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Original Paper

Silage maturity of maize in a foothill area with NIRS method support – part II

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The aim of this work was to identify and quantify the relationships between dry matter content and key energy nutrients (water-soluble carbohydrates and starch) in silage maize hybrids (*Zea mays* L.) during vegetation development in the foothills of Slovakia. This work summarises the results of 9 experiments carried out at three locations (2016 to 2021: 140 different hybrids; 1,320 measured samples). Dry matter content was determined by a combination of gravimetric determination and measurement of water content in dried samples using the NIRS method (prediction model R^2 0.987). We also determined the content of water soluble carbohydrates (prediction model R^2 0.994) and starch (prediction model R^2 0.978) in dry matter using the NIRS method. Using 3rd and 4th degree polynomial models, we evaluated the contents of these nutrients at a dry matter content level of 300 g.kg^{-1}, which we can consider as a basic starting point for reaching maize silage maturity. Across individual years, and across groups of hybrids grouped according to silage maturity determined by dry matter content, water soluble sugar content reached 140 g.kg^{-1} of dry matter and starch content reached 280 g.kg^{-1} of dry matter at a dry matter content of 300 g.kg^{-1}. We found the lowest variation coefficient (7.53%) when evaluating the relationship between dry matter and the sum of water-soluble carbohydrates together with starch. We suggest using this parameter as a second criterion for determining silage maturity, and also for classifying hybrids in silage maturity groups with rounding to a ten day period.

Keywords: maize, silage maturity, dry matter content, water soluble carbohydrates, starch

1 Introduction

The production of maize silage is one of the main pillars of cattle nutrition provision. In the context of ongoing climate change, the need for more detailed knowledge of the characteristics of maize hybrids for silage has come to the fore even more. The classification of hybrids in FAO maturity groups and evaluation of the dairy line on grain are the basic and most frequently used methods in production practice for determining the optimal date for harvesting and ensiling whole maize plants. Both approaches have significant limitations and do not sufficiently describe the silage maturity of whole maize plants.

Dry matter content, nutrient composition, and nutrient digestibility change dynamically during the vegetative development of silage maize stands (Horst et al., 2020; Ferraretto et al., 2018). The goal of silage maize growers is to achieve a dry matter content of about 300 g.kg^{-1} on each plot at the time of harvest. At this dry matter content of the whole plant, the dry matter of the grain reaches 500 g.kg^{-1} (Daynard and Hunter, 1975) and the digestibility of starch and neutral detergent fibre decreases with increasing dry matter content (Di Marco et al., 2002). At that time, the optimal vegetation stage (maturity) is also achieved not only in terms of production efficiency (Ferraretto and Shaver, 2012), but also in terms of minimising losses from silage juices (Bastiman and Altman, 1985). The assessment of the dry matter content of maize crops often uses the „milk line“ assessment of long-lived grains (Crookston and Kurle, 1988) and was developed by Wiersma et al. (1993). The accuracy in the dry matter content estimation is low for this method (Wiersma et al., 1993; Lauer, 1998). Determining the actual dry matter content of whole silage maize plants is mechanically demanding for sample preparation and processing, but it provides a reasonably accurate

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and standard basis for dry matter content assessment and for assessment of nutrient composition (starch, NDF, NDF digestibility, etc.). We discussed the relationship between vegetation development and dry matter in a recently published paper (Mitrík and Mitrík, 2022), and in this paper we supplement and expand upon our results and conclusions.

The aim of this work is to analyse the relationship between dry matter and the contents of water soluble carbohydrates and starch. Relations and evaluations were conducted at the level of individual seasons and at the level of proposed silage maturity groups. The final step and goal is to refine anew the silage maturity assessment system for the conditions of foothill areas in Slovakia.

2 Material and methods

Over six growing seasons, we conducted 9 group experiments at three locations in the Spiš region (central and northern). The hybrids selected for each individual experiment were determined by the sowing plans of seed suppliers (16 companies) and in this respect the selection can be considered random. A total of 140 different silage maize hybrids with FAO group numbers from 120 to 400 were tested and the average repeatability of the hybrids in the experiments reached a value of 1.96 (Mitrík and Mitrík, 2022).

