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STUDY ON STARCH IODINE TEST FOR DETERMINING MATURATION STAGE AT SEVERAL APPLE CULTIVARS IN CORRELATION WITH CLIMATIC FACTORS AT SCDP BISTRITA USING A ONE YEAR MODEL BY MEANS OF IMAGE ANALYSIS IN IMAGEJ

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Abstract

The objective of the present study was to evaluate the possibility to determine the starch index based on the starch iodine test and subsequent image analysis, subsequently to study the changes of the main fruit quality parameters (starch index, soluble sugars, weight, diameter) and the description of the ripening process according to climatic factors. Elaboration of a research model regarding maturation stage and ripening process differentiation among cultivar groups (autumn and winter cultivars) is critical in fruit growing. Results showed that at autumn cultivars 'Auriu', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita') in 2013 starch hydrolysis begun slowly in early September (04.09.2013) presenting incipiently lower level values between 2.1-2.8 starch index and after a month these values increased to 6.2-6.6 due to the increase of mean average temperatures along with genetic factors. At the winter cultivars ('Golden delicious', 'Ionathan', 'Salva', 'Starkrimson') at harvest time it has been observed a small hydrolysis, the values being situated between 1.1-1.2 and after 30 days the values increased slowly to 2.1-2.8, the hydrolysis process being thus much slower when compared with autumn cultivars. The soluble sugar content varied between 12.6-18.4 °Brix at autumn cultivars, the maximum being registered at 'Ionaprim' cultivar (18.4 °Brix), followed by 'Aura' (16.3 °Brix), data registered at harvest day. At 30 days after the harvest day (04.09.13) the soluble sugar content varied between 13.8-18.6 °Brix with a small increasement. At winter cultivars it has been observed a lower degree of starch hydrolysis and implicitly a reduced content of soluble sugars situated between 14.2-15.2 °Brix at harvesting time. At 30 days after harvest day the soluble sugar content varied between 14.6-16.3 °Brix. The ripening processes is primary genetically determined but it is influenced also by the environmental factors, thus an appropriate modeling is a key step in order to interpolate the maturation stage with the sum of active temperatures.

Key words**:** *starch, iodine solution, apple, image analysis, climatic factors, soluble solids*

INTRODUCTION

The starch content from apple fruits accumulate during the vegetation period and it is hydrolyzed in simple sugars in the last period of maturity and development. The starch hydrolysis takes place in the center of fruits and progresses toward to exterior (Philipps and Poapst et al., 1952) or based on recent researches this process has 4 different typology (A type - from the core to the exterior, B - in star shape, C type - little patches, D type – in concentric forms, Szalay et al., 2013).

In order to assess the maturity stage of fruits, the starch from the cut slices of fruit samples when put into reaction with a iodine solution it produces a dark blue-black staining.

Based on the distribution, development and the intensity of coloration Davis and Blair (1936) have elaborated a test guide for the evaluation of starch content for the 'McIntosh' cultivar. The iodine solution test was used in other investigations also (Hesse and Hitz 1938; De Haas and Wennemuth, 1964).

Some researchers (Philipps and Poapst et al. 1952) have affirmed that this test should be done in the early morning because starch content may vary during the day, and probable this test it is not suited for late ripening cultivars because at the harvest it is recorded just a small grade of hydrolysis of the starch. Smock (1948) concluded that the distribution

