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EXTRACTION OF RASPBERRY FRUIT AND EVALUATION OF ITS PHENOLIC COMPOUNDS

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ABSTRACT

The use of natural additives has been increased due to the toxic effects of synthetic antioxidants on the one hand and welcoming natural additives by consumers on the other hand. In recent years, the natural antioxidants have been used to reduce the risk of heart disease, cancer and diabetes, so, the natural antioxidants play an important role in human health. The phenolic compounds of plant samples is one of the best sources of natural antioxidants. The Raspberry is one of the source of phenolic compounds, which have so far received little attention. In this study, at first, ethanol extract of Raspberry was extracted by maceration method with ethanol and the ratio of 1:10 (weight-volume). Then, the phenolic compounds of Raspberry fruit extract was studied in different concentrations (50, 100, 200, 400, 800, 1600 ppm) by the Folin-Ciocalteu method and compared with the control sample. One-way ANOVA method and Mstat c software were used to analyze the data. The results showed that the amount of phenolic compounds, in compared to control sample, increased significantly by increasing the concentration of Raspberry fruit extract ($P < 0.05$) and its maximum amount in 1600 ppm of extract was 7 ppm. The antioxidant activity was due to the much further tocopherol and phenolic compounds of the extract. According to the results, the ethanol

extract of Raspberry fruit can be used as a low cost and available plant antioxidant source in the food industry after performing supplementary tests.

Key words: Raspberry Fruit, Phenolic Compounds, Extraction, Antioxidant Activity.

INTRODUCTION

Oxidation and producing free radicals and peroxides lead to the destruction of food lipids which affect the nutritional quality (fatty acids and fat-soluble vitamins) and food organoleptic characteristics (color, odor, taste, tissue) adversely. In addition, many of the products of lipid oxidation are considered as the dangerous factors for human health (1). In recent years, the use of synthetic antioxidants such as BHT BHA and TBHQ as other chemical additives has been restricted due to their potential toxicity and carcinogenicity. Today, most of the research on this area have focused on the use of new and safe antioxidants from plant, animal and food sources. The phenolic compounds, with several different mechanisms, prevent the lipid oxidation. The most important function of these compounds associated with oxidation is making free radicals inactive and forming Metal Byonne Complex (2). Raspberry is a shrub from Rosaceae which grows widely and are abundant in humid forest areas such as Guilan and Mazandaran. Its stems are red with long thorns. Its leaves are oval, toothed and its flowers are white and pink and also a cluster. Its fruit likes a blackberry and strawberry

which is tart and its color is reddish-black. Also it is called wild berry of three-flower berry (3). Kamkar et al. (2009) evaluated the antioxidant capacity of Iranian peppermint's essence and extract and concluded that the ethanol extract of peppermint has a good antioxidant power against a variety of oxidative systems and can be used as an available natural antioxidant (4). In another research, Sepehrifar et al. (2009) investigated the medicinal plant of Redcurrant's (*Vacanium Arctostaphylos L*) antioxidant properties and concluded that the fruits and leaves of Redcurrant are rich in phenolic compounds, especially anthocyanins and also have antioxidant property (5).

The studies have indicated that no research had performed on the antioxidant activity and assessment of phenolic compounds of ethanol extract of Raspberry fruit (in Iran), so this study has been performed on the use of plant oxidant of Raspberry fruit ,which is the native products of our country, as a suitable alternative of synthetic antioxidants.

MATERIALS AND METHODS

Extraction of Raspberry fruit by maceration method

In this study, a variety of the Raspberry fruits were gathered from the forests of Noshahr City and kept in the freezer in the temperature of -18 C. Some frozen Raspberry fruits were crushed by blender for each tests. In order to extract, 50 gr of crushed Raspberry fruits were mixed with 500 ml of ethanol as a mixing ratio of 1:10 (weight-volume) and put in hotplate with the rate of 250 rpm for 24 hours at ambient temperature and then under vacuum, it was filtrated by Buchner funnel with a Whatman filter paper No. 1. After that, it was condensed by rotary evaporator (LABORATA4000) at 35 C and finally, the extracts were dried under vacuum at 40 C by dryer and kept in container, which is enclosed and impervious to air, at 4 C. All materials used in this study were provided from Merck and Sigma companied with high purity (6).

Measurement of phenolic compounds

Plot a calibration curve

First, the standard solution of Gallic acid in ethanol with various concentrations in the range of 0.04 to 0.4 mg per ml was prepared. Then, 0.5 ml of the standard solution of Gallic acid was mixed with 2.5 ml of the Folin-Ciocalteu reagent (to prepare the Folin-Ciocalteu reagent, the concentrate was diluted with distilled water at a ratio of 1 to 10) by 50ml volumetric flasks. After 10 minutes, 2 ml of 7.5% sodium carbonate solution was

added to them and the final volume was completed with pure water. After one hour at ambient temperature, its absorption was read at the wavelength of 760 nm and absorption curve was plotted to the Gallic acid concentration (mg/ml) and the following equation was obtained with the explanation correlation of 0.99:

$$(1)Y = 1.0776X^2 + 0.2644X + 0.0099$$

Where X is the amount of absorption at the wavelength of 760 nm and Y is the amount of phenolic compounds (mg/ml) (7).

