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Antioxidant Properties of a Parsley (*Petroselinum crispum*) Juice Rich in Polyphenols and Nitrites

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ABSTRACT

Parsley *(Petroselinum crispum)* is an herbaceous vegetable used as foodstuff, spice and medicinal plant because it provides antioxidants especially flavonoids (apigenin), vitamins (K, C and A), and volatile oils, among other compounds. Because parsley has important concentrations of nitrates and flavonoids, very little vegetal pigment and a mild flavour profile, it was chosen for this study. The aim of this study was to obtain parsley juice rich in nitrite and polyphenols and to assess its antioxidant activity. To obtain nitrite from nitrate by enzymatic reaction, *Staphylococcus xylosus* ATCC 29971 was used as nitrate reductase source. To obtain the vegetable juice, fresh roots were minced and homogenized in aqueous solution. The sterile juice was filtered and then inoculated with *S. xylosus* and incubated at 37°C. The nitrate and nitrite concentrations (mg/L) were determined using a colorimetric method using salicylic acid and Griess reagent, respectively. The total polyphenols content (TPC) was measured with Folin-Ciocalteu reagent and expressed as mg gallic acid equivalent / 100 mL (mg GAE / 100 mL). The total flavonoids content (TFC) was measured with aluminium chloride reagent, and expressed as mg catechin equivalent / 100 mL (mg CE / 100 mL). To determine the antioxidant activity of parsley juice, the ability to reduce DPPH synthetic radical, reducing power of Fe^{3+} ion and the capacity of chelating transition metal ions were assessed. Maximum concentration of nitrites was achieved for parsley juice in the presence of *S. xylosus* after 24 hours; TPC was 14.87 mg GAE / 100 mL and TFC was 11.21 mg CE / 100 mL. The ability of parsley fermented juice to reduce DPPH synthetic radical was 79.45%, while the capacity to reduce Fe^{3+} was 0.758 ± 0.14 (absorbance at 700 nm) and to chelate Fe^{2+} ion was 23.64%. Parsley juice represents an important source of natural nitrate and flavonoids, with important antioxidant capacity.

Keywords: Parsley *(Petroselinum crispum)*, vegetable juice, antioxidants, natural nitrate.

INTRODUCTION

Nitrate is naturally present in soils, water and plants (especially in vegetables) as a consequence of nitrogen fixation. Nitrate is reduced to nitrite by nitrate reductase enzymes. Parsley, among other vegetables, possesses the tendency to accumulate nitrate[1].

Parsley *(Petroselinum crispum)* belongs to Apiaceous family and it has been used as food, pharmaceutical, perfume, and cosmetic ingredient[2]. The name *petroselinum* came from the Greek word “petros” which means “stone” and it is referring to the plant’s habit of growing in rocky places[1]. Fejes *et al*. (2000) investigated phytochemical profile of parsley and revealed the presence of several classes

of polyphenols, especially flavonoids[3]. The major flavonoids found in parsley are flavonols (kaempferol and quercetin) and glycosylated flavones (apigenin and luteolin). The sanogenous effects of parsley result from the high content in flavonoids (about 100 mg/100 g fresh weight[4]) with antioxidant activity and the capacity to scavenge free radicals[5].

The purpose of this study was to obtain a fermented juice from parsley roots, rich in nitrites and polyphenols, and to test its antioxidant activity (DPPH radical scavenging activity, Fe^{3+} reducing power activity and Fe^{2+} chelating activity) comparatively with ascorbic acid solution (10 µg/mL) and butylated hydroxyanisole solution (10 µg/mL).

MATERIALS AND METHODS

All chemicals and reagents used in the study were of analytical grade and purchased from Sigma chemicals (Romania). *Staphylococcus xylosus* ATCC 29971 strain used for vegetable juice fermentations was purchased from BioMerieux.

Vegetable juice obtaining

Parsley roots were bought from a local market and frozen until analyses. Unfrozen plant material was cut into pieces and chopped in a laboratory blender. The fresh juices were sterilized for 15 min at 121°C. After sterilization, the juices were filtrated and kept in refrigeration condition, at 4°C, until analyzes were performed.

Bacterial strains

Staphylococcus xylosus ATCC 29971, a coagulase-negative and non-toxigenic strain, used for vegetable juice fermentations, was incubated in nutritive broth for 24 hours and then it was used as nitrate reductase source.

