

Saccharides of yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] tubers and rhizomes and factors affecting their content

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ABSTRACT

Yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson], a native plant of the Andes, belongs to the family *Compositae* (*Asteraceae*). It represents a traditional crop from the original population of Peru. Most of the tuberous root biomass is constituted by water (> 70% of the fresh weight). Saccharides, especially oligofructans, form 70–80% of their dry weight. Four yacon ecotypes originating from Bolivia, Ecuador, Germany and New Zealand were cultivated on the trial fields of the Czech University of Agriculture in Prague in 1995, 1996, 2000 and 2001. Considerable differences among the ecotypes were observed in their content of inulin (141–289 mg/kg d.m.) and lesser for fructose levels (195–217 mg/kg d.m.). No differences were found in glucose and saccharose contents. The highest inulin and fructose contents were found in the harvests from 2001 and 2000, similar trends were found for glucose. Statistically significant effect on the content of all saccharides has the year of cultivation. Tubers contained much higher levels of inulin (179 g/kg d.m.) and fructose (193 g/kg d.m.) in comparison with rhizomes. No significant differences were found for saccharose (higher in rhizomes) and glucose (lower in rhizomes). The contents of inulin and fructose in the upper and lower parts of tubers were reciprocal. During the storage period of 140 days at 10°C and 75% a relative humidity inulin content decreased by 48.7% and monosaccharides content increased (fructose by 9.97%, glucose by 31.4%) due to hydrolysis. Likewise saccharose content increased by 12.9%.

Keywords: yacon; saccharides; effect of ecotype; effect of cultivation year; effect of storage; tubers and rhizomes; different parts of the tubers

Yacon saccharides

Most of the root biomass is constituted by water that usually exceeds 70% of the fresh weight. Due to high water content, root energy value is low. The tuberous roots contain only 0.3–3.7% protein (Table 1), but 70–80% of the dry matter (d.m.) is constituted by saccharides, mainly fructooligosaccharides (Goto et al. 1995). The underground reserve organs of yacon accumulate over 60% (on d.m. basis) of inulin type β (2 \rightarrow 1) fructans, mainly oligomers (GF₂–GF₁₆) (Itaya et al. 2002). The structures of kestose and nystose, the main fructooligosaccharides, are given in Figure 1. Fukai et al. (1993, 1997) determined fructan content and the activities of saccharose: saccharose fructan fructosyltransferase (EC 2.4.1.99), fructan: fructan fructosyltransferase (EC 2.4.1.100), and fructan hydrolase in each part of yacon during the growth period. They found that during the summer the amount of fructans accumulated in each part was minimal despite the existence of relatively high specific activities of both transferases in the stems, tuberous roots,

and rhizomataceous stems. As Goto et al. (1995) confirmed using enzymatic, C-13-NMR, and methylation analyses, the fructooligosaccharides represent mainly oligosaccharides from trisaccharide to decasaccharide with terminal saccharose (inulin type fructooligosaccharides). Hermann et al. (1998) reported that yacon fructans are of low molecular mass. Yacon has also significant fructose (3–22% of root dry matter) and glucose (2–5% of root dry matter) contents (Ohyama et al. 1990). The calculated yacon food energy 619–937 kJ/kg of fresh matter is very low and has similar properties as dietary fibre (Quemener et al. 1994). The content of individual saccharides in yacon tuberous root is given in Table 2 (Valentová et al. 2001).

Factors influencing yields and contents of saccharides in yacon

The highest dry matter and fructan yields were observed in dodecaploid lines as compared with octoploid ones. Cisneros-Zevallos et al. (2002) evalu-

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Table 1. Chemical composition of tuberous roots, leaves and stems from literature data (Lachman et al. 2003)

Compound	Tuberous roots							
	fresh				dry			
	A	B	C	D	A	B	C	D
Water (%)	69.50	92.70	86.6	84.80	–	–	–	–
Ash (%)	2.40	0.26	–	3.50	6.71	3.59	–	23.03
Proteins (%)	2.22	0.44	0.30	3.70	7.31	6.02	2.24	24.34
Lipids (%)	0.13	0.10	0.30	1.50	0.43	1.32	2.24	9.87
Fiber (%)	1.75	0.28	0.50	3.40	5.73	3.88	3.73	22.37
Saccharides (%)	19.67	–	–	–	67.53	–	–	–

