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Yacon (Smallanthus sonchifolius Poepp. & Endl) - the potential of a neglected crop as an alternative sweetener and source of phytochemicals for functional foods

Dissertation

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List of abbreviations and acronyms

% Percent

°S Degree South, latitude °C Degree centigrade

μmol Micromoleμg Micrograma.s.l. Above sea level

ABTS 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

CHAD Convective hot air drying

cm Centimetre
cv. Cultivar
DE Decoction
DM Dry matter

DPPH 2,2-diphenyl-1-picrylhydrazyl

e.g. For exampleEU European UnionFD Freeze drying

FOS Fructooligosaccharides

FRAP Ferric reducing antioxidant power

g Gram
kg Kilogram
m Meter
mg Milligram

OH-DE Ohmic-assissted decoction
TFC Total flavonoid content
TPC Total phenolic content
US/USA United States of America

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1. Introduction

1. 1. Functional and health promoting plant based food products

The growing attention towards health promoting and functional food products in various sectors of the food supply chain, from primary production in the agricultural sector to the consumption point at the tables of the consumers' houses is a reflection of the combination of a number of factors. Firstly, it has been forewarned that the world population will grow to 9 billion by 2050, with implications that strategies need to be developed that assure global food security (Godfray et al., 2010). It has been noted that in order to feed the future population the agricultural production should grow 50 to 70% (Horlings & Marsden, 2011; Karunasagar & Karunasagar, 2016). However, population growth is not the only factor at play in regard to the upward trend for food demand.

The continuously raising level of awareness of the link between health and diet has induced certain alterations in diet, consumers' food choices and raw material demand (Ubeda et al., 2011). Some of the most significant characteristics of modern diets are the high intake of sugar, fat and protein which have been recognized among the contributing factors of specific chronic diseases such as obesity, type II diabetes, coronary heart disease, etc. More precisely, animal protein consumption, particularly meat consumption has been associated with the risk of chronic diseases such as colorectal cancer, cardiovascular diseases, and obesity (Hu, 2011; Godfray et al., 2018; Wang & Beydoun, 2009). Although, meat serves as a source of energy and certain nutrients (iron, zinc and vitamin B_{12}), it is possible to provide most of these nutrients without meat through maintaining a wide variation of food products in the diet (Godfray et al., 2018). The rising pattern of meat consumption has a negative impact on environment and use of water and land. Moreover, studies have shown that plant based diets such as a lacto-ovo-vegetarian diet is a more sustainable diet in terms of usage of resources such as energy, land and water and is healthier as well (Heller & Keoleian, 2015; Ruini et al., 2015). Consequently, the demand for plant based food products is raising, which has led the primary production sector to focus on enhancing the production of crops such as e.g. quinoa, hemp, soy, legumes. These crops can contribute to maintaining plant based proteins and nutrients in the diet (Friedman, 1996; Iqbal, Khalil, Ateeq, & Khan, 2006; Tang, Ten, Wang, & Yang, 2006).

Several studies have shown that the diet plays a crucial role in the occurrence of certain chronic diseases namely, obesity and type II diabetes, that stem from the globalization of a new unbalanced diet consisting of industrialized, high-energy content, and refined food products (e.g. Western diet) (Fardet & Boirie, 2014). The epidemic of obesity and type II diabetes have been associated with high levels of sucrose as a part of modern diet in a wide range of foods and beverages (Laville & Nazare, 2009). In association with the concern over the upward trend of obesity and type II diabetes, policy makers have already started to implement policies such as taxation on food and beverages containing sucrose like the recently imposed tax on sugar products in the UK (Pym, 2018). Some other strategies intended to be

