AMERICAN-EURASIAN JOURNAL OF SUSTAINABLE AGRICULTURE
ISSN: 1995-0748, EISSN: 1998-1074
2015, volume(9),issue(4):pages(31-36)
Published Online May 2015 in http://www.aensiweb.com/AEJSA/

Analysis of Climatic Factor and the Degree of Fruit Ripeness, Affecting the Chemical Composition and Antioxidant Activity of Apples

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Received 28 February 2015; accepted 26 March 2015; published 25 May 2015

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ABSTRACT

In this article you can find the results of the comparative research of different varieties of apples grown on the territory of Samara region as a result of variation of the storage periods regarding the content of the total number of phenolic compounds using the Folin-Ciocalteu method, antioxidant ability using the DPPH free radical (2.2-diphenyl-1-picrylhydrazyl), total amount of flavonoids, restoring force, antioxidant activity in the linolic acid system as well as physical and chemical indices (mass fraction of reducing sugars, soluble dry compounds, titratable acids; the pulp content, the sugar-acid index). This study was financially supported by the Ministry of Education and Science of the Russian Federation within the basic part of the government task number 2014/199 FSBEI HPE "Samara State Technical University" code 974.

KEY WORDS

Apples, climatic factor, antioxidant activity, phenolics, flavonoids, physico-chemical parameters

INTRODUCTION

There still exists an idea that the general definition of fruit quality is difficult to formulate because this definition is subjective and is held on the basis of very different criteria. The gardener is interested in getting fruit, similar in shape, size, color and ripeness. Commerce requires preservation of hardness, reserve of afterripening within a certain time. The buyer needs healthy fruit of good taste. There are the following states of ripeness: unripe fruit, harvest ripeness, consumer ripeness, overripe fruit.

Gardeners and storage specialists are primarily interested in harvest and consumer ripeness. The first - to determine the timing of fruit harvesting in the garden and the latter - to determine storage time. There are major factors affecting plant growth, physiology and quality of fruit. It should be noted that the analysis of the physiological state of fruit must take into account climatic conditions. Among the external factors affecting plant growth, the most powerful are air temperature, solar radiation, soil moisture, air humidity, mineral fertilizing. These factors are uncontrollable and it is necessary to establish the relationship between them and the parameters of the state of the object of study. Other factors - and they are mainly agrotechnical - for example, the trees of some area, row, maybe even individual trees, planting density, which have a definite influence on the apples, - can be controlled throughout the whole study period.

Over the past few years, several studies on the antioxidant activity of apples have been published [1, 2]. Fruit quality is influenced by many factors: variety genotype, environmental and agrotechnical conditions of

growing, physiological status of the fruit - the degree of ripeness, yield size, age of the trees, storage time and conditions [3].

One of the most important factors influencing the chemical composition of apples is the climatic conditions of their growth. However, the influence of climatic conditions of growth on the content of phenolic compounds and the level of antioxidant force of apples still remains an open question. Even the most detailed monograph [4] on the role of phenolic compounds in the life processes of horticultural crops discusses only the problem of changing the content of polyphenols in the process of technological processing.

At the same time, the examples of tomatos [5], almonds [6], pomegranates [7] show that the rates of the content of phenolics and antioxidant activity may differ 1.5-3 times depending on the year of harvesting.

Numerous experiments, as well as practice, have shown that unripe and immature fruit produces juices of poor quality. Juices from such fruit get lower scores at flavour sampling (rating of aromatic substances) than the corresponding juices from full-grown, i.e. ripe and mature fruit. The poor quality of fruit especially affects the quality and quantity of concentrated aromatic substances. Besides, sensory analyses show that the flavour of juices from unripe and immature fruit do not have complete enough flavor – this is usually a result of the low content of sugar and extractive substances, thus lowering the degrees described by Oechsle and Brix [2].

In one and the same kind of fruit, and even within the same variety the content of extractive substances may vary by 10-20% or more. Since sugar and extractive substances are formed while ripening, the commercial value of fruit can be judged by the degree of ripeness [3]. Production of a certain amount of juice or standard juice concentrate with an average dry extractive content will require much more unripe and immature raw fruit than fully ripened and mature. The cost of raw fruit usually makes the most part of the cost of the finished product, so the juice manufacturer is interested in buying more full-grown raw fruit with a high content of sugar, and buying at reduced prices raw fruit with a low content of sugar, i.e. immature and unripe.