Vegetable juice fermentation

Parsley juice was inoculated with *S. xylosus* in concentration of 10^8 CFU/mL and the fermentation was conducted at 37°C for 30 hours.

Determination of nitrate concentration in vegetable juices

For nitrate determination, it was used a colorimetric method described by Cataldo *et al.* (1975)[6]. The complex formed by nitration of salicylic acid under highly acidic conditions absorbs maximally at 410 nm in alkaline (pH>12) solutions. The nitrate concentration was determined from 6 to 6 hours for 30 hours, and expressed as mg nitrate/1000 mL (ppm)[7].

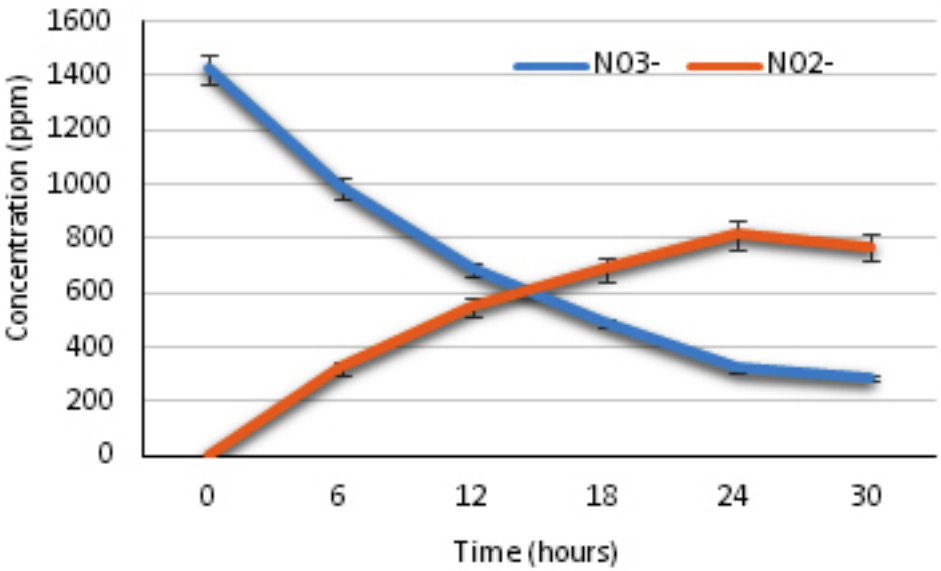

Fig. 1: Nitrates and nitrites concentration in the presence of *Staphylococcus xylosus* nitrate reductase

Determination of nitrite concentration in vegetable juices

Nitrite ions react with Griess reagent and the purple colour that developed after 20 min was read spectrophotometrically at 538 nm[8]. The nitrite concentration was determined from 6 to 6 hours for 30 hours, and expressed as mg nitrite/1000 mL (ppm)[7].

Determination of total phenolic content (TPC)

TPC was measured using the Folin–Ciocalteu colorimetric method[9]. The absorbance of the resulting blue colour was measured at 765 nm. The results were expressed as milligrams of gallic acid equivalents/100 mL juice (mg GAE/100 mL).

Determination of total flavonoid content (TFC)

The total flavonoid content of fermented juice was determined by the aluminium chloride colorimetric method[10]. The absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg catechin equivalents/100 mL juice (mg CE/100 mL).

Determination of antioxidant activity (AA)

Antioxidant activity of parsley fermented juices was compared to the one of ascorbic acid (10 µg/mL) and butylated hydroxyanisole (10 µg/mL).

- DPPH radical scavenging activity. The ability of fermented juices to scavenge DPPH synthetic radical was assessed according to the method of Burits and Bucar (2000), with some modifications[11]. Aliquots of 1.95 mL of 0.2 mM DPPH ethanolic solution were mixed with 50 µL of the samples. The mixture was shaken vigorously and then kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. DPPH radical scavenging activity was expressed as % Inhibition.
- Fe^{3+} reducing power activity. The reducing power of the fermented juices was determined according to the method of Oyaizu (1986)[12]. Fe^{3+} reducing power activity was expressed as absorbance at 700 nm (A700 nm).
- Fe^{2+} chelating activity. Fe^{2+} chelating activity was measured according to the method of Benzie and Strain (1996)[13] and it was expressed as % Chelation [*% Chelation = (A_c – A_s)/A_c x 100*), where A_c is the absorbance of the control and A_s is the absorbance of the sample].