effective in reducing sucrose intake are campaigning for raising public awareness and reducing the availability of food and beverages that contain sucrose, especially for younger people (e.g. no longer offering these products in schools) (Popkin & Hawkes, 2016). As sugar makes up a relevant part of the taste of a food product, the development of alternative non-nutritive and/or artificial resources as sweetener such as stevia, aspartame, acesulphame-K, saccharin, sucralose, and cyclamate have been the subject of various studies (Chattopadhyay, Raychaudhuri, & Chakraborty, 2014; Mattes & Popkin, 2008; Raben & Richelsen, 2012). However, as consumers are worried over the use of food additives, the use of non-nutritive or low calorie natural sweeteners has attracted the attention of various sectors engaged in the food supply chain (Bearth, Cousin, & Siegrist, 2014). Furthermore, there is a rising market with regard to food products that suit the diet for those that are suffering from such digestive diseases (e.g. diabetes and obesity) as well as providing them with variation in food products ("Diabetic Food Market Analysis By Application," 2017). Additionally, certain groups of consumers are interested in maintaining a healthier and more active lifestyle such as dieters and athletes. Others are taking into account their ethical values which are impacting their food choices, like vegetarians and vegans. Therefore, the market of low calorie functional and health promoting food products based on plant foods should fulfil the demand of such consumers.

Besides the classical components of sugar, fat and protein in food products, phytochemicals like phenolic compounds have gained huge attention due to their antioxidant properties (Chirinos, Pedreschi, Rogez, Larondelle, & Campos, 2013). Phenolic compounds have been identified as one of the major groups of non-nutritive phytochemicals in plant foods responsible for beneficial health effects (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Zhang & Tsao, 2016). Phenolic compounds can be divided into three major groups: phenolic acids, flavonoids and non-flavonoids depending on the number of aromatic rings with one or more hydroxyl group in their structure (Tsao, 2010). Phenolic compounds from food plant resources have been investigated and a wide range of biological roles including antioxidant activity has been assigned to them (Crozier et al., 2006; Soto-Vaca, Gutierrez, Losso, Xu, & Finley, 2012). It has been shown that the phenolic compounds possess remarkable antioxidant characteristics depending on the solvent used in their extraction, the plant origin, the environmental conditions during cultivation of the plant, the time of harvest, and the storage conditions after harvest (Avello, Pastene, Bustos, Bittner, & Becerra, 2013; Taârit, Msaada, Hosni, & Marzouk, 2012; Trabelsi et al., 2013). Consequently, investigating the antioxidant characteristics of phenolic extracts of various plant species has been the hot topic in the scientific community in particular with regard to their in vitro antioxidant activity (Martins, Barros, & Ferreira, 2016). The significant in vitro antioxidant activity of phenolic compounds is derived from their ability to scavenge free radicals through electron or hydrogen atom donation to reactive oxygen, nitrogen, and chlorine species. Moreover, they act as metal chelators by directly inhibiting Fe³⁺ reduction to stabilized hydroperoxides or metal peroxidants (Perron & Brumaghim, 2009). In this regard, phenolic acids and flavonoids can act as radical scavengers while their ability as metal-chelating agents and their reducing power depends on their structure (Zhang & Tsao, 2016).

There is a demand for plant foods, including fruits, grains, tubers, and vegetables as fresh and/or as processed food products not only as a nutritive part of a diet, but also due to their non-nutritive health beneficial phytochemicals, specifically their phenolic compounds (Karaman et al., 2014; Zhang & Tsao, 2016). In particular, some of the underutilized fruits, vegetables, roots and tubers have attracted attention in this respect, because of the evidence that suggests the lower incidences of chronic diseases in the native population that consumed them for many decades (De Almeida Paula, Abranches, & De Luces Fortes Ferreira, 2015).

Considering all the above-mentioned aspects, there is no dispute over the fact that there is a demand for functional and health promoting foods, which are produced in an environmentally friendly way.

