Vol.62: e19180439, 2019,
http://dx.doi.org/10.1590/1678-4324-2019180439
ISSN 1678-4324 Online Edition

Article - Biological and Applied Sciences

Relationship Between Parameters of Development and Functional Compounds of Yacon Leaves

Bruna Mayara Roldão Ferreira[1]
https://orcid.org/0000-0002-6980-3181

João Luiz Andreotti Dagostin[1]
https://orcid.org/0000-0002-5366-8712

Eriel Forville de Andrade[1]
https://orcid.org/0000-0002-5572-3690

Thiago Atsushi Takashina[1]
https://orcid.org/0000-0002-9625-3429

Luciana de Souza Neves Ellendersen[2]
https://orcid.org/0000-0001-9248-3203

Maria Lucia Masson[1*]
https://orcid.org/0000-0001-6574-6477

[1]Federal University of Paraná, Department of Chemical Engineering, Curitiba, PR, Brazil; [2]Federal University of Paraná, Department of Forestry Engineering, Curitiba, PR, Brazil;

Received: 2018.08.17; Accepted: 2018.06.17.

*****Correspondence: joaodagostin@hotmail.com; Tel.: +55-41-3361-3232 (J.L.A.D.)

HIGHLIGHTS

- Yacon leaves at the apical and middle of the plant present higher phenolic content.
- Phenolic content showed correlations with the greenness of the leaves.
- The decoction of yacon leaves can extract more phenolics than the infusion.

Abstract: Yacon is a tuberous root from the Andean region being increasingly grown across the world due to the low caloric values of their roots and the functional properties of extractions of its leaves. However, there is still a gap in the knowledge of how the plant develops, and if physical and chemical changes are noticed regarding maturity. The subject of this work was to investigate the dynamics of a group of parameters and to establish correlations across them. To achieve this goal, leaves collected from three different parts of the plant in three months were assessed regarding size (plant height and leaf area), color and the presence of sesquiterpene lactones. Different methods of extraction were also studied and the total phenolic content was analyzed. The analysis of the results revealed different patterns in the quantity, size and leaf distribution according to the age of the plant. From three different methods of extraction studied, decoction seems to be the most appropriate one to obtain phenolic compounds with greater yield and safety. Besides, the greenness of yacon leaves showed a linear correlation to the total phenolic content, being a simple and promising estimator of the corresponding bioactive group. Sesquiterpene lactones were identified in all extracts, indicating the ease of their extractability even in simple procedures of tea-making.

Keywords: *Smallanthus sonchifolius*; sesquiterpene lactones; physical parameters; chemical parameters; phenolic content.

INTRODUCTION

Yacon, *Smallanthus sonchifolius* (Poepp & Endl.) H. Robinson is a herbaceous perennial plant native to South America that belongs to the Asteraceae family. The plant has been used since pre-Columbian times in the Andean region and its cultivation and consumption have expanded in recent decades to several Asian and European countries. The Asian dispersal began in Japan, where the use of a different part of the plant, its leaves, emerged apparently for the first time as a new anti-diabetic herbal tea [1]. Extracts of dried yacon leaves present a variety of pharmacological activities, including antimicrobial [2,3], anti-inflammatory [4], antioxidant and free radical scavenging properties [5,6], in addition to antitumor, analgesic, antiparasitic and cardiotonic properties [7]. Leaves and stems are known to contain significant amounts of sesquiterpene lactones (SLs), flavonoids and phenolic compounds (as chlorogenic, caffeic and ferrulic acids), what makes it a highly appreciated source of biologically functional compounds [4,8–14].

The optimal use of yacon leaves as basis for teas and extracts may depend on plant development and how they are processed. Although there are recommendations regarding plant management, it is still arbitrary the way leaves are collected and consumed, making it necessary to understand the plant development and conditions that may favor economic and functional yield. Some of these recommendations include: **1.** Harvesting should occur preferentially after 2 – 2.5 months after planting; **2.** Leaves should be harvested when they reach maturity; and **3.** One should not collect all leaves from the plant, since this will impart the formation of new leaves and plant development [15]. Although important, the guidelines found for yacon leaves harvesting little say about physical and chemical aspects of the material. In order to improve understanding of how yacon leaves develop and establish base parameters for their harvest, this study aimed to gather data of physical (plant height, leaves count, leaf area and color parameters) and chemical parameters (total phenolic content and indirect SL identification) from *in natura* leaves and leaf extracts of yacon. The period of the study comprised 3 months, starting from the 2^{nd} month after planting, as recommended. Yacon leaves were also categorized in three groups for the analyses, according to their age/position in plant. Response variables were correlated as a means of finding predictive parameters of interest.

MATERIAL AND METHODS

Material and Methods should be described with sufficient details to allow others to replicate and build on published results. Please note that publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information in cover letter. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. This section may be divided by subheadings as reported below.

Plant Material

Yacon leaves were harvested during summer in São José dos Pinhais, Brazil, located at 25º 37' 14'' latitude, 49° 07' 40'' W (960 m). Monthly climate conditions for this area are shown in Table 1. The plants analyzed are of clonal origin, what diminishes issues regarding genetic variability. Fifteen plants were randomly chosen for sampling on each month of the season. All leaves from the selected plants were excised, accounting for a total of 675 leaves (Table 1).

Table 1. Collection periods and amount of leaves collected.

			Climate parameters[a]			
Period	Plants Quantified	Leaves Quantified	Sun hours per month	Total precipitation (mm)	Maximum average temperature	Minimum average temperature
1st month	15	185	158.2	254.6	28.6	18.8
2nd month	15	249	161.5	77.6	25.1	16.7
3rd month	15	241	124.9	197.7	26.5	18.1

[a] INMET – Brazilian National Institute of Meteorology [16]

Prior to excision, each plant was divided into three parts: upper (Group A), middle (Group B) and lower (Group C), being their leaves categorized accordingly. Thus, the analyses performed also took into account the position and maturity of the leaves in relation to sun exposure. Figure 1 illustrates the methodology design performed in this work.

