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The effect of different processing methods on phenolic acid content and antioxidant activity of red beet

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ABSTRACT

Red beet is a rich source of phenolic acids and has high antioxidant capacity. Effect of and different processing methods (microwave, vacuum, boiling, roasting and germination) on phenolic acid (HPLC) content and antioxidant activity of red beet were studied. The measured antioxidant activity included free radical scavenging activities against DPPH and ABTS. Among the phenolic acids from red beet extracts, 4-hydroxy benzoic acid was the major constituent followed by cinnamic acid, vanillic, chlorogenic, trans ferulic acid and caffeic acid. The concentrations of the phenolic acid differ depending on treatments. The present study exhibited that there was increase in phenolic acid concentrations with high vacuum treatments, roasting for 5 min and also with germination. Different phenolic acids seem to be more stable with vacuum treatment. The antioxidant activity has gradually increased to 1.5 to 3 fold during different treatments when compared to control. With high vacuum treatment there was up to 13% increase with DPPH assay and 11% increase with ABTS assay.

1. Introduction

Epidemiological studies have shown that consumption of fruits and vegetables is associated with decreased incidence of cancer and cardiovascular diseases etc (Kähkönen et al., 1999; Liu, 2004). Antioxidant compounds are present in foods as endogenous constituents or phytochemicals (Siddhuraju, Mohan, & Becker, 2002). Consumption of red beet which is a rich source of antioxidants can contribute to protection from age-related diseases. Phenolic compounds presented in red beet decrease oxidative damage of lipids and improve antioxidant status in humans. Antioxidant activity in red beet is associated with involvement of antioxidants in the scavenging of free radicals and consequently in the prevention of diseases like cancer and cardiovascular diseases (Delgado-Vargas, Jimenez, & Paredes- Lopez, 2000). Phenolic acids have attracted interest in the past few years due to their many potential health benefits. Phenolic acids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory actions (Duthie, Duthie, & Kyle, 2000). Phenolic acids are also known to be useful in controlling inflammation, boosting the immune system, and improving blood circulation, all of which produce significant anti-aging benefits in the body. According to Zitnanova et al. (2006), antioxidants that are able to scavenge ABTS were most abundant in red beet than in onion, garlic, brussel sprout, leek, cabbage, potato and peas. Red beet is regarded as a good potential source of antioxidants (Mattila & Hellstrom, 2007). Effect of processing on bioactive phytochemicals would enable better interpretation of data with respect to dietary habits and human health, which is a critical step for formulating dietary recommendations. Information of nutrient content of processed fruits and vegetables is of interest to consumers as they are becoming more aware of health benefits and are also interested in knowing the difference between processed and fresh foods. Although nutritional quality is generally considered to be lost during processing there are instances where chemical reactions, interactions that release bound components during processing can lead to an increase in nutritional quality. Phytochemicals, especially phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for the health benefits.

Phenolic composition and antioxidant activity of fruits and vegetables are widely studied (Kähkönen et al., 1999; Vinson, Hao, Su, & Zubik, 1998). According to Lewis, Walker, and Lancaster (1999)) there were high positive correlations between antioxidant capacity and phenolic acids which suggests that these compounds are mainly responsible for the antioxidant capacity. Phenolic compounds have been associated with health promotion due to their antioxidant activity (Velioglu, Mazza, Gao, & Oomah, 1998). As phenolic acids are susceptible to oxidation and degradation, exposure to light, oxygen and heat, conditions present during food processing, accelerate the

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destruction of phenolic acids (Han & Koh, 2011). The aim of this study was to investigate the influence of different processing methods (microwave, boiling, roasting, vacuuming and germination) on the phenolic acid content and antioxidant activity of the red beet.

2. Materials and methods

2.1. Plant materials

The fresh red beet roots and seeds were obtained from the local vegetable market in Berlin, Germany. The experiments were generally performed immediately after procurement.

2.2. Sample preparation

The red beet roots were washed and chopped into small pieces using *magic mix*, *mechanical chopper*.