1. 2. General Outline and aims

On the account of the rising demand for production of functional and health promoting food products, plants of the Andean region have attracted considerable attention. A divers range of Andean plants including various fruits, herbs, seeds, grains, roots and tubers has been consumed by the native population and are believed to possess nutritional and medicinal attributes (Chirinos et al., 2013). Quinoa, amaranth, chia, maca, oca, yacon, etc. are among the Andean agricultural products, which have gained attention due their significant nutritional values and their health promoting effects (Chirinos et al., 2013). Yacon (Smallanthus sonchifolius Poepp. & Endl.) is a root crop native to the Andean region (Delgado, Tamashiro, Junior, & Pastore, 2013). With its health promoting effects it has gained considerable attention during the past two decades (Ojansivu, Ferreira, & Salminen, 2011). It is an herbaceous perennial plant, which produces large tuberous roots similar to sweet potato tubers while they have a sweet taste and crunchy texture for which they are considered as a fruit (Hermann, 1997; Lachman, Fernández, & Orsák, 2003). The edible tubers are characterized by low energy values, because they mainly consist of water (80-90% of fresh weight), while 60-70 % of their dry matter content is composed of fructooligosaccharides (FOS) as their main carbohydrate. In addition, yacon tubers contain considerable amounts of bioactive compounds including phenolic compounds which further shows their potential health benefits (Campos et al., 2012). Besides, consumption of yacon leaves in form of tea has been recommended in traditional Japanese medicine for those suffering from chronic diseases such as diabetes. On that account, several studies investigated and showed the antidiabetic effects of yacon leaves extract as well as other positive biological effects of it such as its antioxidant activity (Honoré, Genta, & Sánchez, 2015).

The low sugar content and corresponding high FOS and inulin content make this crop a favourable agricultural product as a fresh fruit, or with further processing as food products such as chips or flour to be used independently or as an ingredient in other functional food products (De Almeida Paula et al., 2015). In light of actions taken to reduce the sucrose consumption e.g. taxing of products that contain a certain amount of sugar, campaigning to raise awareness about sugar consumption, the importance of yacon as a plant resource to be used as an alternative to sweeteners and prebiotics may attract even more

attention (Popkin & Hawkes, 2016; Pym, 2018). Consequently, market demand can be predicted for yacon and its products. The main segment of currently available fresh or processed yacon is imported from Peru. The export of yacon and its products - mainly yacon syrup- has doubled (increased by 53%) and risen to 22 tonnes per year from 2011 to 2015, which evidences the growing demand. Yacon can be cultivated under central European environmental conditions and has just started gaining attention. Although, the experimental cultivation of yacon started in Europe in the Czech Republic as the main producer, the local production of yacon and its products are still insignificant (Exporting yacón to Europe, 2016).

On account of that, the foundation of this research was based on an anticipated growing demand for local production of yacon and its products in Europe (Exporting yacón to Europe, 2016). Various factors were taken into consideration to ensure the local production of yacon products of high quality. A significant key factor that influences the chemical quality of yacon tubers and leaves is the cultivar (Lachman, Fernandez, Viehmannova, Šulc, & Eepkova, 2007). Moreover, the environmental conditions during cultivation, time of harvest, the storage conditions after harvest, the conditions during post-harvest handling, and the processing conditions can affect the quality of tubers and leaves. Consequently, derived products vary in terms of sugar, FOS, and phytochemical content. The main goal of this study was to take a holistic approach towards maintaining a local supply chain for yacon and its products in the EU. The overall aims of this study were to 1) investigate the diversity of chemical composition of yacon as a raw food material using different cultivars, 2) determine the potential of application of greener novel technologies for the processing of yacon leaves, and 3) investigate the production of food products from yacon tubers while using simple, green processing technologies.

1. 3. Hypothesis

Although a number of studies have been conducted on the influence of cultivar, environmental conditions during cultivation, post-harvest handling and food processing on certain chemical components of yacon products, namely the sugar and FOS content, there is a lack of information on their influence on phenolic compounds and antioxidant activity in yacon and its products. Therefore, in the present thesis, the effect of cultivar and food processing after harvest on phyto/chemical composition of leaves and tubers of yacon plants grown under environmental conditions of southwestern Germany were investigated. In this thesis the term ''phyto/chemical'' was chosen to address certain groups of components of tubers and leaves, namely, FOS, inulin, sugars, and phenolic compounds. Hereinafter, the main hypotheses on which this thesis was based, are as follows:

