Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 1, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE MEASUREMENTS OF STARCH AND TOTAL SUGARS CONTENT IN SOME GRAPEVINE VARIETIES (*VITIS VINIFERA* L.) DURING DORMANCY THROUGH DIFFERENT METHODS

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Abstract

Two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination. Seven-year-old grapevines (table grapes varieties - 'Muscat de Hamburg', 'Napoca', 'Cardinal', 'Perla of Csaba', and wine varieties - 'Fetească regală', 'Muscat Ottonel', 'Pinot noir', and 'Fetească neagră') were marked before winter. During dormancy (October-February), starch level in canes was appreciated through iodine in the potassium iodide method and the anthrone method, both methods using the same biological material (grape canes). Through the anthrone method, the soluble sugars, starch, and total carbohydrates were quantified as g % dry substance. Among the used methods, there were similarities regarding the results. The presented selected methods for starch content determination in plant materials are commonly used and sufficiently accurate in measurements made of engineering works.

Key words*: anthrone method, dormancy, grapevine, iodine, starch, soluble sugar.*

INTRODUCTION

Starch is the most important carbohydrate stored in woody plants (Chapin et al., 1990), the presence of starch in plants is different depending on species, organs, or seasons. The monitorization of carbohydrate reserves (mostly starch, but we have soluble sugar as well) in the grapevine during dormancy is important because the number of reserves will influence the shoot growth and reproductive development in the following season (Bennett et al., 2005).

In the literature, carbohydrates reserve status from different parts of plants, was described by various authors (Schaefer, 1986; Warmund et al., 1986; Koblet and Perret, 1990; Koblet et al., 1993; Candolfi-Vasconcelos et al., 1994, 1995; Warmund et al., 1986; Schumann and Schaefer, 1988; Vršič, 1996).

The use and storage of carbohydrates (starch and soluble sugars) during the growing season are variable depending on environmental conditions such as temperature -cool or hot climate (Londo and Kovaleski, 2019; Santos et al., 2020), water reserve (Pellegrino et al., 2014), light level (Schreiner et al., 2012 and plant factors such as photosynthetic characteristics of grapevine leaves (optical properties of leaves and leaf morphology (Bates et al., 2002). Winter resistance is related to the accumulation of carbohydrates in grapevine stem tissues depending on the vigor of the shoots or canes (Samra, 2008). The presence of starch indirectly influences winter tolerance, as plants use starch to produce other metabolites or provide energy for cold hardiness. (Noronha et al., 2018). In grapevines, carbohydrates accumulated in perennial organs in the previous year will support the early development of annual organs. Considering the above facts, the aim of this study was to understand the dynamics of starch reserves during dormancy in some grape varieties and to compare two different methods of starch content determination.

Strategies for examination of samples for starch for the most part require a quantitative

breakdown of the polymer to the monomer, glucose, which is at that point quantitatively decided, frequently colorimetric. Different strategies for the starch examination have involved polarographic (Hopkins, 1934) or chromatographic (Englyst et al., 1983) procedures. The starch-iodine response has moreover been consolidated into strategies (Blakeney and Matheson, 1984), but is rarely used individually because is not a quantitative determination.

Considering the above-mentioned facts, the present study aims to understand the dynamics of the starch reserve during the dormancy season of some table and wine grapevine varieties. Two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination. The results obtained by the two methods were compared.

MATERIALS AND METHODS

The experiment was conducted during 2019-2020 dormancy period, on *Vitis vinifera* L., on four table grapes cultivars: 'Muscat de Hamburg', 'Napoca', 'Cardinal', 'Perla de Csaba' and four wine grapes 'Fetească regală', 'Muscat Ottonel', 'Pinot noir' and 'Fetească neagră'. The vineyard was planted in 2013 at the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, Romania (46°46′N; 23°35′E). Grapevines were Guyot pruned (2 canes of 8-9 buds/vine and 2-3 spur) with the same trunk height (0.7-0.8 m). Planting densities were 4545 vines/ha (2.0 m x 1.1 m). The soil of the vineyard is haplic luvisols, deep and fertile, with high water holding capacity. Monthly temperature and precipitation are reported in Table 1.

Samples of one-year-old canes were collected from five vine plants from each variety during dormancy (October, November, December, January, and February).

