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Effect of Seasonal Changes on Content and Profile of Soluble Carbohydrates in Tubers of Different Varieties of Jerusalem Artichoke (*Helianthus tuberosus* L.)

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A high content (60–65% of dry mass DM) of water soluble carbohydrates was found in early harvested varieties (Bella and Bianka) and middle early varieties (Topstar and Gigant) harvested 22–25 weeks after plantation. In late varieties (Waldspindel, Violet de Rennes, Rote Zonenkugel) a similar amount was obtained (55–60% of DM) when harvested 29–33 weeks after planting. There was a distinctive impact on maturing process as well as frost period alterations which resulted in conversion of high polymer inulin to low polymer inulin as well as to sucrose. In early / middle early varieties a correlation between sucrose and inulin level ($r = -0.952$**) with a linear regression of $y = -1.35x + 62.32$ was observed, whereas the dp_n of inulin decreased from 12–14 to 6–8. In late cultivars this correlation was not as exact ($r = -0.502$**); dp_n of inulin decreased from 12–16 to 7–10. This knowledge about carbohydrate profiles for different varieties of Jerusalem artichoke offers the possibility of selecting suitable cultivars and deciding the appropriate harvest time for an optimum processing of tubers for their application as prebiotic and novel food component.

KEYWORDS: Jerusalem artichoke; soluble carbohydrates; inulin; fructo-oligosaccharides; peroxidase activity

INTRODUCTION

Fructans, which are present as storage carbohydrates in a lot of plants, are applied in human nutrition as part of the daily diet in the form of vegetables, spices, and fruits (e.g., globe artichoke, jerusalem artichoke, chicory, asparagus, garlic, onion, chives, cereals, banana) (*1*, *2*). Owing to their health promoting properties, the interest in fructans particularly fructooligosaccharides (FOS) and inulin as a supplement in functional food is increasing. Fructans such as FOS and inulin are highly beneficial in enhancing the quantity of bifidogenic bacteria as well as modulating the benevolent microflora of the human colon. Therefore they are considered as prebiotics (*3*, *4*). Gibson et al. (*5*) found that the substitution of sugar by FOS or inulin (15 g/day) can lead to a significant change in the balance of microbiota in the large intestine (bifidogenic effect). Moreover FOS and inulin have a beneficial effect on human lipid metabolism (*6*). Consequently risk factors for cardiovascular disease can be reduced. Furthermore a decrease in risk factors responsible for osteoporosis and colon cancer has been observed after intake of FOS and inulin rich diet (*7*). Fructosyl units of FOS and inulin have a metabolic caloric value of 1.1–1.7 kcal/g only, thus qualifying fructans as dietary supplements for the fight against obesity (*8*).

The difference between FOS (low molecular weight inulin = inulooligosaccharides) and inulin concerning the healthy effects on the human body is not significant; the higher solubility of FOS compared to polymeric inulin (dp> 10−12) makes it more easily available and therefore more rapidly fermentable by the microbiota in the colon (*9*).

Because of its health promoting properties, FOS and inulin are used as functional food components throughout the world (*4*, *10*, *11*). Because of the better solubility of FOS these are applied mainly in beverages or calorie reduced food supplements, whereas inulin with its tendency to crystallinity owing to its helical structure is more applicable as fat replacer for instance. Presently, the plant raw material for the production of inulin and FOS are roots of chicory. Only a small segment of the inulin market is covered by the production of nonreducing FOS from tubers of Jerusalem artichoke (*12*).

The high content of inulin and a peculiarly high per hectare yield of tubers allow Jerusalem artichoke to be designated as fructan crop with a bright future (*13*, *14*). Semiarid areas with their typical climatic conditions (different seasons with cold

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winters) are suitable for producing tubers having a high inulin content when harvested in fall. The plant is rich in FOS ($3 \leq dp_n \leq 10$) when harvesting is done during spring time (*15–17*).

Tubers of Jerusalem artichoke with their fresh and nutty taste can be applied as raw material for making chips or as powder after drying processes. Jerusalem artichoke powder (JAP) can be added to bread, bakery and other food products (*18*). Alternatively, the tubers can be processed to form juice providing a base for low calorie soft drinks (*17*). For drying processes as well as for juice production browning reactions caused by phenoloxidases activity or Maillard reaction have to be reduced.

Utilization of the whole tubers and making the crop independent from seasonal aspects would further enhance the market potential for Jerusalem artichoke.

To maintain enzymatic activities and carbohydrate profile as well as to avoid the damage of tubers by stress (*19, 20*) during storage time the tubers must be carefully dried or lyophilized for conservation before processing.

Detailed information about the composition of soluble carbohydrates viz. glucose, fructose, sucrose, FOS, and inulin in tubers of different varieties at optimal harvest time allows to make a correct decision for applications in food products. Therefore, in the present work, it was envisaged to perform a detailed investigation about the composition of soluble carbohydrates, protein content, and peroxidase activity in early, middle early, and late varieties at different harvest terms.

Cultivation conditions as well as results from yields of shoot and tubers, dry matter, mean tuber weight, and influence of irrigation on the yields are presented in parts 1 and 2 (*21, 22*).

MATERIALS AND METHODS

Materials. Two early varieties of Jerusalem artichoke (Bella, Bianka), two middle early (Topstar, Gigant), and three late varieties (Violet de Rennes, Waldspindel, and Rote Zonenkugel- Rozo) were grown and harvested at different terms on the Experimental Station of our University, Groβ-Enzersdorf, Vienna (longitude 16° 57′ east, latitude 48° 12′ north), Austria, 1999. Details about agricultural and climatic conditions as well as plant development data have been reported recently (*21, 22*). After harvesting (10 plants of each variety), the tubers were washed, counted, weighed, and finally a representative part of tubers was sliced and freeze-dried. The milled freeze-dried powders were used for further analysis.

