radical stabilizing them, beyond the chelation of metal ions, particularly divalent, such as Cu^{+2} and Zn^{+2}group (Leake, 2001).

It was demonstrated the antioxidant activity of some cruciferous as broccoli, brussels sprouts, cauliflower, kale and cabbage (Cao et al., 1996). A cabbage spectrometric profile indicated the presence of phenolic compounds, flavonoids, triterpenes and steroids in aqueous and ethanolic extracts (Carvalho et al., 2008). Also, Llorach et al (2003) verified the antioxidant activity of cauliflower by-product (leaves and stems) by inhibition of lipid peroxidation.

Since the antioxidant compounds found in plants have different polarities, different solvents are used to extract then. Water, methanol, ethanol, and acetone are solvents commonly used in extraction processes. The antioxidant activity of the extract and the yield depends on the selected solvent (Gong et al., 2012).

Studies for a comparison between agricultural production systems by organic farming and conventional techniques there are few (Bourn and Prescott, 2002; Williams, 2002). However, there seems to be a clearer trend, as for example, a higher percentage of dry matter (Bourn and Prescott 2002; Williams, 2002) and a greater measure of vitamin C (Ren et al., 2001; Williams, 2002) in organic products, especially in legumes and hardwoods. Regarding the presence of phenolic compounds, most studies show a higher content of organic cultivars (Ren et al., 2001).

The antioxidant activity of a substance cannot be measured directly, but through their effects on a substrate or system that can be monitored. Most of these methods use oxidation processes, involving the addition of an initiator, a transition metal or even exposure to light and a source of free radicals. These radicals are then oxidized under standard conditions and the degree of oxidation is measured (Antolovich et al., 2002).

Among the chemicals methods, there is the method which uses the radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), which is a stable free radical. In the presence of antioxidant responsible for the donation of hydrogen (AH) can be reduced by forming picrilhidrazina diphenyl (Koleva et al., 2002). Our study aimed to investigate the influence of cropping systems (organic and conventional), polarities of solvents (water and ethanol: water) and the methods of sample preparation (dried and raw) on the ability of free radical scavenging of extracts prepared from cruciferous by-products (cauliflower, broccoli and white cabbage) by the chemical method which uses the DPPH radical.

Material and methods

Plant material

The *Brassica oleracea* L var *capitata* (cabbage), *Brassica oleracea* L. var. *botrytis* L. subv. *cauliflora* (cauliflower) and *Brassica oleracea* L. var. *botrytis* L. subv. *cymosa* (broccoli) were obtained in the local market.

Each raw by-product (broccoli, cauliflower and cabbage) was washed in sterile water to eliminate contaminating substances and divided into two groups: one for the withdrawal of water (dried) to obtain extracts from dried samples (Simões et al., 2004) and a second one to obtain extracts from raw samples (Balbach & Boarim, 1993), in order to reproduce the conventional methods used to make teas and infusions. In both methods of sample preparation, the samples were triturated and homogenized using a laboratory grinder to obtain a powder with a particle size of 40 µm, which was used to perform the experiments.

Determination of moisture content

An aliquot of each raw by-product (25 g) was taken to dryness in air circulation oven model Fanem 315-SE (50 °C/ 4 h) to determine the moisture content, which was obtained by the difference between the initial weight of the sample and its weight after drying (AOAC, 1998). The dried material was used to obtain the aqueous extract of cruciferous by-products (cabbage, cauliflower and broccoli), in a concentration of 10 % (w/v), which was used for DPPH assay.

Preparation of extracts

Hydroalcoholic extraction

It was used hydroalcoholic solution of ethanol concentration of 50 % (v/v), using absolute ethyl alcohol and distilled water. Thus, five grams of the plant powder was extracted with 50 mL of mixture ethanol: water. The extraction occurred during the period of 3–6 h at room temperature. The extractions were performed in triplicates. The extracts were filtered through Whatman filter paper No. 3 (Maidstone, U.K.) by applying vacuum. The solvent was removed in an oven at 55 °C for 24 h, according to the methodology proposed by Melo (2010), Corrêa and Salgado (2011) and Martins el al. (2012).