Ripeness is reflected by such parameters as acidity and sugar content. For example, the acidity of the apple is almost unchanged during the last stage of ripening on the tree. The situation is different with processing of overripe and stale fruit. Overripe fruit of table apple varieties are only slightly different in sugar content from the fruit of optimum ripeness. However, sampling juice obtained therefrom shows much worse quality, i.e. already slightly disappeared or slightly less fruity aroma and flavour. Furthermore, stale apples are badly pressed: due to overripening, protopectin transforms into soluble pectin, so fruits become soft and the juice produced during pressing becomes viscous. Reduction in the yield of juice during processing overripe fruit can reach 10-20% and more.

The pulp of overripe apples is pressed better if the duration of pressing is increased or if the pulp is exposed to enzymatic treatment to cleave pectin. However, all these methods require additional cost, and may have other drawbacks. With extraction (diffusion) juice yield is less dependent on overripeness, but its quality is lower [3].

The question arose about the study of one of the physiological statuses of fruit – degree of ripeness. To do this, we studied the influence of the time of apple harvesting on the parameters of chemical composition, antioxidant properties and technological parameters of apples.

MATERIALS AND METHODS

2.1. Chemicals and reagents:

Folin-Ciocalteu reagent in sodium carbonate, gallic acid, catechin, were purchased from Fluka (Germany). DPPH (2,2-diphenyl- 1-picrylhydrazyl), Tween 40, hydrogen peroxide, sodium nitrite, aluminum chloride, thiobarbituric acid, trichloroacetic acid were purchased from Sigma-Aldrich Chem. mp. (USA).

2.2. Fruit collection:

As objects of study three apple varieties were selected – Zolotoe Letnee, Spartak, Kutuzovets from the collection of the State Budgetary Institution of the Samara Region "Research Institute of Horticulture and Medicinal Plants" Zhigulevskie Sady"; summer apple varieties Zolotoe Letnee and Vozrozhdenie grown in the Samara Region at various stages of ripeness.

2.3. Determination of total phenols:

Total phenolic content of methanolic fruit extracts was assessed using a modified version of the Folin–Ciocalteu assay (Singelton, Orthofer, & Lamuela-Raventos, 1999). Gallic acid was used as a standard and the aqueous gallic acid solution (200 mg l^{1}) was di-luted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 ll of methanolic fruit extract or gallic acid standard, 100 ll of methanol, 100 ll of Folin–Ciocal-teu reagent and 700 ll of Na_2CO_3 were added into 1.5 ml micro-centrifuge tube. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). The results were expressed in mg gallic acid equivalent/100 g dry weight (mg GAE 100 g^{1} DW) [8].

2.4. Determination of total flavonoids:

The flavonoid content of the methanolic extracts were measured using a colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999). A known volume (0.5 ml) of the extract or standard solution of quercetin was added to a 10 ml vol-umetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v NaNO2 was added to the flask. After 5 min, 0.6 ml of 10% w/v AlCl3 was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 350 nm against the blank (water) and flavonoid content was expressed as mg querce-tin equivalents (QE) in 100 g of fresh material [9].

2.5. DPPH radical scavenging activity:

The scavenging activity of samples was measured in accordance with the method of Brand-Williams (Brand-Williams, Cuvelier, & Berset, 1995). The method was based on the reduction of methanolic DPPH in the presence of a hydrogen-donating antioxidant. DPPH solution was an intense violet colour and showed an absorption band at 515 nm. Adsorption and colour lowered when DPPH was reduced by an antioxidant compound. The remaining DPPH corresponded inversely to the radical-scavenging activity of the antioxidant. DPPH (2 mg) was dissolved in 54 ml of MeOH. Aliquots of investigated extract (50, 100, 200, 300, 500 and 1000 lg) were dissolved in 2 ml of MeOH. Then 1.0 ml of each solution was added to 1.0 ml of DPPH solution at room temperature. The absorbance at 515 nm was measured against a blank (2 ml MeOH in 2.0 ml of DPPH solution) using a UV-1601 Shimadzu spectrophotometer. The results were expressed as percent-age of reduction of the initial DPPH adsorption by test samples:
% of reduction of the initial DPPH adsorption =
ADPPH(t)_A sample (t)/ADPPH (t) _ 100,
ADPPH(t) is absorbance of DPPH at time t and A sample (t) is absorbance of sample at t the same time [10].