Leaf size (area)

Yacon leaves were measured through digital imaging processing. Firstly the samples were subjected to black and white image scanning to obtain the image files. Later, the images were opened using ImageJ software [17], an open platform for scientific image analysis. All images were treated for holes and failures correction using the “Fill Holes” tool of ImageJ software (voids are filled with the same color pattern) and the area of each leaf was calculated.

Color Determination

Color assessments were performed using a MiniScan XE Plus spectrophotometer (Hunter Associates Laboratory, Inc., Reston, USA) set to the CIELab color system (CIE76, International Commission on Illumination).

Leaf processing

Prior to extraction, the yacon leaves of each group and month were dried at 40 °C for 24 hours in a convective oven (Fabbe Ltda, São Paulo, Brazil) applying an average air flow of 18 $m^3.h^{-1}$ in a circulating/recirculating air system [8]. After dehydration, the leaves were mixed and crushed in a four-blade crusher and stored at 10 °C. Three different extraction procedures were used on samples of 2 g of dried yacon leaves: Soxhlet extraction, decoction and infusion (the two last simulating household extraction procedures) according to Genta *et al*. [9], Barcellona *et al*. [8] and Valentová *et al*. [6].

a) *Soxhlet extraction (organic fraction)*. Processed leaves were subjected to Soxhlet extraction using methanol as solvent (300 mL, 120 h). After extraction the solvent was recovered, the solid extract was dispersed in water (100 mL) and chlorophyll was removed by a triple extraction using petroleum ether (50 mL). The aqueous layer was then acidified with 10 mL H_3PO_4 0.01 M and extracted with ethyl acetate (5 × 50 mL)

b) *Decoction*. Dried yacon leaves were kept in 100 mL of boiling distilled water under reflux for 20 min, left to cool to 25 °C and then filtered.

c) *Infusion*. Boiling distilled water (100 mL) was poured onto dried yacon leaves and then allowed to extract until cooling to 25 °C.

Every extract solution was oven-dried at 40 °C until constant weight. Extraction methods were performed in triplicate for each sample. Total phenolic content measurements were used to verify the reproducibility of each extraction method.

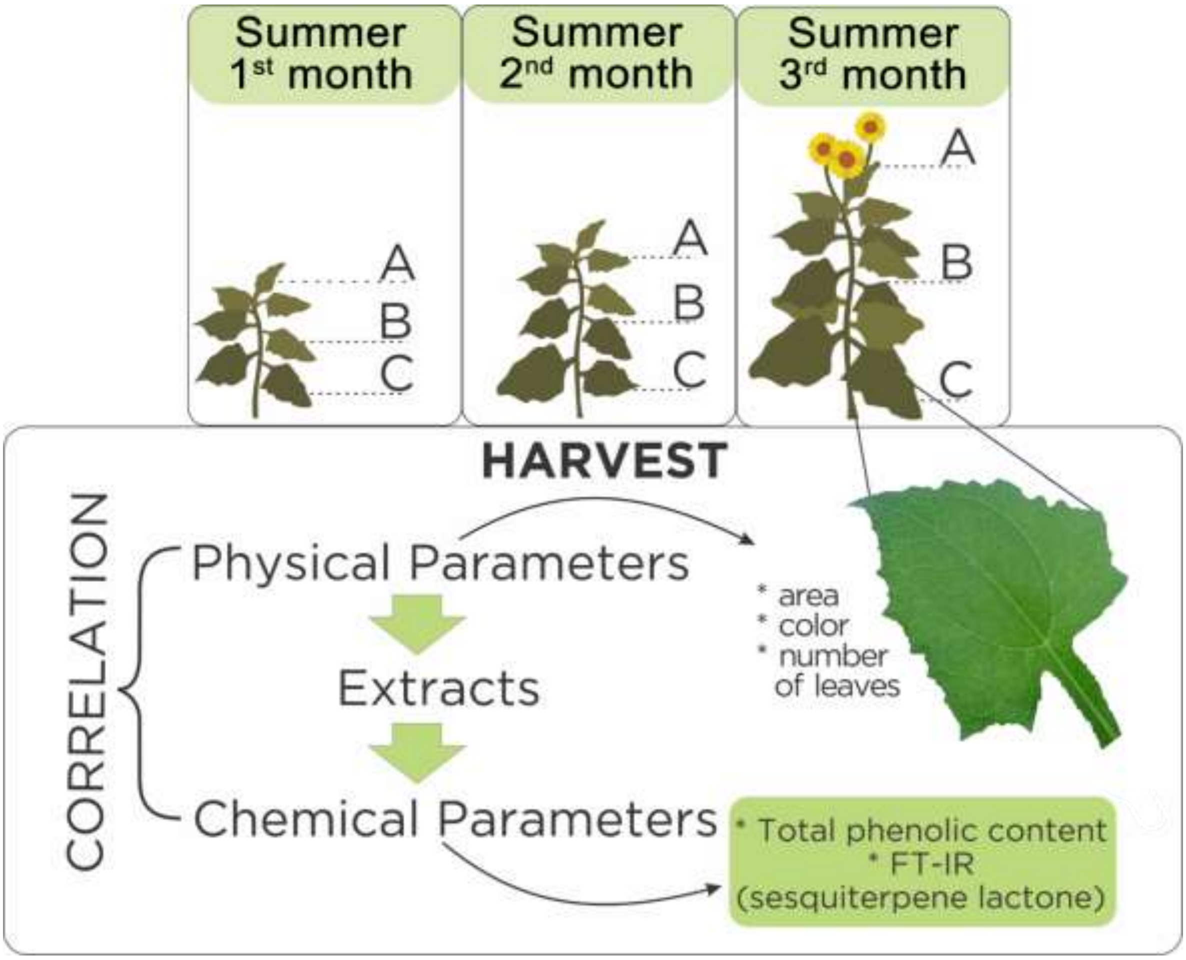

Figure 1. Illustrative scheme of the procedures performed in sample collection and analysis.