Aqueous extraction – infusion

To prepare the aqueous extraction, 50 grams of ground by-product samples were blended with 500 ml of water distilled at 100 °C (10 % – w/v) for 30 minutes. Afterwards, the supernatant was separated from the solids by centrifugation the extract at 930 g for 15 minutes at 4 °C. The supernatant was then collected and filtered using a Whatman filter paper No. 3 (Maidstone, U.K.) by applying vacuum. The filtered extracts were stored in tubes and frozen at –20 °C. The extractions were performed in triplicates. The methodology was adapted from Girolometto (2005) and Corrêa and Salgado (2011).

Determination of free radical scavenging activity by capture of free radical DPPH

The stable free-radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), 95 % pure and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, TrOH), 97 % pure were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France).

The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. (1995). The extracts solutions of cruciferous by-product concentration used in the study prepared at concentrations of 0.100, 0.075, 0.050, 0.025, 0.012, 0.006, e 0.003 mg/mL. The samples were reacted with the stable DPPH radical in an ethanol solution. When DPPH reacts with an antioxidant compound, which can

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donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read Absorbance (Abs) at 517 nm after 30 min of reaction against a blank (Shimada et al., 1992) using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA).

The mixture of ethanol and sample serve as blank. DPPH solution plus ethanol was used as a negative control. Trolox was used as positive control in the test. All experiments were carried out in triplicate.

The ability of free radical scavenging was expressed in two ways: by the amount of inhibitory concentration (IC_{50}), which represents the amount of antioxidant substance required to reduce by 50 % the initial concentration of DPPH and also was expressed as inhibition percentage (IP) of radical oxidation and calculated according to methodology described by Machado et al. (2013) in Equation:

$$IP\ (\%inhibition) = \left[\frac{A(0) - A(t)}{A(0)} \right] * 100$$

Where A(0) was control absorbance and A(t) was sample extract absorbance.

Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The concentration required for 50 % scavenging of the DPPH free radical (IC_{50}) was calculated from the corresponding log-dose inhibition curve.

Statistical analysis

All data were reported as mean ± standard deviation of three replicates. The studied parameters were assessed by analysis of variance (ANOVA), and when differences were significant (P<0.05), the data were subjected regression analysis. All evaluations were performed using the software SAEG.

Results and discussion

The dry matter of by-products of broccoli, cauliflower and white cabbage were 4–5 %. The high content of water in the family *Brassicaceae* vegetables is widely known (Herrmann, 1994; Singh et al., 2006). In a similar manner it was found that *Brassica* by-product extracts showed high moisture content.

Regarding crop systems, the organic vegetables had a lower moisture content compared to plants of the conventional systems resulting in increased dry matter content, ie higher content of nutrients for food weight which probably contributed in a greater antioxidant activity (Table 1 and Figures 1 and 2). Azevedo (2003) reported that vegetables grown in organic systems have greater durability and this fact is associated with the reduction of water activity of organic foods because there is less moisture and reduced free water content, there will be a lesser degree of bacterial growth and early deterioration of food.

With regard to nutritional quality, in general, for most nutrients there is still no consensus on the superiority of organic. However, for some elements, there appears to be a clear trend, for example, a higher percentage of dry matter (Bourn and Prescott, 2002; Williams, 2002) and a greater content of phenolic compounds in products from organic farming (Azevedo, 2003; Ren et al., 2001).

On average, the values of dry matter of the aqueous extracts obtained from dried plant materials were 0.3–0.5 g/100 g. The aqueous extracts of raw by-products had to 0.03–0.07 g/100 g. Thus, the processing mode of samples (raw and dried) interfered with the dry matter content and probably also in the plant tissue structure. Drying led to an increase in nutrient content and probably cooperating disruption of plant tissue. Therefore, the dried aqueous extract showed higher dry matter yield than the raw aqueous extract, which was reflected in the percentage of inhibition percentage of radical oxidation (Fig. 1 and 2) that represents the percentage of DPPH consumed.

Our results showed DPPH value for cauliflower by-product, broccoli by-product and white cabbage by-product of 16.33 %, 15.69 % and 12.89 % at 0.1 mg/mL. These results corroborate similar studies conducted by Arbos (2004) that observed DPPH value for broccoli of 17.1–22.3 at 0.1 mg/mL. Llorach et al. (2003) found that cauliflower and broccoli by-products extracts showed significant antioxidant activity. According to Samec et al. (2011) and Soengas et al. (2011) there are correlation among DPPH values and content of phenolic compounds.