Table 1. The climatic data at UASVM Cluj-Napoca during the analyzed period (2019-2020)

Month/ climate	X	XI	XII	I	II
		2019		**2020**	
Mean T°C	12.1	6.2	-0.1	-1.7	2.0
Min T°C	5.2	-12.0	-13.0	-16.0	-7.0
Max T°C	22,7	13.0	2.5	1.5	7,2
Precipitation (mm)	57.85	17.02	56.14	55.88	17.53

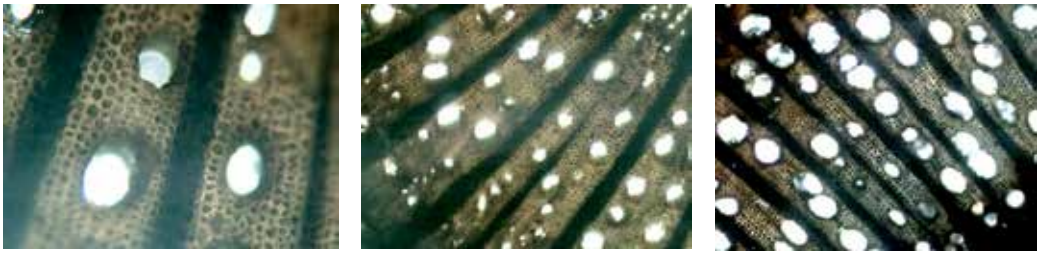
The first method used to determine the insoluble carbohydrate reserve concentrations (starch) was the treating of wood samples with iodine in potassium iodide after cutting into small pieces of 70-90 µm (Cordea, 2014; Călugăr et al., 2019b). The starch content was appreciated by grading the sections on a scale from 0 to 5 depending on the presence of the starch in the canes' tissue as follows:

0 - when there is no starch in any cane tissues;
1 - when the starch is present only in medullary ray up to the xylem area;
2 - when the starch is in medullary ray, a few in xylems and not found in phloem;
3 - when the starch is present in medullary ray, xylem, and a few in phloem;
4 - when the starch can be identified in all tissues section, but without filling up all cells;
5 - when all cells of sections are filled with starch.

All sections were analyzed with a 10x microscope objective and evaluated in a range from 3 to 5 in all eight *Vitis* cultivars that were analyzed (Figure1).

The current experiment did not have any section with 0, 1 or 2. This first method is simple, incipient, low cost, easy, and is used to evaluate the starch content for different grape varieties before spring pruning or inbreeding fields.

The second method was the determination of carbohydrates by the chemical method with the anthrone reagent, described by Cimpoi et al., 2020. This method was also been used by Călugăr et al., 2019a, and Călugăr et al., 2021. The content of soluble sugars, respectively of the starch of the analyzed material expressed as glucose and related in percentage to the dry material.



a b c

Figure 1. The starch content on sections evaluated from 3 (a), 4 (b) to 5 (c) under 10x objectiv

The total carbohydrate was calculated as the sum of the soluble sugars and starch. According to Iliescu et al., 2012, after the calculated total carbohydrate (THC g%) for analyzed material, it can be stated that if:

THC g% < 11 g% = very low content;
11 - 13 g% = low content;
13 - 14 g% = sufficient content;
14 - 16 g% = good content;
>16g% = very good content.

The color intensity obtained (with transparent blue-green color shades) is measured colori-metrically, using UV-VIS spectrophotometer at a wavelength of 620nm (Comsa et al., 2013; Călugăr et al., 2010).

Statistical analysis

The data were analyzed using variance analysis (ANOVA) and the Duncan test ($p \leq 0.05$) was performed to emphasize the significant differences between moments of analysis.

RESULTS AND DISCUSSIONS

The experimental field was settled in Cluj-Napoca area, in central Transylvania in the Somes Mic Corridor, located within three major geographical units: the Transylvanian Plain, the Somes Plateau, and the Apuseni Mountains (Boancă et al., 2018). Voevod et al., 2020 showed that temperature and precipitation in Cluj-Napoca show a clear warming trend over the last three decades. In the summer of 2019, the temperature rose by +2.7% to the mean temperature of the Cluj area. Regarding the precipitations, during the fall of 2019 were registered the lowest amounts of precipitations, with 52.7% smaller than the mean values (Voevod et al., 2020).

