DYNAMICS OF CHANGES OF STARCH AND ITS COMPONENTS IN FRUITLETS AND MATURING 'JONAGOLD' AND 'GALA MUST' APPLES

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ABSTRACT

In 2003 and 2004 the volume of 'Jonagold' and 'Gala Must' (*Malus domestica* Borkh.) apple fruitlets and their concentrations of starch, amylose and amylopectin were assessed at two-week intervals. The investigations started in the 7th or 8th week after full bloom (WFB) and continued until harvest. 'Gala Must' apples contained, on average, about 40% more starch than 'Jonagold' apples. The dominant starch form was amylopectin; its maximum content was 68% in 'Jonagold' and 63% in 'Gala Must' apples. The dynamics of the changes of the starch content and its components, in growing and maturing apples, revealed considerable differences in both years of the experiment. In 2003, the highest starch accumulation occurred in the 7th WFB for both apple cultivars, whereas in 2004, fruits of 'Jonagold' showed the highest starch accumulation in the 14th week, and those of 'Gala Must' in the 12th week. At this stage, apple fruit volume was 2.5 times smaller in 2003 than in 2004. It seems that higher than average temperatures at that time were the main factor determining considerable enhancing of starch accumulation in apple fruitlets in 2003. In both years, the period of starch breakdown in 'Jonagold' apples was two weeks shorter than in 'Gala Must' apples.

Key words: amylopectin, amylose, apples, climatic conditions, starch, volume of fruits

INTRODUCTION

Changes of starch content are a articularly interesting biochemical processes in growing fruitlets and maturing apples. The analysis of starch conversion to monosaccharides is, among others, one of the essential indicators determining the optimum date of apple harvest, to guarantee the best apple post-harvest quality (Kvikliene, 2001). The commonly used starch index, i.e. reference chart based on visual analysis of an apple cross-section area dyed with iodine solution, was recently shown to change in a sigmoidal pattern during fruit development (Peirs et al., 2002).

Starch is a plant reserve polysaccharide; a transient form of carbohydrates in apples. It is composed of amylose, a linear polymer of glucose, and prevailing amylopectin with greater and branched molecules. Starch accumulates in chloroplasts and amyloplasts as granules, characteristic for a given species. In apples they are spherical with a smooth surface, though sometimes cracked (Ohmiya and Kakiuchi, 1990), with the diameter between 2 and 12 μm (Carrin et al., 2004; Stevenson et al., 2006). The greatest number of starch granules is to be found on the outer part of an apple, close to the skin. The least amount of starch granules may be encountered inside the flesh, around the seed socket (Brookfield et al., 1997). It is a fact that starch content in apple flesh is low at the initial phase of fruitlet development, but increases significantly about 30 days after fertilization. Starch content reaches the maximum value several weeks before harvest and then this value gradually decreases. Starch breakdown is controlled by several enzymes, but β-amylase plays a crucial role. It directly attacks starch granules releasing dextrine, which is subsequently hydrolyzed into smaller components. This enzyme activity is low at the early stage of development, intensifies with apple growth, and reaches the peak the final week before harvest. Starch granules in apples gradually become smaller and finally disappear (Kovacs and Eads, 1999). Ohmiya and Kakiuchi (1990) conducted research on 'Jonagold' apples. They found that starch degradation begins mainly in the internal granules, and its rate is faster than the breakdown of starch localized immediately under the skin. Brookfield et al. (1997) observed the same phenomenon in their investigations on 'Fuji' and 'Royal Gala' cultivars.

The course of changes in apple starch content depends on the properties of the apple species and most probably is also modified by climatic conditions during the growing season (Kovacs and Eads, 1999). The beginning of starch accumulation is connected with the blooming date but there is no simple correlation between the blooming date, maximum concentration of this component in fruitlets, and the harvest date (Magein and Lerquin, 2000).

The objective of the paper was the determination of the dynamics of

changes of starch and its components during growth and maturing of 'Jonagold' and 'Gala Must' apples, with reference to climatic conditions in the two growing seasons of 2003 and 2004. The growth of fruitlets and fruit was presented through the changes of their volume.