Total phenolic content

Total phenolic content of all extracts was determined following the Folin-Ciocalteau (gallic acid equivalent) colorimetric method [18]. Firstly, the Folin–Ciocalteu reagent was diluted 10-fold with distilled water and kept protected from light at 4 °C. The diluted reactant was mixed with 0.5 mL of the extract solution and 2.0 mL of Na_2CO_3 (75 $g.L^{-1}$). After incubation for 5 min at 50 °C, the absorbance was measured at 760 nm. Results were expressed as gallic acid equivalents. A standard curve was drawn using gallic acid solutions (8 - 80 mg gallic acid/L distilled water) as reference. Total phenolic content was expressed as gallic acid equivalents (GAE) per gram of dry weight (mg GAE. g^{-1}).

Extracts of sesquiterpene lactones

Sesquiterpene lactones (SLs) were extracted and purified according to Genta *et al*. [9]. The method allows the extraction of the target compounds from glandular trichomes and their subsequent purification [8].

Infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) analyses were performed in a Nicolet 6700 (Thermo Scientific). Samples of 1 – 3 mg were mixed into KBr (150 mg) powder, finely pulverized and put into the die of a 10 ton CrushIR digital hydraulic press (PIKE technologies) to form the pellets. The analysis was performed in triplicate for each sample type.

Statistical Analysis

Results for the quantitative variables were expressed as mean ± SD or SE, as indicated in each case. For physical parameters comparison (plant height, amount of leaves, leaf area, and color-derived parameters) and phenolic content verified in yacon leaves collected during three months, the homogeneity of variances was verified through the Brown-Forsythe test. Significant differences between groups were tested by the analysis of variance for repeated measures (RM-ANOVA). For comparing groups A, B and C in the same month, the one-way ANOVA was used.

Tukey's least significant difference test was used for statistically significant differences ($p<0.05$) between means. Statistical correlations based on Pearson's correlation coefficient (parametric data) were calculated to verify the existence and intensity of association between response variables. A p-value of 0.05 was adopted to reject ($p<0.05$) or accept ($p\geq0.05$) the null hypothesis. All analyses were performed using the software Statistica 7.0 (Statsoft, Tulsa, USA).

RESULTS AND DISCUSSION

Physical Parameters

The physical parameters used in the evaluation of yacon plant development include plant height, number of leaves, leaf size (are), and leaf color.

Plant Height

During the period of analyses, the average height of yacon plants varied considerably (Table 2, $p<0.05$). From the first to the second month of summer they grew approximately 64.66 cm (84% difference), and form the second to the third month their growth decreased by half, corresponding to an average of 30.07 cm (21% difference). As the plants were planted in the beginning of the second month of spring (two months before the first month of analysis), it can be noticed by the data obtained that the substantial growth of the plants occurred right on the period evaluated (summer, third to fifth month after planting). Yacon plants usually achieve 2 meters [19], which indicates that in the end of summer they are reaching their maturity, when flowers start growing. Their cycle ends in the middle to the end of autumn.

Due to the insufficient amount of data, it is impossible to establish a precise correlation of plant growth rate with climate conditions (Table1). However, these data were presented to serve as a basis for future works.

Table 2. Yacon plant height evolution during the period of study.

Month (summer)	Plant height (cm)
1st	77.07 ± 5.02^{c}
2nd	141.73 ± 7.68^{b}
3rd	171.80 ± 9.20^{a}

Different lowercase letters for different months represent statistically different mean values by the SD ($p<0.05$).

Number of Leaves

Yacon plants presented statistically equal ($p<0.05$) amounts of leaves between the second and third months of summer for Groups A and B, which were higher than in the beginning of the season (Table 3). As the two groups include the younger leaves, these data may indicate an accelerated growth towards maturity, since the plant finish its cycle in autumn. No significant differences ($p<0.05$) in the amount of leaves were observed between the three-month analysis for Group C. This result was expected once new leaves can only appear at the superior parts of the plant. It also indicates that there was no significant loss of leaves during the period studied.

Table 3. Statistical comparison between months and groups of yacon leaves: number of leaves, area and color parameters.

	Month (summer)	Group A	Group B	Group C
Number of Leaves	1st	6.80 ± 1.01^{bA}	2.53 ± 0.92^{bB}	3.00 ± 0.93^{aB}
	2nd	8.87 ± 1.46^{aA}	4.00 ± 1.07^{aB}	3.67 ± 1.11^{aB}
	3rd	9.07 ± 1.03^{aA}	4.00 ± 0.00^{aB}	2.93 ± 1.22^{aC}
Leaf Area (cm^2)	1st	229.36 ± 37.51^{bB}	279.91 ± 62.27^{aA}	262.35 ± 54.12^{aA}
	2nd	227.27 ± 23.08^{bA}	233.25 ± 39.91^{bA}	254.97 ± 49.31^{aA}
	3rd	262.82 ± 21.48^{aB}	293.59 ± 17.32^{aA}	261.87 ± 38.22^{aB}
L*	1st	32.80 ± 0.97^{bC}	33.75 ± 1.31^{aB}	36.67 ± 1.20^{aA}
	2nd	33.89 ± 1.36^{aA}	31.70 ± 0.59^{bB}	33.74 ± 1.20^{bA}
	3rd	32.93 ± 0.72^{bA}	33.08 ± 0.74^{aA}	33.20 ± 1.10^{bA}
a*	1st	-7.82 ± 0.39^{aA}	-7.68 ± 0.56^{aA}	-8.28 ± 0.70^{aB}
	2nd	-7.59 ± 0.76^{aA}	-7.34 ± 0.51^{abA}	-7.60 ± 0.49^{bA}
	3rd	-7.49 ± 0.37^{aB}	-7.20 ± 0.56^{bB}	-6.48 ± 0.46^{cA}
b*	1st	14.25 ± 0.51^{cB}	14.64 ± 2.05^{abB}	18.36 ± 1.59^{aA}
	2nd	18.39 ± 1.18^{aA}	13.54 ± 1.11^{bB}	15.77 ± 1.25^{bC}
	3rd	16.88 ± 1.02^{bA}	15.54 ± 1.54^{aB}	13.98 ± 0.76^{cC}

Different lowercase letters in the same column, for different months, represent statistically different mean values ($p<0.05$). Different uppercase letters in the same line, for the same month, represent statistically different mean values ($p<0.05$). Data represent mean ± SD.

