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VARIATION IN CONTENT OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF BASIL (*Ocimum Basilicum*), DILL (*Anethum graveolens*) AND PARSLEY (*Petroselinum sativum*)

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Abstract: *The aim of this study was to determine the content of polyphenolics, antioxidant activity and vitamin C in the extracts of three medicinal and aromatic plants: basil (Ocimum Basilicum), dill (Anethum graveolens) and parsley (Petroselinum sativum).*
The content of total polyphenols determined for the three aromatic plants can be estimated as high. The 1,1´-diphenyl-2-picrylhydrazyl (DPPH) test were applied to determine antioxidant activity, which was also high in comparison with other herbs species. With regard to IC_{50} values (%), the order in DPPH radical-scavenging were dried parsley (14.45) > fresh basil (9.03) > and fresh parsley (8.62).
The ascorbic acid content varied between 347.60 mg/100g in the fresh parsley and 16.53 from dried dill. These results suggest the Ocimum Basilicum extract with highest polyphenolic content has more antioxidant activity against protein oxidation.

Keywords: *Basil, parsle, dill, total polyphenols content, scavening activity DPPH, ascorbic acid.*

1. Introduction

The importance of oxidation processes in the body and the food was widely recognized in many studies. Defense mechanisms against excessive oxidation effect is provided by the action of antioxidants. The oxidative stress plays a role in the development of many diseases with high mortality. According to the Bahramikia S. et al., 2009 oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system, which results in functional tissue damage.

It has long been recognized that naturally occurring substances in higher plants have antioxidant activities [2]. Antioxidants such as vitamin C and phenolic compounds, vitamin E and carotenoids present in vegetables contribute to the reduce oxidative stress. As a result, they protect the cell from oxidative damage, and thus can prevent chronic diseases, such as cancer, cardiovascular diseases, diabetes [3].

In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases[4].

The most effective components seem to be flavonoids and phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits [4].

Many studies have been shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis [2]. Polyphenols are considered protective factors against degenerative diseases and cardiovascular disease.

Herbs may contain phenolic compounds that contribute to the intake of natural antioxidants that protect important cellular components such as ADN, proteins and lipid membranes against the action of reactive oxygen species.

Levels of phenolic compounds from plants, fruits, vegetables and herbs are influenced by genotype, agronomic practices, maturity at harvest and post-harvest storage, climate, region and processing conditions [5].

Parsley (*Petroselinum crispum L.*), dill (*Anethum graveolens*) and basil (*Ocimum Basilicum*), are the most important Romanian seasoning herbs. The aromatic herbs most consumed Romania are parsley, dill and lovage and Romania is one of the leading European spice consumer, according to EUROSTAT and FAOSTAT [6].

It is widely used, both fresh and dry, to enhance the flavor of many different foods [7]. Spices leaves deterioration occurs during processing and storage and is related to oxidative processes.

Amounts of nutrients are removed or destroyed components during storage of raw materials or technological processing (drying, grinding). Spices and dry herbs are used as ingredients in a variety of products prepared in different ways, this fact suggests the need to provide a control system to improve the quality of herbs and spices [8].

Essential oils are known to possess multifunctional properties other than their classical roles as natural food additives and/or fragrances [2]. The commercial essential oil of parsley is largely derived from the seed or the herb harvested at seed formation, prior to ripening [2].

Wahba NM et all., 2010 evaluated the addition of some edible plants including cayenne, green pepper, parsley, and dill to Kareish cheese and to evaluate the antimicrobial activity of these plant materials against natural microflora, coliforms, molds, and *Staphylococcus aureus*.

Therefore, this study revealed that pepper, parsley, and dill exhibited antibacterial activity against natural microflora in Kareish cheese, and the addition of these plants is acceptable to the consumer and may contribute to the development of new and safe varieties of Kareish cheese [9].

Lisiewska Z. et al., 2003, evaluate the effect of temperature and storage period on the preservation of vitamin C, thiamine and riboflavin in leaves and whole plants (leaves with petioles and stems) of dill, harvested with a plant height of 25 cm. The treatment of blanching affected a decrease in the level of vitamin C by 35-48% [10].

The purpose of this paper is to evaluate and compare the total polyphenol content, antioxidant capacity and the content of vitamin C parsley, dill and basil fresh and dry.