In general, cruciferous by-products aqueous extracts studied (broccoli, cauliflower and cabbage) had anti-

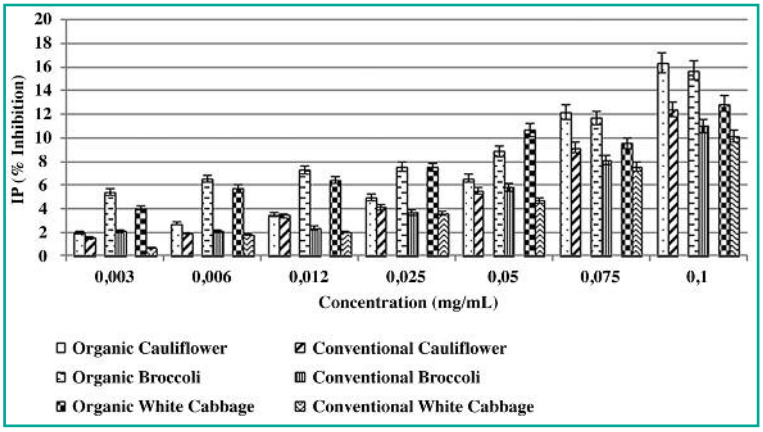

FIGURE 1: *Free Radical Scavenging Activity (DPPH Assay) by dried cruciferous by-products aqueous extracts obtained from different cropping systems (Organic and Conventional). Error bars indicate standard deviation (n = 3).*

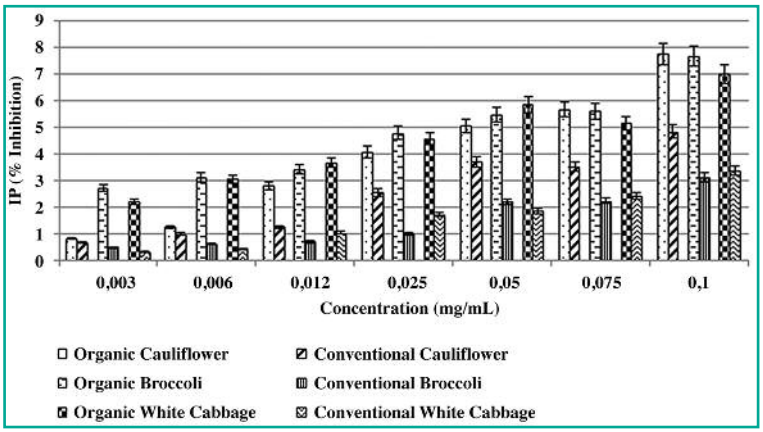

FIGURE 2: *Free Radical Scavenging Activity (DPPH Assay) by raw cruciferous by-products aqueous extracts obtained from different cropping systems (Organic and Conventional). Error bars indicate standard deviation (n = 3).*

oxidant activity, whereas the absorbance after reaction of DPPH with different concentrations of the samples tested were significantly lower compared to the absorbance obtained for the negative control. Generally this antioxidant activity has been correlated with the presence of phenolic compounds among others. The antioxidant capacity of *Brassica* vegetables has been associated with their phenolic content that represents the major water-soluble antioxidant of these plants (Podsedek et al., 2007; Jahangir et al., 2009; Soengas et al., 2011). Phenolics range from single, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols (Crozier et al., 2009; Crozier et al., 2006).

When the DPPH method is used, marked variation in the submission of the results concerning the investigated antioxidant activity is observed, complicating comparisons. For example, one may express the results as the ability to sequester/reduce DPPH * percentage (Choi et al., 2002), the IC_{50} value, ie the required amount of antioxidant substance to reduce by 50 % the initial concentration of DPPH * (Brand-Williams et al., 1995) or also the antioxidant power or antiradical power, which expresses the inverse ratio IC_{50} (Brand-Williams et al., 1995).