The pattern presented between groups A, B and C on every month shows a very higher concentration of leaves at the superior part of the plant: two to three-fold the amount of leaves from the lower parts. While group A results were all significantly higher, groups B and C presented practically the same amount of leaves.

Leaf size (area)

Yacon leaves presented a wide variation in size, from 132.62 to 438.95 cm^2, and an average area of 256.53 cm^2 during the whole period of the study (Table 3). This variability can be seen in the resulting standard deviations – mainly for groups B and C -, which varied from 6 to 22% of the mean values. In general, as the plant develops it was possible to verify that the leaf size growths accordingly from group A to group B. Once yacon plants possess large leaves, this rapid increase is a mean of achieving the ideal size to capture adequate amounts of sunlight.

Reaching the final plant height (month 3), younger leaves (group A) were not only present in higher counts but also presented higher areas. At this stage the plant slows down its growth, and those younger leaves tend to remain at the top for longer. This justifies the higher mean leaf size found on the last month for group A samples. Data relating groups B and C indicate the leaves had already achieved their final conformation. Differences found in group B over time are probably related to the spatial position of the group: it has its limits with younger leaves and older leaves. Moreover, this group presents a higher variation due to plant growth, which makes its limitations to oscillate more dynamically.

Color parameters

Different shades of green were found in yacon leaves for different groups and periods of the study. Table 3 shows the different values found in their color by the CIELab color map.

Higher variations were found in Group C for all parameters in general, probably a result of the aging of leaves. Regarding parameter a*, higher values were found for every group of leaves within time, indicating the loss of greenness as the plant reaches maturity. Despite being a common practice, the single use of values from color parameters (Table 3) as means of discussion is a difficult task, with little intuitiveness. To actually observe color variations, CIELab values were converted to the sRGB color space (to the most approximate values possible), as shown in the Figure 2. In this image it is possible to check how the color of yacon leaves are perceived and differentiated by the eye. Placing the colors side by side it is possible to verify some very close hues, such as those of Group A – 1st month and Group B – 2nd month, and also the pair of Group B- 1st month and Group C – 2nd month. This trend may suggest that the color of yacon leaves do not change substantially during their rapid growing (first to second months studied), since similar colors were verified from Group A to B and from Group B to C during this period. Evidently, this is an assumption that should be explored in a broader way to guarantee its veracity. When reaching maturity (3rd month) the color of leaves seem to have stabilized, appearing as virtually indistinguishable colors between all groups analyzed.

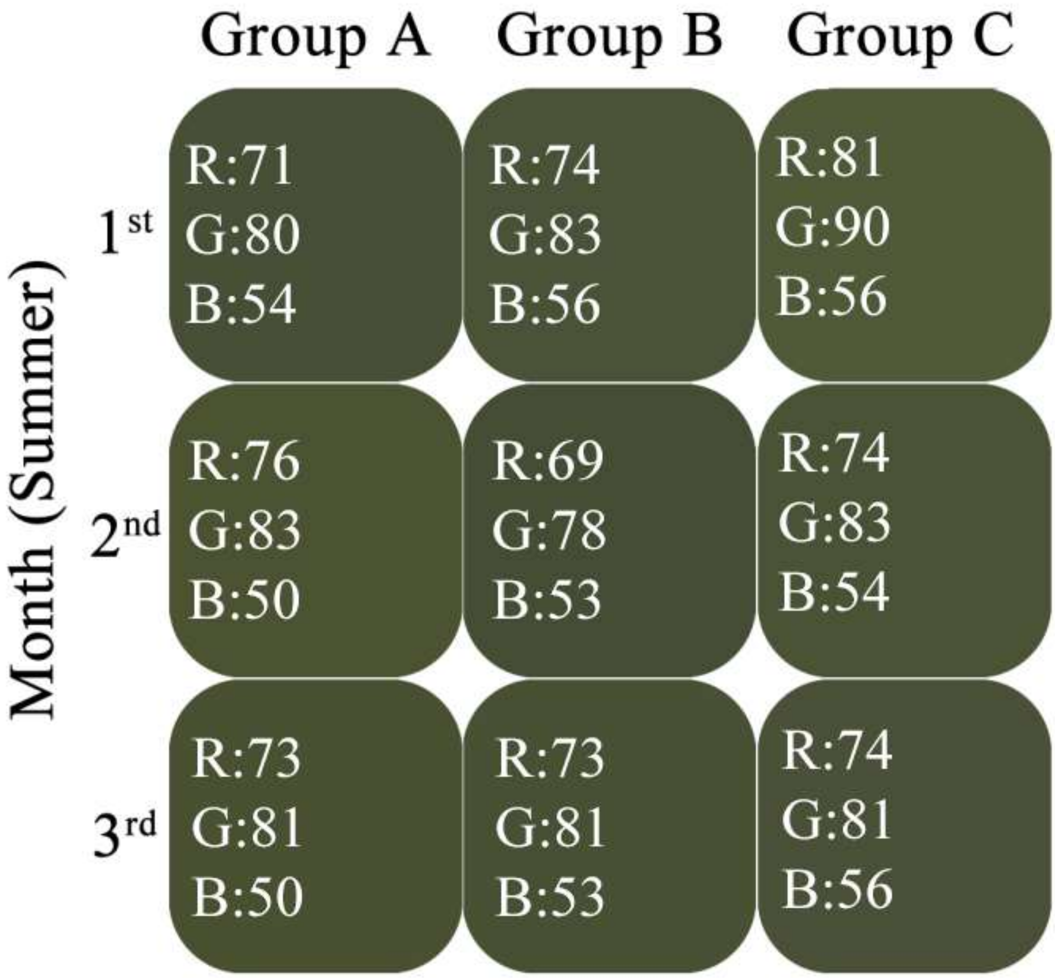

Figure 2. Approximate color of yacon leaves (3 to 5-month old) verified in summer for Groups A, B and C. Colors represent approximations of the CIELab values to the sRGB color system.

