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ANTIOXIDANT CHARACTERISTICS OF DIFFERENT VARIETIES OF APPLES DISTRIBUTED IN BULGARIA

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Abstract-

Background: Because of its wonderful taste, as well as numerous nutritional and medicinal qualities valued especially in the 20th century apple fruit is preferred worldwide.

Objective: The current work presents the antioxidant specifications of several varieties of apples distributed in Republic of Bulgaria and also methodology/equipment and method for the preparation of the apples for the experiment/ used to determine the antioxidant characteristics.

Methods: We use the following methods: spectrophotometric method; systematic approach and a critical analysis of the accessible Research Periodicals

Results: We obtain nectar from apples by their immediate squeezing prior the experiment using a juicer. (1) Mix the three tubes with electromagnetic stirrer VORTEX 2 GENIE for 10 seconds. (2) Place the tubes in a water bath for 10-15 minutes at 380C. (3) Put the tubes in cold water for 5 seconds to stop the reaction. (4.) Identification of the activity of the samples with a UV-VIS spectrophotometer; Thermo-Scientific; Used length λ = 560 nm.

CONCLUSION:

1. Choose fully ripe fruits to generate the highest amount of antioxidants.

2. The studied varieties of apples can be arranged according to their antioxidant activity as follows: Grey smith, Aidere, Golden Delicious, Jonah Gold, Golden Delicious, Red Delicious, Chadeo, Mutsu, and Florina.

Keywords: apple, antioxidant activity

I. INTRODUCTION

The apple is well known fruit since ancient times. Besides its marvelous taste, it is preferred for its numerous nutritious and healing qualities that are most valued in the twentieth century[5,6]. We believe that there is more to learn about its origin and benefits

OBJECTIVE: This paper presents:

- The antioxidant properties of several varieties of apples grown in Republic of Bulgaria
- Methodology (instrumentation and method for preparing the apples for experiment) used to determine the antioxidant characteristics.

Object of the survey are the following sorts of apples: Golden Delicious, Red Delicious, Grey-Smith, Mutsu, Florina, Chadeo, JonaGold, Aidere.

Information sources

Time of the study spans from September the 12 to October the 30 2013.

Place of study: Medical University of Plovdiv - Faculty of Pharmacy and College of Medicine, University of Thrace

Methods applied:

- Spectrophotometric method[8,10]
- Systemic approach and critical analysis of the available scientific periodicals[11]

Apparatus:

- UV-VIS spectrophotometer
- Thermo scientific
- Electromagnetic stirrer VORTEX 2 GENIE
- Centrifuge Centronix Microcentrifugo 1236V
- Juicer

II. RESULTS AND DISCUSSION

GENERAL - a brief review of the available scientific periodicals

Apple refers to the Rosaceae family /Rosaceae/, apple subfamily /Pomoidae/, genus Malus Mill. There are about 30 sorts of apples, but only few of them are progenitors of modern varieties. The apple was known to prehistoric man. Remains of apple fruits were found during excavations of the pile dwellings that existed some 5,000 years ago. Its homeland is considered to be an area in the northwestern Himalayas. Cultivation of apple originated in Asia Minor, and then its path leads to the Caucasus, Egypt and Palestine. More than 600 BC Apples were cultivated in ancient Greece, where it was taken to Rome and thence to Europe. This culture is mentioned in ancient manuscripts in China, Persia, India and Rome. Although it was known from ancient times, great economic importance the apple gained in twentieth century, when it was valued for its nutritional and medicinal qualities[2,10].

The apple is among the best fruit foods for human. This is due to its diverse chemical composition: dry matter content - 8.0-13.0%; sugars; Organic acids - 0.11-0.49%; minerals, vitamins, enzymes, fiber, mainly in the form of pectin and cellulose. Vitamin C content varies from 4 to 80 mg. So besides being a nourishing food, the fruits have valuable medicinal and dietary properties[9,10].

Apples are a great source of vegetable matter - i.e. flavonoids. Particularly valuable is quercetin, which reduces the risk of lung cancer by 46%. In 2001, American Laboratory studies have shown that apples stop the growth of prostate cancer cells and the risk of cancer of the digestive system decreases by 30-40%. Due to the anti-inflammatory properties of quercetin, apples improve lung functioning and relieve asthma. The usage of only five apples per week improves lung function by 3%. Another

flavonoid, which can boast the apple, is catechin, and studies confirm that people where catechin content is high in the blood rarely suffer heart attack[3,4,7].

The amount of cellulose in apples is greater than in other fruits and varies from 0.8 to 1.2%. Cellulose is concentrated in the skin and seed of the fruit. Pectin (from 0.08 to 1.77%) makes apple fruit strong and gives them very good gelling properties, which is why they are very important for the processing industry.