These plants were chosen because they are represented as native herbs, and spices that presents a common variable consumption also shows a strong pharmacological activity, and have high antioxidant content.

2. Materials and Methods

2.1. Plant material

A fresh spice (table 1) was purchased at a commercial establishment in Suceava in the spring 2013. The leaves were removed from the stems and were refrigerated at 5 °C for analysis fresh. Dried vegetables were obtained from a local market and are from different companies.

Table 1

Botanical and common names of spices used for the analysis

Botanical name	***Common name***
Ocimum Basilicum	basil
Anethum graveolens	dill
Petroselinum sativum	parsley

2.2. Chemicals

All chemicals used for experiments were of analytical grade and procured from Sigma Merck, Aldrich and Fluka. Deionizer water was used. Absorption determination for total polyphenols content was made using UV–VIS spectrophotometer.

2.3. Chemical analyses

The determination of moisture in samples was effectuated according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103 °C.

Total polyphenol were assayed according to the Folin-Ciocalteu method. Fresh herbs was cleaned, carefully washed with tap water was extracted with 80% ethanol (0.1 g sample/10 mL of 80% ethanol), and total phenol contents (TPC) of the extracts were determined. The results were expressed as mg of gallic acid equivalents (mg GAE).The correlation coefficient (r^2) for the calibration curve was 0.9954.

2,2-Di (4-tert-octylphenyl) -1-pycrilhydra - zyl (DPPH) scavenging capacity assay

The method used for determining the antioxidant activity of spices herbs extracts is based on scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The spices samples aliquot (0.5 mL) was added to freshly prepared DPPH reagent. After incubating for 5 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer. The control was conducted in the same manner, except that distilled water was used instead of sample.

The IC_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given.

The DPPH scavenging capacity assay value is calculated according to the formula:

$$IC_{50}\,\% = [1 - (A_{samples}/\,A_{control})] \times 100.$$

Ascorbic acid determination was done on acid extracts of samples.

Extraction of ascorbic acid from samples

The extracts were obtained following Rodriguez-Saona and Wrolstad (2001) modified protocol: 4 gram of parsley was extracted with 12 ml of acidified solutions (Perchloric acid and o- Phosphoric acid 1%) using a ceramic mortar and a pestle. The residue was re-extracted until the extraction solvents remained colorless (the total solvent volume was 50 ml).The extract was filtered through a Whatman no. 1 filter paper.

The extracts were kept at -20°C until further analysis.

Ascorbic acid separation, identification and dosage

The ascorbic acid in the samples was separated, identified and dosed in a HPLC

SHMADZU system coupled with UV–VIS detector (DAD). ZORBAX - C18 column (5μm, 250x4,6) was used. The column was eluted in isocratic system with a mobile phase consisted of phosphate buffer pH = 3.5 (TFA): solution 0.02 m/l of monopotassium phosphate and orthophosphoric acid 10%, adjusted to pH = 3.5. At a flow rate of 0, 6 ml/min. The chromatograms were registered at 245 nm.

For ascorbic acid identification standard L-ascorbic acid (Sigma 99% standard L ascorbic acid) was used. For dosage of ascorbic acid in the samples, a calibration curve was constructed using dilutions of standard L-ascorbic acid in bidistilled water.

3. Results and Discussions

Phenolic compounds widely exist in plants and they are highly effective antioxidants [1,11]. The contents (mg/g of dried extract) of phenols in ethanolic extracts of dill flower, leaf and seed was higher in the order of flower > leaf > seed extracts [11].

12 samples were formed, and table 2 shows the values obtained for moisture, total phenols and DPPH values of aromatics herbs.

Table 2.

Moisture, phenolic, and free Radical Scavenging Activities (DPPH) values in selected herbs

Samples			**Moisture (g/100 g)**	**TP[a] (mg GAE/100g)**	**IC_{50}**
Basil *(Ocimum Basilicum)*	S1	Fresh	79.82	245.2	9.03
	S2	Dried	0.97	109.6	29.07
	S3		0.98	92.8	55.63
	S4		0.98	63.2	67.05
Dill *(Anethum graveolens)*	S5	Fresh	82.25	228.1	26.51
	S6	Dried	0.99	92.8	84.24
	S7		0.98	111.8	92.77
	S8		0.99	57.7	86.56
Parsley *(Petroselinum sativum)*	S9	Fresh	83.24	211.9	8.62
	S10	Dried	1.00	181.9	14.45
	S11		0.99	178	23.84
	S12		1.00	160.3	16.76