Chemical parameters

Total phenolic content (TPC)

Total phenolic content of yacon leaves are shown in Table 4. Mean values for Soxhlet, decoction and infusion were 14.22 to 27.13, 13.46 to 24.65, and 5.98 to 13.97 mg GAE.g^{-1}, respectively, during the whole period of study. These results are in accordance with those of Russo *et al*. [20], who investigated the total phenolic content (TPC) of leaves from five yacon varieties. They have found 14.0 to 37.4 mg GAE.g^{-1} and a mean 25.08 mg GAE.g^{-1} in the extract obtained by decoction. Moreover, another group of researchers found 24.6, 10.7 and 11.8 mg GAE.g^{-1} in extracts of yacon leaves obtained by methanol, decoction and infusion, respectively [6,13].

Table 4. Total phenolic content (mg GAE.g^{-1}) of yacon leaves for the three groups evaluated.

	Month (summer)	**Method of extraction**		
		Soxhlet	**Decoction**	**Infusion**
Group A	1st	27.13 ± 0.23^{aA}	20.92 ± 0.50^{bB}	10.93 ± 1.08^{aC}
	2nd	21.86 ± 0.42^{bB}	23.61 ± 0.12^{aA}	8.54 ± 0.65^{bC}
	3rd	20.15 ± 0.35^{aB}	23.50 ± 0.64^{aA}	13.97 ± 0.53^{aC}
Group B	1st	18.41 ± 0.42^{bB}	22.73 ± 0.59^{abA}	11.95 ± 1.69^{aC}
	2nd	24.66 ± 0.52^{aA}	18.45 ± 0.29^{aB}	9.25 ± 0.57^{abC}
	3rd	19.05 ± 0.31^{bA}	16.56 ± 0.05^{bB}	10.67 ± 0.55^{bC}
Group C	1st	26.78 ± 0.39^{aA}	24.65 ± 0.65^{aB}	11.26 ± 0.42^{aC}
	2nd	24.82 ± 0.62^{aA}	19.25 ± 0.87^{aA}	11.38 ± 1.06^{aB}
	3rd	14.22 ± 0.14^{cA}	13.46 ± 0.35^{cA}	5.98 ± 0.17^{cC}

Lowercase letters indicate significant differences ($p<0.05$) among different study periods for the same type of extraction, whereas capital letters represent statistically significant differences ($p<0.05$) between different extraction methods for the same evaluation period by Tukey's *post-hoc* test. Data represent mean ± SE of triplicate analyses.

The choice of a method and type of solvent is crucial in any process of extraction. In this case, more phenolic compounds were obtained in the processes of solvent extraction (Soxhlet, methanol) and aqueous decoction than in infusion. A very lower amount of phenolics was found in leaves of group C in the last month of summer when compared to all other groups and periods, regardless the method of extraction employed. This is the opposite of what was found by de Andrade *et al*. [21], where a much lower TPC was found (approximately 10% lower) in yacon leaves extracted by methanol. Despite the significant differences found on different periods and groups, in the present study it was not found a trend in phenolics variation related to plant growth, maturity or sun exposal. These results corroborate with a previous study, where the TPC of young and old leaves from yacon plants were analyzed at harvest time [22]. The authors investigated two different methods of extraction (regular decoction and ohmic-assisted decoction) and found no trend in phenolic content regarding the age of leaves or method of extraction. Total phenolic content analyses are considered fairly common in literature, but no comparative data between total phenolic content and plants development were found.

Infrared Spectroscopy

Extracts of samples from all groups and periods were subjected to FT-IR. This analysis was performed in order to verify the presence of sesquiterpene lactones and estimate if there are changes in their production related to plant maturity/leaf age. Sesquiterpene lactones were isolated and purified using a standard method [8] and those bands corresponding to 2000-1500 cm^{-1} were analyzed as indicatives of the presence of SLs, as discussed below.

It is known that depending on the type of extraction, operational conditions and solvent used, there will be a specific selectivity ruling the composition of final extracts. Therefore, it is of most interest to check if benefic or functional compounds can be brought from the raw material to the product of consumption. In this sense, sesquiterpene lactones are subjected to be or not be available if a solvent is not appropriate [9]. The FT-IR spectra of all extracts tested (Figure 3) displayed strong absorptions at 1770 cm^{-1} (α-methylene-γ-lactone moiety), 1739 cm^{-1} (saturated ester carbonyl) and 1717cm^{-1} (α,β-unsaturated ester carbonyl), suggesting the presence of sesquiterpene lactones in all extracts obtained [3,4,8,9,23]. This result indicates that SLs were present no matter the type of extraction applied in this study, and also reveals that young or even older leaves contain these compounds. The type of SLs present in yacon (and *Smallanthus* species) leaves and their amount are a consequence of several factors, such as cultivar and latitudinal gradients [23–26]. How the fractions of each SL vary with time is still unknown and could be better explored in the future, being out of the scope of the present work.