2.3. Microwave irradiation

10 g red beet was treated at 450, 900 and 1800 W for 10, 20, and 30 s using Microwave-Panasonic NE-1846.

2.4. Boiling treatment

50 g of sample was treated at medium direct heat at 80 °C over a pan and stirred frequently at 30 rpm. Equal quantity of water was added to samples and heated for 60, 120, and 180 s.

2.5. Roasting process

50 g of sample was treated at medium direct heat over a pan and stirred frequently at 30 rpm for uniform heat distribution for 60, 120, and 180 s.

2.6. Vacuum treatment

30 g sample was packed in polypropylene bags and vacuumed at medium 94% and high 99% vacuum conditions using Komet plus Vacu 23.

2.7. Germination experiments

The red beet seeds were divided to two parts: one soaked and another non-soaked. The soaked red beet seeds were soaked for 12 h and the two parts of seeds were allowed to germinate for 12 days and sprayed with water every day at room temperature of 27 °C. Soaked seeds were lighter in color and also were better grown than the non-soaked one. Treated samples were harvested in liquid nitrogen.

All the samples after the respective treatment were cooled to room temperature, homogenized and freeze dried. The dried samples were taken for further extraction studies. All the experiments were done in triplicates.

2.8. HPLC analysis

A total of 20 mg grounded dried samples was extracted for 15 min using 750 μL 70% methanol (v/v, pH 4, phosphoric acid) in an ultrasonic water bath (Sonorex digital 10p, Bandelin) on ice. Samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice more with 500 μL 70% methanol. Cinnamic acid (40 μL of 3 mM solution) was added as internal standard to the first extraction. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac, SC 110) at 25 °C. Samples were then made up to 1 mL with 40% acetonitrile. The samples were filtrated using 0.22 μm filter, and then analyzed with HPLC.

Extracts (10 μL) were analyzed at a flow rate of 0.4 mL min^{-1} and a column temperature of 35 °C. A 30 min gradient program was used with 1% (v/v) phosphoric acid in ultrapure water (eluent A) and of 40% (v/v) acetonitrile in ultrapure water (eluent B) as follows: 0–1 min: 0.5% B, 1–10 min: 0–40% B, 10–12 min: 40% B, 12–18 min: 40–80% B, 18–20 min: 80% B, 20–24 min: 80–99% B, 24–30 min: 99–100% B. The gradient program was followed by a 4 min period to return to 0.5% B and a 5 min equilibration period resulting in a total duration of 39 min. The eluent was monitored at 290, 330, and 254 nm. Phenolic acid quantity was calculated from HPLC peak areas at 290 nm against the internal standard and external standards.

Identification and quantification of phenolic acids present were done by comparing retention time and area of the peaks in the extracts with that of the standard phenolic acids (chlorogenic acid, caffeic acid, cinnamic acid, coumaric acid, rosmarinic acid and sinapic acid).

2.2. Extraction of samples

0.100 g of freeze dried samples was dissolved in 10 mL of 50% ethanol, was agitated for 10 s and then centrifuged at 6000 rpm for 10 min. The supernatant was collected and the same procedure was repeated for 2 more times.

3. Determination of antioxidant activity

The antioxidant activity of the extracted samples was determined by DPPH and ABTS methods. The DPPH assay (Lee et al., 2003) was utilized with some modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at −20 °C until use. The working solution (6×10^{-5} M) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using a spectrophotometer. Extracts each of 0.1 mL were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. The DPPH solution with no added extract was analyzed as control.

Scavenging activity was calculated as follows:

$$\text{DPPH radical}-\text{scavenging activity } (\%) = [(A\text{control}-A\text{sample})/A\text{control}] \times 100$$

where *A* is the absorbance at 515 nm.

For the ABTS assay the method (Re et al., 1999) was adopted. The stock solutions were 7 mmol L^{-1} ABTS solution and 2.4 mmol L^{-1} potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. Then 1 mL of the resulting ABTS• + solution was diluted with 60 mL of methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm, as measured using a spectrophotometer. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

$$\text{ABTS radical}-\text{scavenging activity } (\%) = [(Abs \text{ control}–Abs \text{ sample})/Abs \text{ control}] \times 100$$

where *A* is the absorbance at 735 nm.

