Development of a rapid analytical method for determination of total polyphenols in plant material used for meat production

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Abstract

Polyphenols are chemical compounds, belonging in the class of antioxidants, which have attracted extensive attention as natural bioactive materials. The availability of polyphenols in cheap natural sources such as tissues of several plants makes their utilization more attractive and favourable. The aim of the proposed research work was to develop a new technique for measurement of total polyphenolic compounds as Gallic acid and to recover these polyphenolic compounds from tissues of fobber plants such as *Petroselinum crispum*, *Poa pratensis, Vicia sativa, Medicago sativa, Trifolium sp., Hordeum vulgare, Avena sativa* and *Triticum cereale.* There is a significat difference between various plant tissues and pasturage products in the percentage of total polyphenols contained in their tissues. From the eight different plants tested for polyphenolic compounds, *Petroselinum crispum* and *Poa pratensis* was found to have the highest concentrations while the lowest polyphenol concentration was found to be in *Triticum cereale* tissues. According to the developed UV method the peak of absorption presented at 765nm and the linear range was from 0-50 ppm. The validation tests of the developed rapid method proved that the repeatability was satisfactory so that this can be suggested for routine analysis of several plant tissues.

Introduction

The interest for the role of the natural antioxidant compounds in human and animal health has been increased the last few years. The interest of the researchers is focused in the creation of natural plant products which contains high concentrations in antioxidant substances, adding additional nutritious value of foods. Among the substances that have been found to appear antioxidant action are various phenolic and polyphenolic compounds such as simple phenols, flavonoids terpenoids etc found in several common plant tissues and products.

Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of oxidizable matters by scavenging free radicals and diminish oxidative stress. Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress. The intake of natural antioxidants has been associated with reduced risk of cancer, cardiovascular disease, diabetes and diseases associated with ageing. Studies on dietary free radical scavenging molecules have attracted the attention to characterize phenolic compounds and other naturally occurring phytochemicals as antioxidants (Ani et al., 2006).

Moreover, plants encounter numerous pests and pathogens in the natural environment. An appropriate response to attack by such organisms can lead to tolerance or resistance mechanisms that enable the plant to survive. Thus, most plants produce a broad range of secondary metabolites that are toxic to pathogens and herbivores, either as part of their normal program of growth and development or in response to biotic stress. Among the metabolites in nature with the above characteristics are Polyphenols. It is agreed that phenolic compounds are widely distributed in plants used for defensive functions showing antimicrobial activities (Boudet 2006; Xia et al., 2010) controlling bacteria [Baydar et al., 2006; Taguri et al., 2004; Ani et al., 2006), fungi (Bruno and Sparapano 2007) and viruses (Chavez et al., 2006). Thus, there is currently an increasing interest in the isolation, examination and exploitation of agricultural wastes or inexpensive plant sources, rich in polyphenols such as tissues of *Olea europaea, Prunus amygdalus, Stevia rebaudiana* and in their wastes such as olive mills waste waters. Phenolics are a class of plant secondary metabolites that contain one or more hydroxyl derivatives of benzene rings.

Furthermore, according to Manach et al., (2005), polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are considered to contribute to the prevention of various degenerative diseases, including cardiovascular diseases. Two reasons in particular can be inferred. The first one is that the polyphenol family encompasses very diverse compounds with highly different bioavailabilities. Hence the results obtained for one polyphenol cannot be generalized to others. The second point is that polyphenols are now known to be largely metabolized in the body and native compounds most often tested in *in vitro* studies are virtually absent in the tissues.

The study of polyphenols has begun in 1950 and up to the beginning of next decade an important part of chemistry of the polyphenolic compounds had become acquaintance. Nevertheless, the available analytic techniques and the limited knowledge of these compounds did not allow a completed approach on to this subject. Hardly in 1976 was analyzed the quantity of polyphenols in the foods with chromatography of thin stack (Thin Layer Chromatography, T.L.C), while enough later, in 1992, were studied the total content in aglicones from five flavonoids and flavones in fruits and vegetables and tea. Since then they have been recognized thousands flavonoid compounds and continuously are isolated new. Since then high concentrations of catechins, flavonols and depsides were found to be restricted to the young vegetative and floral shoots, whereas leucoanthocyanins r flavylogens were characteristic of the more bulky axial tissues of the plant.