Compound	leaves			stem			leaves and stem	
	A		E	A		E	B	
	fresh	dry	dry	fresh	dry	dry	fresh	dry
Water (%)	83.20	–	–	86.70	–	–	92.00	–
Ash (%)	2.68	15.98	12.52	1.35	10.23	9.60	1.03	14.49
Proteins (%)	2.87	17.12	21.18	1.51	11.37	9.73	1.13	15.97
Lipids (%)	1.24	7.40	4.20	6.30	2.26	1.98	0.22	3.04
Fiber (%)	1.68	10.04	11.63	3.57	26.85	23.82	1.11	15.69
Saccharides (%)	1.44	8.58	–	1.55	11.70	–	–	–

Source: A = Calvino (1940), B = Bredemann (1948), C = León (1964), D = Nieto (1991), E = Frček et al. (1995)

ated three accessions of yacon from Huanco (Peru) for their saccharide distribution and stability after 0, 15, 30, 45 and 90 days of storage at 4°C and at

room temperature (25°C). Their results indicated high variability in fructooligosaccharide content (2.1–70.8 g/100 g d.m.) and a reverse relationship

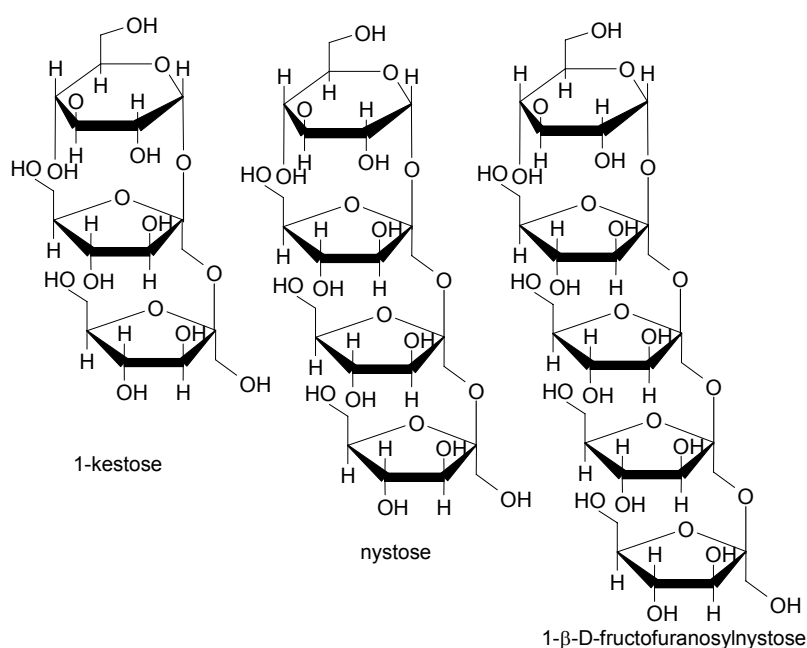


Figure 1. Chemical structure of three main fructooligosaccharides (GF₂–GF₄)

GF₂ – trisaccharide consisting of one molecule of glucose and two molecules of fructose
 GF₃ – tetrasaccharide consisting of one molecule of glucose and three molecules of fructose
 GF₄ – pentasaccharide consisting of one molecule of glucose and four molecules of fructose

between fructooligosaccharide and reducing sugars contents. Moreover, they estimated in three accessions a decrease in the order of 46.5, 32.8 and 21.6% of initial amount at 25°C after 15 days and 73.3, 56.5 and 76.8% after 90 days of storage at 25°C and coincidentally an increase in reducing sugar contents (mean value 42%). Fructooligosaccharide content also decreased at 4°C, however, in less proportion in comparison with the temperature 25°C (1.65, 2.94 and 3.6% after 15 days of storage and 27, 17 and 21% after 90 days of storage). Asami et al. (1991) investigated the changes of the contents of fructose, glucose, saccharose and oligofructans (GF₂-GF₉) during growth and storage of the yacon tubers. The average polymerisation degree of saccharides increased during the growth stage (maximum 4.3 at the date of the harvest) and then it decreased during storage. During the two-week storage of yacon tubers in the soil holes in the field, and at 5 or 25°C, the contents of oligofructans decreased to 21, 33 and 41% of the amount determined at the harvest. Coincidentally the contents of fructose, glucose and saccharose increased. Bagautdinova et al. (1999) observed a tendency of a decrease of polymerisation degree of oligofructans related to the cultivation of yacon in the regions situated more to the North (at 56° of north latitude no inulin content was found). Tjukavin (2001) found that the content of monosaccharides in the yacon tubers cultivated in a plastic greenhouse was higher (+ 37%) as compared with yacon tubers cultivated in the field. The content of monosaccharides increased by 2.78–3.38% during the six-month storage of frozen tubers. If the tubers were stored at + 4°C, an increase of monosaccharides content was found (from the field 3.3 fold, from the plastic greenhouse 2.1 fold) with the maximum during the period of 2–4 months of storage.