MATERIAL AND METHODS

'Jonagold' and 'Gala Must' apples harvested from seven-year-old trees grafted on M.26 rootstock were the subject of the investigation. The orchard was situated by the University of Agriculture, in the Krakow research unit. Tree spacing was 4 x 1.5 m. The canopies were trained according to a super spindle system. The soil cultivation system included sward in the interrows and herbicide fallow in tree rows. Fertilization was applied according to the recommendations for fully fruiting orchards.

In 2003, full bloom of the analysed tree varieties occurred between 4 and 6 May, and in 2004 between 3 and 5 May. After the period of fruitlet drop, 50 fruits of each analysed variety, growing on different tree parts, were collected at fortnight intervals. The samples were collected until the apples reached harvest maturity.

At each harvest date, fruit volume as well as the tissue content of starch, amylose and amylopectin were determined. The latter were assessed colorimetrically using iodine solution in potassium iodide and applying the formulae stated by Magel (1991).

The results were verified statistically with ANOVA in a completely randomized design and the differences between means were estimated on the basis of Duncan's test at the confidence interval $\alpha = 0.05$.

RESULTS

The volume of 'Jonagold' apples significantly increased in 2003, between the successive dates of analyses (Fig. 1). The greatest absolute increase in the volume was noted during the period between 13th and 15th WFB (by c.a. 62.5 cm^3) and between 17th and 19th (by c.a. 63.4 cm^3). Apples of this variety showed an average volume of 281.6 cm^3.

Starch content in 'Jonagold' apple fruitlets, on the first date of observation, was 26.16 mg g^{-1} FW. Its significant decrease was registered only in 17th WFB, when starch level declined to about 77% of its initial content. Starch broke down fast over the subsequent period. At the time of harvest 5.66 mg g^{-1} FW of starch was found in the apples, which constituted 28% of the content noted at the beginning of the experiment.

On the first date of analysis (7th WFB), 19.81 mg g^{-1} FW of amylopectin was registered in 'Jonagold' apple fruitlets. During the first two weeks of observations, the amylopectin level declined to 84.5%, and during the next four weeks to 69.1% of the initial content. The degradation was the fastest in the two final weeks preceding harvest. At this period, the harvested apples contained 5.20 mg g^{-1} FW of amylopectin, i.e. 26% of its initial content.

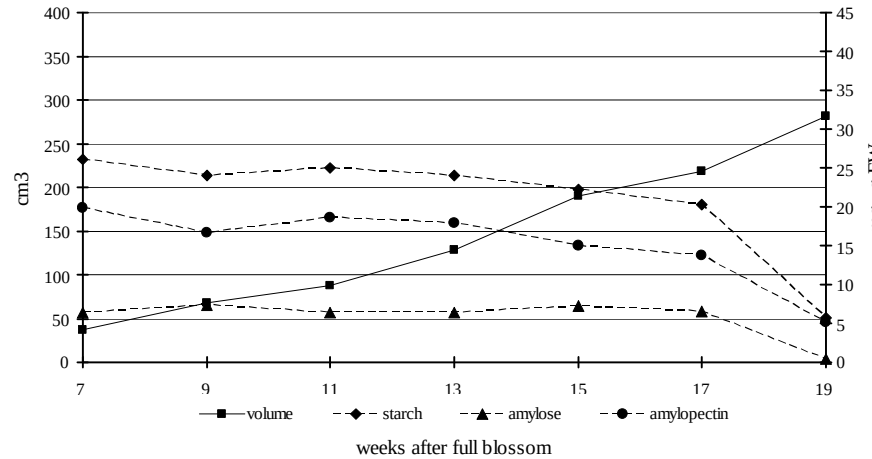

Figure1. The volume and content of starch, amylopectin and amylose in 'Jonagold' apples during growth and fruit maturation period in 2003