The flavor of apples is largely dependent on the amount and the ratio of the content of sugars, organic acids and tannins. The aroma is due to the essential oils.

The content of sugars in apple fruit is 9.24 to 12.71%.

The fructose is dominant - 6.8%, glucose - 2.7%, and sucrose - 2.2%.

The total acidity is 0.17% at the sweet varieties to 1.2% at the sour ones.

The pleasant apple flavor is owed to the many essential oils that are available in apples. Different tastes of individual fruit species are dependent on the ratio of the quantity of sugars and organic acids. Interestingly, the vitamin C content is different with different varieties of apples, such as certain sorts may contain up to 3 times more Vitamin C than the others.

Apples combine flavonoids, fibers and antioxidants in unique and incomparable way. The greatest amount of antioxidants contains in fully ripe fruits. Research conducted at the University of Innsbruck, Austria, shows that with the full ripening of apples, just before they spoil their antioxidant levels increase.

Red and Golden Delicious are among the sweetest varieties, Gray Smith and Gravenstein are the tartness and during the processing retain their best texture.

The phytonutrient content of apple did not change notably throughout the period of their storage. After 100 days, the content of phenolic compounds decreased slightly, but even after 200 days kept cold, the overall amount of these compounds remains about the same as it was in the day of their retraction.

Nutritional values per 100 grams raw apple with peel: energy - 218kj (50kal.) Carbohydrates - 13.81g., Starch - 0.05g., Sugar - 10.39g., Sucrose - 2.07g., Glucose - 2.43g., Fructose - 5.9g., lactose - 0g., maltose - 0g., fiber - 2.4g., fat - 0.17g., cholesterol- 0g., protein-0.26g., water-85.56g. ash - 0.19g[1].

Vitamins per 100 grams of raw apples cortex: vitamin A - 0 µg (0%), alpha carotene - 0 µg (0%), beta-carotene - 27µg (3%); Thiamine (B1) - 0,017mg (1%); Riboflavin (B2) - 0.026 mg (2%); niacin (B3) - 0.091 mg (1%); pantothenic acid (B5) - 0.061 mg (1%); Pyridoxine (B6) - 0.041 mg (3%); folic acid (B9) - 3 µg (1%); cobalamin (B12) - 0mg (0%); Vitamin C - 4.6 mg (5%); vitamin D - 0 mg (0%); Vitamin E - 0.18 mg (1%); Vitamin K - 2.2 µg (2%); lycopene - 0 µg (0%).

Minerals per 100 grams raw apple with peel: calcium Ca - 6 mg (1%); Iron Fe - 0.12 mg (2%); Magnesium Mg - 5mg (1%); Phosphorus P - 11 mg (2%); potassium K - 107 mg (2%); sodium Na - 1 mg (0%); zinc Zn - 0.04 mg (0%); Copper Cu - 0.027 mg (3%); Manganese Mn - 0.035 mg (2%); Selenium Se - 0 mg (0%)[1].

Amino acids per 100 grams raw apple with peel: tryptophan - 0,001g; threonine - 0,006g; threonine - 0,006 g; isoleucine - 0,006g; leucine - 0,013g; lysine - 0,012g; methionine - 0,001g; cystine - 0,001g; phenylalanine - 0,006g; tyrosine - 0,001g; valine - 0,012g; Arginine - 0,006g; histidine - 0,005g; alanine - 0,011g; aspartic acid- 0,07g; glutamic acid - 0,025g; glycine - 0,009g; proline - 0,006g; serine - 0,01g; choline - 0,003g[1].

Other ingredients in 100 g raw apple with peel: alcohol - 0 g.

III. METHODS OF DETERMINATION

For the experiment using:

- 9 varieties of apples (Table. 1).
- Hipoknantin oxidase
- phosphate buffer

№	Variety apple	Origin
1	Golden Delicious	Katunitsa village
2	Golden Delicious	Ognianovo village
3	Red Delicious	Ognianovo village
4	Grey smith	Ognianovo village
5	Mutsu	Katunitsa village
6	Florina	Katunitsa village
7	Chadeo	Katunitsa village
8	Jonah Gold	Katunitsa village
9	Aidere	Katunitsa village

Table 1. Sorts of apples and place of cultivation

The experiment is based on the ability of antioxidants (products with antioxidant properties) to trap free radicals - super oxide anion (super oxide radical). The mechanism of formation of the super oxide radical is the following:

Hypoxanthine + hypoxanthine oxidase + uric acid + O2-super oxide radical

$$O_2^0 + O_2^0 + H_2 \xrightarrow{SOD-Super\ oxide\ dismutase} H_2O_2 + O_2$$

We evaluate the degree of antioxidant activity by the difference in the degree of staining of the solution.

The degree of staining is determined by UV-VIS spectrophotometer.