The date chosen for sowing the hybrids was dependent upon the contemporaneous conditions during the given growing season and was implemented at the first possible date with conditions suitable for sowing maize. Sowing was performed over a period of 35 calendar days (from April 24 to May 20, i.e. between the 108th and 143rd calendar day) using pneumatic seed drills in the range of 4–8 rows with a spacing of 75 cm for a length of at least 100 metres and with a sowing density of 75–85 thousand individuals per hectare.

Sampling of whole plants began at the onset of milk maturity and continued at 7- to 10-day intervals until the highest possible stage of vegetative development and maturity. The average range of the sampling period was 45 days (from 29 to 59 days). In 2017, heavy frosts ended sampling opportunities in the second half of September, while in other cases technical and technological limitations were the limiting factors. The sampling periods were spread from the 78th to the 171st vegetation day, which in the interpretation for individual seasons represented the 217th to 294th calendar day.

Individual samples consisted of 5–20 consecutive representative plants in the middle rows of the sowing row cut 15–20 cm above the ground (average number of plants in 1 sample: 8.78). The average weight of the samples was 6.16 ±2.02 kg. The samples were immediately transported to the laboratory, where they were mechanically cut into particles with a maximum length of 3 cm on proprietary technical equipment of our own design. The chopped mass was thoroughly mechanically homogenized (mixed), and only then were laboratory samples weighing 500–750 grams taken and dried at 60 °C in Memmert UFE 500 and UFE 700 dryers. After drying (16–24 hours), the samples were weighed, and the laboratory dry matter content was calculated. The dried samples were ground on RETCH SM-100 and TWISTER mills (passing through a 1 mm sieve).

Nutritional parameters were analysed using an NIRS Antaris II FT-NIR Analyzer (manufactured by THERMO SCIENTIFIC) using our own validated calibration models (Table 1) based on the wet chemistry methods of the time. The total dry matter content was evaluated on the basis of the laboratory dry matter content and the dry matter content measured by the NIRS method. Water soluble carbohydrates (WSC): a method for the rapid determination of carbohydrate concentration using a 315 nm UV spectrophotometer with a 50/50 glucose-fructose standard solution (Albalasmeh et al., 2013); starch: polarimetric method according to AOAC 996.11.

We defined the term „silage maturity“ as being the calendar day in which the dry matter content of 300 g.kg^{-1} was reached (Mitrík and Mitrík, 2022). For the calculation, we used equations from non-linear polynomial regressions by year, and by silage maturity group.

Silage maturity groups (SM) are defined by the calendar day on which 300 g.kg^{-1} of dry matter content was reached with ten-day steps. Hybrids were classified into SM groups using the achieved silage maturity value rounded to whole tens.

We performed statistical evaluations with the program NCSS 12 (64 bit) – version 12.0.18 – NCSS LLC (used methods: ANOVA, linear regression, non-linear polynomial regression).

Table 1 Parameters of the NIRS calibration models

	RMSEC*	R	RMSEP*	R
DM	5.65	0.990	5.55	0.987
WSC	4.39	0.998	7.98	0.992
	7.05	0.994	6.61	0.995
	6.49	0.996	5.68	0.996
Starch	18.30	0.983	17.60	0.981
	20.70	0.979	19.20	0.979
	20.30	0.983	23.10	0.974

DM – dry matter; WSC – water soluble carbohydrates; RMSEC – mean quadratic calibration error; RMSEP – mean quadratic prediction error; * g.kg^{-1} DM

3 Results and discussion

We evaluated the relationship between dry matter content and individual nutrient parameters (WSC, starch, WSC + starch) using 3rd and 4th degree non-linear polynomial regression models. We also carried out evaluations at the level of individual years and also at the level of silage maturity groups.

3.1 *Water soluble carbohydrates in relation to dry matter content*

The measured sugar contents are in accordance with the findings of other authors (Jarrige et al., 1995). We found a high degree of mutual determination (R^2 0.685) between dry matter and WSC content (Table 2, Figure 1A). The lower rate of determination may also be due to the fact that the samples were taken at different stages of the day, which is also indicated by the results of Liang et al. (2019).

WSC content could also have even been affected to some extent by the respiration of the sampled plants during transport, which lasted from 4 to 8 hours after the processing of the samples, but we did not measure and evaluate these changes.