During the dormancy period 2019-2020, in Cluj Napoca area, the lowest temperatures were registered at the end of November (-10…-12°C), in the middle of December (-12...-13°C), in January (-11...-16°C). During the dormancy of the analyzed period, there were very short periods with temperatures below -10°C, far from grapevine temperature frost resistance (-18±3°C). Cluj-Napoca is not included in the wine-growing areas of Romania, according to Order no. 225/2006 regarding the approval of the zoning of the *Vitis vinifera* grape varieties admitted for planting. In time, there were some table grape varieties ('Napoca', 'Cetățuia', 'Timpuriu de Cluj') created for this area to satisfy consumers' need for local products given the appropriate climate conditions of the area.

The determination of carbohydrates is a reliable laboratory indicator for assessing the degree of maturation of rootstock and scion cuttings for grafting and for obtaining a good planting material (Iliescu et al., 2011; Călugăr et al., 2019a; Călugăr et al., 2021).

In Figure 2 and Figure 3, the two extraction and measurement methods usually employed to analyze the total sugar and starch contents in plant tissues were investigated with the view to streamlining the process of total sugar and starch determination obtained results were similar. Using the first method (treating of wood samples with iodine in potassium iodide) by analyzing each grapevine variety, it has been evaluated that the starch content level varied between varieties. During October, the highest starch level was recorded in 'Napoca' and 'Fetească regală' varieties studied were statistically equal (4.90) (Figure 2).

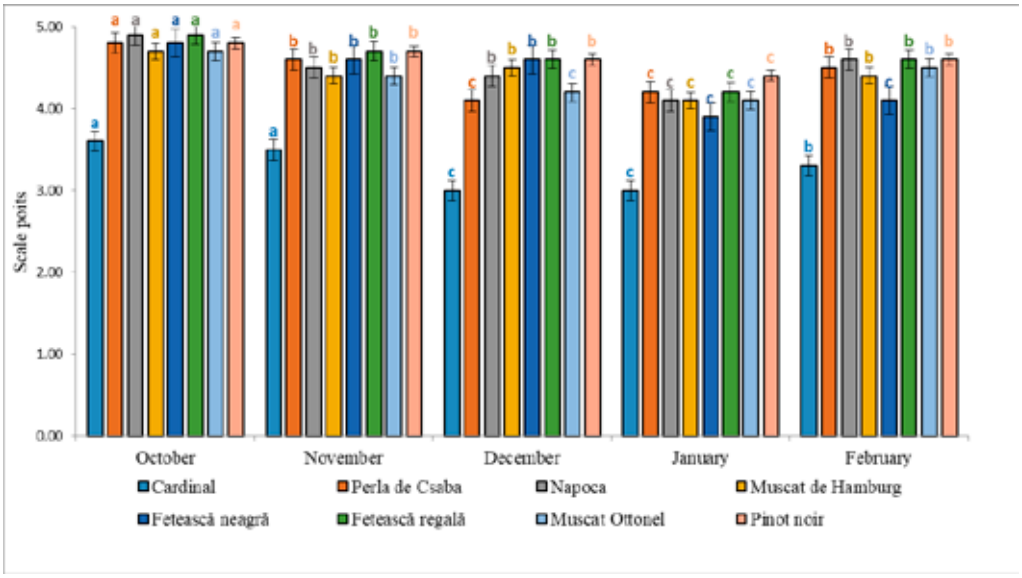


Figure 2. Starch level in canes over the 2019/2020 winter conditions according to the method 1 (the treating of cane with iodine in potassium iodide) The difference between any two columns with the same color, followed by at least one common letter, is insignificant ($p \leq 0.05$). Error bars indicate the standard error of the mean at the 5 % level of probability (n=5)

