

P55: OPTIMISATION OF ASSAY CONDITIONS FOR THE DETERMINATION OF ANTIOXIDANT CAPACITY IN PLANT FOOD MATRICES BY USING PHOTOCHEMILUMINESCENCE

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Abstract –In this study, we optimized the extraction conditions (solvents, time of extraction, solvent-to-solid ratio, and sample storage) in order to measure the antioxidant capacity of fruits and vegetables extracts by using photochemiluminescence PCL assay.

Photochem apparatus (Analytic Jena, Germany) employing the built-in protocols for water-soluble (ACW) and lipid-soluble (ACL) bioactive compounds was used for determination. We performed experiments on kiwi, lemon, lemon peel, and broccoli.

Keywords: photochemiluminescence, PCL, superoxide radical, antioxidant activity

1. INTRODUCTION

Antioxidants are chemical substances that can interfere with the chain reaction by forming less reactive radicals or significantly lowering the number of free radicals; both of these actions effectively prevent, delay, or inhibit the oxidation reaction. [1-3].

Depending on their nature, antioxidants can be classified into two main categories: enzymatic (which includes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and non-enzymatic (which includes vitamin E, vitamin C, glutathione, carotenes and retinols, as well as some transition metals :Zn, Cu and Se).

The terms “antioxidant activity” and “antioxidant capacity” have different meanings: antioxidant activity deals with the kinetics of a reaction between an antioxidant and the prooxidant or radical it reduces or scavenges, whereas antioxidant capacity measures thermodynamic conversion efficiency of reactive species by antioxidants, such as the number of moles of reactive species scavenged by 1 mol of antioxidant during a fixed time period [4].

In the absence of antioxidants, reactive oxygen/nitrogen species (ROS/RNS) can cause diseases such as cancer, cardiovascular diseases, diabetes, arteriosclerosis, etc. Reactive oxygen species (ROS) can be divided into: radicals (e.g., superoxide, hydroxyl, peroxy) and non-radicals (e.g., hydrogen peroxide, ozone, and singlet oxygen).

Antioxidants can be classified in water soluble and fat soluble compounds. Water soluble or hydrophilic antioxidants reacts with oxidants present in the cell, while fat soluble or lipophilic antioxidants scavenge the unpaired electron that can induce lipid peroxidation on cell membrane. Different methods are used for the determination of antioxidant activity and considering the mechanism of action, the methods for assessing antioxidant activity can be divided into ET (electron transfer) and HAT (hydrogen atom transfer) mechanisms, the result of which can be similar, while the accompanying reaction kinetics varies. [4-5].

The Photochemiluminescence assay (PCL assay) is performed in the presence of a superoxide anion-radical ($O_2^{\bullet-}$), which is one of the most reactive oxygen species present in the human body [6]. Photochemiluminescence assay principle is based on generating superoxide anion-radicals ($O_2^{\bullet-}$) under the influence of UV light ($h\nu_1$), and a photosensitizer, followed by their detection during the reaction with a chemiluminogenous compound (luminol). The measure of radical quantity in the system is the intensity of the emitted light ($h\nu_2$), and the radical scavenging compounds attenuate the photochemiluminescence intensity in proportion to the amount and activity of the tested antioxidant [7-9].

The aim of the present work was to establish an antioxidant extraction method applicable to various food matrices. To optimize the extraction procedure, we focused mainly on solvent composition, the extraction time (duration), the

solvent-to-solid ratio, and the sample storage conditions. These parameters could influence the extract amount and composition, and thus the measured antioxidant capacity.

2. EXPERIMENTAL

2.1. Materials

Kiwi, lemon, and broccoli were purchased from Romanian local supermarkets. The fresh material was stored for no more than 3 days at 4-8 °C prior to analysis.

2.2. Sample preparation

For each experiment (comprising the whole set of extraction conditions: solvent for extraction; solvent-to-solid ratio; time of extraction; the samples storage conditions), we used kiwi and lemon without the peel, same-sized external heads from one broccoli. Each extraction was done in triplicate and analyses were performed on the day of extraction. Within each experiment, all the tests on a given matrix were done on the same day.

2.3. Determination of total antioxidant capacity using the PCL assay

The procedure was based on the methodology published by Popov and Lewin. The principle of the PCL assay is based on the fact that the superoxide anion radicals ($O_2^{\bullet-}$) generated upon exposure to light and the presence of a photosensitizer are detected by their reaction with a photosensitizer, chemiluminogenous, compound-luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). Analyses are conducted using a Photochem® apparatus (Analytik Jena, Germany). PCL evaluations are precise, reproducible and rapid analysis of antioxidant capacity in both lipid-soluble (ACL) and water-soluble (ACW) extracts due to kits provided by the manufacturer (Analytik Jena, Germany). The presence of an antioxidant in the reaction solution results in a retardation of the luminescence. PCL assays were carried out in triplicate for each sample, using HPLC grade water for ACW and HPLC grade methanol for ACL measurements. Results were expressed as μmol Vitamin C equivalents per 1 g sample and as μmol Trolox equivalents per 1 g of tested sample.