Compared to dry matter content and starch content, WSC in dry matter is the most dynamic nutrient, the content of which has a significant upward and downward phase (Figure 1A).

At a dry matter content of 150 g.kg^{-1}, WSC reached around 200 g.kg^{-1} of dry matter, and a WSC content of 260 g.kg^{-1} of dry matter was the culminating point at a dry matter content level of 193 ±6 g.kg^{-1}. Maximum WSC concentrations (350 g.kg^{-1} of dry matter) were also measured at this level of dry matter.

At a dry matter content of 300 g.kg^{-1}, the average WSC content reached 139.57 g.kg^{-1} of dry matter (Table 2, 3). In individual years, this value oscillated in a relatively wide range of 44.90 g.kg^{-1} of dry matter, and we found statistically significant differences in WSC concentration between individual years (*P* <0.01).

In individual SM groups (Table 3) at a dry matter content of 300 g.kg^{-1}, WSC was in a narrow range of 120.55–144.85 g.kg^{-1} of dry matter, and silage maturity groups

Table 2 Relationship between WSC (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg^{-1} in years

Year	n	WSC	s	CV	R^2
2016	151	128.40^{cef}	28.36	22.09	0.881
2017	92	131.10^{ce}	25.05	19.11	0.696
2018	175	116.02^{abde}	21.01	18.11	0.668
2019	193	160.92^{c}	23.31	14.49	0.799
2020	325	159.11^{abc}	30.30	19.04	0.698
2021	384	133.59^{ac}	31.37	23.48	0.651
all	1,320	139.57	35.19	25.21	0.685

3rd degree polynomial model; CV – variation coefficient (%); index values are significantly different (*P* <0.01)

Table 3 Relationship between WSC (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg-1 by SM (silage maturity) groups

SM	n	WSC	s	CV	R^2
250	64	120.55^{bcde}	29.60	24.55	0.694
260	219	136.27^{acde}	34.60	25.39	0.717
270	454	144.85^{ab}	35.22	24.31	0.693
280	380	143.21^{ab}	35.62	24.87	0.591
290	203	137.33^{ab}	33.14	24.13	0.573
All	1,320	139.57	35.19	25.21	0.685

3rd degree polynomial model; CV – variation coefficient (%); index values are significantly different (*P* <0.01)

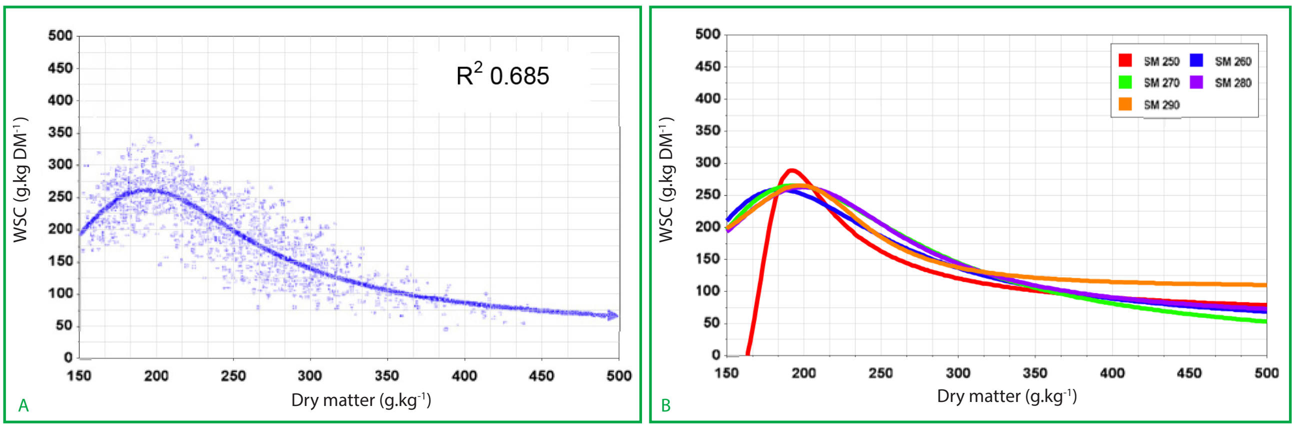

Figure 1 WSC and dry matter content

were statistically significantly different from each other (P <0.01).