Polyphenols are enough widespread in the plant foods such as vegetables, cereals, legumes, dry fruits, fruits as well as in the drinks such as wine, beer, tea, cocoa etc. The quantity of total polyphenols presented varies even between cultures of the same type. It is known that the quantity of the total polyphenols in plant tissues is depending on genetic factors and by the environmental conditions. Other factors as the degree of maturation the variety, the treatment and the storage, influence the content of phenolic compounds derivatives of plants. The bitter flavor of foods and drinks depends from their content in polyphenols. In the legumes and the cereals the most common polyphenolic compounds are flavonoids, phenolic acid and tannines. Furthermore, in the legumes the higher amount of content in polyphenolic compounds appeared to be in the dark varieties such as red and black beans. The legumes contain also isoflavones while the vegetables contain mainly flavonoids. The roots and the tubers have low concentrations of flavonoids, with the exception of certain plants as the onions and the liquorice.

In the past, analytical methods used for search of total phenols in the animal diet have demonstrate many examples of inadequate use of them. Precautions should be taken at the export of phenols (McLeod 1974, Gartlan et al., 1980). Many phenolic unions affected by the sunlight, react with oxygen in the alkaline solution and with methanol in the temperature of room and the pH 6 (Haslam, 1966). The existence of phenols in the plants has been measured colorimetrically using the reaction agent folin Denis (Herdsman and Hillis, 1959). Furthermore, this reaction agent has been often used in order to measure the content in tannin in the harvests of fodder crops due to its simplicity in use (Burn, 1963).

The reaction agent of folin as it is modified from Folin and Ciocalteau (1927) gives a better estimate of total phenolic compounds (Singletone and Rossi, 1965). This reaction agent gives a better answer of colour with phenols and a smaller answer in the not-phenolic unions. (Price and Butler, 1977).

Materials and Methods

For the determination of total polyphenols extracted for the plant tissues were used the following materials:

Folin-Ciolteau: Phenol reagent 100 ml F-9252 Lot 52K3671
Gallic acid: $C_7H_6O_5H_2O$ 1-hydrate chemically pure 500g 2311K Lot 219862100
Sodium carbonate: Na_2CO_3 anhydrous (Reag. Ph. Eur) PA-ACS-ISO 1000g
Ethanol: absolute PA-ACS-ISO CH_3CH_2OH 2.5 L
Distilled water

The plants that they were used is this study, are mainly veterinary plants used for the diet of animals which are either cultivated or are found in the countryside. These plants participate also in the human diet. The samples that were taken were fresh plan tissues and no seeds at all. The plants used in this study were species of *Medicago sativa*, *Hordeum vulgare*, *Vicia sativa*, *Petroselinum crispum*, *Avena sativa*, *Poa pratensis*, *Trifollium* sp and *Triticum* spp.

The determination of total polyphenols becomes with the method known as Folin Ciocalteu. At this method is prepared solution Gallic acid and sodium carbonate.

Preparation of solution of Gallic acid: In volumetric bottle of 100ml they were dissolved: 0,5gr Gallic acid in 10ml of ethanol. Complete with distilled water up to final volume.

Preparation of solution of sodium carbonate: In 2 lt glass volumetric flask were dissolved: 200gr of arid carbonic sodium and 800ml of distilled water. Furthermore, the solution was placed on a thermal plate, and brought to boiling continuously stirred. Consequently the solution was cooled down and then certain crystal of sodium carbonate was added. After 24 hours the solution was filtered and distilled water was added in the volumetric flask of final volume of 1lit.

After this procedure it followed dilution of the initial solution in volumetric flasks of 50ml where they were placed 10ml from the solution of Gallic acid. Finally the 50ml flask was filled by distilled water. Then, in 5 volumetric flasks of 25ml they were placed 1ml 2ml 3ml 4ml 5ml from the diluted solution of Gallic acid respectively and distilled water was supplemented up to final volume. In 5 test pipes they were placed 1,6ml from each volumetric flask (1,2,3,4,5), 0,1ml Folin- ciolteau which was left for 8 min, 0,3gr of sodium carbomate. All samples were stirred and churned in the vortex. From the above solution 5 samples were made and theywere placed in dark environment with temperature 20°C for 2 hours. It follows churn in the vortex. Afterwards, the UV absorption was measured at 765nm compared against a blank solution which corresponded in the volumetric flask that did not contain solution of Gallic acid.