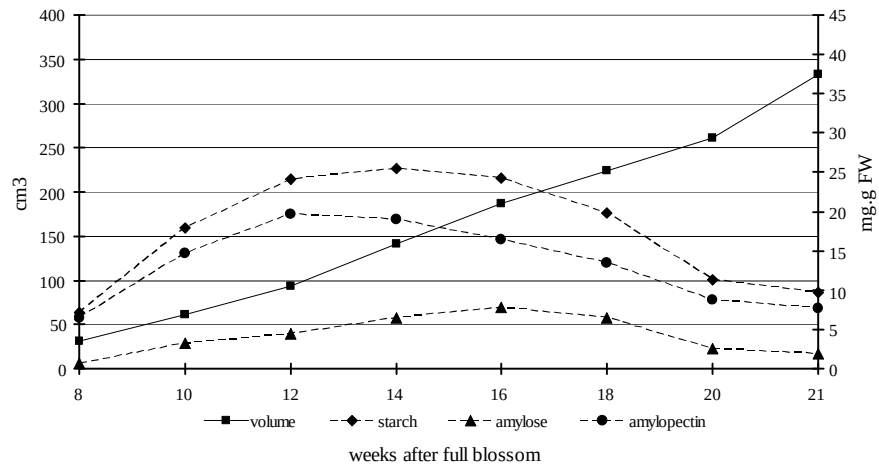

Figure 2. The volume and content of starch, amylopectin and amylose in 'Gala Must' apples during growth and fruit maturation period in 2003

Amylose concentration in the seven-week-old 'Jonagold' apple fruitlets was 6.35 mg g^{-1} FW. Until the 17th WFB, amylose remained constant but was almost completely degraded to 7.2% of its initial content within the last two weeks before harvest. Harvested apples contained only 0.46 mg g^{-1} FW of amylose.

In 2004, the highest absolute increase in the 'Jonagold' apple volume, by 72.0 cm^3, occurred in the final week before harvest (Fig. 2). Mean volume of harvested apples in that year was 332.6 cm^3.

Initially apple fruitlets contained 7.12 mg g^{-1} FW of starch. During the first four weeks, fruitlets constantly accumulated starch. They reached

approx. 340% more than their initial content the 14th WFB, which was the highest result in that season. Starch breakdown started in 16th WFB. Within 2 weeks, starch content decreased to 78.0 and 44.1% of its maximum content, respectively. The final starch analyses revealed 9.66 mg g^{-1} FW of starch, which corresponds to 38% of the initial content.

Eight-week old fruitlets contained 6.47 mg g^{-1} FW of amylopectin. During the first two periods of measurements, amylopectin content increased to 304% in comparison with the initial period of analyses. Amylopectin breakdown started in the 14th week of fruitlet growth. The level of amylopectin declined in the subsequent periods respectively to 253, 207 and 136% of the initial content. Mature apples showed 7.74 mg g^{-1} FW of amylopectin.

Eight weeks after full bloom, 'Jonagold' apple fruitlets contained 0.65 mg g^{-1} FW of amylose. During the period between the 8th and 10th week, amylose content increased five times. The next apparent growth (to 995%) occurred between the 12th and 14th WFB. Over the subsequent four weeks, amylose concentration reached the highest level that year. In 16-week-old fruitlets, 7.84 mg g^{-1} FW of amylose was assessed. The amylose breakdown period fell mainly between the 18th and 20th WFB. Between these dates, two thirds of the amylose content broke down. At harvest the apples contained 1.92 mg g^{-1} FW of amylose.

The highest absolute increase in the 'Gala Must' apple volume in 2003 occurred between the 11th and 13th WFB (on average by 36.8 cm^3) and between the 13th and 15th week (by 38.6 cm^3) (Fig. 3). The average apple volume at harvest was 191.0 cm^3.

Starch content in the 'Gala Must' apple fruitlets at the beginning of the experiment was 38.5 mg g^{-1} FW and was the highest in that vegetation season. In the period between the 7th and 9th WF, the level of starch declined to c.a. 87%. Most of the starch broke down during the four final weeks before harvest, while starch content diminished to about 66% and then to 36% between the 15th and 17th WFB. At harvest, the apples contained 14.17 mg g^{-1} FW of starch.

Initially amylopectin concentration in the 'Gala Must' apple fruitlets was 27.97 mg g^{-1} FW. In the initial period of research it decreased to 88%. Another, substantial decline (to c.a. 81%) in this amylopectin content was registered in the subsequent four weeks. During the next two week period of measurements a constant diminishing in the amylopectin amount was 70%, 57% and 36%, respectively. In the harvested apples, 10.04 mg g^{-1} FW of amylopectin was assayed.