of the starch is irregular in the fruit flesh and Blanpied (1960) affirmed that the starch content and the conversion into sugar is influenced by the climate, yield per tree and the cultural techniques.
Despite the fact that there are a series of contradictions in these research conclusions regarding this test, it is widely used in the fruit growing due to its simplicity in the practice, it is a rapid test and is the most important parameter in the monitoring process of maturation (Szalay et al., 2013).
Later these starch staining tests were completed for other cultivars also like Red Delicious, Northern Spy (Smith, 1974) and nowadays it is used also a German system (ART System-Apple Ripening Test). The value of the starch index is situated between 1 and 10 in the German system and 1 to 8 in the American system. Note 1 represents the hydrolysis of the lower grade and note 10 represent the most intense hydrolysis.
During the maturation process, the starch is converted to simple sugars. In the starch test the starch granules from the fruits are binding with iodine and it is formed the dark blue-black staining. If the dark blue-black staining is more intense means that the hydrolysis is in its incipient phase and if the dark blue coloring is more shifted to light blue-white-transparent state than maturation process is in its developed phase (starch converted to simple sugar molecules). In the southern zone of the USA it is used the starch index elaborated by Blanpied and Silsby (1960). In our studies we compared our results with the German system of estimation of starch hydrolysis due to the fact that this system allows the percent evaluation of the maturation grade, thus results being more accurate.

MATERIALS AND METHODS

Starch index quantification

In 04.09.2013 there have been sampled 10 fruits/variant at the 8 studied cultivars (4 autumn and 4 winter cultivars) grafted on rootstock M26 ('Aura', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita', 'Golden delicious', 'Ionathan', 'Salva', 'Starkrimson', Fig. 1) and these were cut transversally in order to perform the staining procedure with a iodine solution (iodine in iodine potassium medicinal solution). The cut apple surfaces were impregnated with the iodine solution and placed on a filter paper for 10 minutes. Subsequently there have been effectuated photos with the transverse colored apples and stored on the PC. Colored images were transformed to binary images (black and white – black coloration meaning the starch area not hydrolyzed from the fruit, white meaning unstained fruit flesh area) using Image J software. Quantification of the starch percent not hydrolyzed (black) meaning the fruit surface occupied by the not hydrolyzed part or contrary the amount of white area was visually compared with the ART System chart in order to determine a proper starch index note.

Soluble sugars determination

In order to perform the dry matter determination it has been used a manual prism refractometer which measures the soluble sugar content in Brix grade.

RESULTS AND DISCUSSIONS

At the late ripening winter cultivars (Fig. 1) sampled in 04.09.2013 at harvest day, the starch index was of the lesser grade among the studied variants situated between 1.0-1.2, these cultivars having the highest starch content (Table 1). In Fig. 2 it is presented the variation of the starch index at four autumn apple cultivars with early maturation (‘Aura’, ‘Starkprim’, ‘Ionaprim’, ‘Auriu de Bistrita’), respectively 4 late ripening winter apple cultivars (‘Golden delicious’, ‘Ionathan’, ‘Salva’, ‘Starkrimson’) at the early phase of maturation (04.09.2013) and after 30 days after the first day of harvesting. In the case of the autumn cultivars at the harvest date 04.09.2013 the starch index was relatively reduced, but the hydrolysis process was much more advanced in comparison with the winter cultivars. The value of the starch index in the case of the winter cultivars varied between 2.1-2.8 (Table 1).