2.6. *Determination of Antioxidant Activity in a Linoleic Acid System:*

The total antioxidant activity of FEHP was carried out by use of a linoleic acid system (26). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5-mL ethanol solution of different concentration of FEHP (50-500 íg/mL) was mixed with linoleic acid emulsion (2.5 mL, 0.2 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C in the dark to accelerate the peroxidation process.

2.7. FRAP assay

The FRAP assay was carried out according to Stratil *et al*. (2006) with slight modifications. The FRAP solution was freshly prepared on the day of use, by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. Following this the FRAP solution was heated, while protected from light, until it had reached a temperature of 37 LC. Appropriate dilutions of methano-lic fruit extracts were prepared. One hundred microlitres of the di-luted sample extract (or for blank 100 ll methanol and for Trolox standard curves 100 ll Trolox of appropriate concentration) and 900 ll of FRAP solution were added into a micro-centrifuge tubes. The tubes were vortexed and left at 37 LC for exactly 40 min, and the absorbance was measured at 593 nm. The Trolox standard curves were used to calculate the antioxidant activity of the sam-ples in relation to Trolox and were expressed as mg Trolox equiv-alent/100 g dry weight sample (mg TE 100 g^{1} DW).

2.8. Total sugar content:

The Luff–Svhoorl technique was used as described in NP-1420 and samples were analyzed as quadruplicates. This method is based on the amount of cuprous oxide obtained after reduction of a cuprous sulphate solution (copper II) by sugars in an alkaline environment. An iodometric titration is used for the determination of cuprous oxide.

2.9. Acidity:

A potentiometric titration using a combined glass membrane electrode was used according to NP-1421:1977.

2.10. Mass concentration of soluble solids:

This is an important indicator nowadays. It is the content of soluble solids that determines the status of the drink as nectar, restored juice or 100% directly squeezed juice. To study this indicator, indirect determination of the refractive index of the soluble non-volatile substances is performed. The determination is performed using a refractometer in accordance with the State Standard Specification 28562-90.

3. Results:

The results of studies of physical and chemical parameters, chemical composition and antioxidant characteristics of the selected objects are presented in Table 1-3.

Table 1: Results of the study of the chemical composition and antioxidant activity of apples.

Apple variety	Parameters				
	Total phenols content, mg of gallic acid / 100 g of raw fruit	Total flavonoids content, mg catechine/ 100g of raw fruit	E_{c50}, mg/cm^3	FRAP assay millimole Fe^{2+}/ 1 kg of raw fruit	Antioxidant activity in a linoleic acid system, % of inhibition of linoleic acid oxidation
Zolotoe Letnee, juice 2011	203	24	65,0	9,09	6,9
Zolotoe Letnee, juice 2012	630	141	18,0	7,42	Not found
Zolotoe Letnee, pulp 2011	282	76	33,0	12,33	8,4
Zolotoe Letnee, pulp 2012	832	164	16,4	9,83	10,3
Spartak Letnee, juice 2011	125	53,0	39,0	3,96	Not found
Spartak Letnee, juice 2012	400	98	11,0	9,36	79,7
Spartak, pulp 2011	147	109,0	15,0	7,29	Not found
Spartak, pulp 2012	467	129	8,0	12,41	90,5
Kutuzovets, juice 2011	221	236	89,0	7,74	15,4
Kutuzovets, juice 2012	232	269	31,0	8,64	85,8
Kutuzovets, pulp 2011	354	244	29,0	11,61	19,6
Kutuzovets, pulp 2012	456	284	10,0	13,41	94,6

The results of the study of the chemical composition and antioxidant activity of apples at different stages of ripeness are presented in Table 2.

Table 2: Results of the study of the chemical composition and antioxidant properties of the apple varieties Zolotoe Letnee, Vozrozhdenie at different stages of ripeness.