Amylose content in 'Gala Must' apples on the first date of analyses was, on average, 10.38 mg g^{-1} FW and declined to 85% between the 7th and 9th week of fruitlet development. Between the 13th and 15th WFB, amylose content increased again to 113% of its initial

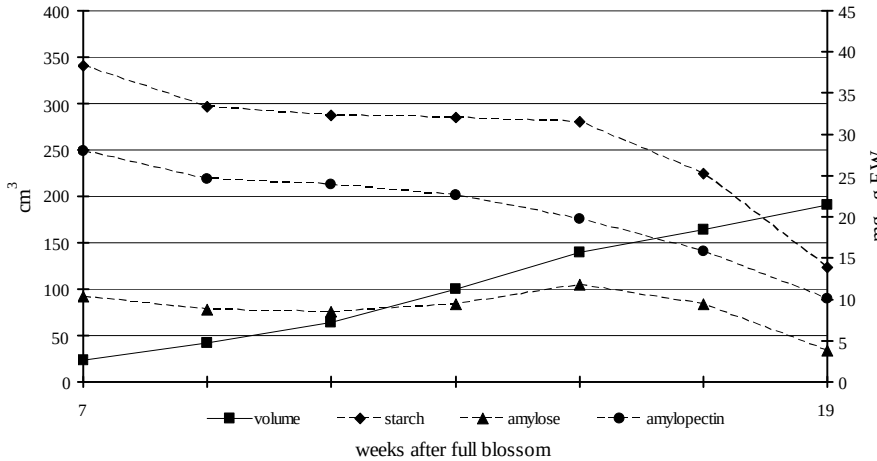

Figure 3. The volume and content of starch, amylopectin and amylose in 'Jonagold' apples during growth and fruit maturation period in 2004

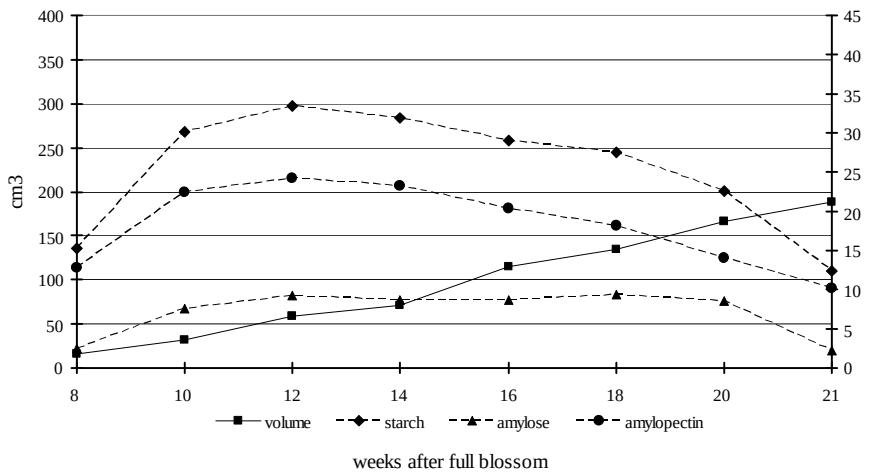

Figure 4. The volume and content of starch, amylopectin and amylose in 'Gala Must' apples during growth and fruit maturation period in 2004

value but afterwards started to decline to 91% and 37%, respectively. After harvest, the apples contained 3.80 mg g^{-1} FW of amylose.

In 2004, the highest absolute increase in the 'Gala Must' apple volume – 43.4 cm^3 was registered between 14th and 16th WFB (Fig. 4). The average volume of harvested 'Gala Must' apples was 188.9 cm^3.

Eight weeks after full bloom, 'Gala Must' apple fruitlets contained 15.29 mg g^{-1} FW of starch. During the first two weeks, starch content in

the fruitlets increased twice as much. Between the 10th and 12th WFB, a subsequent increase amounted to 219% of the initial value. This meant that the amount of starch reached the maximum level in that year, of 33.47 mg g^{-1} FW. A significant decline in starch content occurred between the 18th and 21st WFB when its value decreased to 81% of the content registered at the beginning of the experiment. Harvested fruit showed 12.35 mg g^{-1} FW of starch.

In the 'Gala Must' apple fruitlets, amylopectin content rapidly increased to 176% of its initial content between the 8th and 10th WFB. In the 12th WFB, amylopectin accumulation in the fruitlets was at its maximum (24.24 mg g^{-1} FW). It started to gradually degrade after 14th WFB. Breakdown accelerated as apples reached their harvest maturity. After harvest, they retained 79% of their initial amylopectin content.