The aim of this work was to determine saccharide composition of yacon tubers and rhizomes of four different ecotypes, mainly inulin, saccharose, fructose and glucose and to determine the effect of the year of cultivation on their content in yacon tubers as well as the changes of the contents of these saccharides during storage of tubers.

MATERIAL AND METHODS

Material. Tubers of four yacon ecotypes were analysed: Bolivia (BOL, 1995, 1996, 2000, 2001), Ecuador (ECU, 2000, 2001), New Zealand (NZ, 1995, 1996, 2000, 2001), and Germany (GER, 2000, 2001). Cultivated material of Bolivia ecotype was originally introduced to the Czech Republic from Bolivia (natural habitat San Pedro – Potoci at the altitude 2800 m above sea) in the year 1995, of Ecuador ecotype in the year 1994, of New Zealand ecotype

from Auckland in the year 1993, and of Germany ecotype from Stückborstel in the year 1994. The plants were cultivated in the mentioned years on trial field plots of the University of Agriculture in Prague-Suchdol in barley-beet region at the altitude 286 m above sea with average year temperature 9.5°C and vegetation temperature 14.5°C and total sum of precipitation about 525 mm. Yacon was harvested in October/November 1995, 1996, 2000 and 2001 years after 149–167 days of vegetation period. After harvest they were analysed for dry matter content and saccharides content: inulin, fructose, saccharose and glucose. Rhizomes were also analysed in 1995 and 2000. Tubers were cut into three horizontal parts designated as A, B and C (Table 6).

Preparation of extracts. Samples of tubers were frozen in liquid nitrogen and then freeze-dried using Lyovac GT2, Finn-aqua freeze-drier. Freeze-dried samples were extracted with 80% methanol in a water bath at 75°C for 10 min. Methanol was removed by centrifugation in vacuum (Speedvac, Savant). Redistilled water was then added in ratio 0.5 ml to 10 mg dry matter (d.m.). Soluble saccharides were sonicated (Julabo USR) for 15 min. The extracts were then centrifuged in a microcentrifuge Eppendorf 5415 for 10 min at 14 000 rpm for separation of coarse solid particles and filtered through membrane filters (0.45 µm Millipore). The frozen samples were then stored at –18°C for a few days before HPLC determination.

Table 2. Contents of saccharides in yacon tuberous roots (Valentová et al. 2001)

Saccharide	Content (mg/g d.m.)
Fructose	350.1 ± 42.0
Glucose	158.3 ± 28.6
Saccharose	74.5 ± 19.0
GF ₂	60.1 ± 12.6
GF ₃	47.4 ± 8.2
GF ₄	33.6 ± 9.3
GF ₅	20.6 ± 5.2
GF ₆	15.8 ± 4.0
GF ₇	12.7 ± 4.0
GF ₈	9.6 ± 7.2
GF ₉	6.6 ± 2.3
Inulin	13.5 ± 0.4

GF_x = glucofructans, where index _x is a number of fructose units in the molecule

Table 3. Contents of inulin, fructose, saccharose and glucose in dry matter of yacon tubers in different ecotypes and years of cultivation