Object of study	Total phenols content, mg of gallic acid / 100 g of raw fruit	Total flavonoids content, mg catechine/ 100g of raw fruit	E_{c50}, mg/cm^3	FRAP assay millimole Fe^{2+}/ 1 kg of raw fruit	Antioxidant activity in a linoleic acid system, % of inhibition of linoleic acid oxidation
Zolotoe Letnee					
unripe juice	157	110	13,6	12,06	Not found
unripe pulp	558	117	7,5	17,44	Not found
ripe juice	630	141	18,0	7,42	Not found
ripe pulp	832	164	16,4	9,83	10,3
overripe juice	613	108	41,5	5,94	40,9
overripe pulp	730	112	31,2	7,65	81,0
Vozrozhdenie					
unripe juice	168	220	20,4	14,62	Not found
unripe pulp	424	254	8,0	17,93	Not found
ripe juice	416	290	26,5	10,89	3,9
ripe pulp	512	310	10,0	12,87	6,8
overripe juice	397	212	31,7	8,74	42,4
overripe pulp	498	260	17,4	9,23	78,0

The results of studies of the technological properties of Zolotoe Letnee, Vozrozhdenie apple varieties at different stages of ripeness are presented in Table 3.

RESULTS AND DISCUSSION

4.1 Determination of phenolic compounds:

Studying the experimental data in Table 1 can confirm the idea that the pulp contains more active substances, in comparison with the juice. Leaders in the content of phenolic compounds for the year of 2011 are the pulp of Kutuzovets apple variety, juice of Zolotoe and Spartak varieties (respectively 354, 282, 147 mg of

gallic acid/100 g of raw fruit), for the year of 2012 the leading group includes the juice and pulp of Zolotoe apple variety.

Table 3: Results of studies of the technological properties of Zolotoe Letnee, Vozrozhdenie apple varieties at different stages of ripeness.

Objects of study	Parameters		
	Mass concentration of titratable acids in malic acid equivalent, %	Mass concentration of soluble solids, %	Mass concentration of reducing sugars, %
Unripe Zolotoe Letnee	0,60	11,00	9,50
Ripe Zolotoe Letnee	0,40	11,50	12,34
Overripe Zolotoe Letnee	0,40	12,00	14,27
Unripe Vozrozhdenie	0,60	10,50	10,20
Ripe Vozrozhdenie	0,55	11,0	11,74
Overripe Vozrozhdenie	0,40	11,50	18,92

Considering the data in Table 2 on the content of phenolic compounds, the following can be observed - the lowest content of these compounds is observed in unripe fruit of Zolotoe Letnee apples variety (157 and 558 mg of gallic acid/100 g of raw fruit), then when the fruits are at the stage of ripeness, these compounds are accumulated (630 and 832 mg of gallic acid / 100 g of raw fruit). The objects studied at the stage of overripeness showed a slight decrease in the number of objects of phenolic structure (613 and 730 mg of gallic acid/100 g of raw fruit).

4.2. Flavonoids content:

According to Table 1, the pulp and juice of Kutuzovets apple variety are characterized by the highest flavonoids content for the year of 2011 (244 and 236 mg of catechine/100g of raw fruit). In 2012, the leading apple variety is again Kutuzovets.

Studying the data of Table 2, we can see a mixed picture. The total flavonoids content in the varieties changes like the total content of phenolic compounds, with the only difference being that unripe and overripe fruit both show equally low levels of these substances - 110 mg and 108 mg of catechine/ 100 g of raw fruit in the juice, 117 and 112 mg of catechine/ 100 g of raw fruit in the pulp of Zolotoe Letnee apple variety, respectively. Apples at the stage of optimum ripeness continue holding the leading position - 141 and 164 mg of catechine/ 100 g of raw fruit.

4.3. Assessment of the level of antioxidant activity in apples:

According to Table 1, Spartak apple variety shows the highest antiradical activity for the year of 2011 (15 mg / ml). For the year of 2012, Kutuzovets and Spartak apple varieties show the highest antiradical activity as well (10 and 8 mg / ml).