In the 8-week-old 'Gala Must' fruitlets, 2.51 mg g^{-1} FW of amylose was found. Its content increased over three fold during the first two weeks of the research. The highest accumulation of this compound (9.35 mg g^{-1} FW) was found in the 18th WFB, at which time the amylose level was 372% of the initial value. Over the subsequent three weeks, this component concentration diminished to one fourth and at harvest to 2.23 mg g^{-1} FW.

DISCUSSION

'Jonagold' apples picked in 2004 had on average, an 18% bigger volume than those harvested in 2003. 'Gala Must' apples did not differ in their volume in either year of our research. The fruit increased their volume sigmoidaly during the period of observations, which was typical for both varieties (Welte, 1990, Greene and Autio, 1993, Zude-Sasse et al., 2000). The highest absolute increment of 'Jonagold' apples occurred in the final period of growth. For 'Gala Must' apples, the highest absolute increment was noted in the month preceding harvest.

In both years of observations, 'Gala Must' apples contained on average, 39 and 44% more starch in 2003 and 2004, respectively. Amylopectin content was also higher in 'Gala Must' apples, by 35 and 37%, in respective years. 'Gala Must' apples also accumulated bigger amounts of amylose in both growing seasons; 53 and 64% in 2003 and 2004, respectively.

The maximum concentration of amylose in the starch of 'Jonagold' apples has been estimated as 40-43% (Magein and Lerquin, 2000), whereas in 'Gala Must' apples, it was 40% (Stevenson et al., 2006). In 2003, initial analyses revealed that starch of 'Jonagold' apple fruitlets contained 24% of amylose, whereas starch of 'Gala Must' fruitlets had 27% of amylose . In 2004, when the starch accumulation process was considerably less advanced, the share of amylose in starch of the 'Jonagold' fruitlets was 9% and in the 'Gala Must' fruitlets 15%. Percent content of amylose in starch rose as apple size grew. Percent of amylose in starch reached the highest level

about four weeks before harvest – 32% in 'Jonagold' apples and 37% in 'Gala Must' apples. The amylose results were identical in both years. In the final weeks before harvest amylose content in starch decreased. In harvested apples, amylose content reached between 10 and 27%.

Dynamics of changes in the starch content, and its components in growing and ripening apples revealed considerable similarities between the analyzed cultivars and great differences between the years of the experiment. In 2003, the content of starch, amylopectin, and amylose in the seven-week-old fruitlets was the highest measured value in that season (except for amylose in 'Gala Must' apples). At that time, apples already reached the maximum content of these compounds and further weeks of growth did not bring any more increase in their content. In 2004, the content of the analyzed carbohydrates in apples assessed after eight weeks of fruitlet development increased considerably, generally even several times, during the first two weeks of investigations. On the other hand the level of starch and amylopectin kept increasing for the subsequent two weeks. Maximum content of starch and its components became established in about 12^{th} WFB. Only after a longer or shorter stabilization period did they start to gradually decrease. Former research demonstrated that apple fruitlets show the highest starch content between the 70^{th} and 100^{th} day after F.B. (Brookfield et al., 1997; Zhang DaPeng and Wang Yong Zhang, 2002; Soung Kwan Jeong et al., 2003). In the presented experiment, the maximum starch content in 2003 was registered 49 days after F.B. for both cultivars, i.e. 3 weeks earlier. In 2004, the highest starch content was noted after 98 days in 'Jonagold', and after 84 in 'Gala Must'.

The presented results showed that in addition to the apple cultivar properties, other factors of fruit growth also significantly affected the dynamics of starch synthesis and breakdown. At the time of the greatest accumulation of starch and its components, the volume of the 'Jonagold' fruitlets was on average, 37 cm^3 in 2003, and 94 cm^3 in 2004. At the same time, the 'Gala Must' apple fruitlets showed a volume of about 23 and 59 cm^3, so in 2003 they were 2.5 times smaller than in 2004. The data show that the highest starch content stage is not connected with a rise in the volume of amylopectin and amylose in growing apples.