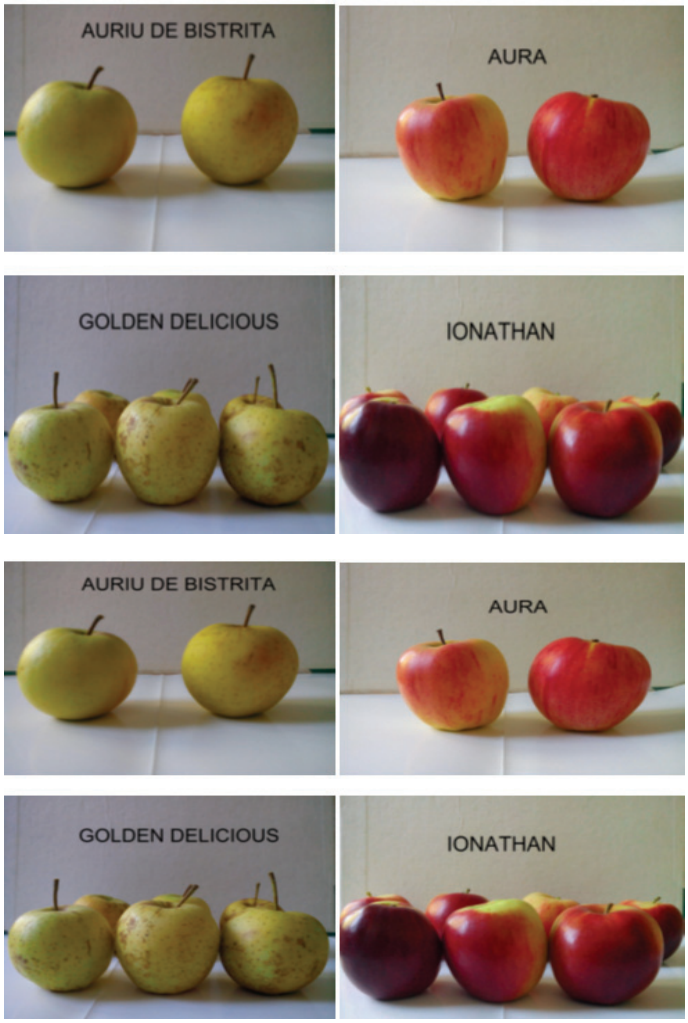


Figure 1. Autumn cultivars and winter cultivars studied in the experiment
(Photo: Jakab-Ilyefalvi Zsolt, 2013)

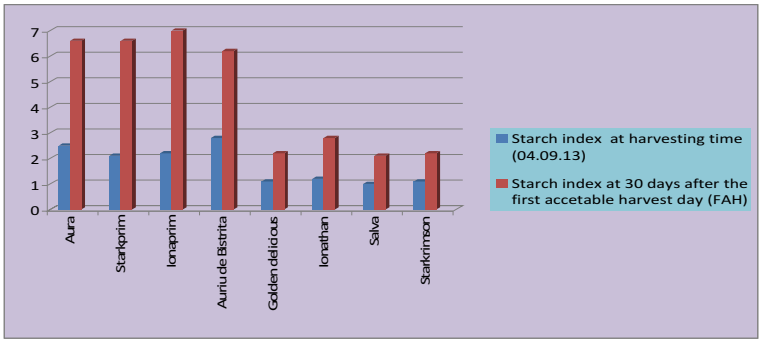


Figure 2. Variation of starch index of 4 autumn cultivars and 4 winter cultivars at the harvesting time and after 30 days of storage in classical storage house

Table 1. Pomologic characteristics and total soluble sugars of the studied apple cultivars at 30 days after harvest - SCDP Bistrita 2013

Cultivar	Mean weight (g)	Diameter (mm)	Total soluble solids (°Brix)	Starch Index
Aura	162	82	16,3	6,6
Starkprim	155	81	12,6	6,6
Ionaprim	110	73	18,4	7
Auriu de Bistrita	170	90	15,7	6.2
Golden delicious	125	86	14,2	2,2
Ionathan	110	76	15,2	2,8
Salva	130	82	14,5	2,1
Starcrimson	92	68	14,2	2,2
Treatment Average	131,8	79,8	15,1	4,5
Standard deviation	28,0	7,1	1,7	2,3

At 30 days after the first harvest date (04.09.13) fruits were again sampled from the same trees. In the case of the autumn cultivars ('Aura', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita') it has been observed a more progressed maturation process and thus a much more advanced hydrolysis, the starch index was higher being situated between 6.2-7.0 (Fig. 3). At the winter cultivars ('Golden delicious', 'Ionathan', 'Salva', 'Starkrimson') it has been observed a smaller hydrolysis, the values being situated between 2.1-2.8.