Extraction of polyphenols from plant tissues: 1 g of sample was extracted for two hours with 20ml of solution of 80% of ethanol that contains 1% HCl under continuous stir at 200 rpm in room temperature. The mixture was centrifuged at 3000 rpm for the 20 minutes and the supernatant solution was transported in the test pipe Falcon (50ml). The solid residual in the Falcon tube was treated once more by centrifugation in a similar way. The two supernatant solutions were transferred into 50ml volumetric flask and alcoholic solution was added up the volumetric line.

Preparation of the sample: In volumetric flask of 25 ml 2,5 ml of the diluted ethanolic extract of the plant tissue were added. The solution supplemented with distilled water up to final volume. Afterwards, in test pipes of 5 ml, 1,6ml of the final alcoholic solution – prepared sample 0,1ml Folin-Ciocalteu reagent and within a period of 8min 0,3ml of solution of sodium carbonate was also added. The samples brought up well and were placed in the dark for one hour. Afterwards, the absorption was measured at 765nm compared against a blank solution which corresponded in the volumetric flask that did not contain solution of Gallic acid.

For the calculation of the concentration of total polyphenols in the plant tissue the following formula was used: ppm of total polyphenol in the plant tissue = 500 x concentration of measured solution.

Results and Discussion

From the eight different plants tested for polyphenolic compounds, *Petroselinum crispum* and *Poa pratensis* was found to have the highest concentrations while the lowest polyphenol concentration was found to be in *Triticum cereale* tissues. According to the developed UV method the peak of absorption presented at 765nm and the linear range was from 0-50 ppm. The validation tests of the developed rapid method proved that the repeatability was satisfactory so that this can be suggested for routine analysis of several plant tissues.

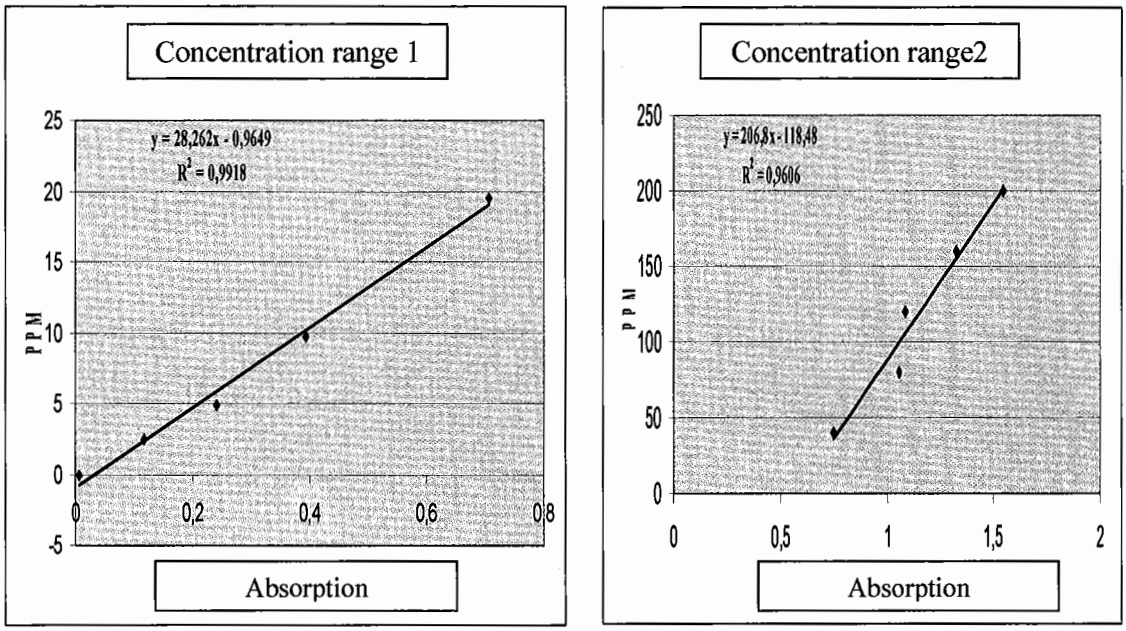

Figure 1. The calibration curves for two respective polyphenol concentration ranges