Ecotype/year	Inulin	Fructose	Saccharose		Glucose
			(g/kg d.m.)		
BOL/95	236	113	20.1		45.8
BOL/96	120	124	19.4		102
BOL/00	169	295	35.5		113
BOL/01	460	250	19.5		122
BOL/AVE	246	195	23.6		95.4
ECU/96	215	115	20.4		91.5
ECU/00	231	275	37.4		114
ECU/01	420	217	19.7		104
ECU/AVE	289	203	26.2		103
NZ/95	117	184	23.1		80.7
NZ/96	204	113	20.9		77.0
NZ/00	243	287	49.8		54.8
NZ/01	375	283	27.5		106
NZ/AVE	235	217	30.4		79.5
GER/96	181	104	20.9		67.8
GER/00	80	90.2	19.7		20.4
GER/01	163	432	26.1		179
GER/AVE	141	209	22.2		89.0

BOL = Bolivia; NZ = New Zealand; ECU = Ecuador; GER = Germany; AVE = average value

Determination of soluble saccharides by HPLC.

Soluble saccharides were determined using Spectra Physics SP 8800 series high performance liquid chromatograph with refractometric detector Shodex RI-71. Isocratic separation was performed at 80°C on the column OSTION LGKS Ca²⁺ with water as the mobile phase and isocratic pump at flow rate 0.5 ml/min. Mobile phase was adjusted by ultrafiltration on Milli Q, Millipore. Precolumns Hema-Bio 1000Q and Hema-Bio 1000SB were used. The detection limit for fructose was approx. 20 µmol/l.

Determination of dry matter (d.m.). Samples were weighed up and dried at 105°C for 3 h.

Storage of tubers. Tubers were stored for 140 days in a climatic box at a storage temperature of 10°C and relative humidity 75% in paper sacks.

Statistic evaluation. The results (mean values from three parallel determinations) were statistically evaluated by ANOVA and Tukey's methods with Statgraphics program by the analysis of variance with multiple and single grouping. More detail evaluation was performed by Scheffe's test.

RESULTS

The results are given in Tables 3–7, statistical evaluation in Tables 8 and 9.

The effect of ecotype. The highest content of inulin in the tubers was found in the ecotype from Ecuador (289 g/kg d.m.), while the ecotype from Germany had the lowest content (141 g/kg d.m.). Comparable mean values were estimated

Table 4. Content of saccharides in dry matter of yacon tubers grown in different years

Year	Inulin	Fructose	Saccharose		Glucose
			(g/kg d.m.)		
1995	177	149	21.6		63.2
1996	180	114	20.7		84.5
2000	181	237	35.6		75.4
2001	354	296	23.2		128.0

Table 5. Content of saccharides in dry matter of yacon tubers and rhizomes in 1995 and 2000

Analyzed yacon part/ year of cultivation	Inulin	Fructose	Saccharose	Glucose
Tubers/95	177	149	21.6	63.2
Tubers/00	181	237	35.6	75.4
Tubers/AVE	179	193	28.6	69.3
Rhizomes/95	97.4	124	23.8	56.4
Rhizomes/00	194	134	53.2	65.1
Rhizomes/AVE	146	129	38.5	60.8

Table 6. Contents of saccharides in dry matter of yacon tuber horizontal parts

Part of tuber	Inulin	Fructose	Saccharose	Glucose
A – upper	292	196	23.3	74.9
B – middle	188	198	28.3	82.4
C – lower	209	211	26.1	97.0

Table 7. Effect of storage for 140 days on content of saccharides in dry matter of tubers of the ecotype from Bolivia

Days of storage	Inulin	Fructose	Saccharose	Glucose
0	236	113	20.1	45.8
140	121	125	44.4	60.2

in the ecotypes from Bolivia and New Zealand. The highest content of fructose was determined in the ecotype from New Zealand (217 g/kg d.m.), the lowest one in the ecotype from Bolivia (195 g/kg d.m.). The highest glucose content was observed in the ecotype from Ecuador (103 g/kg d.m.), contents in the other ecotypes were comparable. Statistically significant differences among the ecotypes were found only for glucose ($P = 0.000054$).