[a]Values are referred to mg/100 g fresh weight vegetable. TP = content of Total Phenols (mg GAE/100 g).
IC_{50} = Free Radical Scavenging Activities

Moisture from dried samples presented the lower values. Important to maintain the quality of dried spices herbs is low moisture content, which decreases the likelihood of microbial growth and late biochemical reactions associated with these processes. Moisture content from fresh samples was higher in S9 parsley (83.24 %) and lower in S1 basil (79.82 %).

The use of freshly harvested vegetables in human nutrition is of fundamental importance today as modern storage or transforming systems allow the conservation of fresh vegetables for long periods of time in refrigerated cells under a controlled atmosphere, although the long-storage or mildly processed vegetables possess a significantly lower phenolic and ascorbic acid content than fresh vegetables and their antioxidant capacity decreases proportionally [12].

Total phenols. The comparison of selected aromatics herbs reveals impressive differences. From Table 2, total phenolic contents of three dried basil extracts were relatively lower and their total antioxidant capacities were weaker. The three dried dill spice extracts contained lower poliphenols, (mean = 87.43 mg/100 g of DW) than the three dried parsley spice extracts contained higher poliphenols, (mean=173.4 mg/100g of DW). Basil had the highest content of poliphenols 245.2 mg GAE/100 g.

The correlation value for the parameters moisture and total phenols measured in selected aromatics herbs is 0.740, reveled a stronger correlation between these two parameters (table 3).

Table 3
Correlation matrix for the variablesmeasured in aromatics herbs: Moisture(g/100 g), TP (mg GAE/100g) and IC_{50}

	Moisture (g/100 g)	TP (mg GAE/100g)	IC_{50}
Moisture (g/100 g)	**1**		
TP (mg GAE/100g)	**0.740**	**1**	
IC_{50}	-0.333	**-0.753**	**1**

Values in bold are different from 0 with a significance level alpha=0,05

The highest negatively correlation have been observed in the case of total poliphenols with antioxidant activity against the DPPH* free radical IC_{50} (r = -0.753).

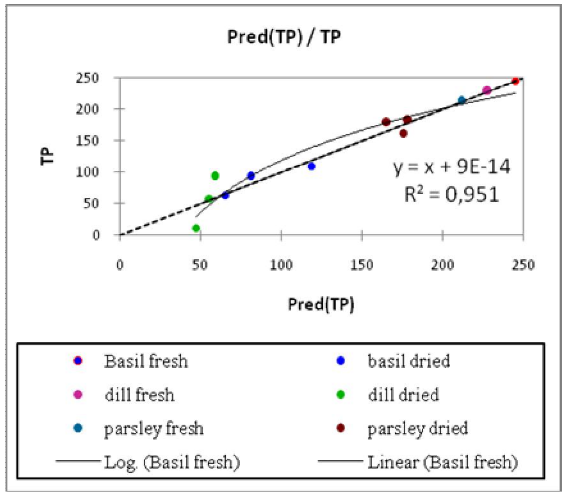

Figure 1. The relationship between the content of Total phenols of analyzed samples

The analyses show a significant relationship between total phenols content values on samples of study (see figure 1).

DPPH scavenging capacity assay
Methanolic extracts of fresh basil, dill and parsley had exceptionally high scavenging activity. A stronger radical-quenching agent resulted in a lower IC_{50} value (scavenging of 50% DPPH radical).
Table 2 shows that the DPPH radical scavenging ability of samples can be ranked as dried parsley > dried basil> dried dill. The scavenging abilities on DPPH radicals at 0.1 mg of dried extract/mL were 14.45%, 16.46% and 23.84% for the dried parsley, and 29.07%, 55.63% and 67.05% for the dries basil.

The ascorbic acid extracts of samples are presented in figure 2. The ascorbic acid content in aromatics herbs has recorded different discounts, depending the samples analyzed fresh or dried. Thus, the greatest values of acid ascorbic concentration were registered in the fresh parsley 347.60±6.2 mg/100g. The greatest reduction in the content of ascorbic acid was in the dried dill samples (89.33 %), followed by parsley (75.41 %). As seen in the figure 2, the ascorbic acid content in samples analyzed was higher in fresh samples.