These results significantly indicate that with a dry matter content of 300 g.kg^{-1} in maize silage, the WSC level reaches around 140 g.kg^{-1} of dry matter (Table 3, Figure 1B). The significantly different curve of the SM 250 group in the area below 200 g.kg^{-1} is due to the fact that we captured the lowest dry matter content at a level of 180 g.kg^{-1} in this group.

3.2 Starch in relation to dry matter content

We found a very high degree of mutual determination (R^2 0.885) between dry matter and starch (Figure 2, Table 4). Starch started to form at a dry matter content level of 150 g.kg^{-1}, rising dynamically after dry matter content exceeded 200 g.kg^{-1} on up to a level of 300 g.kg^{-1}. In the next phase, the dynamics of starch formation lose significant intensity. The course and dynamics of formation are in full accordance with the published results of the work of Khan et al. (2014), who analysed the results of studies from 1992 to 2013 and with results Colonna et al. (1995).

Table 4 Relationship between starch (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg^{-1} by year

Year	n	STARCH	s	CV	R^2
2016	151	291.46^{ce}	31.02	10.64	0.929
2017	92	294.44^{ce}	28.15	9.56	0.865
2018	175	312.48^{abdef}	36.07	11.54	0.830
2019	193	246.66^{ce}	24.85	10.08	0.933
2020	325	282.93^{bcdf}	32.17	11.37	0.914
2021	384	282.07^{ce}	30.64	10.86	0.910
All	1,320	287.38	38.36	13.34	0.885

3rd degree polynomial model; CV – variation coefficient (%); index values are significantly different (P <0.01)

Table 4A Relationship between starch (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg^{-1} in Wisconsine

Year	n	R^2	STARCH	s*	CV
2016	263	0.450	284.85^{bde}	19.74	6.93
2017	282	0.346	271.47^{acf}	22.64	8.34
2018	266	0.553	275.95^{bde}	14.85	5.38
2019	285	0.431	272.70^{acf}	22.79	8.36
2020	245	0.588	266.11^{acf}	16.56	6.22
2021	191	0.538	287.35^{bde}	18.43	6.41
Σ	1,532	0.550	274.71	20.39	7.42

Source: Kohn et al., 2016–2021
3rd degree polynomials model; CV – variation coefficient (%); index values are significantly different (P <0.01)

Table 5 Relationship between starch (g.kg^{-1} of dry matter) and dry content of 300 g.kg^{-1} by SM group

SM	n	STARCH	s	CV	R^2
250	64	291.79^{bcde}	42.16	14.45	0.797
260	219	298.26^{acde}	39.36	13.20	0.881
270	454	282.22^{abde}	37.97	13.45	0.880
280	380	280.05^{abc}	39.66	14.15	0.851
290	203	289.16^{abc}	31.70	10.96	0.892
All	1,320	287.38	38.35	13.34	0.885

3rd degree polynomial model; CV – variation coefficient (%); index values are significantly different (P <0.01)

The beginning of starch formation coincides with the culmination of WSC content, which is completely logical and natural. The dynamics of WSC transformation to starch and their mutual relationships within the vegetative development (Figure 1A, Figure 2) are consistent with the findings of Chen et al. (2014). At a dry matter content of 300 g.kg^{-1}, the average starch content reached 287.38 g.kg^{-1} of dry matter (Table 4). In individual years, this value oscillated compared to the WSC content in a much narrower range of 21.24 g.kg^{-1} of dry matter, and we found statistically significant differences in starch concentration in dry matter between individual years (P <0.01).

Starch in individual SM groups (Table 5) at a dry content of 300 g.kg^{-1} reached a more narrow range than WSC (18.21 g.kg^{-1} of dry content: 280.05 to 298.26 g.kg^{-1} of dry content) and we found statistically significant differences between individual groups (P <0.01).