Analysis of Water Soluble Carbohydrates. Dry matter was determined by drying thoroughly weighed milled freeze-dried samples for 18 h at 105 °C and specifying the weight loss.

Extraction of soluble carbohydrates: 200 mg of freeze-dried powder of Jerusalem artichoke tuber was mixed with 20 mL distilled water in a 25 mL flask and extracted for 60 min at 80 °C on a water bath. After making the volume up to 25 mL with distilled water, a part of the supernatant was centrifuged at 13000 rpm for 10 min (Biofuge in 1.5 mL vials).

Free glucose, fructose, and sucrose were determined in the clear solution by enzymatic assay (Boehringer-Mannheim; test kit no. 716 260).

For the determination of fructan (inulin) content, the samples were chemically hydrolysed with acid. In brief, 200 μL of clear extract was mixed with 800 μL of 3% w/w HCl in screwable tubes and heated in an aluminum block at 80 °C for 3 h. After cooling, 200 μL of the hydrolysed samples were mixed with 800 μL of 2% w/w $NaHCO_3$ for neutralization. From this solution the total content of glucose and fructose was determined by enzymatic assay (Boehringer-Mannheim; testkit no. 716 269).

The content and the number average degree of polymerisation (dp_n) of fructan (inulin) was calculated in the following way:

$$\text{g Fructan} = (\text{gFru}_{corr} + \text{gGlu}_{corr})\, f_A$$

f_A: 0,9 (correction factor of glu to anhydro-glu)

$$\text{g Fru}_{corr} = \text{gFru}_{total} - (\text{gFru} + \text{g Suc}/2)$$

$$\text{g Glu}_{corr} = \text{gGlu}_{total} - (\text{gGlu} + \text{gSuc}/2)$$

$$dp_n = (\text{gFru}_{corr} + \text{gGlu}_{corr})/\text{gGlu}_{corr}$$

This calculation is only valid when it is assumed that each fructan molecule contains only *one* glucose unit.

Analysis of Total Protein (Dumas Method). The content of nitrogen in freeze-dried powder of Jerusalem artichoke was determined with the help of a fully automated Analyser CN-2000 from LECO (Leco Instruments GmbH, D- 85551 Kirchheim). Exactly 0.6 g samples were weighed into sliding ceramic shuttles and brought into the combustor by means of an autoloader. After combustion, the amount of resulting nitrogen was calculated automatically by means of computer aided software. Investigation for all the samples was repeated three times. The nitrogen content was multiplied with 6.25 to estimate the crude protein content.

Analysis of Water-Soluble Protein (Bradford Method). Soluble protein content was determined by the ability of Coomassie pigments (Brilliant Blue G-250) to bind proteins (*23*).

Analysis of Peroxidase Activity (Pod). For the determination of peroxidase activity the method of Sigma (*24*) was applied. In this case, one unit of peroxidase is defined as the amount of enzyme that converts 1 mg pyrogallol to purpurogallin at 20 °C in 20 s.

The specific activity (units/mg soluble protein) of peroxidase was obtained by dividing the units of peroxidase activity by the amount of total soluble protein in the sample.

Statistical Analysis. Statistical analysis of the obtained data was carried out by variance analysis (ANOVA). When significant differences ($p < 0.05$) were found between the data means during the harvest time the results were compared by Tukey's-test. First results were calculated with Pearson correlation coefficient. All statistical analysis was performed using SPSS software package version 10.07.

RESULTS AND DISCUSSION

Nearly 65 different varieties of Jerusalem artichoke are available in Austria for utilization in food and feed products. From this collection, two early (Bella, Bianka), two middle early (Topstar, Gigant), and three middle late or late (Violet de Rennes, Waldspindel and Rozo) varieties predefined by the date of flowering and tuber maturity were chosen. Detailed information about yields of green biomass and tubers per ha, status of tubers (weight and dry matter) and yield of inulin/ha at different harvest terms and weather conditions (temperature sum) as well as different irrigation conditions are reported in parts 1 and 2 (*21, 22*).

The highest yield for tubers of early varieties was found to be at the end of September with 17.5 t/ha of DM and in continuation a similar level of yield for middle late and late varieties was obtained at the end of November. Furthermore, the knowledge of the profiles of soluble carbohydrates, in particular inulin, sucrose, and monosaccharides in the tubers is especially important for the utilization of Jerusalem artichoke as source for inulin and FOS and for the application of tubers as a whole in reduced calorie and health promoting products. The determination of soluble carbohydrate profiles in the tubers was carried out simultaneously along with the determination of agricultural parameters at the chosen harvest terms during the vegetation period of Jerusalem artichoke varieties.

Total Content of Soluble Carbohydrates. During the period of high photosynthetic activity between 12 and 22 weeks after planting (summer and early fall) the transport of carbohydrates from leaves and stalks to the tubers takes place immediately, thus stimulating the germination of new sprouts and tubers (*15*). Therefore, the total content of soluble carbohydrates in the developing tubers is generally high but their variation is mainly based on the genetic predisposition of the different varieties.

In the early varieties, Bella and Bianka, the maximum level of total soluble carbohydrates, which was 55.2% and 68.6% of DM was found in the small unripe tubers 14 to 17 weeks old. After 22 weeks of plantation the highest yield per ha (19.5 t/ha of DM) was obtained. The content of carbohydrates in mature Bella was similar as in unripe tubers, whereas the carbohydrate content in mature Bianka was 62.3% being slightly reduced. Similar content of soluble carbohydrates between 60 and 65% of DM during the riping process of tubers was found in the middle early varieties, Topstar and Gigant, 14 to 25 weeks after planting. The mature tubers contained 62.1% (Topstar) and 62.8% (Gigant) total soluble carbohydrates at the optimal harvest term, i.e., twenty-five weeks after planting with a 17.5 t/ha of DM yield. The results demonstrate a high content of soluble carbohydrate in the developing and mature tubers for early and middle early varieties and thus offer a long period of harvesting for industrial utilization.