 Ohmic-assissted decoction (OH-DE) of yacon leaves will lead to higher amounts of phenolic compounds and antioxidant activities in comparison to conventional decoction (DE). OH-DE takes advantage of the volumetric heating mechanism, which leads to a sharp temperature rise in mixture of leaves and water compared to DE, that works according to conduction and

- convection heating mechanism during which temperature rises gradually. Consequently, fast destruction of cellular structure and release of phenolic compounds due to thermal shock during OH-DE leads to extraction of higher amounts of phenolic compounds.
- The energy consumption during OH-DE is lower in comparison to the case in which DE is applied for extraction. Moreover, the rapid heating mechanism of OH-DE reduces the time for processing which reduces the energy consumption.
- Leaves of different cultivars of yacon (red and white) contain different amounts of phenolic compounds and the profile of individual phenolic compounds in them may be different. Moreover, the phenolic content of old leaves which are mature and are collected from the lower part of the yacon stem contain lower amounts of phenols compared to young leaves collected from the upper stem. The biosynthesis of phenolic compounds and their accumulation may be significantly slowed down as leaves reach maturity and expand. Therefore, expansion of leaves may induce a dilution effect on phenolic content of leaves. Also, young leaves are collected from upper part of stems that are more exposed to sun light compared to large leaves at lower part of stems. Exposure to sunlight may induce biosynthesis of phenolic compounds to protect the leaves from harmful effects of ultra-violet light.
- The phyto/chemical content of various parts of yacon tubers (peel, flesh and whole tuber) varies among various cultivars of yacon which are grown under the same environmental conditions in southwestern Germany. The variation in amount of phyto/chemicals in different parts of yacon tubers from different cultivars, is due to the differences in expression of enzymes responsible for biosynthesis of these compounds.
- The phyto/chemical content of various parts of yacon tubers, namely, peel and flesh, is different. The peel of tubers contains higher phenolic compounds and exhibits higher antioxidant activity, compared to the flesh of the tubers. Higher concentration of phenolic compounds in the peels of tuber may enhance the defence system of them against potential harmful effects of environment as phenolic compounds possess various biological activity such as antioxidant, antimicrobial, pesticide properties, etc.
- The storage condition affects the phyto/chemical content and antioxidant activity of yacon tubers. In this regard, the longer the duration of storage after harvest and before processing, the more the loss of cell integrity and release of enzymes, and eventually the more the changes in their phyto/chemicals and antioxidant activity.
- Pre-treatment of yacon slices before drying using diluted lime juice will enhance the quality of
 final yacon chips in terms of their phyto/chemical content and antioxidant activity. Lime juice
 contains vitamin C and citric acid which act as an antioxidant and an inhibitor, respectively, for
 indigenous polyphenol oxidase of yacon tubers during the drying process and protects the
 phyto/chemicals from oxidation reactions.
- Drying of yacon slices by means of freeze drying (FD) will result in yacon chips with a higher phyto/chemical content compared to yacon chips which are dried using convective hot air drying

(CHAD) method due to the following: absence of oxygen, lower temperatures of processing and absence of liquid water. Higher drying temperatures will induce adverse effects on the phyto/chemical content of yacon chips as it may have a detrimental effect on heat sensible compounds of yacon slices.

To achieve the targeted objectives, cultivation of two yacon cultivars (red and white) was carried out during 2015 at the experimental station "Kleinhohenheim" of Hohenheim University (Stuttgart, Germany) in southwestern Germany. The tubers of red and white yacon cultivars that were harvested at October 2015, were stored under the same conditions, at ambient temperature for one and three weeks after harvest, before being used for drying experiments. The young and old leaves of red and white yacon cultivars were collected at harvest time (October 2015) to evaluate the extraction of their phyto/chemicals. The collected leaves were then dried at 40 °C and kept in a dark and cool place before being used for extraction of phyto/chemicals by means of OH-DE and DE. Furthermore, seven cultivars of yacon, namely, Cajamarca, Cusco, Early White, Late Red, Morado, New Zealand and Quinault, were purchased from Cultivariable (Moclips, WA, USA) and cultivated at "Ihinger Hof" experimental research station of University of Hohenheim (Stuttgart, Germany) in the year 2016 using the same agricultural practices to ensure ceteris paribus during primary production. After harvest in October 2016, tubers of each cultivar where collected from individual plants and samples of peel, flesh and whole tubers were immediately frozen by means of liquid nitrogen before freeze drying. The freeze-dried powder of samples was used to study the differences between phyto/chemical content of different parts of tubers in the tested yacon cultivars. The detailed description of all field experiments is noted in chapter "publications" which presents the three publications including the findings of this work under three chapters, namely, chapter I, II and III.