We statistically processed an extensive database of results from the monitoring of the starch content in silage maize in Wisconsin (Kohn et al., 2016–2021; Table 4A), where in

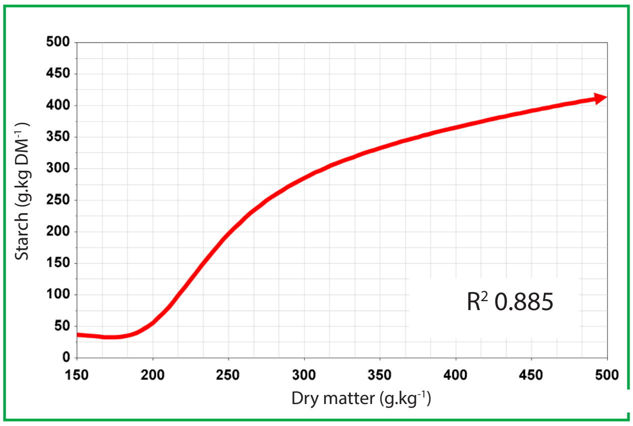

Figure 2 Starch and dry matter content

individual years starch content was measured from 271.47 to 287.35 g.kg^{-1} of dry matter at a dry matter content of 300 g.kg^{-1}, with an average of 274.71 g.kg^{-1} of dry matter. These results are consistent with our findings (Table 4). Relatively lower determination values in Wisconsin may be due to the geographic extensiveness of the distribution of experimental units. Our measurements describe a much more geographically compact and less variable environment.

Starch at a dry matter content of 300 g.kg^{-1} in silage maize reaches a constant concentration at the level of 280 g.kg^{-1} of dry matter content.

3.3 *Amount of water-soluble carbohydrates and starch in relation to dry matter content*

Glucose, representing monosaccharides, is the basic building block of water-soluble carbohydrates and starch. Therefore, we also evaluated the dynamics of the time dependence of the sum of these two nutrients together (WSC + STARCH) at the level of calendar days.

Between dry matter content and WSC + STARCH (Figure 3; Table 6) we found a very high rate of mutual determination (R2 0.776) over the entire period of observation, and in individual years (R^2 0.610–0.877). Between individual years, we found statistically significant differences in the WSC + STARCH content (P <0.01), which indicates differing dynamics of silage maize maturation in individual years.

At a dry matter content of 300 g.kg^{-1}, the WSC + STARCH content reached 429.52 g.kg^{-1} of dry matter (412.90–440.02). In further development, i.e. after exceeding a dry matter content of 300 g.kg^{-1}, the dynamics of starch formation drop significantly in intensity (Figure 3), and therefore this dry matter content represents a turning point that limits the silage maturity of silage maize (Figure 3).

Table 6 WSC + STARCH (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg^{-1} by year

Year	n	WSC + STARCH	s	CV	R^2
2016	151	425.85^{cdf}	30.55	30.55	0.618
2017	92	433.99^{cf}	29.22	29.22	0.610
2018	175	430.11^{abdef}	31.60	31.60	0.709
2019	193	412.90^{ace}	25.16	25.16	0.881
2020	325	440.02^{cdf}	23.21	23.21	0.872
2021	384	417.75^{abce}	21.82	21.82	0.877
All	1,320	429.52	32.36	32.36	0.766

Quadratic model; CV – variation coefficient (%); index values are significantly different (P <0.01)

Table 7 WSC + STARCH (g.kg^{-1} of dry matter) and dry matter content of 300 g.kg^{-1} by SM group

SM	n	WSC + STARCH	s	CV	R^2
250	64	414.92^{cde}	33.16	7.99	0.570
260	219	430.50^{cde}	33.40	7.76	0.710
270	454	428.59^{abde}	31.88	7.44	0.741
280	380	426.33^{abc}	32.59	7.64	0.762
290	203	440.14^{abc}	28.49	6.47	0.810
All	1,320	429.52	32.36	7.53	0.766

Quadratic model; CV – variation coefficient(%); index values are significantly different (P <0.01)

The average WSC + STARCH content at a dry matter content of 300 g.kg^{-1} is in a very narrow range (414.92 to 440.14 g.kg^{-1} of dry matter) and the coefficients of determination according to individual years are high to very high. Between the individual silage maturity groups (Figure 4), we found statistically very significant differences (P <0.01), similar to the individual nutrients, which indicates that there are differences between SM groups during maturation, but the relationship between