The growth of fruitlets in 2003 and 2004 occurred under diversified climatic conditions. In 2004, the appointed F.B. date was only one day earlier than in 2003. For this reason, the weather in both years may be compared, since the age of fruitlets was then identical. Meteorological measurements in 2003 showed that May and June were warmer by 1.2 ^oC than the multi-annual average, which is 14.2 ^oC for May and 16.6 ^oC for June. The temperature in July was close to the multi-annual average 18.3 ^oC. The rainfall in May was slightly heavier than usual, whereas June was very dry although it is

normally the month with the greatest amount of rainfall in the year (on average 96.2 mm). In 2003, rainfall was only 39.0 mm. Total precipitation in July was 63.8 mm and was typical for that month. In 2004, the period from May to July was cooler than usual with the mean temperature in May lower by 2.5 °C, in June by 1.0 °C, and in July by 2.6 °C than the multi-annual average for this period. May and June were dry months, particularly June with total rainfall 45 mm lower than average. On the other hand, July was rainy with rainfall total exceeding the multi-annual average by 60 mm. The analysis of the quoted data shows that the first three months of apple growth in 2003 were much warmer than the following year. Average temperatures in May, June and July that year were 3.5 °C, 2.2 °C and 2.6 °C higher, in comparison with 2004. This meant the mean temperature for this period was 2.8 °C higher in the first year of the experiment than in the second. It seems that higher temperature was the factor which determined such a faster accumulation of starch and its components in apple fruitlets. The diversification of rainfall during this period was lesser, and both years and June were exceptionally dry. July 2004 was characterized by heavy rains in comparison with 2003, but starch accumulation at that time was already completed.

The period of starch degradation in 2003 lasted for 2 weeks in 'Jonagold' apples and 4 weeks in 'Gala Must' apples, whereas in 2004 the period lasted for 5 and 7 weeks, respectively. August and September in both years did not differ from the multi-annual mean with respect to the temperature. These two months were not diversified in respect to rainfall, either. So, the weather conditions in this period did not significantly affect the dynamics of starch breakdown in the analyzed apples. However, weather might be connected with the course of starch accumulation.

In both years of the investigations, the period of starch destruction in 'Jonagold' apples was about 2 weeks shorter than in 'Gala Must' apples. These findings may be due to a lower content of starch in the flesh of this cultivar. Peirs and others (2002) defined the rate of starch degradation in six popular cultivars of apples. The rate of degradation of starch in 'Gala Must' apples was moderate, while in 'Jonagold' apples as fast. Our research, which took place in southern Poland led to similar conclusions.

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DYNAMIKA ZMIAN ZAWARTOŚCI SKROBI I JEJ SKŁADNIKÓW W ZAWIĄZKACH I DOJRZEWAJĄCYCH JABŁKACH ‘JONAGOLD’ I ‘GALA MUST’

Maria Gawęda i Jan Ben

S T R E S Z C Z E N I E

W latach 2003 i 2004 w odstępach dwutygodniowych określano objętość zawiązków jabłek odmian ‘Jonagold’ i ‘Gala Must’ (*Malus domestica* Borkh.) oraz zawartość w nich skrobi, amylozy i amylopektyny. Badania rozpoczęto w 7 lub 8 tygodniu po pełnym kwitnieniu i wykonywano do zbioru. Jabłka ‘Gala Must’ zawierały średnio o około 40% więcej skrobi niż ‘Jonagold’. Dominującą formą skrobi była amylopektyna, maksymalna jej zawartość w jabłkach ‘Jonagold’ wynosiła 68%, a w ‘Gala Must’ 63%. Dynamika zmian poziomu skrobi i jej składników w rosnących i dojrzewających jabłkach wykazała znaczne różnice w obu latach doświadczenia. W roku 2003 okres największej kumulacji skrobi przypadł w 7 tygodniu po pełnym kwitnieniu dla obu odmian jabłek, natomiast w roku 2004 w 14 tygodniu dla odmiany ‘Jonagold’ i w 12 tygodniu dla odmiany ‘Gala Must’. Objętość jabłek w tym stadium była 2,5 razy mniejsza w roku 2003 niż w roku 2004. Wydaje się, że decydującym czynnikiem znacznego przyspieszenia kumulacji skrobi w zawiązkach jabłek w roku 2003 były wyższe niż przeciętne temperatury w tym okresie. W obu latach badań okres destrukcji skrobi w jabłkach ‘Jonagold’ był o 2 tygodnie krótszy niż w jabłkach ‘Gala Must’.

Słowa kluczowe: amylopektyna, amyloza, jabłka, objętość owoców, skrobia, warunki klimatyczne