4. Result and discussion

The effect of processing methods on phenolic acids content of red beet root is shown in Fig. 1. Similar HPLC patterns of phenolic acids were observed for all processing samples however, their contents

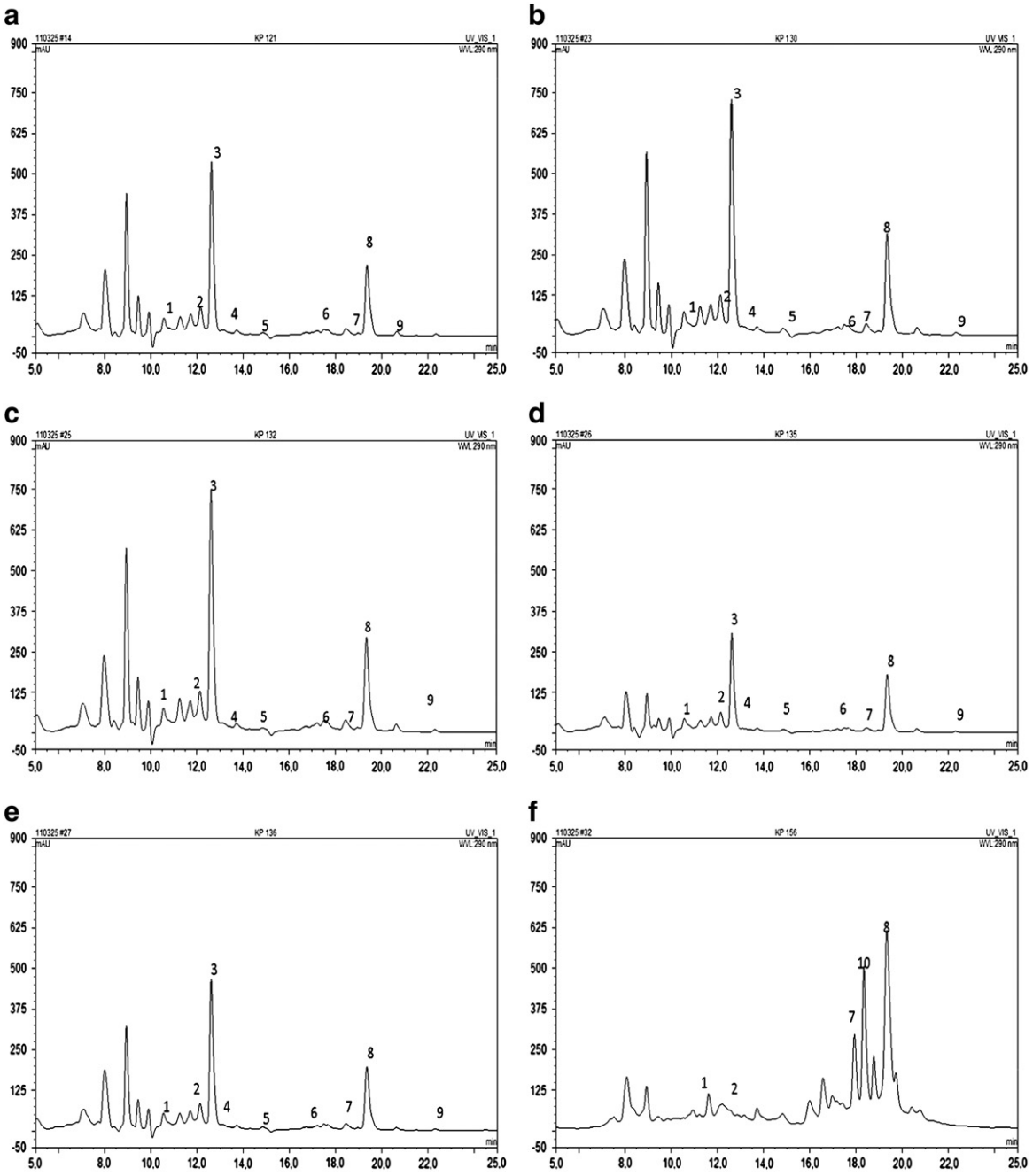

Fig. 1. HPLC chromatogram for phenolic acids for red beet after treatments. a). Microwave 450 W 10 s b) Vacuum treatment c) Control d) Boiling 1 min. e) Roasting 1 min f) Germination.1. 4-Hydroxy benzoic acid. 2. Vanillic acid 3. Chlorogenic acid 4. Caffeic acid 5. P-Coumaric acid 6. Trans ferulic acid 7. Trans-o-hydroxy cinnamic acid 8. Cinnamic acid 9. 4-Methoxy cinnamic acid.

differed depending on treatments. From the results in Fig. 1, it was noticed that 4-hydroxy benzoic acid was the major constituent followed by cinnamic acid, vanillic, chlorogenic, trans ferulic acid and caffeic acid. However, anis acid and coumaric acids were present only in trace amounts. According to Georgiev, V. G. W. J. K. E. M. D. P. N. B. T., and P. A. L. (2010) in red beet plants and hairy root cultures 4-hydroxyl benzoic acid and caffeic acid were detected.

4-Hydroxy benozoic acid seems to be more stable at 1800 W treatment than with 450 and 900 W while, the mild vacuum treatment enhanced 4-hydroxyl benzoic acid from 23.3 to 24.94 μmol/g DM. With boiling and roasting for 1 and 3 min, 4-hydroxyl benzoic acid decreased but with roasting for 5 min there was an increase of 70% when compared to control. Germination samples have reduced the content of 4-hydroxy benozoic acid up to 90% when compared to the control.

Vanillic acid reduced with different microwave treatments compared to control, but it increased from 8.9 to 9.15 μmol/g DM, 12.13 μmol/g DM with high vacuum and roasting for 5 min treatments respectively, while cholorogenic acid content was reduced with all processing methods.

Caffeic acid decreased with microwave treatments by up to 40% when compared with control, but it was stable with vacuum treatments. Re, Bramley, and Rice-Evans (2002) have observed increase of up to 30% in the content of caffeic, p-coumaric and ferulic acids of super cold break process used for tomato sauce at 65 °C under vacuum. Roasting for 5 min seems to increase the caffeic acid from 1.49