In the late varieties—Violet de Rennes, Rozo and Waldspindel—the highest level of soluble carbohydrates (55–67% of DM) was found predominantly in adult tubers (9.8–18.4 t/ha of DM) lasting from the end of November to the midst of December (29–33 weeks after planting). Although, harvesting of tubers in the end of November can be a problem because of frost.

The content of soluble carbohydrates in the tubers staying in the soil during fall and winter time changed specifically (*25–27*). In the early (Bella, Bianka) and middle early varieties (Topstar and Gigant) the content of soluble carbohydrates was reduced continually from optimal harvest term at maturity of tubers (60–63%) to about 51–55% when harvested in spring. The content of soluble carbohydrates in the late varieties, Violet de Rennes and Waldspindel did not change during the winter period and exhibited similar results with 65 and 55% in the spring. The carbohydrate content was reduced only in the variety Rozo and was found to be 50%.

Seasonal Changes of Soluble Carbohydrate Profile. The profile of soluble carbohydrates reflects the assimilation activities during developing, maturing and storing of the tubers in the soil (*15*, *16*, *27–31*). The content of monosaccharides, glucose and fructose, was reduced in all varieties from about 1.6% in unripe (14–17 weeks after planting) to 0.1–0.5% in mature tubers as well as in tubers, stored in the soil during winter (detailed results not shown). In context to the building process of inulin during the period of high photosynthesis, sucrose, transported from the leaves and stem, is immediately metabolized in the cells of tubers by the enzyme SST (sucrose–sucrose–transferase) to 1-Kestose and subsequently transformed with FFT (fructosyl–fructosyl–transferase) to higher polymers of inulin (*2*, *26*). In this context, the level of sucrose in growing tubers as well as in adult tubers of all varieties was low (1.8–7% of DM). The content of inulin, however, was primarily influenced by the genotype of tubers.

In the early variety Bella, the content of inulin was in the range of 53–55% of DM during the growth stage up to maturity (14–22 weeks after planting). In the Bianka variety, there was 64% inulin of DM (14 weeks) at the start of tuber development, getting slightly reduced in ripe tubers (22 weeks) to 58%. In both cultivars the dp_ns (number average degree of polymerization) were in the range of 11 and 13. These dp_ns calculated from the enzymatic analysis can be regarded as an indicator for the polymeric profile of inulin in the tubers. A $dp_n > 8$ reflects a higher percentage of polymers above dp_n12 usually called inulin, whereas a $dp_n < 8$ indicates a polymeric profile of $3 < dp_n < 12$ being still inulin (β−2,1 linked fructofuranosyl residues) but usually termed as fructooligosaccharides (FOS); this statement is confirmed by the results of polymer distribution by calibrated SEC as published recently (*2*).

Furthermore, the middle early varieties Topstar and Gigant, possessed a relatively high content of inulin with 55 and 60% during unripe stage up to 61 and 64% of DM during the mature status (14–25 weeks). Likewise the dp_ns were high and were found to be in the range of 12–15 promising a very high polymeric distribution of inulin.

In the late varieties, the inulin content during ripening of tubers was contrastingly different. In this case, the building process as well as the storage of the carbohydrates, sucrose, and inulin in the green biomass is preferred and thus a reduction in the transport of assimilates to the tubers before flowering time is observed.

The genotype Waldspindel, which is often defined as a middle late variety showed values of inulin continually rising from 43 to 48% of DM during the development of tubers till maturity (17–29 weeks). In these tubers, the polymerization of inulin was genetically determined to high polymeric components. The tubers at early stage of development showed a dp_n of about 18 for inulin being reduced slowly to the normal level of 11 in adult tubers.

The late variety Violet de Rennes started with a low content of inulin (42–46% of DM) in the early development period of tubers during the highest photosynthetic activity in summer time (14–19 weeks), but the content increased to 59% of DM during the developing process of tubers up till maturity (19–29 weeks), which is acceptable for industrial utilization. Compared to the foregoing these tubers differ showing a nearly constant dp_n of 14 for inulin during the developing process, only in adult tubers was the dp_n, reduced to 10.

Contrarily, in the Rozo variety, the biosynthesis started rather early with a high level of inulin between 59 and 63% of DM after 14–22 weeks of plantation. This was reduced slowly to a level of 47% of DM in the mature tubers 29 weeks old. Simultaneously the dp_n of inulin was reduced from 13 in unripe tubers to 8 in adult tubers.

In adult tubers the activity of SST was reduced and subsequently disappeared in the tubers remaining in the soil particularly during a frost period in winter time (*16*, *32*, *33*). Contrarily, the activity of FFT (for depolymerization of high polymer inulin to lower polymer inulin and sucrose) was induced in the cells of tubers at low temperature periods (<4 °C) in the soil. Due to this process, the increasing molar concentration of low molecular weight sugars supports the osmotic regulation of cells against damage and destruction. Additionally, sucrose can be transferred to the cell for metabolism as well as for stabilizing living processes of tubers (e.g., supporting processes of lignification to strengthen the skin and thus protecting the tubers). In this context the changes in the composition of sucrose and inulin can be expected for tubers stored in the soil after maturity.