2.4. Optimization of extraction for the determination of the antioxidant capacity of

water-soluble bioactive compounds by the ACW-PCL

We used the food matrices of kiwi, lemon and broccoli. Lemon and kiwi fruits were selected because they contain large amounts of ascorbic acid. The variations were trailed for extraction of samples as follows: water extraction, extraction with water/methanol mixture (80/20; vol/vol) and extraction with water/methanol/acetic acid mixture (80/15/5; vol/vol/vol). Fresh samples were immediately ground with a blender before addition of solvent mixture. The mixture was shaken for 15 min, 30 min and 1 h using vortex at 800-900 rpm and centrifuged at 8,000g for 20 min. The supernatant was used for ACW-PLC assay and the results were expressed as μmol vitamin C per 1g fresh weight or dried matter.

2.5. Optimization of extraction for the determination of the antioxidant capacity of lipid-soluble bioactive compounds by the ACL-PCL

For the extraction of lipid-soluble bioactive compounds by ACL- PCL assay, we used the same matrix, kiwi, lemon and broccoli, respectively, as ACW-PCL assay. Experimental works consisted in: extraction of bioactive compounds (in methanol and ethanol); extraction from fresh and dried products; the solvent-to-solid ratio; extraction using a vortex and centrifugation. The supernatant was used for ACL-PLC assay and the results were expressed as μmol Trolox per 1g fresh weight or dried weight.

3. RESULTS AND DISCUSSION

The solvent extraction in various hydrophilic systems has not influenced significantly the ACW measurements. The results of these three different extraction showed that different solvents: water, water/methanol mixture (80/20; vol/vol) and water/methanol/acetic acid mixture (80/15/5; vol/vol/vol) have not significantly influenced the extraction of antioxidant compounds. Average values for kiwi were, respectively, 9.85 ± 0.09 , 9.71 ± 0.14 and 9.27 ± 0.198 μmol vitamin C/g fresh weight (FW).

The optimum solvent-to-solid ratio was 1 g fresh sample and 20 ml solvent, the value of antioxidant capacity was 9.79 ± 0.11 μmol equivalent vitamin C /g FW, by comparison with 9.35 ± 0.49 μmol equivalent vitamin C /g product obtained using 1g fresh sample and 10 ml solvent.

Considering the extraction time (duration) we performed experiments at 15 min, 30 min and 1 h, the optimal extraction time using a vortex, was 30 minutes at 800-900 rpm. Also, for centrifugation used was conducted at 8,000g for 20 min.

There were significant differences for values obtained from the extraction of fresh samples in relation to the samples dried at 50°C, meaning that the values of antioxidant capacity for dried samples were lower. For fresh kiwi, the mean value obtained for the antioxidant capacity, was 9.748 µmol equivalent vitamin C /g, and by reporting to the dry matter (dm), the value was 70.88 µmol equivalent vitamin C /g dm; dried kiwi samples had an average of 57.7 µmol equivalent vitamin C /g dm.

Experiments to determine the antioxidant capacity of lipid systems by the ACL-PCL assay, have led to optimal extraction variants as follows: optimal extraction solvent was methanol, solvent-to-solid ratio 1g/20 ml solvent, extraction time 30 min. The results of different extraction showed that methanol extract had a better antioxidant capacity, 8.87±0.11 µmol equivalent Trolox/1g FW, compared with ethanol extract, respectively, 6.76±0.09 µmol equivalent Trolox/1gFW. Also, the extraction time (duration) we performed experiments at 15 min, 30 min and 1 h, the optimal extraction time using a vortex, was 30 minutes at 800-900 rpm. Average values for kiwi were, respectively, 8.61± 0.26, 9.77± 0.10 and 9.64± 0.14 µmol equivalent Trolox/g FW.

There were significant differences for values obtained from fresh samples in relation to the dried samples at 50°C, meaning that the dried samples had lower values of antioxidant capacity, expressed in µmol equivalent Trolox/g product. For example, fresh kiwi presented 62.79 µmol equivalent Trolox/g dm and 58.31 µmol equivalent Trolox/g dm after drying.

4. CONCLUSIONS

The optimized procedures for the extraction of bioactive compounds consist in: solvent-to-solid ratio of 20 mL per 1g sample, extraction time 30 min (vortex), fresh sample use, extraction with water for ACW protocol and extraction with methanol for ACL protocol.

We performed experiments on kiwi, lemon, lemon peel, and broccoli.

The antioxidant capacity of extracts was, in

descending order, as follows: kiwi > lemon > broccoli > lemon peel for water-soluble antioxidants, respectively, 70.88; 59.66; 56.07; 13.67 (expressed as µmol Vitamin C equivalents/g dm) and lemon peel > broccoli > lemon > kiwi for lipid-soluble antioxidants, respectively, 115.65; 68.4; 65.13; 62.79 (expressed as µmol Trolox equivalents/g dm).

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