All determinations were made in triplicate.

RESULTS AND DISCUSSION

Determination of nitrate and nitrite concentration in vegetable juices

During the 30 hours of fermentation, the nitrate was reduced to nitrite. The initial concentration of nitrate in parsley juice was 1425.01 ± 54.94 ppm. In the presence of the microbial nitrate reductase, the nitrate concentration decreased to 287.19 ± 14.11 ppm, after 30 hours of fermentation (Figure 1).

After 6 hours of fermentation, the nitrite concentration was 321.32 ± 36.46 ppm. At the end of fermentation experiment, the nitrite concentration increased up to 767.25 ± 51.13 ppm (Figure 1). The conversion rate of nitrate to nitrite in the reduction reaction was calculated: after 24 hours of fermentation, the conversion of nitrate to nitrite was 77.01% and after 30 hours it was 72.56%; after 24 hours of fermentation, a secondary reaction occurred (Table 1).

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

Parsley roots contain high levels of phenolics, some of them soluble in aqueous media. For this reason, in the fermented juice these compounds with antioxidant activity were recovered. Total phenolic content (TPC) and total flavonoid content (TFC) found in fermented parsley juice are presented in Table 2; total flavonoids represent 75.39% from total phenolic content.

Determination of antioxidant activity

As expected, due to high content in flavonoids, fermented parsley juice exhibited an important antioxidant activity (Table 3). $DPPH^{\cdot}$ scavenging activity of a product depends on the ability of its antioxidant compounds to lose hydrogen and the structural conformation of these components[14]. Parsley fermented juice contains phenolic compounds able to convert the free radical in a stable diamagnetic molecule, which causes discoloration of $DPPH^{\cdot}$ solution. DPPH radical scavenging activity, expressed as % Inhibition,

Table 1: Nitrate-nitrite conversion rate (%) in the presence of *Staphylococcus xylosus* nitrate reductase

Time (hours)	Conversion rate (%)
6	30.37
12	51.75
18	65.18
24	77.01
30	72.56

Table 2 : The total phenolic content (TPC) and total flavonoid content (TFC) concentration of fermented parsley juice

Sample	TPC (mg GAE/100 mL)	TFC (mg CE/100 mL)
Parsley	14.87 ± 1.03	11.21 ± 1.11

Table 3 : Antioxidant activity of fermented parsley juice compared to ascorbic acid and butylated hydroxyanisole

Sample	DPPH· scavenging activity(% Inhibition)	Fe^{3+} reducing power(A 700 nm)	Fe^{2+} chelating activity%Chelation
Parsley	79.45 ± 6.23	0.758 ± 0.140	23.64 ± 2.14
Ascorbic acid (10 µg/mL)	59.36 ± 4.25	0.644 ± 0.071	66.36 ± 5.47
Butylated hydroxyanisole (10 µg/mL)	77.12 ± 6.47	0.760 ± 0.078	78.36 ± 7.14

found in parsley fermented juice, was 79.45 ± 6.23. Fe^{3+} reducing power activity of a juice containing antioxidants reflects the ability of antioxidants to donate electrons, to be reducer compounds. Fe^{3+} reducing power activity of the fermented parsley juice, expressed as absorbance at 700 nm, was 0.758 ± 0.14, which demonstrates an appreciable antioxidant activity. Fe^{2+} is pro-oxidant due to its implication in generation of HO· in Fenton reaction. Therefore, the compounds able to chelate this cation are strong antioxidants because they prevent HO· generation in Fenton reaction, which is the most aggressive free radical. Fe^{2+} chelating activity of fermented parsley juice, expressed as % Chelation, was 23.64 ± 2.14, which indicates the presence of phenolics with hydroxyl groups in the vicinal positions, able to chelate Fe^{2+}.

The results showed that fermented parsley juice represents an important source of natural nitrate. The conversion of nitrate to nitrite was maximum after 24 h of fermentation. Also, fermented parsley juice contains high level of flavonoids with antioxidant activity. DPPH· scavenging activity and Fe^{3+} reducing power activity of fermented parsley juice was approximately equal with those found for butylated hydroxyanisole solution and slightly higher than those found for ascorbic acid. Fe^{2+} chelating activity found for parsley fermented juice was much lower than the values found for standard solutions.

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