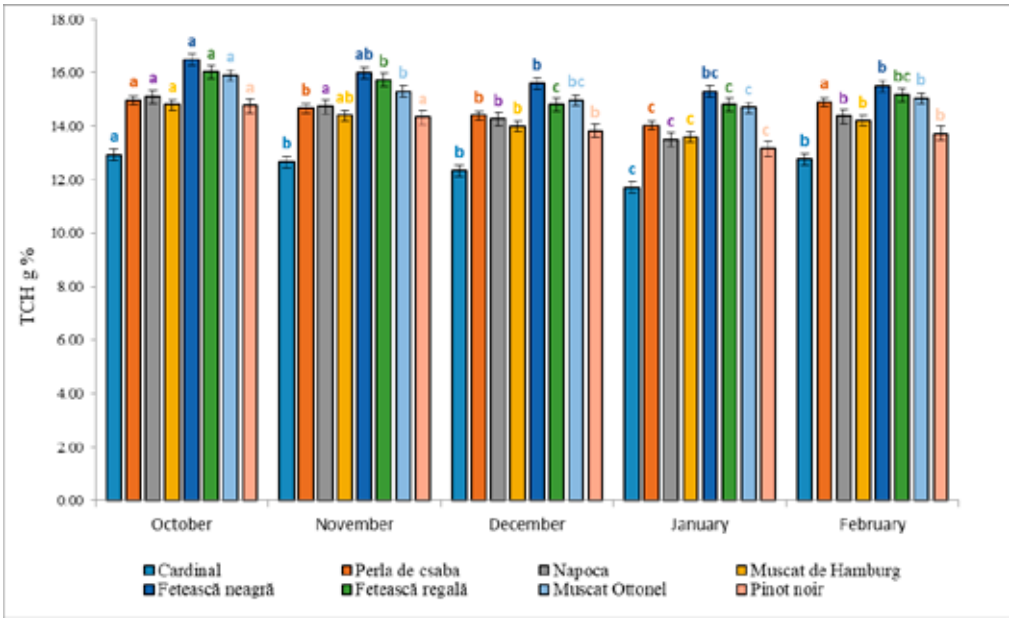


Figure 3. THC % level in canes over the 2019/2020 winter conditions according to the method 2 (the determination of carbohydrates by the chemical method with the anthrone reagent) The difference between any two columns with the same color, followed by at least one common letter, is insignificant ($p \leq 0.05$). Error bars indicate the standard error of the mean at the 5 % level of probability

Using the second method (chemical method with the anthrone reagent) showed a similar result as the previous method, with the highest content of TCH g% for 'Fetească regală' (16.03 g%) and for 'Napoca' (15.03 g%) as in Figure 2. A lower starch content level was recorded in