In tubers of early varieties respiration and assimilation processes start immediately after maturity (indicated by the loss of green biomass) during the storage in the soil (*13*, *29*). Therefore, the sucrose level was increased and the content of inulin reduced in the tubers 25 weeks after plantation. In the Bella variety, the profile of soluble carbohydrate changed significantly to 18% sucrose and 44% inulin with a dp_n of 6.7 in the following weeks (29 weeks); after the first frost period at a harvest in the midst of December (33 weeks) the content of sucrose increased to 23.5% of DM, whereas the inulin content dropped down to 32.8% of DM with a dp_n of 5.3 indicating a very low polymeric distribution; at a harvest in spring after

Table 1. Content of Soluble Carbohydrates (Inulin, Sucrose) and Dp_N of Inulin in Early Varieties Bella and Bianka[a]

harvest time		BELLA				BIANKA			
weeks after planting	temp-sum[b] [°C]	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average
14	1733	2.7 ± 0.1 a	53.5 ± 0.4 a	57.8 ± 0.6 ad	11.6 ± 0.8 ab	3.5 ± 0.2 a	63.5 ± 0.5 a	68.1 ± 0.8 a	11.2 ± 0.1 ad
17	2138	3.6 ± 0.1 a	55.2 ± 0.6 a	60.1 ± 0.8 ab	11.9 ± 0.2 ab	4.0 ± 0.1 a	63.0 ± 0.9 ab	68.6 ± 1.1 a	12.2 ± 0.1 ab
19	2444	3.2 ± 0.2 ab	54.8 ± 0.8 a	58.2 ± 1.1 ad	13.2 ± 0.1 b	3.9 ± 0.1 a	62.5 ± 0.2 ab	66.8 ± 0.4 b	13.2 ± 0.1 b
22	2823	6.0 ± 0.1 c	54.2 ± 0.9 a	60.4 ± 1.2 b	10.5 ± 0.3 a	3.9 ± 0.1 a	58.3 ± 0.3 b	62.3 ± 0.5 c	13.1 ± 0.4 b
25	3053	8.5 ± 0.2 d	49.2 ± 0.3 b	58.1 ± 0.6 abd	9.9 ± 0.3 a	9.1 ± 0.6 b	47.7 ± 0.8 c	57.5 ± 1.3 d	10.5 ± 0.9 a
29	3282	18.0 ± 0.3 e	44.4 ± 0.5 c	62.7 ± 0.8 c	6.7 ± 0.9 c	11.6 ± 0.1 c	43.4 ± 0.2 d	55.3 ± 0.4 d	8.6 ± 0.1 c
33	3328	23.5 ± 0.7 f	32.8 ± 0.4 d	56.5 ± 1.3 d	5.3 ± 0.3 c	10.6 ± 0.8 c	45.2 ± 0.7 cd	55.9 ± 1.4 d	10.0 ± 0.9 cd
44	3476	21.4 ± 0.6 g	37.6 ± 0.8 e	59.1 ± 1.5 a	5.6 ± 0.1 c	18.9 ± 0.2 d	35.2 ± 0.5 e	54.3 ± 0.8 d	6.5 ± 0.1 e
47	3578	23.2 ± 0.3 f	30.8 ± 0.4 d	54.1 ± 0.8 e	5.5 ± 0.2 c	20.0 ± 0.4 d	35.3 ± 0.6 e	55.5 ± 1.0 d	6.3 ± 0.2 e

[a] Data are presented as mean ± SD (n = 4); values with different letters within the same column are significantly different at the α = 0.05 level by Tukey's multiple range test. [b] Sum of average daily temperature over 4 °C (from day of planting).

Table 2. Content of Soluble Carbohydrates (Inulin, Sucrose) and Dp_N of Inulin in Middle Early Varieties Topstar and Gigant[a]

harvest time		TOPSTAR				GIGANT			
weeks after planting	temp-sum[b] [°C]	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of i nulin average	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average
14	1733	1.9 ± 0.2 a	57.5 ± 0.6 ab	60.3 ± 0.9 abc	12.5 ± 0.1 ac	1.8 ± 0.1 a	61.9 ± 0.1 a	64.5 ± 0.2 a	13.2 ± 0.1 ab
17	2138	2.5 ± 0.1 a	58.2 ± 0.3 a	61.3 ± 0.5 abc	15.1 ± 0.3 b	2.7 ± 0.1 b	58.4 ± 0.3 bcd	62.2 ± 0.5 bc	14.4 ± 0.9 a
19	2444	3.5 ± 0.4 b	60.9 ± 0.7 a	64.7 ± 1.0 a	13.2 ± 0.2 ab	3.2 ± 0.1 b	57.7 ± 0.7 c	61.1 ± 0.9 b	13.1 ± 0.8 ab
22	2823	4.3 ± 0.1 c	56.3 ± 0.9 abc	60.8 ± 1.2 abc	12.4 ± 0.1 ac	4.0 ± 0.2 c	59.6 ± 0.5 d	63.8 ± 0.8 ac	13.4 ± 0.7 ab
25	3053	6.8 ± 0.2 d	55.0 ± 0.6 abc	62.1 ± 0.8 ab	10.3 ± 0.3 c	6.7 ± 0.4 d	55.9 ± 0.2 e	62.8 ± 0.7 abc	10.9 ± 0.4 b
29	3282	5.4 ± 0.4 e	50.6 ± 0.4 bc	56.1 ± 0.8 bc	6.5 ± 0.1 d	10.6 ± 0.3 e	52.4 ± 0.4 f	63.2 ± 0.8 ac	7.4 ± 0.1 c
33	3328	7.4 ± 0.1 d	49.7 ± 0.8 c	57.3 ± 1.0 b	5.5 ± 0.1 d	14.9 ± 0.2 f	41.3 ± 0.3 g	56.6 ± 0.6 d	7.7 ± 0.2 c
44	3476	15.9 ± 0.4 f	41.5 ± 0.5 d	57.7 ± 1.1 abc	6.5 ± 0.2 d	22.0 ± 0.4 g	31.4 ± 0.6 h	53.6 ± 1.1 e	7.4 ± 0.3 c
47	3578	14.3 ± 0.2 g	39.5 ± 0.4 d	54.2 ± 0.7 c	6.8 ± 0.4 d	20.9 ± 0.3 h	30.0 ± 0.2 h	51.1 ± 0.6 f	7.7 ± 0.2 c

[a] Data are presented as mean ± SD (n = 4); values with different letters within the same column are significantly different at the α = 0.05 level by Tukey's multiple range test. [b] Sum of average daily temperature over 4 °C (from day of planting).