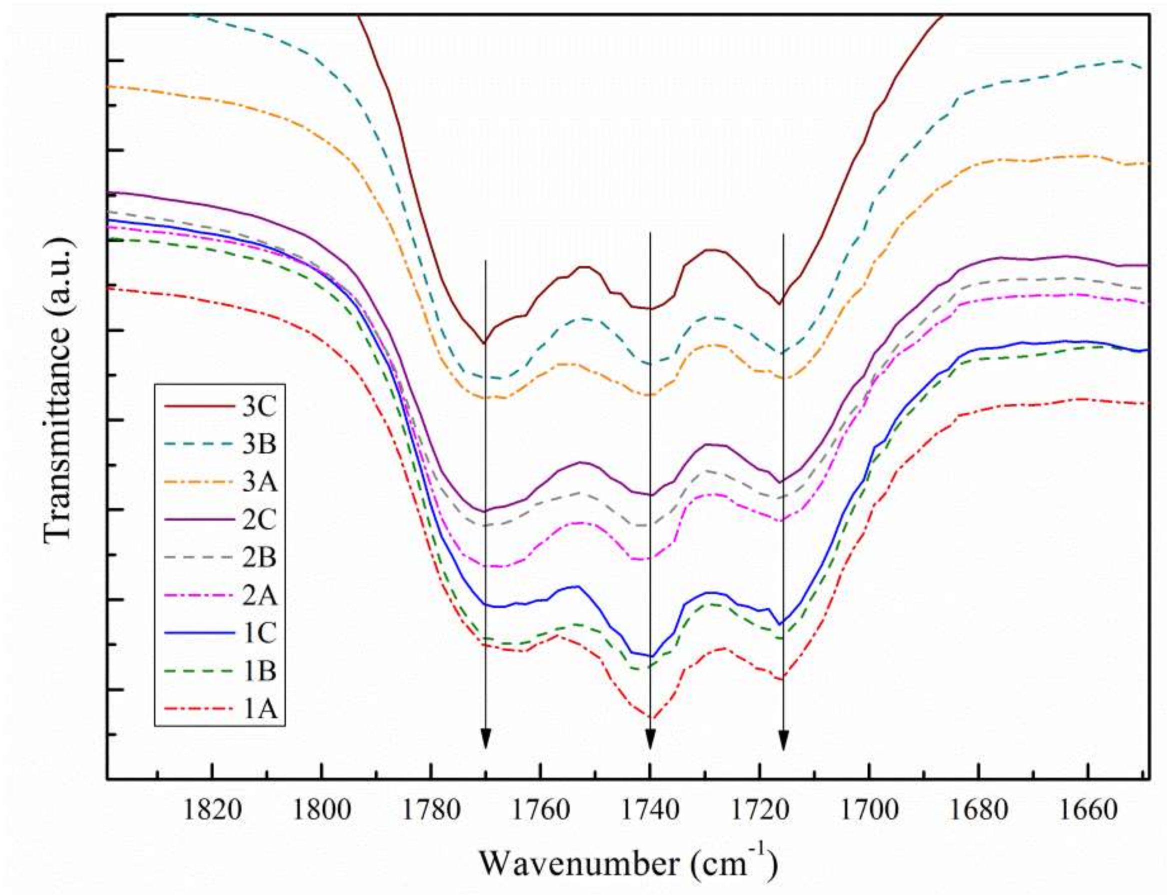

Figure 3. FT-IR spectra of the nine yacon samples (months and groups) at the 2000-1500 cm^{-1} range (KBr pellets).

Correlations between physical and chemical parameters

For each group (A, B and C), the variables month, plant height, number of leaves, leaf area and the color parameters L*, a*, and b* were checked for the existence of linear correlations over pairs. To accomplish this analysis all data obtained from the samples of 15 plants, for each group, were fed into the data processing software. Table 5 present the results obtained. As expected, for all groups there was a high and positive linear correlation between plant age (month) and plant height. Another positive correlation was found between plant height and number of leaves, only for groups A and B. As no correlation was found in this case for group C, it just confirms that the plants raise the amount of leaves produced with time (as also verified for mean values, group A, Table 3).

Table 5. Analyses of linear correlations between pairs of the variables 'plant height', 'number of leaves', 'leaf area', and the color parameters L*, a* and b*.

	Month	Plant height	No. leaves	Leaf area	L*	a*
Group A						
Plant height	**0.9624 (p=0.00)**	-	-	-	-	-
No. leaves	**0.6020 (p=.000)**	**0.6684 (p=.000)**	-	-	-	-
Leaf area	**0.4287 (p=.003)**	**0.3885 (p=.008)**	-0.0552 (p=.719)	-	-	-
L*	0.0460 (p=.764)	0.1381 (p=.366)	**0.3350 (p=.024)**	**-0.4247 (p=.004)**	-	-
a*	0.2489 (p=.099)	0.2527 (p=.094)	**0.5539 (p=.000)**	-0.2716 (p=.071)	0.1937 (p=.202)	-
b*	**0.5548 (p=.000)**	**0.6856 (p=.000)**	**0.3705 (p=.012)**	0.2197 (p=.147)	**0.3432 (p=.021)**	-0.1512 (p=.322)
Group B						
	Month	**Plant height**	**No. leaves**	**Leaf area**	**L***	**a***
Plant height	**0.9596 (p=0.00)**	-	-	-	-	-
No. leaves	**0.5347 (p=.000)**	**0.5192 (p=.000)**	-	-	-	-
Leaf area	0.1519 (p=.331)	0.0203 (p=.897)	-0.0874 (p=.577)	-	-	-
L*	-0.1177 (p=.452)	-0.2644 (p=.087)	**-0.3149 (p=.040)**	**0.5244 (p=.000)**	-	-
a*	0.2757 (p=.074)	0.2765 (p=.073)	**0.3691 (p=.015)**	-0.1078 (p=.491)	**-0.4455 (p=.003)**	-
b*	**0.3837 (p=.011)**	0.2759 (p=.073)	0.0470 (p=.765)	**0.4926 (p=.001)**	**0.6035 (p=.000)**	**-0.6058 (p=.000)**
Group C						
	Month	**Plant height**	**No. leaves**	**Leaf area**	**L***	**a***
Plant height	**0.9697 (p=0.00)**	-	-	-	-	-
No. leaves	0.0483 (p=.764)	0.1400 (p=.383)	-	-	-	-
Leaf area	-0.0316 (p=.844)	-0.0822 (p=.610)	-0.1306 (p=.416)	-	-	-
L*	**-0.7458 (p=.000)**	**-0.8122 (p=.000)**	**-0.3948 (p=.011)**	0.1847 (p=.248)	-	-
a*	**0.7920 (p=.000)**	**0.7336 (p=.000)**	0.1556 (p=.331)	0.0929 (p=.564)	**-0.7026 (p=.000)**	-
b*	**-0.8197 (p=.000)**	**-0.8053 (p=.000)**	-0.1895 (p=.235)	-0.0594 (p=.712)	**0.8619 (p=.000)**	**-0.8732 (p=.000)**

Linear correlations were considered for p-values < 0.05. Significant values are marked in red.