the case Cardinal variety with 3.60 using the first method and a TCH g% of 12.93 g% which means a low content of total carbohydrates.
In the following months, the level of starch was dropping, no matter the used method, with the lowest values in January for all grapevine varieties. Between the level of starch determined in October and the level of starch determined in November, there is a significant difference for all analyzed varieties, according to the Duncan test (Figure 2 and Figure 3). The same pattern is maintained till January when the lowest level of starch was determined. In the middle of dormancy, the content of starch was appreciated with 3.0 for 'Cardinal' variety (when the starch is present in medullary ray, xylem, and a few in phloem) and was determined the content of 11.70 g% TCH. Between grape varieties, in the middle of January, the highest content of starch was for 'Pinot noir' (4.40), followed by 'Fetească regală' and 'Perla of Csaba' (4.2) using the first method of determination. Interesting, there were some differences in results between the two methods, during testings in January. If through the first method, the level of starch for 'Pinot noir' was evalueted with 4.40 (a good appreciation of starch), the results for the second method, were 13.70 g% TCH which means sufficient content of carbohydrate.
In a study on the same varieties and on the same location, but in a previous dormancy period, Călugăr et al., 2019b state that, due to the temperature during winter in Cluj-Napoca, between 0 and -5…-7°C, and the abundant snow precipitations, surprisingly, starch content in canes starts to resynthesize, thus, in the middle of January, it was significantly higher than in November. In the middle of January (2019), the highest content of starch was appreciated for 'Perla de Csaba', 'Muscat Ottonel', and 'Fetească regală' (4.90), significantly higher than for 'Napoca' (4.68), 'Cardinal' (4.63), and 'Pinot noir' (4.55). After another month, in the middle of February (2019), the level of starch appreciated was the highest for 'Napoca' and 'Fetească neagră' (5.00), very close to 'Perla of Csaba' (4.97) and 'Muscat de Hamburg' (4.95). The lowest level of starch appreciated in canes in February was for 'Muscat Ottonel', 'Pinot noir' (4.86), and 'Fetească regală' (4.84).
The results obtained in the following dormancy period (2019/2020) on the same varieties as presented by Călugăr et al. 2019b are lower regarding the appreciation of starch content, mainly due to the climatic condition during the vegetation period, the phytosanitary state, and also, during winter (with lower temperatures).
Air temperature, relative humidity, daily thermal amplitude, and genotype are considered to be the main triggering factors of this pattern according to the studies undertaken by Londo and Kovaleski, 2017; Antivilo et al., 2017. Starch accumulation in grape canes and buds is associated with dormancy. Starch breakdown and the subsequent increase in sugar content are associated with cold hardiness (Rubio et al., 2015).
Grapevine varieties that genetically tend to have more carbohydrates also tend to be more frost resistant during the winter.
During February, the content of starch starts to increase, the highest value was recorded for 'Napoca', 'Fetească regală' and 'Pinot noir' (4.6) and the highest content of TCH g% (14.37 g% for 'Napoca' and 15.17 g% for 'Fetească regală'). In this month, 'Cardinal' (table grape variety) was evaluated with 3.30 as the first method determination and 12.77 g% TCH (low content carbohydrate) according to the second method.
This could be considered as a new polymerization process that takes place due to increasing temperatures, thus in spring, the amount of starch and soluble sugars would be in balance. Kovaleski et al., 2018, has demonstrated that wild grapevine species with northern spreading tend to exhibit higher cold resistance and responsiveness to temperature fluctuations than southern species. The same authors suggest that future grapevine improvements can be sustained through the investigation and mapping of temperature responsiveness. Wood ripening of grape canes has been related to resistance to winter frost and bud viability, but it was also associated with the biological material (Dobrei et al., 2015).
Our results can be compared with those by Cimpoi et al., 2020, for the three varieties, the best results were obtained by the 'Golia' grape variety, which recorded a TCH of 15.64 g% followed by 'Aromat de Iași' (TCH of 14.97% and 'Chasselas doré' (TCH of 13.83 g%).

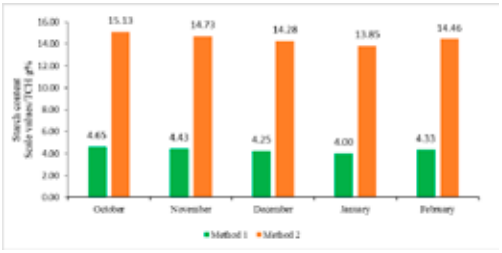


Figure 4. The appreciation of wood ripening through two different methods (Method1 - treating of wood samples with iodine in potassium iodide (with scale from 0-5); Method 2 - the chemical method with the anthrone reagent - results expressed as TCH g%)

As seen in Figure 4 between the two methods of testing starch content are similarities in results. The differences between methods are from an economically and time-consuming point of view. The first method of treating wood samples with iodine in potassium iodide (scale from 0-5) is more rapid in obtaining the results, but it needs a special microscope and a trained person. The second method (the chemical method with the anthrone reagent) is time-consuming and may pollute the environment by using several chemical substances (as presented in chapter Materials and Methods) but is more precise in the result as the researcher can find the starch and soluble sugar content as g%.

The results of the present study indicate that, although the studied varieties are table and wine grape, there is no distinct difference depending on the direction of production as for the starch content. Therefore, the results show the different potential of the genetic determinants for wood ripening which may be correlated with frost resistance as demonstrated by Dobrei et al. (2015).

CONCLUSIONS

The presented selected methods for starch content determination in plant materials are commonly used and sufficiently accurate in measurements made of engineering works. The starch analysis involves mainly determinations of its content as well as the shape and size of its granules.

The findings of the present research show the different potential of the genetic determinants for wood ripening which may be correlated with frost resistance. Also, the study presents the results regarding the two methods used for testing wood ripening. The results obtained through both methods are similar, but the difference arises from working methods (time, materials and environmental).

ACKNOWLEDGEMENTS

This research work and publication was supported by funds from the National Research Development Projects to finance excellence (PFE)-14/2022-2024 granted by the Romanian Ministry of Research and Innovation.

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