1. 4. Outline and structure of the thesis

Overall, the body of the present thesis consists of a literature portion giving further information on yacon and its compounds in relation to agronomic and processing parameters, and the three scientific peer-reviewed publications which are presented in the chapter ''publications'' followed by a general discussion and a summary.

In chapter I, the results of investigating the phyto/chemical content including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of young and old leaves of red and white yacon cultivars extracted by means of OH-DE and DE are reported. Moreover, the results of quantifying the individual phenolic compounds, namely, caffeic acid, ferrulic acid, gallic acid, kaempherol, myricetin, p-coumaric acid, rutin, and quercetin, in the yacon leaves are presented in Chapter I. The yield of extraction and energy consumption of each extraction method is evaluated. This chapter has been published in ''Molecules '' in the special issue of ''Green Extraction of Natural Product: Innovative Techniques, Alternative Solvents and Original Procedures '' as an original research article

entitled ''Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction '' (Khajehei, F., Niakousari, M., Seidi Damyeh, M., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2017). Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction. *Molecules*, 22(12), 2043.).

Chapter II presents the results of differentiating the phyto/chemical content, in particular TPC, TFC, and simple sugar (fructose, glucose and sucrose) content as well as antioxidant activity of the peel, flesh and whole yacon tubers of seven cultivars. The presented findings can be used to determine the suitability of each cultivar for specific food product developments with regard to nutritional and functional quality of tubers. The results regarding the evaluation of the phyto/chemical content of the peels show the potential of peels to be used in other products. The reported results may be used to develop strategies for waste reduction during processing of yacon tubers. This chapter has been published in ''Molecules '' in the special issue of ''The Antioxidant Capacities of Natural Products '' as an original research article entitled ''Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars '' (Khajehei, F., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2018). Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars. *Molecules*, 23(2), 278.).

Chapter III focuses on the results of drying yacon slices of two cultivars for production of chips. Differences in quality of chips produced using various hot air temperatures (40, 50 and 60 °C) in the convective hot air drier in association with their antioxidant activity and TPC, are compared with those of yacon slices dried using freeze drying (FD) techniques. Additionally, the effects of storage duration after harvest of yacon tubers and the influence of pre-treating yacon slices with diluted lime juice before drying on the overall quality of chips were investigated. This study aimed to provide insight regarding processing conditions for production of yacon chips with premium antioxidant activity and TPC using convective hot air drying (CHAD) technique as a simple drying method that can be used by farmers and small producers as well. Production of yacon chips may serve as a good strategy to maintain and preserve the quality of harvested tubers as they can be consumed directly as final product or be milled to yacon flour. This chapter has been published in the Journal of "Agriculture" in the special issue of "Food for Future" as an original research article entitled "Total Phenolic Content and Antioxidant Activity of yacon (smallanthus sonchifolius poepp. and endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying " (Khajehei, F., Hartung, J., & Graeff-Hönninger, S. (2018). Total Phenolic Content and Antioxidant Activity of Yacon (Smallanthus Sonchifolius Poepp. and Endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying. Agriculture, 8(12), 183.).

2. Literature review

2. 1. Origin of yacon (Smallanthus sonchifolius Poepp. & Endl.)

Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) is an underutilized tuberous crop from the family of Asteraceae, classified under the genus *Smallanthus*, and belongs to the species *Smallanthus sonchifolios* (Delgado et al., 2013; National Research Council, 1989). It has its origins in South America where it is grown widely and has been cultivated by smallholders for subsistence in backyard gardens or in corners of farms for centuries (Delgado et al., 2013; Hermann, Freire, & Pazos, 1997). More specifically, it is native of the Andean region including Ecuador, Peru, Bolivia and North Argentina (Delgado et al., 2013). The richest region with regard to germplasm diversity of yacon plant is noted to be the eastern Andean slopes, expanding from the Apurimac river basin (12°S) located in Peru to the location of La Paz river basin (17°S) in Bolivia (Gurung, 2018). In Peru, the cultivation of yacon takes place at 900 to 3500 m above the sea level, while it is grown in a lower range of altitudes in Bolivia and Ecuador at 600 to 2500 m above sea level (Delgado et al., 2013; National Research Council, 1989). Yacon is grown in other parts of the world in large scales including New Zealand, Japan, Brazil, South-Korea, Hainan, Taiwan, the Philippines and the Czech Republic (Figure 1) (Ojansivu et al., 2011). The latest is the main local producer of yacon in the EU region (Exporting yacón to Europe, 2016).

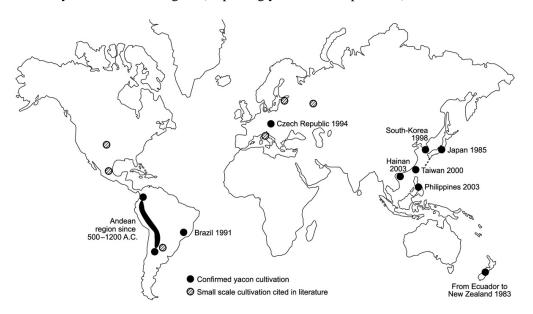


Figure 1. Locations of yacon cultivation (Ojansivu et al., 2011).

2. 2. Botanical and morphological characteristics of yacon (*Smallanthus sonchifolius* Poepp. & Endl.)

Figure 2 shows the botanical and morphological characteristics of a yacon plant. It is an herbaceous perennial plant, which occurs less frequently as a shrub or small tree (Honoré et al., 2015). The plant may reach maturity between 6 to 12 months. At maturity stage, its height can vary between 1 to 2.5 meters with simple leaves that are largely crossed opposite, ovate to ovate-lanceolated in shape and have a jagged-serrated margin (Honoré et al., 2015; Manrique, P'arraga, & Hermann, 2005). The plant has tuberous roots that are similar to a sweet potato. Tubers may vary in colour, shape and size. Yacon tubers are in average 15-20 cm long and 10 cm thick at harvest time while having different peel colours of brown, pink, purple, cream, and tan (Ojansivu et al., 2011). They may weigh between 200 and 500 g on average, but may also reach up to 2 kg. In general, each yacon plant may produce between 5 to 20 units of tubers with an overall weight of 5 to 10 kg (Caetano et al., 2016; Delgado et al., 2013).

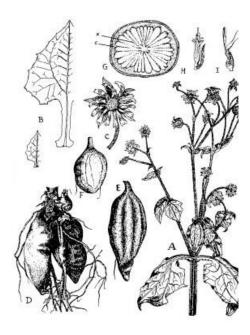


Figure 2. Botanical and morphological attributes of yacon (Lachman et al., 2003; León, 1964). A = flowering branches, B = leaves, C = flower head, D–F = tuberous roots, G = tuberous root in cross-section (x = xylem, c = cortical tissues), H = staminal disk flower, I = pistillate ray flower.

Today, some of the cultivars of yacon are supplied by small producers under different names according to their main origin and categorized in terms of the physical attributes of leaves and tubers, for example the colour of peel and flesh of tubers. Table 1 lists certain of the yacon cultivars supplied from an independent plant breeder based in USA including the colour of their tubers' flesh and peel, some of which have been used in this study (Yacon (*Smallanthus* sonchifolius) and Relatives, 2018).

Table 1. Categorizing yacon tubers according to the colour of their peel and flesh (Yacon (*Smallanthus sonchifolius*) and Relatives, 2018).