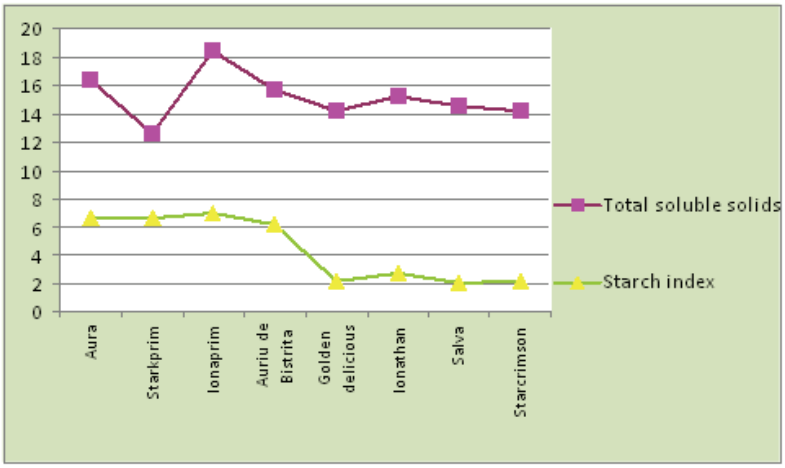
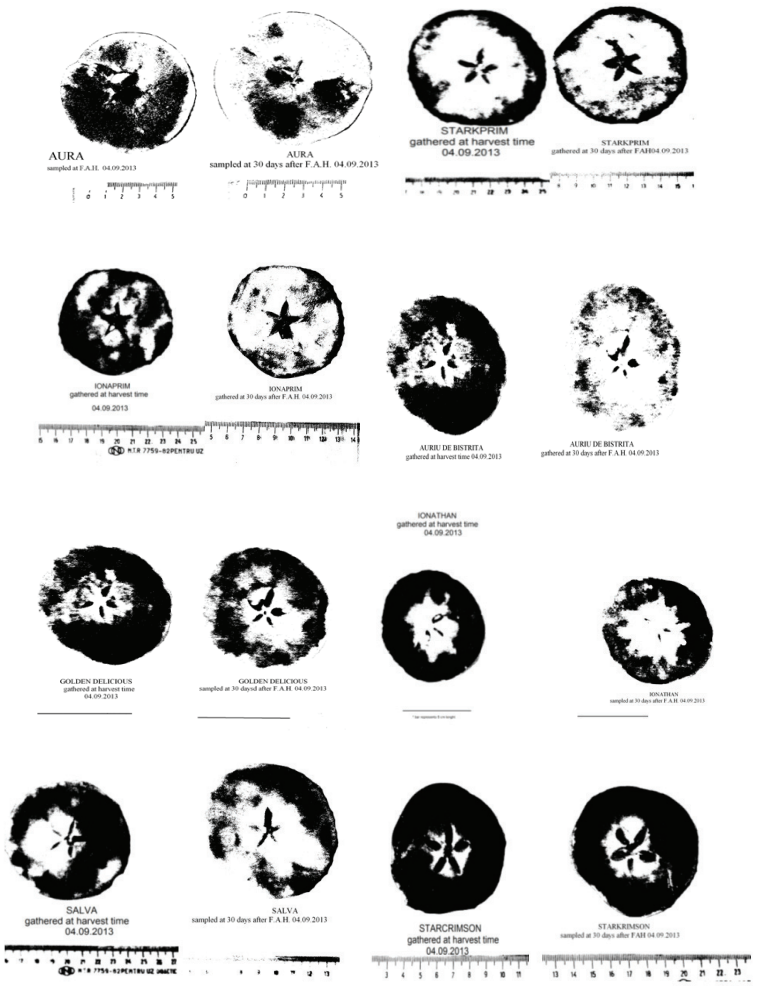


Figure 3. Total soluble sugars, Starch index variation of the studied apple cultivars at 30 days after harvest - SCDP Bistrita 2013

The data from Table 2 shows that there is a correlation between the area percent of the hydrolyzed starch (starch colored in dark blue-black) and the USA and the German noting system. The German system seems to be more sensible with fine starch changes, showing more subtle modifications of starch hydrolysis, although there are little differences between the two noting systems.