Studying the data in Table 2, it should be noted that the study of antiradical activity showed the results which are completely different from the previous studies. Here, in the process of fruit ripening there is a steady decline in all parameters. The analysis of antioxidant activity in a linoleic acid system showed its absence in unripe fruit, its presence in the pulp of ripe fruit, and its stable presence in overripe fruit.

4.4. FRAP assay:

Considering the results of FRAP assay in 2011, you will notice that Zolotoe and Kutuzovets apple varieties are almost at the same level, showing a high restoring force. For the year of 2012, Kutuzovets apple variety together with Spartak apple variety show the most striking properties.

Considering the data on the degree of ripeness in Table 2, we can see that here, in the process of fruit ripening, there is a steady decline in all parameters.

4.5. Composition analysis:

Studying the data in Table 3, the following pattern can be traced. By the content of soluble solids, all the parameters comply with the State Standard Specification R 5284-2003. It confirms the literature data on the accumulation of extractive substances during the ripening process. The gradual accumulation of the mass concentration of reducing sugars is observed. Titratable acidity at fruit ripening, as can be seen from Table 3, is being reduced, primarily due to the diminution of malic acid.

5. Conclusions:

A sufficient amount of Russian and foreign publications are devoted to the study of the influence of climate change on agricultural production. It is stated that the main reason for field crop production loss is the frequent occurrence of large-scale and prolonged arid wether conditions.

The weather in the Samara Region is determined by the location of the city in a temperate continental climate zone. Winters are quite frosty here, with heavy snowfalls. Summers are hot in Samara; periodic droughts are possible.

July 2011 takes the second place among the warmest years and the sixth place among the driest years over the 75-year observation period. In July, there were recorded 19 days with temperatures above +30 °C at the norm of 7 days, in August there were 8 such days (the norm is 4 days). Abnormally hot weather and drought were recorded. Rainfall in July was 35 mm below the norm.

Studying the 2011 harvest data and comparing them with the climatic conditions, we can easily identify the reasons for such low values of antioxidant activity. The first viewpoint is hypothermic as originally the winter of 2010-2011 was unusually warm. Then there was a sharp drop in temperatures to abnormally low temperatures in February together with the subsequent cold and wet period in May. The sharp fluctuation of temperature in winter is fraught with extremely dangerous consequences. Abnormally low temperatures in February caused freezing of blossom buds and shoots tissues. The second viewpoint is associated with a decrease in the activity of the polyphenol oxidase enzyme due to unfavourable weather conditions in the spring, leading to a decrease in the resistance of plants and the emerging epiphytotics causing necroses. One should not forget about the abnormally hot and dry summer of 2010, which led to a general weakening of life processes and plant resistance to various types of stress.

Studying the 2012 harvest data and comparing them with the climatic conditions, we can reveal the following regularities. In general, the conditions for the normal passage of the period of deep and induced dormancy in apple trees were favorable. Steady snow cover formed from 1-16 days before the norm and at the end of February the amount of snow was above the norm. The air temperature in winter was 0.8 °C below normal, but 0.5 °C higher than the previous year. There were no sudden changes in temperature. May frosts were short and caused no loss to agriculture. The spring and summer were warmer than usual. The amount of rainfall in July was 71% of normal. This affected the phenes of apple-trees: fruit obtained for analysis was smaller than expected.

If you link the data on climatic conditions and the analysis of the chemical composition and antioxidant activity determination, it can be noted that all apple varieties have higher values of the parameters in 2012 as compared with 2011. Thus, we can assume that the more extreme climatic conditions contribute to the increase in the values of all the parameters.

Thus, summarizing the data of all parameterrs, the following conclusions can be made:

1) regardless of the harvest year, apple pulp is a more active component than juice;
2) the climatic factor is not given enough attention in studies on the antioxidant activity. The antioxidant activity of the apple harvest in 2011 is lower than of the same apple varieties analyzed in 2012.
3) combining the data of chemical analysis and antioxidant activity, it can be said that the most advantageous choice for the manufacturer of the functional product is the selection of fruit at the stage of optimum fruit ripeness or at unripe stage, as at these stages the objects show strongly marked antioxidant properties;
4) in the studied apple varieties the most intense accumulation of sugars and solid substances occurs during the period of active mass increase and fruit size growth;
5) the content of organic acids increases at the fruit growth stage and decreases at fruit ripening stage.

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