Cultivar	Peel colour	Flesh colour
Bekya	Tan	White
Blanco	Tan	White
Cajamarca	Tan	White
Kalaloch	Tan ,White	White
Late Red	Red, Tan	Orange, Yellow
Morado	Purple	White
New Zealand	Purple, Tan	White
Quinault	Tan, White	White

2. 3. Phyto/chemical characteristics of yacon leaves and tubers

Yacon tubers and leaves can be used fresh or processed into food or nutraceutical products. Yacon has been listed as a medicinal plant with antidiabetic potential in accordance with the health promoting attributes of its tubers and leaves (Mentreddy, 2007).

On one hand, yacon leaves are dried and are used in infusions to make herbal teas, which is assumed to be beneficial in combating chronic diseases such as diabetes and renal disorder. Yacon leaves have been used as an herbal tea against diabetes for the first time in Japan (Aybar, Riera, Grau, & Sanchez, 2001; Genta et al., 2010; Hermann, 1997; Honoré et al., 2015; Lachman et al., 2003; Ojansivu et al., 2011). The extract of yacon leaves exerts different biological activities such as anti-inflammatory, antioxidant activity, antimicrobial activity, antifungal activity, antibacterial activity, and antidiabetic activity (De Andrade, De Souza Leone, Ellendersen, & Masson, 2014; Honoré et al., 2015; Joung et al., 2010; Lin, Hasegawa, & Kodama, 2003; Oliveira et al., 2013; Padla, Solis, & Ragasa, 2012). It has been noted that yacon leaves contain various bioactive substances including phenolic acids and flavonoids, namely, chlorogenic acid, caffeic acid, ferulic acid, quercetin, rosmarinic acid, luteolin, gallic acid, rutin, and myricetin which are responsible for such biological activities. Previous studies determined the positive effect of yacon leaves extract consumption on hyperglycemia (Santos et al., 2017), hepatic aspartate aminotransferase (AST) activity (Baroni et al., 2014), and circulating insulin levels and blood glucose in diabetic rats (Aybar et al., 2001; Honoré, Cabrera, Genta, & Sánchez, 2012). This might be due to the phyto/chemical content of yacon leaves such as polyphenolic compounds (e.g. chlorogenic acid) with biological effects (Arion et al., 1997). The antioxidant activity of yacon leaves has been investigated by several researchers using various solvents, by fractionating the extracts, and by various in vivo methods. This recommended the potential of yacon leaves for preventing chronic diseases that are associated with oxidative stress such as arteriosclerosis, coronary artery disease, diabetes, etc. (De Andrade et al., 2014; Chang & Chuang, 2010; Heitzer, Schlinzig, Krohn, Meinertz, & Münzel, 2001; Valentova, Cvak, Muck, Ulrichova, & Simanek, 2003).