Table 3. Content of Soluble Carbohydrates (Inulin, Sucrose) and Dp_N of Inulin in Late Varieties Violet De Rennes and Rozo[a]

harvest time		VIOLET de RENNES				ROTE ZONENKUGEL			
weeks after planting	temp-sum[b] [°C]	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average
14	1733	2.9 ± 0.2 a	46.2 ± 0.9 a	50.1 ± 1.2 a	13.5 ± 0.8 a	3.0 ± 0.1 a	63.4 ± 0.1 a	66.9 ± 0.3 a	13.2 ± 0.1 a
17	2138	3.5 ± 0.1 a	45.3 ± 0.1 a	49.4 ± 0.3 a	14.4 ± 0.4 a	3.1 ± 0.3 a	58.9 ± 0.1 b	63.0 ± 0.5 bc	11.3 ± 0.2 b
19	2444	3.2 ± 0.1 a	42.5 ± 0.4 b	46.2 ± 0.6 b	14.7 ± 0.9 a	3.1 ± 0.8 a	58.6 ± 0.9 b	62.7 ± 1.7 bc	10.2 ± 0.7 b
22	2823	3.9 ± 0.1 b	50.1 ± 0.9 cd	54.5 ± 1.1 c	14.6 ± 0.3 a	2.1 ± 0.1 a	59.0 ± 0.3 b	61.4 ± 0.5 c	11.3 ± 0.1 b
25	3053	4.7 ± 0.3 c	52.2 ± 0.1 d	57.0 ± 0.5 d	13.8 ± 0.2 a	3.5 ± 0.4 ab	52.4 ± 0.2 c	56.0 ± 0.7 d	10.7 ± 0.2 b
29	3282	7.5 ± 0.5 d	59.1 ± 0.6 e	66.9 ± 1.2 e	10.3 ± 0.3 b	5.2 ± 0.1 b	47.1 ± 0.7 d	52.5 ± 0.9 e	8.1 ± 0.4 c
33	3328	11.7 ± 0.4 e	54.0 ± 0.6 d	65.9 ± 1.1 e	7.4 ± 0.1 c	16.1 ± 0.4 cd	48.0 ± 0.9 d	64.2 ± 1.4 ab	9.3 ± 0.3 d
44	3476	15.4 ± 0.6 f	48.9 ± 0.2 c	64.4 ± 0.8 e	6.6 ± 0.2 c	16.9 ± 0.9 d	32.9 ± 0.1 e	49.9 ± 1.2 e	10.3 ± 0.4 b
47	3578	14.0 ± 0.4 g	50.5 ± 0.2 cd	64.7 ± 0.7 e	6.9 ± 0.4 c	15.5 ± 0.6 c	36.6 ± 0.3 f	52.3 ± 1.1 e	8.2 ± 0.6 c

[a] Data are presented as mean ± SD (n = 4), values with different letters within the same column are significantly different at the α = 0.05 level by Tukey's multiple range test. [b] Sum of average daily temperature over 4 °C (from day of planting).

hibernation of tubers in the soil (week 44 and 47) the level of sucrose and inulin was hardly changed compared to the previous harvest in December (week 33). The reduction of yield and dry matter in the tubers during hibernation evidenced the use of soluble carbohydrates for respiration and lignification processes (*19*). The change of the carbohydrate profile in the early cultivar Bianka was more continuous during the storage of the adult tubers in the soil: In the 25. week—similar to Bella—the level of sucrose increased to 9.1% of DM, but in the following weeks this value rose only slowly to 11.6% (29 week) and stayed stable during the frost period resulting in 10.6% of DM after 33 weeks; in this period, the content of inulin was reduced to 43–47% with a dp_n between 8 and 11; but after hibernation the profile of carbohydrates had changed to a high level of sucrose (20% of DM) and a distinctly reduced level of inulin (35% of DM); also in Bianka the assimilation processes could be recognized by yield and dry matter changes in the tubers (*34*).

Middle early varieties, Topstar and Gigant, showed different effects on carbohydrate profile during the storage of tubers in the soil; Topstar achieved a low sucrose level in the fall period (29–33 weeks) with an acceptable content of inulin in the range of 50% of DM; however, a definitely reduced dp_n of 6 for inulin; during hibernation in the soil the level of sucrose increased to the double amount of about 15%, and accordingly, inulin was reduced to a level of about 40% of DM with the same dp_n of 6; in the Gigant cultivar, sucrose level arose noticeably to 15% during the fall period, and at the same time inulin level was reduced to about 40% of DM; during hibernation the effect of

Table 4. Content of Soluble Carbohydrates (Inulin, Sucrose) and Dp_N of Inulin in Late Variety Waldspindel[a]

harvest time		WALDSPINDEL			
weeks after planting	temp-sum[b] [°C]	sucrose [g/100 g]	inulin [g/100 g]	Σsoluble carbohydrate [g/100 g]	dp_n of inulin average
14	1733	nd	nd	nd	nd
17	2138	2.7 ± 0.1 a	43.4 ± 0.6 a	46.4 ± 0.9 a	18.4 ± 0.6 a
19	2444	2.8 ± 0.1 a	44.3 ± 0.9 ab	47.4 ± 1.0 abc	17.8 ± 0.5 a
22	2823	2.5 ± 0.2 a	48.7 ± 0.2 b	51.4 ± 0.5 c	15.5 ± 0.9 b
25	3053	6.2 ± 0.3 b	44.1 ± 0.8 b	50.5 ± 1.2 ac	14.2 ± 0.9 b
29	3282	7.6 ± 0.2 c	43.6 ± 0.9 a	51.3 ± 1.3 bc	11.3 ± 0.6 c
33	3328	12.6 ± 0.7 d	43.9 ± 0.9 a	56.6 ± 1.8 d	7.0 ± 0.3 de
44	3476	11.4 ± 0.5 e	43.3 ± 0.5 a	54.9 ± 1.3 cd	7.3 ± 0.1 d
47	3578	10.6 ± 0.2 f	30.6 ± 0.4 c	53.9 ± 0.9 cd	5.5 ± 0.2 e

[a] Data are presented as mean ± SD ($n = 4$); values with different letters within the same column are significantly different at the $\alpha = 0.05$ level by Tukey's multiple range test. [b] Sum of average daily temperature over 4 °C (from day of planting).

depolymerization resulted in a high content of sucrose (22%) and extremely reduced level of inulin (31%); but the dp_n of inulin with 7.7 was relatively high and constant during 29–47 week after planting the tubers.