We determine the antioxidant activity by the difference of absorption of the control sample and the sample with the corresponding apple juice.

To determine the antioxidant activity of each apple variety we use 3 tubes.

"0" - zero tube

"K" - a control tube

"1" - test tube

We obtain nectar from apple by squeezing them prior the experiment using a PHILIPS ..... juicer.

To prepare the juice for the experiment we centrifuge it for 5 minutes at 14000 rpm in centrifuge Centronix Microcentrifugo 1236V. The goal is to settle the nectar particles and to remain pure juice.

In all tubes we put in 1,5ml kit (hypoxanthine + nitrobluetetrasole).

In tube, "0" we set 100µl sample (juice) + 400ml buffer solution (phosphate buffer) instead of hypoxanthine.

In test tube "K" we put 400µl hypoxanthine oxidase +100 ml buffer instead of sample.

In test tube "1" we put 400µl hypoxanthine oxidase +100 ml sample.

Or:

$$0 \rightarrow 1.5ml\ kit + 400\mu l\ buffer + 100\mu l\ semple = 2ml$$

$$K \rightarrow 1.5ml\ kit + 400\mu l\ hypoxanthine\ oxidasa + 100\mu l\ buffer = 2ml$$

$$1 \rightarrow 1.5ml\ kit + 400\mu l\ hypoxanthine\ oxidasa + 100\mu l\ semple = 2ml$$

Tube "0" is used to remove the difference in color.

Tube "K" has the highest activity of free radicals.

Tube "1" has a lower activity of free radicals, as some of them are caught by the sample.

Sequence of actions:

$$1.5ml\ kit + 400\mu l\ hypoxanthine\ oxidasa + 100\mu l\ buffer$$

3806

5. Mix the three tubes with electromagnetic stirrer VORTEX GENIE 2 for 10 seconds.

6. Put the tubes in a water bath for 10-15 minutes at

7. Put the tubes in cold water for 5 seconds to stop the reaction.

8. Determination of the activity of the samples with a UV-VIS spectrophotometer

Thermo Scientific. Working length λ = 560 nm.

First we measure the control tube with maximum activity.

For our 9 cases (9 varieties of apples) we measure 3 controls and take the average. The controls are the same.

1 – 1,528 nm
2 – 1.450 nm
3 – 1.379 nm
} Average value 1, 4523 nm

The difference in the three measurements should be no greater than 30%

We measure the absorbance of the nine samples "1". For each we make two measurements and take the average. (Table 2)

Before each measurement, we reset the apparatus with a solution of test tube "0", to avoid loss of the absorbance due to the coloration.

Table2. Absorption of test tube „1"

	Measurement 1	Measurement 2	A tube.1	ΔA = A max.-A tube.1
1	0.594	0.664	0.629	0.823
2	0.504	0.515	0.510	0.942
3	0.652	0.716	0.684	0.768
4	0.014	0.019	0.017	1.435
5	0.714	0.611	0.662	0.790
6	0.823	0.844	0.833	0.619
7	0.782	0.763	0.772	0.680
8	0.952	0.835	0.893	0.559
9	0.489	0.504	0.497	0.955

For antioxidant activity we judge by the change in absorbance ΔA between tube "K" and tube "1".

Antioxidant activity (or SOD like activity) is calculated by the following formula:

$$SOD = \frac{\Delta A}{\frac{A_{max}}{2}} . dilution = \frac{\Delta A.2}{A_{max}} . \frac{2}{0,1} = \frac{\Delta A.2.2}{A_{max}.0,1} = \frac{\Delta A}{A_{max}.40}$$

For 0,1 ml juice:

$$SOD = \frac{\Delta A.2}{A_{max}} . \frac{2}{0,1}$$

2ml is the full volume of the tube, and 0,1 – the volume of the juice sample.

In each tube „K" and „1" we have the following volume:

$$1,5ml\ kit + 0,1ml\ sample + 0,4ml\ XOD = 2ml$$

A_{max} – A of test tube "K"

ΔA - A_{max} – A of test tube "1"

In %:

A_{max} = 100%

And tube"1" = X %, where X are the percents of absorption of the tube.

The subtraction 100 % - X % results in antioxidant activity measured in %, which is given respectively for each variety in table 3.

Table3. Antioxidant activity of the apple sorts.

SOD	ANTIOXIDANT ACTIVITY IN %
22.67	56.68
25.95	64.88
21.16	52.89
39.53	98.83
21.76	45.59
17.05	42.63
18.73	46.83
15.40	61.50
26.31	65.77

CONCLUSION

1. Choose ripe fruit to generate the highest amount of antioxidants.
2. The studied varieties of apples can be arranged according their antioxidant activity as follows: Grey smith, Aidere, Golden Delicious, Jonah Gold, Golden Delicious, Red Delicious, Chadeo, Mutsu, and Florina

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