The effect of the year of cultivation. The highest level of inulin was found in 2001 (mean value 354 g/kg d.m. in the ecotypes from Bolivia, Ecuador and New Zealand). The highest contents of fructose were estimated also in 2001 (mean 295 g/kg d.m., especially in the ecotype from Germany – 432 g/kg

d.m.). These levels were higher in comparison with those in 2000 (mean 237 g/kg d.m., high in ecotypes from Bolivia, New Zealand and Ecuador). Differences among saccharose contents in samples cultivated in 1995, 1996, 2000 and 2001 were negligible. Similar trend was observed for glucose, with the highest levels estimated in 2001 (mean 128 g/kg d.m. – with high content in the all ecotypes), while the highest amounts were found in the ecotypes from Ecuador and Bolivia in 2000. Regarding the weather factors, the highest amounts of saccharides were found in 2001 and 2000, as compared with the 1995 and 1996. Statistically significant differences were found for all investigated saccharides. Year \times ecotype differences were significant for fructose ($P = 0.009709$) and glucose ($P = 0.000218$), lesser they were for inulin ($P = 0.058703$) and non-significant for saccharose.

Content of saccharides in tubers and rhizomes.

Tubers have considerably higher contents of inulin (179 g/kg d.m.) and fructose (193 g/kg d.m.) in comparison with rhizomes (146 and 129 g/kg d.m., respectively). On the contrary, the contents of saccharose and glucose are comparable and the differences are not significant. Rhizomes have somewhat higher content of saccharose and lower levels of glucose as compared with tubers. Applying the more detail evaluation of variance analysis of single grouping by Tukey test, significant differences between tubers and rhizomes in saccharose content were found ($P = 0.000113$).

Content of saccharides in different parts of tubers. The analysed tubers were cut and divided into three parts – A (upper), B (middle) and C (lower). The highest inulin content was determined in the part A – 292 g/kg d.m. ($A \gg C \gg B$). Conversely, the highest content of fructose was found in the part C – 211 g/kg d.m. ($C \gg B \geq A$). As results from these data, contents of inulin and fructose in the parts A and C of tubers are reciprocal. No statistically significant differences in the contents of saccharides in the different tuber parts were found.

Effect of storage on saccharides content. Yacon tubers of the Bolivian ecotype were stored for 140 days at conditions cited above. The content of inulin decreased during storage from 236 to 121 g/kg d.m. On the contrary, the content of monosaccharides increased (fructose from 113 to 125 g/kg d.m., glucose from 45.8 to 60.2 g/kg d.m.) as well as the content of saccharose (from 20.1 to 44.4 g/kg d.m.). The decrease of inulin type fructans (by 115 g/kg d.m.) was only partially equilibrated by an increase of saccharose (by 24.3 g/kg d.m.) > fructose (12 g/kg d.m.) > glucose (4.4 g/kg d.m.) indicating their losses by respiration. Using the method of variance analysis of a single grouping by Tukey test, a significant effect of storage was found only for inulin ($P = 0.035787$).

Table 8. Variance analysis of multiple grouping for inulin and fructose content

Effect	Degree of freedom	Inulin			Fructose				
		sum of squares	mean square	F	P	sum of squares	mean square	F	P
Year	2	198 548	99 274.08	8.402293	0.000487*	267 856.3	133 928.1	22.70795	0.000000*
Ecotype	2	53 403	26 701.58	2.259950	0.110979	4 952.4	2 476.2	0.41984	0.658588
Year × ecotype	8	187 728	23 466.04	1.986103	0.058703	129 958.3	16 244.8	2.75436	0.009709*
Error	80	945 209	11 815.12			471 828.1	5 897.9		
Total	94	1 880 334				897 879.3			

* statistically significant differences at $P < 0.05$

Table 9. Variance analysis of multiple grouping for saccharose and glucose content

Effect	Degree of freedom	Saccharose			Glucose				
		sum of squares	mean square	F	P	sum of squares	mean square	F	P
Year	2	17 974.32	8 987.161	41.38252	0.000000*	31 862.6	15 931.31	11.14098	0.000054*
Ecotype	2	541.11	270.557	1.24581	0.293227	10 559.6	5 279.81	3.69224	0.029257*
Year × ecotype	8	2 416.48	302.060	1.39087	0.213249	49 743.3	6 217.91	4.34827	0.000218*
Error	80	17 373.83	217.173			114 397.9	1 429.97		
Total	94	42 814.75				206 211.6			

* statistically significant differences at $P < 0.05$

DISCUSSION

There were found significant differences among ecotypes regarding the contents of glucose, whereas in the saccharose content no significant differences were observed. The highest contents of inulin and glucose were found in the tubers originating from Ecuador. Free glucose and fructose contents were reported among the most variable root parameters (Hermann et al. 1998). Significant differences in ecotype × year of cultivation were found for glucose and inulin levels.