For the late varieties, a decrease of sucrose to about 12–16% of DM was found while harvesting in the midst of December (33 weeks); simultaneously the content of inulin was in the range of 44–54% of DM, similar to the results at earlier harvest terms; in the cultivars Waldspindel and Violet de Rennes, the dp_ns of inulin were on a level of 7, compared to Rozo where a noticeable increase of the dp_n of inulin to 9.3 was found. During hibernation in the soil, the carbohydrate profile changed depending on the cultivar: In Waldspindel similar levels of sucrose, inulin and dp_n of inulin were found at the beginning of March (44 weeks) compared to the last harvest term in fall (33 weeks). Later on, 47 weeks after planting of tubers, the profile changed drastically to the doubled level of sucrose (23% of DM) and an extensively reduced content of inulin (30%) with a dp_n of 5.5. In the beginning of March (44 weeks) the levels of sucrose and inulin in Violet de Rennes were found to be 15 and 50% of DM, respectively with an inulin dp_n of 7 and were constant up till the end of March (47 weeks). In Rozo, the level of sucrose (16%) remained constant, whereas the content of inulin was reduced to 35% with a relatively high dp_n of about 9.

Statistical Evaluation of Soluble Carbohydrates. The statistical evaluation of soluble carbohydrates - total content, sucrose, inulin (dp ≥ 3, fructooligosaccharides included), and dp_n of inulin, in the tubers of Jerusalem artichoke during different seasons, was done by Tukey's multiple range test (**Tables 1–4**). Keeping in mind the fact that the concentration of monosaccharides remained extremely low independent of the seasonal variation, the changes of soluble carbohydrate profiles were mainly determined by calculating sucrose and inulin levels in the tubers. At first instance a significant correlation between sucrose and inulin values (determined by linear regression analysis) was found when all the cultivars were taken into account ($r = -0.804$**). Sucrose and inulin content of the individual varieties, however, showed very different interactions (**Figure 1**). To interpret more precisely the cultivars were divided in early and middle early varieties on the one side and late varieties on the other side for the regression analysis. For the first group—Bella, Bianka, Topstar, Gigant—a highly significant relationship between the content of sucrose and inulin in the tubers ($r = -0.952$**) was found (**Figure 2**). Determination of the mathematical equation for this linear regression $y = -1.35x + 62.32$ thus opens the possibility to estimate the inulin content directly from the amount of sucrose and vice versa. For the second group—Waldspindel, Violet de Rennes and Rozo—the significance of the relation between the content

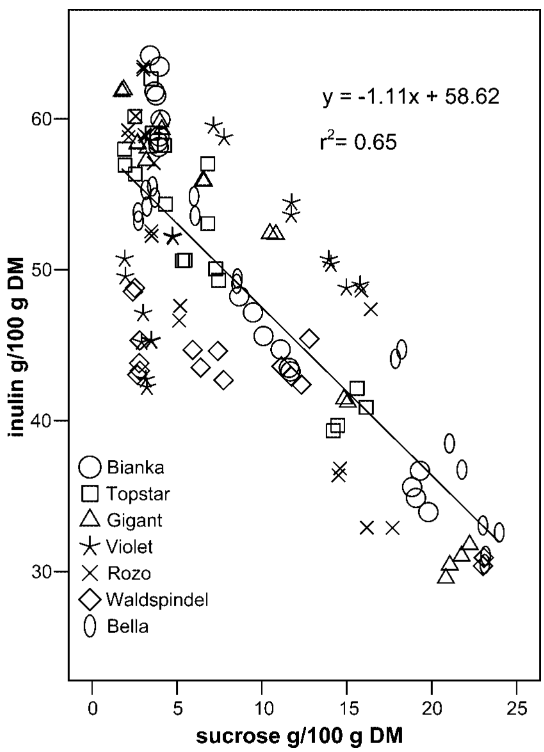

Figure 1. Linear regression between content of sucrose and inulin in tubers taking into account all investigated varieties of Jerusalem artichoke.

of sucrose and inulin was distinctly reduced compared to the earlier cultivars ($r = -0.502$**). **Figure 3** presents the linear regression between the concentration of sucrose and dp_n of inulin ($r = -0.806$**) applied for all the cultivars. The significance of correlation between sucrose and dp_n of inulin was slightly higher ($r = -0.845$**) in the first group (early varieties) and slightly lower ($r = -0.767$**) in the group of late varieties than in the group comprising all the cultivars.

Yield of Soluble Carbohydrate/ha with Respect to Dp_N of Inulin. The yield of soluble carbohydrate/ha and the profile of carbohydrate are essential for the utilization of Jerusalem artichoke tubers in industrial processes (*13, 29–31*). Apart from the *content* of sucrose and inulin the *quality* of inulin indicating the distribution of low to high polymeric inulin represents a further important factor for tailor-made products from Jerusalem artichoke.