Table 2. Variation of starch index at 8 cultivars (image analysis by Image J), by means of USDA Cornell University starch index (1-8 index) and Art System (1-10)

Cultivar	Area of the fruit surface (cm^2) ImageJ	% area of hydrolised starch fruit surface ImageJ	% area of iodine colored fruit surface ImageJ	USDA Cornell starch index scale 1-8	Apple Ripeness Test Art System 1-10
Aura	55,06	82,10	17,90	6.8	6.6
Starkrpim	57,50	72,87	27,13	6.0	6.6
Ionaprim	46,72	78,12	21,88	6.7	7
Auriu de Bistrita	55,06	82,10	17,90	6.8	6.2
Golden delicious	41,05	42,05	57,95	2	2.2
Ionathan	55,11	36,47	63,53	3	2.8
Salva	55,95	43,49	56,51	3	2.1
Starkrimson	41,35	22,82	77,18	2	2.2



Digital image: Image J, Jakab-Ilyefalvi Zsolt, 2013
Figure 4. Starch index determination of apple cultivars by means of image analysis by Image J

There have been studied also the variation of the soluble sugar content of the 8 cultivars at the harvest day 04.09.2013 and after 30 days after the first acceptable harvest day (FAH) (Fig.4). In the case of the autumn cultivars harvested in 04.09.2013 there have been registered a soluble sugar content between 12.6-18.4 Brix, the maximum being registered at 'Ionaprim' cultivar (18.4 Brix), followed by 'Aura' (16.3 Brix) (Table 1). At 30 days after FAH the starch hydrolyzed stepwise, the soluble sugar content varied between 13.8-18.6 Brix. At winter cultivars it has been observed a lower grade of starch hydrolysis and implicitly a reduced content of soluble sugars situated between 14.2-15.2 Brix at harvesting time. At 30 days after FAH the soluble sugar content varied between 14.6-16.3 Brix.

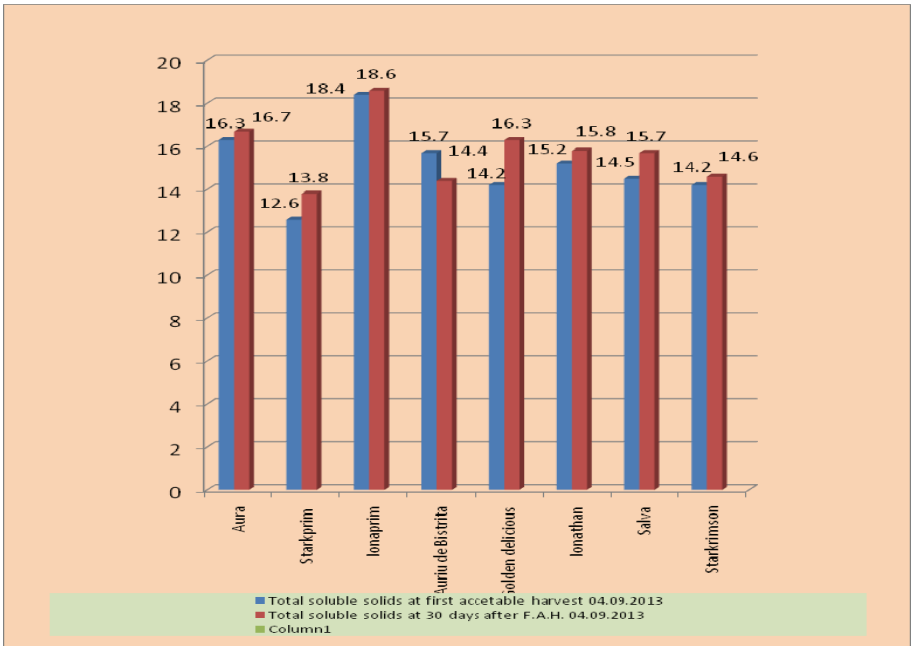


Figure 5. Variation of total soluble solids (Brix) at the harvesting time 04.09.2013 and after 30 days of storage in classical storage house

Studying the pomologic characteristics (Table 1) average weight, diameter it has been observed a higher dimension of fruits at autumn cultivars (73-90 mm) when compared with the winter cultivars (68-86 mm) respectively the weight parameter shows the same tendency (155-170 g).