TABLE 1: *Antioxidant capacities of cruciferous by-products aqueous extracts (mg/mL) obtained from different cropping systems and processing forms.*

Cruciferous by-products	Processing forms	Cropping systems	Equation of the line formula – R^2	IC_{50} (mg/mL)[1]
Cauliflower[2]	Dried	Organic	y = 147.19x + 0.8446 R^2 = 0.9651	0.33
		Conventional	y = 104.91x + 1.5028 R^2 = 0.9628	0.46
	Raw	Organic	y = 67.229x + 1.4428 R^2 = 0.927	0.72
		Conventional	y = 42.504x + 0.9863 R^2 = 0.9277	1.15
Broccoli[2]	Dried	Organic	y = 97.089x + 5.3656 R^2 = 0.9535	0.45
		Conventional	y = 93.641x + 1.4495 R^2 = 0.9936	0.51
	Raw	Organic	y = 49.267x + 2.9393 R^2 = 0.966	0.95
		Conventional	y = 28.756x + 0.4141 R^2 = 0.9685	1.72
White cabbage[2]	Dried	Organic	y = 85.62x + 5.0616 R^2 = 0.9253	0.52
		Conventional	y = 91.02x + 0.8412 R^2 = 0.9811	0.54
	Raw	Organic	y = 45.917x + 2.9031 R^2 = 0.8972	1.02
		Conventional	y = 29.42x + 0.5161 R^2 = 0.9342	1.68
Control			y = 9.5081x + 0.0682 R^2 = 0.9749	0.005

[1]) IC_{50}: The required amount of antioxidant substance to reduce by 50 % the initial concentration of DPPH. [2]) Linear Regression ($P < 0.05$).

In our study, the results of the antioxidant capacity of the cruciferous by-products aqueous extracts are shown in two forms: (a) by the percentage reduction of the initial amount of DPPH; (b) the IC_{50}. It is necessary to clarify that in the present work, plotting, we obtained the equation of the type y = ax + b, which served as the basis for calculation of IC_{50} through of first degree equation (Tab. 1).

The IC_{50} was variable and following an increasing scale, the most potential antioxidant has been demonstrated through the use of dried organic cauliflower by-product extract (IC_{50} = 0.33 mg/mL), followed by the dried organic broccoli by-product extract (IC_{50} = 0.45 mg/mL) and dried organic cabbage by-product (IC_{50} = 0.52 mg/mL). These results corroborate the findings of similar studies conducted by Arbos (2004) that verified IC_{50} for organic broccoli of 0.53 and for conventional broccoli of 0.62, presenting organic farming superior antioxidant capacity to conventional extract.

In the review made by Podsedek et al. (2007) it was stated that brussel sprouts, broccoli and red cabbage had highest antioxidant capacity whereas cabbage demonstrated low antioxidant activity.

Additionally, Llorach et al. (2003) verified that water, ethanolic and purified fractions of cauliflower by-products there are antioxidant activity although the aqueous fraction showed less antioxidant activity.

The organic vegetables have lower IC_{50} values as compared with the conventional products (Tab. 1). It is known that quality and quantity of phytochemicals can vary according to the (1) genetic determinants (Vallejo et al., 2003a; Vallejo et al., 2003b), environmental status (temperature, light, water availability, nutrient availability) (Rosa et al., 1996); (3) agricultural management practices (date of harvest, etc.); and (4) post-harvest storage conditions (Rodriguez and Rosa, 1999; Ou et al., 2002). Specifically regarding crop systems, Naguib et al. (2012) verified enhancement of phenolics, flavonoids and glucosinolates of Broccoli as antioxidants in response to organic and bio-organic fertilizers. Barański et al. (2014) carried out meta-analyses based on 343 peer-reviewed publications that indicate statistically

TABLE 2: *Antioxidant capacity of cruciferous by-products extracts from traditional cropping systems obtained by different solvent polarity.*

Cruciferous by-products	Polarity of the solvent	Equation of the line formula – R^2	IC_{50} (mg/mL)[1]
Cauliflower[2]	Hydroalcoholic	y = 241.84x + 3.9126 R^2 = 0.9953	0.19
	Aqueous water	y = 104.91x + 1.5028 R^2 = 0.9628	0.46
Broccoli[2]	Hydroalcoholic	y = 122.88x + 0.1123 R^2 = 0.9869	0.40
	Aqueous water	y = 93.641x + 1.4495 R^2 = 0.9936	0.51
White cabbage[2]	Hydroalcoholic	y = 90.782x + 1.6621 R^2 = 0.9569	0.51
	Aqueous water	y = 91.02x + 0.8412 R^2 = 0.9811	0.54
Control		y = 9.5081x + 0.0682 R^2 = 0.9749	0.005

[1]) IC_{50}: The required amount of antioxidant substance to reduce by 50 % the initial concentration of DPPH. [2]) Linear Regression ($P < 0.05$).