to 2.06 μmol/g DM. Interestingly, with germination treatments there was no caffeic acid present in the samples.

p-Coumaric acid content increased with microwave treatments at 450 W for 30 s, 1800 W for 30 s and 1800 W 20 s by up to 5.6%. Also, it increased with the high vacuum conditions and was stable with the mild vacuum. Also with roasting for 5 min it increased to 5 times. p-Coumaric acid was also present with sprouting treatments in higher concentration than in plants. In plants there was 0.53 μmol/g DM, but with germination there was higher concentration of 0.77 μmol/g. Trans ferulic acid decreased with microwave, vacuum, boiling and roasting treatments and there it was absent in germination treatments.

Trans 3 hydroxy 4 methoxy acid decreased with microwave, vacuum, boiling treatments but with roasting for 5 min it increased up to 7 times with boiling. p-Anis acid increased up to 5 folds.

Trans-0-hydroxy cinnamic acid slightly increased with microwave treatment at 1800 W for 20 s more than with roasting for 5 min up to 7 folds. Trans-0-hydroxy cinnamic acid content was high in sprouts by 4 times compared to plants.

Cinnamic acid seems to be generally decreased with microwave, boiling treatments, except at microwave treatment with 900 W for 10 s treatment and also with high vacuum it was increased by about 7%. And also with roasting for 5 min up to 3 fold increase was there. Germination samples have 2 to 3 times higher concentration of cinnamic acid. 4-Methoxy cinnamic acid seems to be mostly stable with all treatment conditions.

According to Kujala, Loponen, Klika, and Pihlaja (2000) cold storage seems to have influence on the phenolic acid and there was significant differences in the content of phenolic. Phenolic acids are mainly present in the bound form, linked to cell-wall structural components. Food processing, such as thermal processing, pasteurization, fermentation, and freezing, contributes to the release of these bound phenolic acids (Dewanto, Wu, & Liu, 2002).