As first observation we can conclude that the petal fall phenophase did not occurred differentiated as specific for every cultivar probable due to more special climatic conditions of the past years (climatic changes) oppositely almost it occurred in the same time frame during two-three days having as central petal fall day 05.05.2013 with a total sum of active temperatures of 326^{0}C [(Tmax+Tmin)/2-Tbase (5^{0}C)]. It is clearly evident that it is taking place a fine tuned shifting of the phenophases, at the end of flowering. At the first day of harvest 04.09.2013 the sum of active growing degree days (active temperatures, Table 3) was 1774^{0}C and at the second harvest session in 01.10.2013 and at circa a month after the first acceptable harvest day this sum totalized 2003^{0}C. At the first day of harvest 04.09.2013 the sum of active growing degree days (active temperatures, Table 3) was 1774^{0}C and at the second harvest session in 01.10.2013 and at circa a month after the first acceptable harvest day this sum totalized 2003^{0}C.

Table 3. Sum of active temperature degrees (GDD-growing degree days) between petal drop phase (05.05.2013) and first acceptable harvest date (FAH) 04.09.2013

Cultivar	Sum of active temperature degrees Petal drop 05.05.2013	Sum of active temperature degrees FAH 04.09.2013	Sum of active temperature degrees 01.10.2013	Starch index 04.09.2013	Starch index 01.10.2013
Aura	326	1774	2003	2.5	6.6
Starkprim	326	1774	2003	2.1	6.6
Ionaprim	326	1774	2003	2.2	7
Auriu de Bistrita	326	1774	2003	2.8	6.2
Golden delicious	326	1774	2003	1.1	2.2
Ionathan	326	1774	2003	1.2	2.8
Salva	326	1774	2003	1	2.1
Starkrimson	326	1774	2003	1.1	2.2

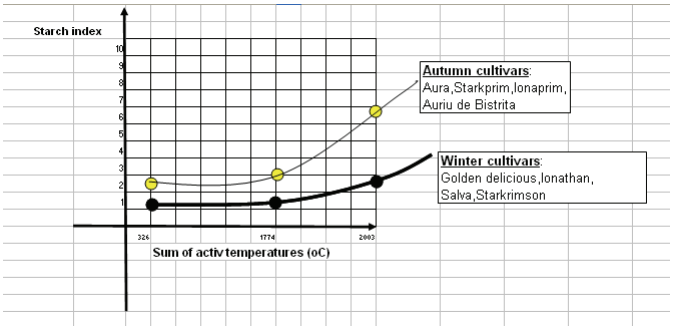


Figure 6. Variation of starch index maturity notes according to GDD

The chart from Figure 6 shows an ascendant trend of the hydrolysis of starch into soluble sugars (2.5-2.8 starch index) and after one month the starch index realized values between 6.2-6.6 the accelerated tendency of hydrolysis of the autumn cultivars being evident.

CONCLUSION

Research results distinguish clearly the fact that autumn cultivars ('Aura', 'Starkprim', 'Ionaprim', 'Auriu de Bistrita') must be gathered at optimal harvest date, when starch hydrolysis is in its incipient phase because fruits need to have firmness. The ripening processes is primary genetically determined but it is influenced also by the environmental factors, thus the using of the present results should be handled with precautions, when taking into account the optimal harvest because the study is a one year model, further multi-annual researches will be effectuated in order to evaluate the ripening stage of the apple cultivars in the northern zone of Bistrita hills region and the starch hydrolysis process in the harvest window.

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