Measurement of phenolic compounds sample

First, the standard solutions of Raspberry fruit extract were prepared with a solvent (ethanol), which has extracted it, with various concentrations in the range of 50 to 1600 ppm and also the control sample was prepared. Then, 0.5 ml of the standard solution of Gallic acid was mixed with 2.5 ml of 0.2 normal Folin-Ciocalteu reagent (to prepare the Folin-Ciocalteu reagent, the concentrate was diluted with distilled water at a ratio of 1 to 10) by 50ml volumetric flasks. After 10 minutes, 2 ml of 7.5% sodium carbonate solution was added to them and the final volume was completed with ethanol. After two hours at ambient temperature, its absorption was read at the wavelength of 760 nm. The total amount of phenolic compounds was measured based

on Gallic acid in percentage by the calibration equation (as a standard for Gallic acid) (7).

Statistical analysis

To analyze the results, one-way ANOVA method was used. The data was analyzed by statistical software of Mstat c. The averages were compared with each other and control sample by LSD-test at the alpha level of 0.05. The Microsoft Excel software was used to plot the charts.

RESULTS AND DISCUSSION

The phenolic compounds of Raspberry fruit extract

Since phenols and phenolic compounds have been widely found in the food and plant products and due to the fact that the phenolic compounds are the main cause of the medicinal plants' antioxidant properties, the phenolic compounds of Raspberry fruit extract were measured (8). The one-way ANOVA analysis was performed on the effect of various concentration of Raspberry fruit extract on the phenolic compounds of ethanol Raspberry fruit extract, which measured by Folin test and the standard curve equation of Gallic acid. The results were shown in Figure1. As can be seen, the phenolic compounds of Raspberry fruit extract has increased by increasing the concentration of extract from 50 to 1600 ppm, it leads to the increase of antioxidant property and thus the

increase of oxidative stability. Based on the results, the antioxidant compounds has increased from 1.4 ppm in the concentration of 50 ppm to 7 ppm in the concentration of 1600 ppm of Raspberry fruit extract by increasing the different concentrations of Raspberry fruit extract. To the concentration of 400 ppm, the increase was taken with a gentle slope, but, then, the rate of increase was more. All concentrations were compared to the control sample and there were a significant differences between them (except the concentration of 50 ppm) at the level of 95% ($P < 0.05$) and the concentration of 1600 ppm had the most phenolic compounds.

More studies were performed on the antioxidant properties of phenolic compounds and isolated flavonoids of fruits. In a study, Senorans et al. (2001) extracted and identified the natural antioxidants of the orange juice (9). In another study, Zarban et al. (2007) evaluated the antioxidants properties of pomegranate concentrate and 9 other commercial juices and concluded that the pomegranate juice had more phenolic compounds than other studied juices and therefore, its total antioxidant capacity was more than them (10)., Sepehrifar et al. (2009) investigated the antioxidant properties of Redcurrant (*Vaccinium Arctostaphylos L*) and concluded that the fruits and leaves of

Redcurrant are rich in phenolic compounds, especially anthocyanins and also have antioxidant property, so, it can be used in food and pharmaceutical industries as a plant source with antioxidant compounds (5). Singh

et al. (2002) studied on antioxidant properties of extracts from the skin and seeds of pomegranate and concluded that its skin and seeds have significant amount of phenolic acids (11).

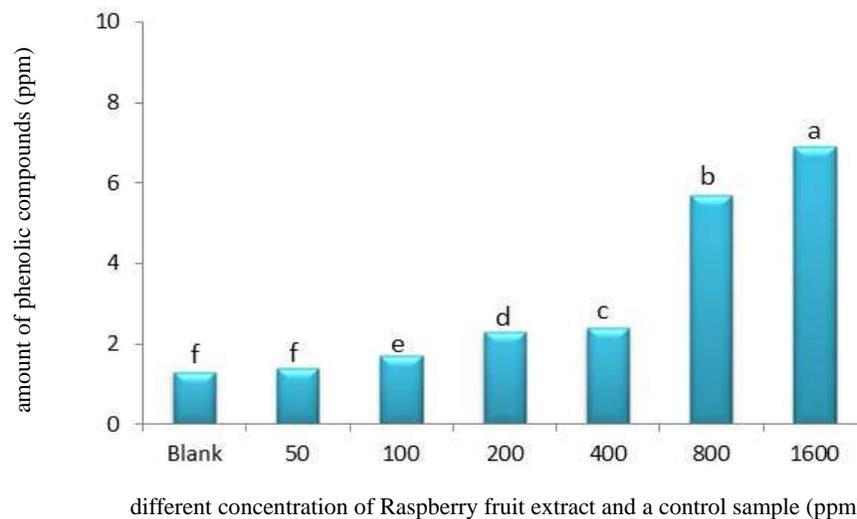


Figure 1: Changes in the amount of phenolic compounds in different concentration of the Raspberry fruit extract

CONCLUSION

Since the synthetic antioxidants have undesirable effects on the human body, they have been gradually removed from the list of consumption antioxidants, so, it is necessary to investigate the sources of natural antioxidants to use instead of synthetic compounds. In this study, the antioxidant properties of the Raspberry fruit extract as a natural antioxidant were investigated in different concentration by measuring phenolic compounds and comparing with control sample. The results showed that the Raspberry fruit extract has the good antioxidant effects.

Compared with the similar studies of other researchers, its antioxidant power is less than some cases and is more than some other cases. In total, inconsistency in the results of the research can be associated with a variety of chemical compounds found in plants, their different reaction mechanisms and kinetics in selected methods. The measured antioxidant capacity of a sample is in relation with the used method and an oxidizing agent. Therefore, Raspberry fruit extract (in Iran) can be used in food and pharmaceutical industries after performing supplementary tests.

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