According to Crozier, Lean, McDonald, and Black (1997) cooking may also have a major effect. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying.

According to Spanos and Wrolstad (1990) processing and storage of grapes have great influence on the phenolic acid composition. Storage of concentrates resulted in decrease of phenolic levels as measured by HPLC.

According to Diaz-Batalla, Widholm, Fahey, Castano-Tostado, and Paredes-Lopez (2006) germinated bean seeds exhibited lower p-hydroxybenzoic acid content, higher vanillic acid and p-coumaric acid content than raw seeds, but ferulic acid content was the same. During the soaking period prior to germination, the content of phenolic acids in legume seeds decreased. During germination, the increase in the phenolic acids showed different behavior patterns in those seeds (Lopez-Amoros, Hernandez, & Estrella, 2006). In our results the germination exhibited changes in content of some phenolic acids. In our results the germination exhibited changes in content of some phenolic acids. Cooking affects antioxidant status of tropical green leafy vegetables due to the release of more phenolic compounds (Adefegha & Oboh, 2011). Considering the results from the two assays, results clearly indicate that all extracts exhibited antioxidant activity. Antioxidant activity was high with microwave treatments at higher temperature and times such as 1800 W for 30 s, roasting for 5 min. With high vacuum treatment there was up to 13% increase with DPPH assay and 11% increase with ABTS assay.

With germination there was up to 21% increase in antioxidant activity when compared to control extract from red beet plant.

The extracts demonstrated relatively high antioxidant activity with ABTS up to 1.5 fold increase in antioxidant activity with 1800 W for 30 s (Fig. 2). In the case of boiling and roasting there was similar increase of up to 1.5 fold increase with roasting for 5 min. Antioxidant activity of red beet after treatments shows that there was slight reduction in initial treatments and with increased temperatures and treatment time the antioxidant activity was increased.

In our previous results with DPPH assay there was gradual increase and up to 3 fold increase of antioxidant activity. Antioxidant activity of red beet after treatments showed that slight reduction in initial treatments and with increased temperatures and time the antioxidant activity was increased. There was an increase of up to 2 folds after microwave treatments. In the case of boiling and roasting there was gradual increase and up to 3 fold increase of antioxidant activity and up to 15% in full vacuum treatment pv1. Treatment of samples has led to better extractability and increased antioxidant activity after treatments. Except for treatments with (Fig. 4) 450 W for 20 s, 900 W for 10 s and 1800Wfor 10 s and also during roasting for 1 min, there was an increase in antioxidant activity.

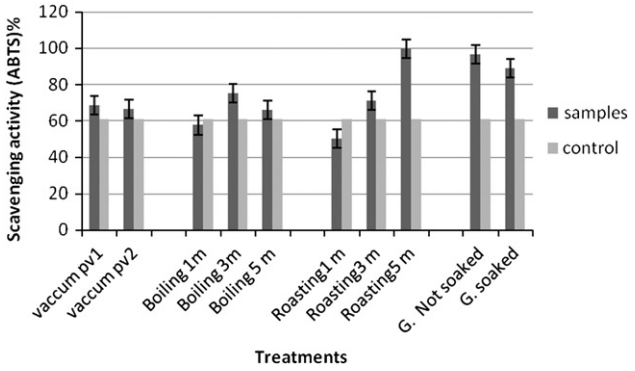

Fig. 3. The effect of different processing methods on antioxidant activity (ABTS assay) of red beet.

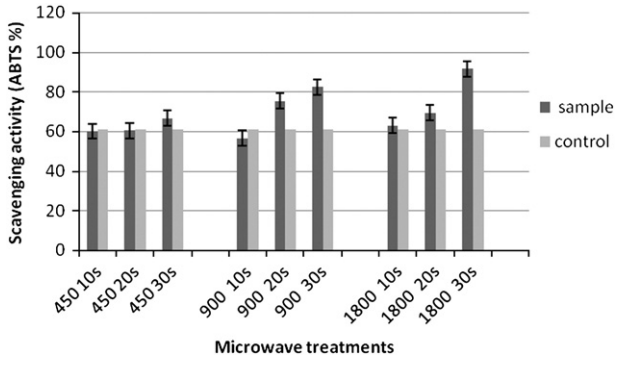

Fig. 2. Effect of microwave treatments on antioxidant activity (ABTS assay) of red beet.

