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Comparison of different solvents for isolation of antioxidant compounds of horseradish

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Abstract

Horseradish (*Armoracia rusticana*) is a perennial herb belonging to the *Brassicaceae* family and contains biologically active substances. Since horseradish has long been used as a spice for meat and fish products, the Food and Drug Administration (FDA) approved it as seasoning, spice, and flavoring and affirmed it as Generally Recognized As Safe (GRAS). Scientists are interested in horseradish because it is a rich source of peroxidase, a heme-containing enzyme that utilizes hydrogen peroxide to oxidize a wide variety of organic and inorganic compounds. Also horseradish is rich in other valuable substances – vitamins, minerals, phenolic compounds and also isothiocyanates.

The aim of the current research was to determine best solvent for extraction of phenolic compounds from horseradish roots showing high antiradical activity. From horseradish roots were extracted with four different ratio of solvent: ethanol/ water (80/20, 70/30, 60/40, 50/50 v/v) using conventional methods and then concentrated to rotary evaporator. Preliminary tests showed that the best solvent ethanol/ water (80/20 v/v) solutions can be chosen.

Keywords: horseradish roots, extraction of phenolic compounds, antiradical activity

1. Introduction

Plants provide abundant natural antioxidants, which are vitally important for human health. Phenolic compounds commonly found in plants are biologically active substances having antiseptic, vitamin activity. It is known that phenolic compounds are very effective antioxidants. Based on these statements, it can be concluded that it is very important to develop the best method for extraction of these compounds from plants.[6-9]

Many researchers reported influence of different extraction solvents, techniques on the content of natural antioxidants in extracts [1-5].

Efficiency of solvents and methods are strongly dependent on plant matrix used. Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh product.The properties of extracting solvents significantly affected the measured total phenolics content and antioxidant capacity in fruits and vegetables.

The highest extract yields were obtained with polar alcohol based solvents [9]. Addition of water to ethanol improves extraction rate, but too high water content brought an increased concomitant extraction of other compounds, and, then to lower phenols concentrations in the extracts.[10-15]

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Literature data shows that extraction efficiency of solvents is strongly dependent on food matrix and the aim of current research was to determine best solvent for extraction of phenolic compounds from horseradish roots showing high antiradical activity [17].

Horseradish peroxidase is one of the most used peroxidase due to wide application in various fields such as analytical chemistry, environmental chemistry or clinical trials. The enzyme is used for many purposes and applications are found at reasonable prices. Generally, the enzyme shows a number of features that make its use beneficial to the common catalysts, namely the ability to operate under conditions of mild reactions, as the processes are ecological in terms of environmental development [14-18]. However there are a number of constraints in using the enzyme, being sensitive, unstable and having to be used in water, features that are ideal for a catalyst but undesirable in most syntheses.

Great importance has to be granted to functional supplements based on horseradish used in cardiovascular diseases because cardiovascular diseases are the leading cause of death and disability worldwide, accounting for 17 million deaths each year. Globally, Romania stands in the first 4 places in terms of cardiovascular mortality.

In horseradish, seven isoenzymes were identified of horseradish peroxidase (HRP), among which the c isoenzyme of HRP (HRPc) is the most abundant and has been successfully isolated, purified and characterized. It has a cardiotonic effect and is recommended to the people that suffer from high blood pressure.

2. Materials and methods

Materials. The vegetables used for this study: horseradish roots, were purchased from a local market. After the preliminary operations like washing, peeling and slicing, the vegetables were mixed with a centrifugal food processor.

Methods. From horseradish roots were extracted with four different solvents: ethanol / water (80/20, 70/30, 60/40, 50/50 by volume) using conventional methods and then concentrated to rotavapor, and were as follows:

- determination of the water content according to the AOAC - 1995 method
- determination of polyphenols - Folin Ciocalteu method
- determination of flavonoids – spectrophotometric method, reference substances are rutine and quercetin
- determination of antioxidant capacity by DPPH method.

3. Results and Discussion

Flavonoids as antioxidants may inhibit the oxidation of LDL cholesterol, reduce platelet aggregation, or reduce ischemic damage. Since flavonoids have good antioxidant property, they are referred to as ―nature‘s biological response modifiers‖, because they modify the body‘s reaction to pathogens as well as compounds such as allergens and carcinogens. They are powerful antioxidants giving protection against oxidative and free radical damage. They prevent formation of oxidized cholesterol through antioxidant effects. Flavonoids exert greater antioxidant effects than vitamin C, vitamin E, selenium, and zinc. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks, and that certain flavonoids can protect LDL from being oxidized and prevent atherosclerosis [19].

Phenolic compounds, the most important antioxidants, include two groups of substances which show strong antiradical action: flavonoids and phenolic acids, which are both present in horseradish. The results obtained from the analysis of horseradish samples on flavonoid content are presented in Figure 1.