Regarding the correlations between leaf area and color parameters, positive and linear correlations were found for groups A and B regarding parameters L* and b*. There was no correlation between parameter a* and leaf area for all groups. Group C did not present any correlation between leaf area and color variation. These results suggest the development of color is a function of leaf growth. As leaves get older and bigger, their color present slight shifts to lighter hues, increasing the amount of yellow. On the other hand, mature and old leaves loose yellowness, greenness and lightness within time, indicated by strong correlations of plant age (month) and height with color parameters b* (-0.8197), a* (0.7920) and L* (-0.7458), possibly as a result of their senescence. For all groups a negative linear relationship was found between a* and parameters L* and b*. In the correlations of b* and L* a positive linear trend was found. This indicates that as lighter the leaves, there will also be a tendency of greenish and yellowish hues.

The results of TPC were also used to investigate if there is a possible linear correlation phenolics content with the other parameters investigated in the present work (Table 6). In this case there was no assessment of the TPC for each plant, but a mixed sample was used to verify the phenolics of the whole group in determinate months. In this sense, mean values of all parameters had to be used in the calculations. More and higher linear correlations were found for results of TPC obtained from extracts produced by decoction and Soxhlet methods. It is understandable that while infusion comprises a quick percolation method, the other two methods are capable of higher solvent-solute contact, allowing higher extraction. This becomes evident comparing the results of TPC from infusion and decoction. So if phenolic compounds could not be completely extracted by the infusion method, most likely there will be no or low correlation to be made with any other parameter. The most important linear correlations found for TPC of extracts obtained by both decoction and Soxhlet methods was a*, i.e., the greenness of the plant seems to be related to the amount of phenolics in leaves. Other parameters that may present some importance, but less pronounced than the previous one, are plant age and plant height, which were all negatively correlated to the TPC of extracts obtained by decoction and Soxhlet.

Table 6. Linear correlations between the total phenolic content of yacon leaves obtained by three methods of extraction and the variables ‘month’, ‘group’, ‘plant height’, ‘number of leaves’, ‘leaf area’, L*, a* and b*.

TPC* method	Month	Group	Plant height (mean)	No. leaves (mean)	Leaf area (mean)	L* (mean)	a* (mean)	b* (mean)
Decoction	**-0.5326 (p=.004)**	**-0.385 (p=.047)**	**-0.5176 (p=.006)**	**0.4057 (p=.036)**	-0.1882 (p=.347)	**0.4648 (p=.015)**	**-0.8028 (p=.000)**	**0.5957 (p=.001)**
Infusion	-0.1937 (p=.333)	-0.265 (p=.182)	-0.2317 (p=.245)	0.2122 (p=.288)	0.2397 (p=.228)	0.1238 (p=.538)	**-0.5494 (p=.003)**	0.2133 (p=.285)
Soxhlet	**-0.6249 (p=.000)**	-0.1095 (p=.586)	**-0.5447 (p=.003)**	0.1451 (p=.470)	**-0.5342 (p=.004)**	0.2363 (p=.235)	**-0.7854 (p=.000)**	0.2555 (p=.198)

*TPC: Total phenolics content. Linear correlations were considered for p-values < 0.05. Significant values are marked in red.

The identification of correlations between physical and chemical leaves parameters becomes relevant to future studies aimed to improve the already existent yacon leaf products and to the development of alternative foods and beverages. While yacon tubers are available commercially in various forms (*in natura*, dried as chips, powders, syrups, etc), there are still few uses for yacon leaves. Knowing maturity parameters of yacon leaves may help to increase its potential as a source of antioxidants and bioactive elements. As a source of such compounds, dried or extracts from leaves may emerge as functional ingredients, which could be added in a great variety of foods, but especially in drinks and even in yacon tuber-derived products.

CONCLUSION

Although collecting leaves, herbs, flower or any other plant intended to make teas and extracts seems to be an unpretentious task in terms of economic and functional benefits, there are parameters to be considered in raising yield in general. For yacon leaves harvesting, knowing plant growth allowed verifying how new leaves sprout accordingly, which is a valuable information for collectors. If the plant loses too many leaves, their development may be impaired. Leaves also should be removed when achieving a proper size, e.g. >~240 cm^2, allowing a higher yield. In addition, it became evident that simply knowing yacon plant age and height may be a suitable first step to establish periods to collect leaves. Collectors should avoid harvesting yacon leaves located at the basal part of the plant, especially on the 4^{th} month after planting (Group C, 3^{rd}), since a significantly lower amount of total phenolics were found on these conditions (older leaves). Furthermore, the total phenolic content showed to be correlated to the greenness of the leaves (-a* value, for any method of extraction), which should be taken into account on harvesting. According to our data, yacon leaves reach maturity when their size are 200 cm^2 or higher, which is suggested as an approximate limit for the collection.

Despite yacon leaves present interesting bioactive compounds, the method of extraction applied may not allow a complete removal of these components. Considering that teas are commonly produced by infusion and decoction, the last should be the method chosen for superior phenolic content, being capable of extracting twice or more phenolic compounds than the former (although decoction is usually performed for roots and hardy wood substances in general). In a preliminary screening it seems that among the methods of extraction used in this work, none was incapable of extracting sesquiterpene lactones, which is a good characteristic when thinking in aqueous extraction systems.