Regarding a year of cultivation, the highest contents of inulin, fructose and glucose were found in 2001 and 2000 in comparison with 1995 and 1996, whereas saccharose levels were comparable. It is in accordance with the results of Tjukavin (2001) who demonstrated that favourable year conditions strongly affected the yield and contents of saccharides of yacon tubers. Our results prove a significant effect of the cultivation year on the content of all four investigated saccharides (Tables 4 and 5). Higher average temperature in the year 2000 (9.6°C) and sunshine (1801 h) increased saccharose content.

Yacon tubers contain much higher levels of inulin and fructose as compared with rhizomes, whereas differences among saccharose and glucose contents were not significant. Itaya et al. (2002) determined fructosyl transferase and hydrolase activities in yacon rhizophores and tuberous roots during their complete cycle under field conditions. Their results showed that synthesising activities were higher in rhizophores than in the tuberous roots, while hydrolysing activities predominated in tubers, which confirms the higher fructose contents. There was reported a significant and highly negative correlation between fructans and free fructose (Hermann et al. 1998).

Changes of yacon tubers oligosaccharides during storage have been of great importance. Decreasing levels of inulin and in contrary enhancing contents of fructose and glucose confirm the hydrolysing process during yacon storage. The higher the temperature of storage, the greater the decrease of inulin and increase of saccharose and especially of fructose and glucose could be observed (Tjukavin 2001).

The highest content of inulin was found in the upper parts of tubers, whereas in the lower parts, the highest contents of fructose were determined. These differences could be related to specific and total activity of fructan hydrolase in different parts of tubers (Fukai et al. 1997). It appears that yacon tubers belong to the group, which accumulates low-molecular fructans, especially in the lower parts of tubers (Ohyama et al. 1990). Tubers and tuberous roots contained about 57 and 66%, respectively, of

oligofructans, especially of 1-kestose (Asami et al. 1991, Fukai et al. 1993).

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ABSTRAKT

Sacharidy v hlízách a stonkových hlízách jakonu [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] a faktory ovlivňující jejich obsah

Jakon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] – rostlina pocházející z And – patří do čeledi *Compositae* (*Asteraceae*) a představuje známou plodinu původního obyvatelstva Peru používanou v tradičním lékařství. Většinu biomasy kořenů tvoří voda (> 70 % čerstvé hmoty), 70–80 % sušiny představují sacharidy, zvláště fruktany. Při porovnání ekotypů pocházejících ze čtyř zemí (Bolívie, Ekvádor, Německo a Nový Zéland), pěstovaných v letech 1995, 1996, 2000 a 2001 na pokusném poli ČZU v Praze-Suchdole, jsme zjistili malé rozdíly v obsahu inulinu (Ekvádor 289 g/kg sušiny, Německo 141 g/kg sušiny) a fruktózy (Nový Zéland 217 g/kg sušiny, Bolívie 195 g/kg sušiny), zatímco v obsahu glukózy a sacharózy jsme rozdíly nezaznamenali. Nejvyšší obsahy inulinu a fruktózy byly zjištěny u sklizní v letech 2001 a 2000. Podobný trend byl zaznamenán i u glukózy, zatímco rozdíly v obsahu sacharózy byly zanedbatelné. Statisticky významný vliv na obsah všech sacharidů měl ročník. Hlízy obsahují mnohem vyšší podíl inulinu (179 g/kg sušiny) a fruktózy (193 g/kg sušiny) ve srovnání se stonkovými hlízami. Statisticky významné rozdíly nebyly nalezeny u sacharózy (vyšší obsah ve stonkových hlízách) ani glukózy (nižší obsah ve stonkových hlízách). Obsahy inulinu a fruktózy ve vrchní a spodní části hlíz byly nepřímo úměrné. Během skladování po dobu 140 dní se hydrolytickými procesy obsah inulinu snížil o 48,7 % a obsah monosacharidů se zvýšil (fruktózy o 9,97 %, glukózy o 31,4 %). Rovněž obsah sacharózy se zvýšil o 12,9 %.

Klíčová slova: jakon; sacharidy; vliv ekotypu; vliv ročníku; vliv skladování; hlízy a stonkové hlízy; různé části hlízy

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