From Figure 1 it is noticeable that, depending on the condition of horseradish samples, the content of flavonoids increases. Horseradish extract obtained by conventional extraction but using different ratio of solvent showed the highest values for both flavonoids (Rutin) and flavones (Quercetin). In the current research four different ratio of solvents were used and the lowest is 50:50 ratio, but the highest is 80:20 ratio.

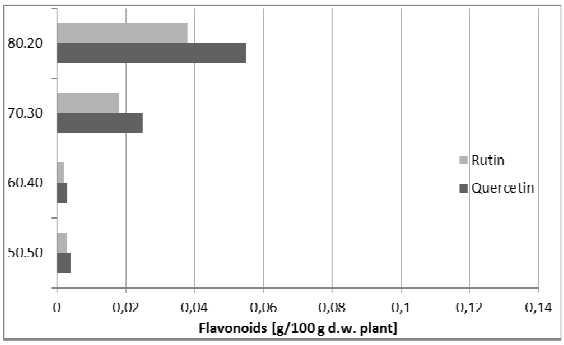

Figure 1. Evolution of flavonoids in different solvents 80 :20 – solvent ethanol/ water; 70 :30 - solvent ethanol/ water, 60 :40 - solvent ethanol/ water, 50:50 - solvent ethanol/ water

Phenolic composition of plants extracts is affected by different factors – variety, climate, storage, processing. Extracts of horseradish roots were prepared using conventional extraction, and total phenolic content was determined using Folin-Ciocalteu reagent, that reacts nonspecifically with phenolic compounds.

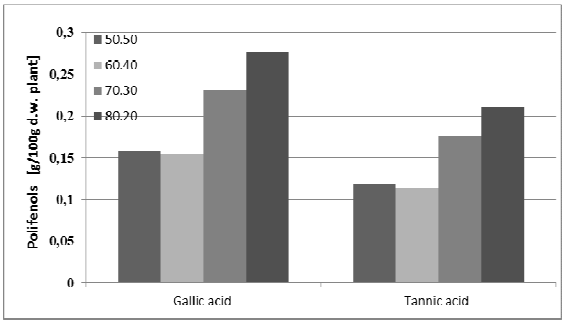

Figure 2. Evolution of polyphenols in different solvents 80 :20 – solvent ethanol/ water; 70 :30 - solvent ethanol/ water, 60 :40 - solvent ethanol/ water, 50:50 - solvent ethanol/ water

The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. In the current research four different ratio of solvents were used, and they can be arranged as follows: 50:50, 60:40, 70:30, 80:20. From selected solvents the lowest is 50:50 ratio, but the highest is 80:20 ratio.

Ethanol and water mixtures are commonly used phenols was found in extracted using 80% ethanol, which agrees with horseradish results.

This is due to the wide range of phenols that the aqueous ethanol mixtures can dissolve. Furthermore, ethanolic mixtures have acceptability for human consumption models. Contrary results can be found in literature.

The scavenging activity of DPPH˙ radicals has been widely used to determine the free radical-scavenging activity. DPPH˙ is a stable free radical that is dissolved in methanol and its colour shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the color from the DPPH˙ assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [17].

There are variations of antioxidants contained in horseradish roots obtained using conventional extraction. The results showed differences in DPPH˙ scavenging activity between rotio of solvents

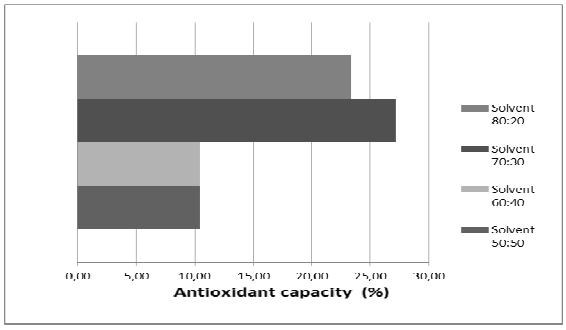

Figure 3. Antioxidant capacity in different solvents 80 :20 – solvent ethanol/ water; 70 :30 - solvent ethanol/ water, 60 :40 - solvent ethanol/ water, 50:50 - solvent ethanol/ water

. Also antiradical activity of horseradish differed significantly depending on different ratio of solvents used and the highest activity was determined in 70:30 (Fig.3) and the lowest is 50:50 and 60:40 ratio.

Analysis of the phenols, flavonoids and free radical scavenging activity of horseradish extracts showed differences depending on different ratio of solvent used. As the best solvents ethanol / water (80:20) solutions can be chosen.It can be concluded that using conventional extraction method more compounds that are not effective antioxidants, but react with Folin–Ciocalteu reagent, are extracted.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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