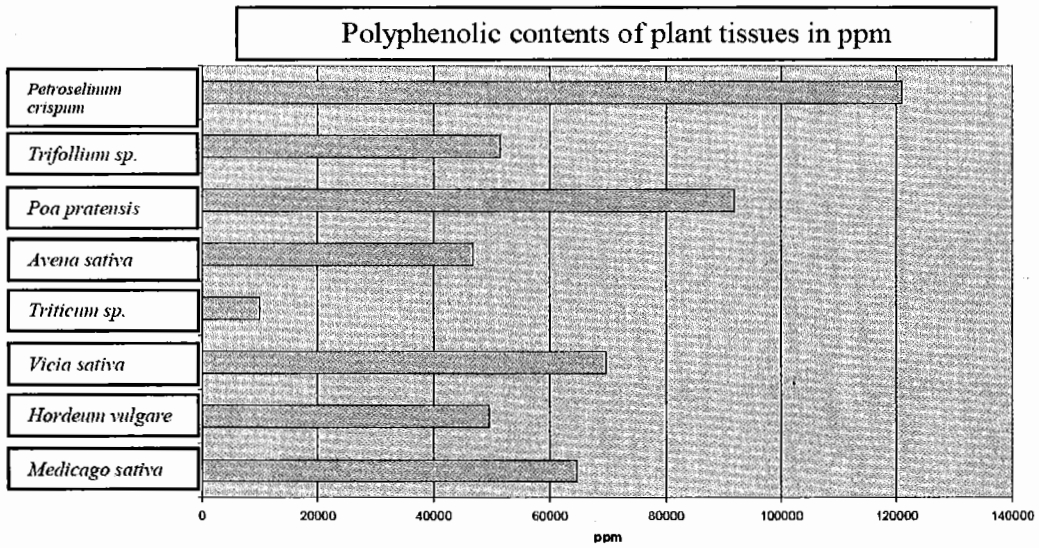

Figure 2. Polyphenol concentration measured in various plant tissues.

Conclusions

1. There is statistically important difference in the percentage of the polyphenolic compounds between various plant tissues.
2. The higher concentration of polyphenolic compounds was observed in the parsley and was 120880ppm on dry bases and the lower in wheat sample and it was 9900ppm on dry bases.
3. The region of linearity of curve of absorption was from 0 up to 50ppm nevertheless can be used with linear adaptation and other regions of curve. Ideally in future measurements the samples should diluted so as to they are in region 0-50 ppm
4. The highest absorption was observed at the 765nm and it is the wave length that should used for the method.

References

Ani V., Varadaraj M. C. and Naidu A. K. (2006). Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *Eur Food Res Technol* 224: 109-115.

Baydar N.G., Sagdic O., Ozkan G., Cetin S. (2006). Determination of antibacterial effects and total phenolic contents of (*Vitis vinifera*) seed extracts. *Int. J. Food Sci.* 41: 799-804.

Boudet A. (2006). Evolution and current status of research in phenolic compounds. *Phytochemistry* 68: 2722–2735.

Bruno G. and Sparapano, L. (2007). Effects of three esca-associated fungi on *Vitis vinifera* L:V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. *Physiol. Mol. Plant Pathol.* 71: 210–229.

Chavez J.H., Leal P.C., Yunes R.A., Nunes R.J., Barardi C.R., Pinto A.R., Simoes C.M. and Zanetti C.R. (2006). Evaluation of antiviral activity of phenolic compounds and derivatives against rabies virus. *Vet. Microbiol.* 116: 53–59.

Manach C., William G., Morand C., Scalbert A., Remesy C., Bioavailability and bioefficacy of pholyphenols in humans I Review of 97 bioavailability studies Am j Clin Nutrition 2005 JAN 81 230S-242S.

Xia E, Deng G., Guo Y. and Li H. (2010). Biological Activities of Polyphenols from Grapes. *International Journal of Molecular Science*, 11: 622-646.

Taguri T., Tanaka T., and Kouno I. (2004). Antimicrobial Activity of 10 Different Plant Polyphenols against Bacteria Causing Food-Borne Disease. *Biol. Pharm. Bull.* 27(12): 1965-1969