In **Figure 4**, the yield of soluble carbohydrates in t/ha of DM and the dp_n of inulin from the applied varieties during the investigated seasons can be seen. The early varieties, Bella and

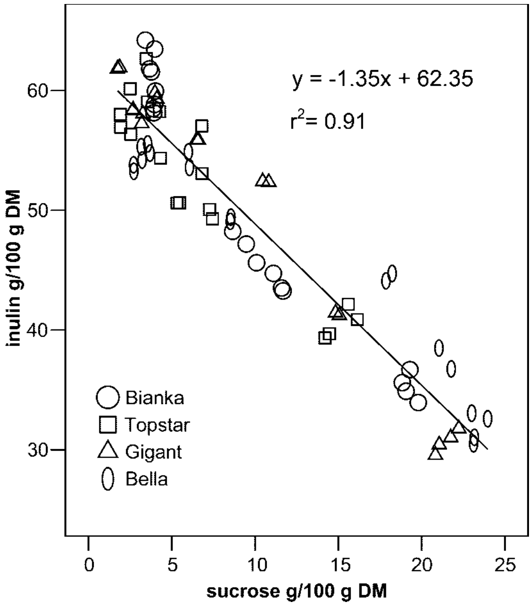

Figure 2. Linear regression between content of sucrose and inulin in tubers taking into account early and middle early varieties of Jerusalem artichoke.

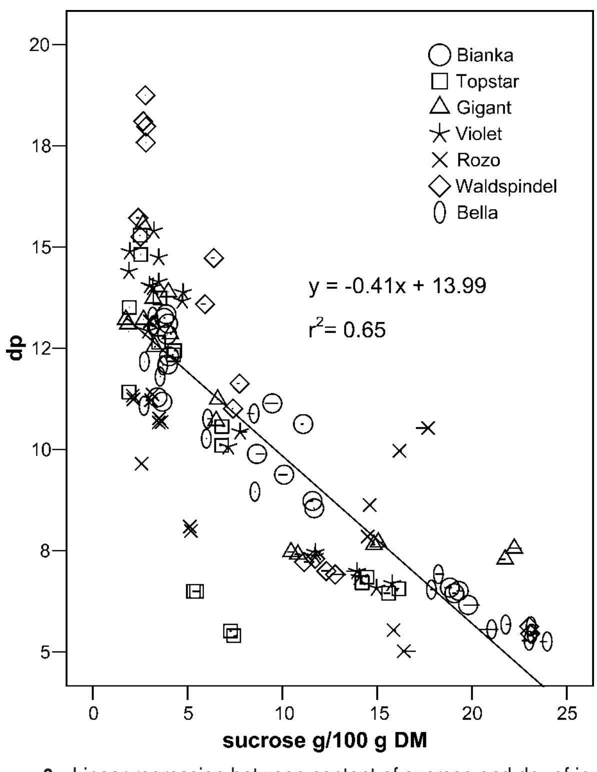

Figure 3. Linear regression between content of sucrose and dp_n of inulin in tubers taking into account all investigated varieties of Jerusalem artichoke.

Bianka, possessed the highest yield of soluble carbohydrates with a high dp_n of inulin in the weeks 19–26 after planting as expected.

The middle early varieties, Topstar and Gigant, showed high differences in the yield but possessed characteristics similar to the earlier varieties. The content of soluble carbohydrates in Gigant was twice as high as in Topstar.

The late varieties differed clearly from the earlier varieties as well as amongst each other: Waldspindel showed an acceptable yield of carbohydrates (weeks 25–33 after planting) with higher dp_n of inulin at the beginning of the harvest period. Violet de Rennes and Rozo started extremely late with higher yields of carbohydrate (29 weeks after planting). Clear difference between both varieties was the distinctly higher dp_n of inulin in Rozo during the winter time and thus in the spring harvest.

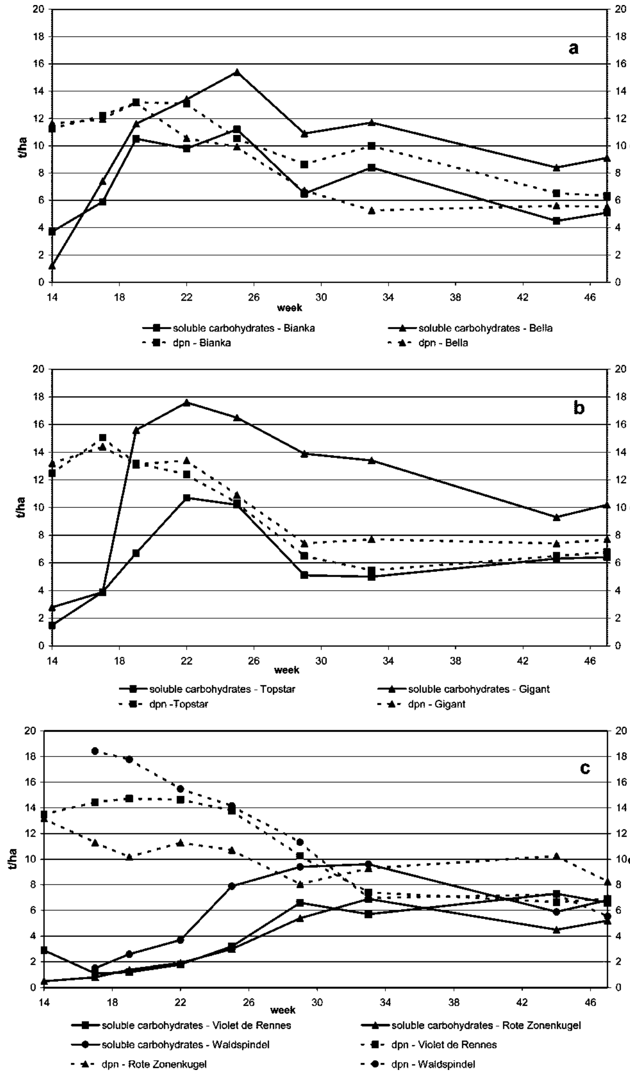

Figure 4. Seasonal change of soluble carbohydrates in tubers (yield t/ha of DM) and dp_n of inulin in early (a), middle early (b) and late (c) varieties of Jerusalem artichoke.