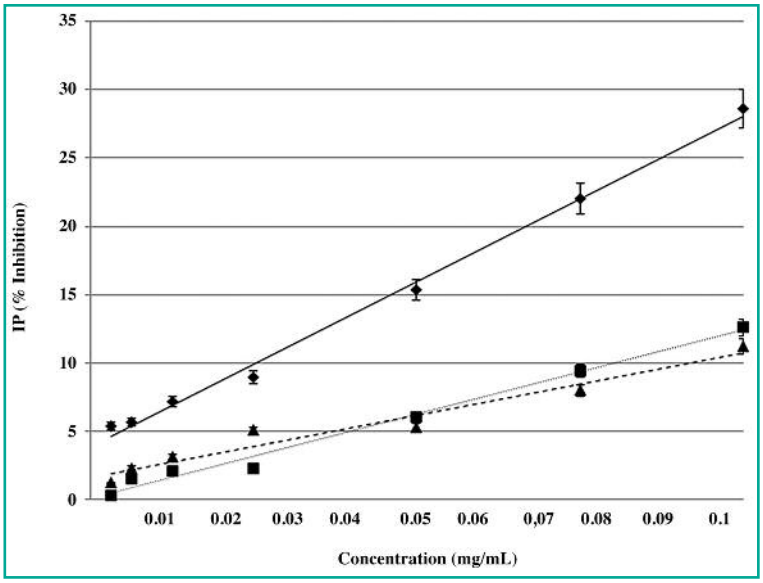

FIGURE 3: *Evaluation of the antioxidant capacity of the hydroalcoholic extracts of cauliflower by-product (◆), broccoli by-product (■), white cabbage by-product (▲) through DPPH radical reduction test. Each point represents the average percentage of free radical consumed at the indicated concentration, obtained from three replicates, which is obtained straight line equation. Error bars indicate standard deviation (n = 3).*

significant and meaningful differences in composition between organic and non-organic crops/ crop-based foods. Most importantly, the concentrations of a range of antioxidants such as polyphenolics were found to be substantially higher in organic crops/crop-based foods, with those of phenolic acids, flavanones, stilbenes, flavones, flavonols and anthocyanins being an estimated 19 %, 69 %, 28 %, 26 %, 50 % and 51 % higher, respectively.

Regarding to the polarity of the solvent, the extracts solutions obtained by hydroalcoholic extraction had a higher antioxidant capacity compared to those that used aqueous water for extraction ($P < 0.05$) (Tab. 2). The most potential antioxidant has been demonstrated by the hydroalcoholic extract of cauliflower by-product (IC_{50} = 0.19 mg/ mL, Fig. 3), followed by the hydroalcoholic extract of broccoli by-product (IC_{50} = 0.40 mg/mL, Fig. 3) and hydroalcoholic extract of cabbage by-product (IC_{50} = 0.51 mg/mL, Fig. 3). Therefore, the antioxidant activity of extracts was strongly dependent upon the solvent, probably due to different antioxidant potential of compounds with different polarity as reported by Soong and Barlow (2004). By polar solvents, the extraction of hydrophilic antioxidants from the food matrix have been done. Wachtel-Galor et al. (2008) extracted hydrophilic antioxidants from broccoli, cauliflower, and cabbage. Other way to extract hydrophilic antioxidants is by use of ethanol and methanol. Ethanol 95% and methanol:ethanol (80:20) was used as solvents to extract antioxidant components from *B. rapa* (Viña et al., 2007; Cefola et al., 2010). Therefore, the type of solvent has significant influence in the antioxidant potential of extracts (Tab. 2).

Conclusions

It was concluded that there is variation in antioxidant potential between species of cruciferous by-products; that the farming systems exert significant influence in this activity; that the sample preparation methods (dried and raw) exert significant effect in this activity; the antioxidant effect is dose-dependent; the type of solvent has significant influence in the antioxidant potential of extracts.

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Conflict of interest

The authors declare that they have no conflicts of interest in this research.

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