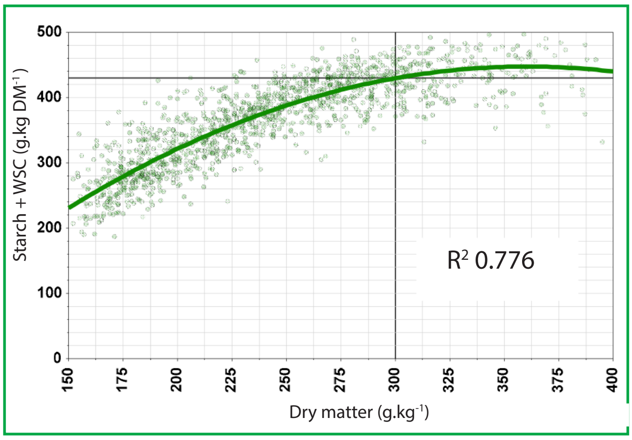

Figure 3 WSC + STARCH and dry matter content

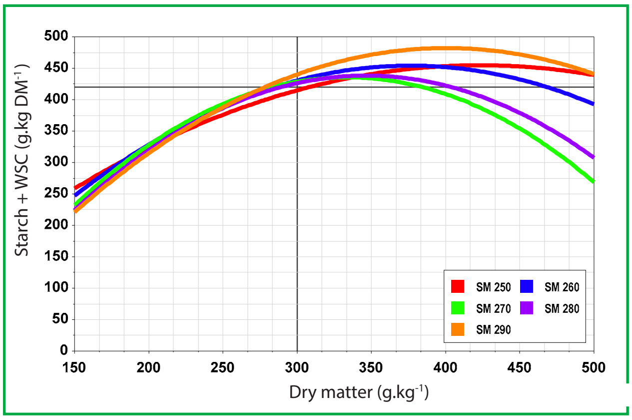

Figure 4 Development of WSC + STARCH content by SM group

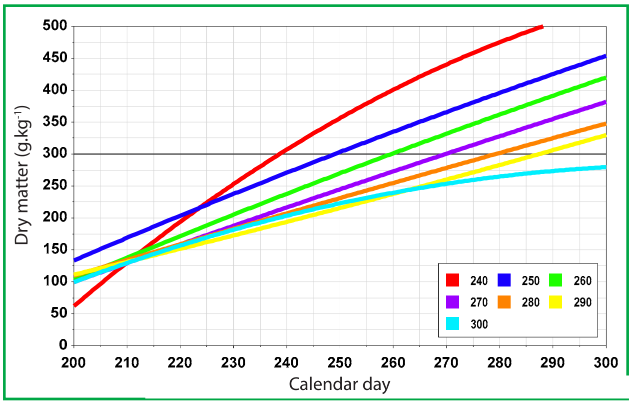

Figure 5 Development of dry matter content by SM group

the content of these two nutrients and a dry matter content of 300 g.kg^{-1} is relatively strong (Table 8).

Both at the level of individual years and at the level of individual SM groups, it follows that the relationship between a dry matter content of 300 g.kg^{-1} and the WSC + STARCH content is stable. In comparison with the starch content (Table 4) or with the WSC content (Table 2), based on the significantly lower coefficient of variation of this parameter (Table 7) we can use the sum of WSC + STARCH as the second criterion for determining maize silage maturity.

3.4 Silage maturity (SM) groups and development of dry matter content

We evaluated the development of dry matter content in relation to the specific calendar day according to silage maturity (SM) groups (Figure 5) by classifying hybrids into SM groups using the date on which a dry matter content of 300 g.kg^{-1} was reached, with rounding to the nearest 10 day period. In the period between the 200th and 210th calendar day, all SM groups have a very narrow and close range of dry matter content. In further vegetation development, the rate of increase in dry matter content among the individual groups diverges greatly and characteristically breaks down as development progresses. Based on these relationships, we consider the classification system in use with rounding by tens to be more suitable compared to the original proposal (Mitrík and Mitrík, 2022), which is illustrated by the course and dynamics of the curves (Figure 5).

4 Conclusions

The results show that there is a strong and firm relationship between WSC (140 g.kg^{-1} of dry matter content), starch (280 g.kg^{-1} of dry matter content) and WSC + STARCH (420 g.kg^{-1} of dry matter content) in the foothills of Slovakia at a dry matter content of 300 g.kg^{-1}. We recommend total WSC + STARCH content as a second suitable criterion for determining maize silage maturity and for evaluation and classification of hybrids into SM groups.

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