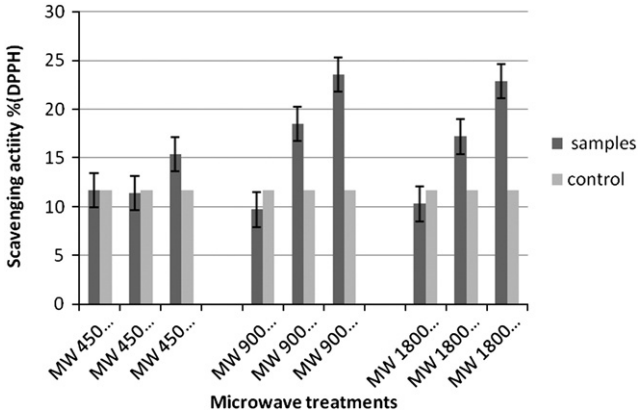

Fig. 4. The effect of microwave processing methods on antioxidant activity (DPPH assay) of red beet.

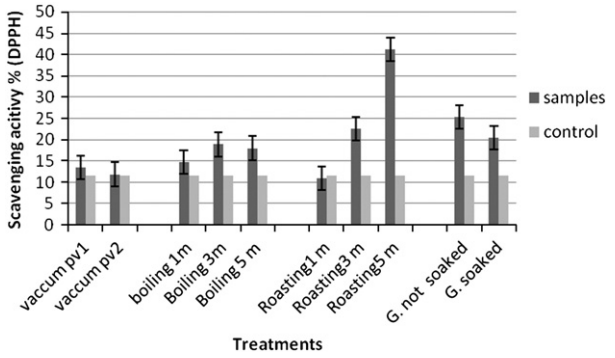

Fig. 5. The effect of different processing methods on antioxidant activity (DPPH assay) of red beet.

The increase in antioxidant activity germination with soaking is comparatively lesser than non-soaked samples. But the antioxidant activity seems to be more than the plant counterpart by up to 59% with ABTS assay (Fig. 3) and more than two folds with DPPH assay (Fig. 5) than the control.

Similar results showing significant correlation between AOA and total phenolic content in the potato were reported (Reddivari, Vanamala, Chintharlapalli, Safe, & Miller, 2007). But Valyova et al. (2009) did not observed correlation between phenolic content and antioxidant activity.

It has been proven that the antioxidant activity is mainly ascribable to the concentration of phenolic compounds in the plant (Heim, Tagliaferro, & Bobilya, 2002). According to Arabshahi-Delousee et al., antioxidant activity was correlated with the amount of total phenolics present in the extracts. With our present experiments antioxidant activity seems to increase with mild vacuum treatment which also correspond with 4-hydroxyl benzoic acid, vanillic acid, caffeic acid, cp-coumaric acid, anis acid and cinnamic acid results.

5. Conclusion

Phenolic acid concentration seems to differ with individual treatments and with some treatments it correlates with antioxidant activity results. The various extracts showed varying degrees of antioxidant activity. Antioxidant activity of red beet is partly due to phenolic acids in them. Different phenolic acids seem to be more stable with vacuum treatment than with other treatments. The antioxidant activity with DPPH and ABTS assay showed that there was gradual increase and up to 1.5 to 3 folds with different treatments compared to control. Cinnamic acid, trans ferulic acid and trans-o-hydroxyl cinnamic acids is present in higher quantities in germination samples than in the control. Treatment process modifies the content of phenolic acids in the plant, there was a decrease as well as an increase in their content that was observed. The additive and synergistic effect of different phytochemicals which include phenolic compounds is responsible of the antioxidant activity.

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