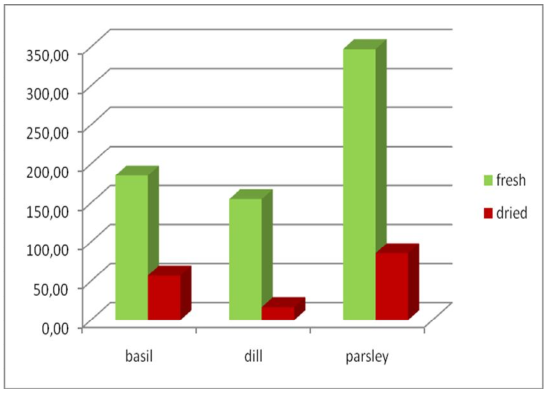

Figure 2. Ascorbic acid concentrations mg /100g of analyzed samples

Cătunescu G.M. et al., 2012 evaluate the ascorbic acid in parsley, dill and lovage samples packed in poliethylene stored in bags at 4°C for up to 12 days. The results showed the content of ascorbic acid diminished by 18% for parsley samples, 8% for dill and 3% for lovage after 12 day of storage.
Our results illustrated that ascorbic acid should be responsible for the effective antioxidant properties of the parsley fresh extract.

Principal component analysis (PCA) was used to test for the correlation between the variables: moisture (g/100 g), TP (mg GAE/100g) and IC. The variation of the parameters studied implies the existents of 3 factors. The principal component that has the eigenvalue more than 1 is F1 (2.235). The eigenvalue for F2 and F3 are less than 1 that implies that these factors are not important. The percentage of variability represented by the first two factors is high (96.75 %).

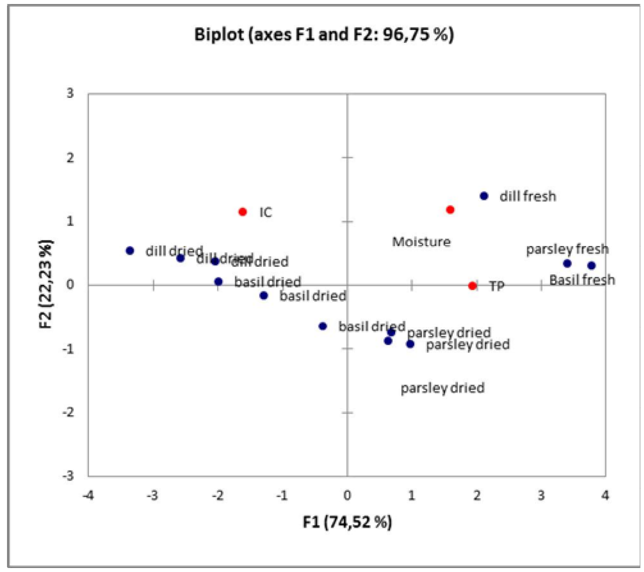

Figure 3. Biplot graphic for the parameters studied in analyzed samples

The first factor (F1) explains 74.52 % of the total variance with a significant parameter TP, having a factor loading 0.871. An important contributions of the observations (%) for the factor F1 have basil fresh (23.9 %), parsley fresh (19.3 %) and dill dried (18.7 %). The second factor (F2) explains 22.23 % , of the total variance with a significant parameter Moisture(g/100 g). An important contributions of the observations (%) for the factor F2 has dill fresh (36.2 %).

4. Conclusions

Antioxidant capacities of basil, dill and parsley fresh and dried were analyzed. During storage important quality losses occur.
Spices herbs are important sources of valuable and essential nutrients such as

vitamins and minerals. These are the richest potential sources containing different antioxidants. Various research studies have shown that a higher consumption of plants containing antioxidant sources is associated with a lower incidence of cardiovascular disease, cancer and other chronic diseases. Following the analysis of samples, total phenols content was 245.2±2.3mg/100 g in the fresh basil but the value decreased to 63.2±3.6mg/100 g. in the dried basil. Both remarkably high phenolic content and radical scavenging activities were found in the fresh dill.

In conclusion, this study suggests that the processing and storage temperature significantly affect the antioxidant capacities of spices herbs analyzed.

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