Content of Total Protein and Peroxidase Activity. The total content of protein in the varieties also showed significant differences at the different harvest terms (**Table 5**). For the early varieties, Bella and Bianka, protein levels were in a similar range between 9 and 12% of DM with average values of 9.7 and 10.5%. In the middle early varieties significant differences were found between the cultivars. Topstar delivered results between 6.3 and 8.4% with a mean of 7.8% of DM, however Gigant contained more protein between 7.8 and 11.1% with a mean of 9.7% of DM. In the late varieties the middle late cultivar Waldspindel showed clearly higher content of protein (range: 10.6–12.2%, mean: 11.3% of DM) compared to the late cultivars Violet de Rennes (range, 7.1–9.4%; mean: 8.4% of DM) and Rozo (range, 7.7–11.6%; mean, 8.9% of DM).

The increase in the activity of polyphenoloxidase or of peroxidase in the tubers can be applied as indicator for stress (*19*). But in general, the level of activities is genetically

Table 5. Seasonal Change of Protein Content in the Investigated Varieties [G/100 G Dm][a]

weeks after planting	temp-sum[b] [°C]	Bella	Bianka	Topstar	Gigant	Violet de Rennes	Waldspindel	Rozo
14	1733	9.6 ± 0.1 a	10.5 ± 0.1 a	8.3 ± 0.2 a	8.5 ± 0.2 a	8.0 ± 0.4 a	n.d.	10.0 ± 0.4 a
17	2138	8.1 ± 0.2 b	9.4 ± 0.6 b	6.7 ± 0.1 b	7.8 ± 0.3 b	7.7 ± 0.4 b	11.3 ± 0.4 a	8.2 ± 0.8 b
19	2444	8.4 ± 0.1 b	9.3 ± 0.2 b	6.3 ± 0.1 c	9.6 ± 0.1 c	9.4 ± 0.1 c	10.6 ± 0.1 b	7.9 ± 0.1 b
22	2823	9.8 ± 0.2 b	10.2 ± 0.1 c	8.0 ± 0.3 d	9.9 ± 0.4 d	8.9 ± 0.4 d	11.7 ± 0.4 c	7.7 ± 0.4 c
25	3053	10.8 ± 0.1 c	9.9 ± 0.1 d	8.4 ± 0.1 a	9.8 ± 0.1 d	9.3 ± 0.2 c	11.1 ± 0.1 d	8.5 ± 0.2 d
29	3282	10.7 ± 0.1 c	12.4 ± 0.4 e	8.3 ± 0.2 a	10.8 ± 0.3 e	8.5 ± 0.4 e	10.9 ± 0.8 e	9.0 ± 0.1 e
33	3328	10.2 ± 0.1 d	9.0 ± 0.2 f	7.9 ± 0.6 d	9.7 ± 0.1 d	9.1 ± 0.1 c	11.3 ± 0.1 a	11.6 ± 0.4 f
44	3476	9.5 ± 0.1 a	10.8 ± 0.1 a	8.1 ± 0.7 e	11.1 ± 0.4 e	7.4 ± 0.8 f	12.2 ± 0.7 f	9.2 ± 0.3 e
47	3578	10.6 ± 0.1 cd	12.8 ± 0.3 e	7.9 ± 0.4 d	10.3 ± 0.1 e	7.1 ± 0.1 g	11.0 ± 0.1 d	8.2 ± 0.1 b

[a] Data are presented as mean ± SD ($n = 4$); values with different letters within the same column are significantly different at the $\alpha = 0.05$ level by Tukey's multiple range test. [b] Sum of average daily temperature over 4 °C (from day of planting).

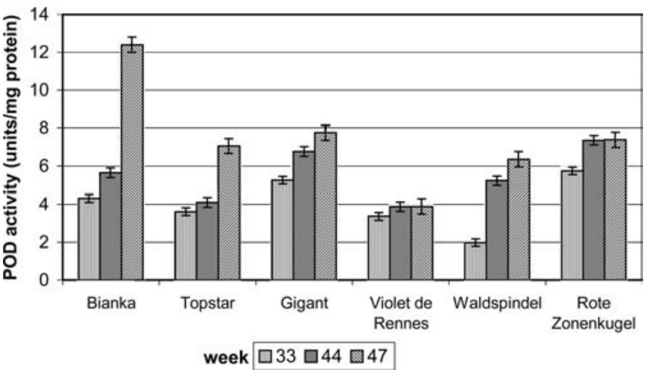

Figure 5. Peroxidase activity (U/mg soluble protein) in tubers before (week 33) and after wintertime (week 44, week 47).

predestined and therefore often extremely different within the varieties. In the first phase of development of tubers during a high accumulation of fructan the peroxidase activity is generally reduced to minimum. When inulin starts to depolymerize and respiration increases the activity of peroxidase is enhanced in adult tubers (*32*). For more information concerning chilling stress peroxidase activity (units/mg soluble protein) of tubers of early and late cultivars were investigated before and after winter time (**Figure 5**). The middle early varieties, Topstar and Gigant, showed a slightly increased activity during winter time. The Topstar cultivar doubled its activity in the spring period, Gigant, however, hardly raised the activity of peroxidase till spring. The middle late variety, Waldspindel, initially had low activity, which changed rapidly to higher levels during winter time and showed only a slight increase in the spring period. The late varieties, Violet de Rennes and Rozo, showed both minor changing of activities during winter and spring period each, but the peroxidase activity in Violet was only half of that in Rozo. This minor increase of peroxidase activity indicates a high stability of these tubers against chilling stress favouring their application after spring harvesting.

Concluding the obtained informations on carbohydrate composition of Jerusalem artichoke considering the yield (t/ha of DM) and on seasonal dependence of carbohydrate profile in different varieties allow a definite choice of harvest time for a suitable processing of tubers proposing a broad range for application as prebiotic and novel food.

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