Funding: This research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.
Acknowledgments: The authors thank Prof. Carlos Itsuo Yamamoto (UFPR, Curitiba, Brazil) for the technical assistance with FT-IR in LACAUT (Laboratory of Automotive Fuel Analyses).
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Grau A, Rea J. Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson. In: Hermann M, Heller J, editors. Andean roots and tubers: Ahipa, arracacha, maca and yacon Promoting the conservation and use of underutilized and neglected crops. Rome, Italy: International Plant Genetic Resources Institute; 1997. p. 199–242.
2. Joung H, Kwon D-Y, Choi J-G, Shin D-Y, Chun S-S, Yu Y-B, et al. Antibacterial and synergistic effects of Smallanthus sonchifolius leaf extracts against methicillin-resistant Staphylococcus aureus under light intensity. J Nat Med. 2010 Apr 30;64(2):212–5.
3. Lin F, Hasegawa M, Kodama O. Purification and Identification of Antimicrobial Sesquiterpene Lactones from Yacon (Smallanthus sonchifolius) Leaves. Biosci Biotechnol Biochem. 2003 Jan 22;67(10):2154–9.
4. Hong SS, Lee SA, Han XH, Lee MH, Hwang JS, Park JS, et al. Melampolides from the leaves of Smallanthus sonchifolius and their inhibitory activity of LPS-induced nitric oxide production. Chem Pharm Bull (Tokyo). 2008;56(2):199–202.
5. Valentova K, Cvak L, Muck A, Ulrichova J, Simanek V. Antioxidant activity of extracts from the leaves of Smallanthus sonchifolius. Eur J Nutr. 2003 Feb 1;42(1):61–6.
6. Valentová K, Šeršeň F, Ulrichová J. Radical Scavenging and Anti-Lipoperoxidative Activities of

Smallanthus sonchifolius Leaf Extracts. J Agric Food Chem. 2005 Jul;53(14):5577–82.

7. Picman AK. Biological activities of sesquiterpene lactones. Biochem Syst Ecol. 1986 May;14(3):255–81.
8. Barcellona CS, Cabrera WM, Honoré SM, Mercado MI, Sánchez SS, Genta SB. Safety assessment of aqueous extract from leaf Smallanthus sonchifolius and its main active lactone, enhydrin. J Ethnopharmacol. 2012 Nov;144(2):362–70.
9. Genta SB, Cabrera WM, Mercado MI, Grau A, Catalán CA, Sánchez SS. Hypoglycemic activity of leaf organic extracts from Smallanthus sonchifolius: Constituents of the most active fractions. Chem Biol Interact. 2010 Apr;185(2):143–52.
10. Schorr K, Da Costa FB. Quantitative determination of enhydrin in leaf rinse extracts and in glandular trichomes of Smallanthus sonchifolius (Asteraceae) by reversed-phase high-performance liquid chromatography. Phytochem Anal. 2005 May;16(3):161–5.
11. Schorr K, Merfort I, Da Costa FB. A Novel Dimeric Melampolide and Further Terpenoids from *Smallanthus sonchifolius* (Asteraceae) and the Inhibition of the Transcription Factor NF-kB. Nat Prod Commun. 2007;2(4):367–74.
12. Simonovska B, Vovk I, Andrenšek S, Valentová K, Ulrichová J. Investigation of phenolic acids in yacon (Smallanthus sonchifolius) leaves and tubers. J Chromatogr A. 2003 Oct;1016(1):89–98.
13. Valentová K, Moncion A, de Waziers I, Ulrichová J. The effect of Smallanthus sonchifolius leaf extracts on rat hepatic metabolism. Cell Biol Toxicol. 2004 Mar;20(2):109–20.
14. Xie W, Li J, Li HM. Study on chemical constituents of Smallanthus sonchifolius. J Chinese Med Mater (Zhong Yao Cai). 2008;31(10):1510–2.
15. Seminario J, Valderrama M, Manrique I. El yacón: fundamentos para el aprovechamiento de un recurso promisorio. Centro Internacional de la Papa (CIP), Universidad Nacional de Cajamarca, Agencia Suiza para el Desarrollo y la Cooperación (COSUDE). Lima, Peru: Centro International de la Papa (CIP), Universidad Nacional de Cajamarca, Agencia Suíza para el Desarrollo y la Cooperación (COSUDE); 2003. 60 p.
16. INMET. Historical Climate Data of the Brazilian National Institute of Meteorology [Internet]. Brasília: Instituto Nacional de Meteorologia. [updated 2019 Sep 30; cited 2019 Apr 20] Available from: http://www.inmet.gov.br [Internet]. [cited 2019 Apr 20]. Available from: http://www.inmet.gov.br
17. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012 Jul 1;9(7):671–5.
18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144–58.
19. Graefe S, Hermann M, Manrique I, Golombek S, Buerkert A. Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. F Crop Res. 2004 Mar;86(2–3):157–65.
20. Russo D, Malafronte N, Frescura D, Imbrenda G, Faraone I, Milella L, et al. Antioxidant activities and quali-quantitative analysis of different Smallanthus sonchifolius [(Poepp. and Endl.) H. Robinson] landrace extracts. Nat Prod Res. 2015 Sep 2;29(17):1673–7.
21. de Andrade EF, Leone R de S, Ellendersen LN, Masson ML. Phenolic profile and antioxidant activity of extracts of leaves and flowers of yacon (Smallanthus sonchifolius). Ind Crops Prod. 2014 Dec;62:499–506.

22. Khajehei F, Niakousari M, Seidi Damyeh M, Merkt N, Claupein W, Graeff-Hoenninger S. Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (Smallanthus sonchifolius Poepp.) Leaves: Comparison with Conventional Decoction. Molecules. 2017 Nov 23;22(12):2043.
23. Inoue A, Tamogami S, Kato H, Nakazato Y, Akiyama M, Kodama O, et al. Antifungal melampolides from leaf extracts of Smallanthus sonchifolius. Phytochemistry. 1995 Jul;39(4):845–8.
24. de Andrade EF, Carpiné D, Dagostin JLA, Barison A, Rüdiger AL, de Muñiz GIB, et al. Identification and antimicrobial activity of the sesquiterpene lactone mixture extracted from Smallanthus sonchifolius dried leaves. Eur Food Res Technol. 2017 Dec 30;243(12):2155–61.
25. Coll Aráoz M V, Mercado MI, Grau A, Catalán CAN. Intraspecific variation of sesquiterpene lactones associated to a latitudinal gradient in Smallanthus macroscyphus (Heliantheae: Asteraceae). Chemoecology. 2016 Aug 16;26(4):143–51.
26. Mercado MI, Coll Aráoz MV, Manrique I, Grau A, Catalán CAN. Variability in sesquiterpene lactones from the leaves of yacon (Smallanthus sonchifolius) accessions of different geographic origin. Genet Resour Crop Evol. 2014 Aug 11;61(6):1209–17.