Yacon tubers are considered as main product of the plant. They are tasteless at harvest with a crunchy texture. In their origin, they are consumed as fresh fruit after being exposed to sunlight for three to five days to ripen and become sweet. It has been mentioned that sweet fresh vacon tastes like a fresh apple or pear (Hermann, 1997; Lachman et al., 2003; Manrique, Hermann, & Bernet, 2012; Shin, Hyun, Kuk, Shin, & Chun, 2015). The tubers are traditionally consumed at religious festivals (e.g. at Corpus Christi and All Souls' Day in Argentina, Bolivia, Ecuador and Peru) or fresh in salads. In addition, they may be processed to various food products such as syrup, flour, sweeteners, marmalade, juice, etc., which can be used for baking and cooking (Delgado et al., 2013). Fresh yacon tubers are characterized by their low energy values, because they mainly consist of water (80-90% of fresh weight). The main component of their dry matter content is carbohydrates including simple sugars, namely, fructose, glucose, and sucrose while they store FOS as their dominant polysaccharides (Lachman et al., 2003). It is noted that 60-70 % of the dry matter content of yacon tubers is composed of FOS of low polymerization degree such as 1-kestose (GF2), Nystose (GF3), and Fructofuranosyl-nistose (GF4) (Delgado, Thomé, Gabriel, Tamashiro, & Pastore, 2012; Delgado et al., 2013; Fernández et al., 2013; Moscatto, Borsato, Bona, De Oliveira, & De Oliveira Hauly, 2006). FOS are sweet tasting non-digestible oligosaccharides in the human small intestine (Losada & Olleros, 2002). Consequently, their consumption does not elevate the blood glucose level, which is why yacon tubers may be attractive for the development of low sugar food products and natural sweeteners. Furthermore, the caloric value of FOS is approximately 25-35% lower than normal starchy carbohydrates. That makes yacon an appealing food source for weight loss and control and in general for dieters (Roberfroid, 1999; Shoaib et al., 2016). In addition, FOS are prebiotic carbohydrates, which can promote a better health of the intestinal tract by enhancing and modulating the lactobacillus and bifidobacteria population in the microflora of the colon (Brownlee, 2011; Losada & Olleros, 2002; Tuohy, Rouzaud, Bruck, & Gibson, 2005). FOS are considered as dietary fibres that can contribute to various health beneficial effects, namely, reducing the time of intestinal transit, increasing the intestinal peristaltic movements, reducing hyperlipidemia and hypercholesterolemia (Brownlee, 2011; Tungland & Meyer, 2002; Kaczmarczyk, Miller, & Freund, 2012). This makes yacon even more appealing for people with digestion disorders. Moreover, consumption of FOS has been associated with reducing the risk of obesity, osteoporosis, colon cancer, intestinal infections and irritable bowel disease (Roberfroid, 2007). During the past decade several studies focused on health benefits of consumption of yacon tubers and their products in accordance with their FOS content (Caetano et al., 2016; Campos et al., 2012; Delgado et al., 2012; Dionísio et al., 2015; Lobo et al., 2011; Satoh, Kudoh, Hasegawa, Hirai, & Watanabe, 2014; Scheid, Genaro, Moreno, & Pastore, 2014). Yacon tubers can serve as a comparable source of FOS compared to other well-known plant resources of FOS, such as onions, garlic, chicory and Jerusalem artichoke (Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). Tubers of various yacon cultivars have been reported to contain comparable and higher amounts of FOS in comparison to Jerusalem artichoke grown under the same environmental conditions (Douglas, Scheffer, Sims, & Triggs, 2002). Studies reporting the FOS content of garlic and onions also indicated that yacon tubers may contain comparable or higher amounts of FOS depending on the chosen cultivar (Campos et al., 2012; Modler,1994). Yacon tubers are more suitable for the development of low calorie sweetener syrups, bakery products and jams compared to other sources FOS, as their consumption is limited or undesirable due to their strong aroma (garlic, onions). Moreover, in case of chicory and Jerusalem artichoke, the tubers/roots have to undergo an extraction process for FOS and inulin before further processing into other food products is feasible.

Therefore, based on the findings of the above-mentioned studies, yacon can be considered as a promising plant food source with multiple positive effects on health. Consequently, it comes as no surprise that conservation and promotion of yacon and its products has attracted the attention of those working on functional foods, nutraceuticals and in the pharmacological supply chain (Pedreschi et al., 2003; Zhuo-ya, 2007).

2. 4. Phyto/chemical characteristics of yacon leaves and tubers under the influence of genotype and environmental conditions

Similar to other agricultural products the phytochemical quality of yacon leaves and tubers are significantly affected by several factors. The phyto/chemical content of yacon tubers highly depends on cultivar, geographical origin and cultivation conditions (Campos et al., 2012; Lock, Perez, Villar, Flores, & Rojas, 2016; Pereira et al., 2016; Silva, Lima, Oliveira, Teixeira, & Machado, 2018). Particularly, the phyto/chemical content may differ to a significant degree among various yacon cultivars, which can be a key factor to determine the suitability of cultivars to be served as products for a targeted group of consumers (e.g. diabetics) (Hermann et al., 1997). Herman et al. (1997) reported that the fructan content among ten accessions of yacon grown in Ecuador varied between 32 and 66 % of dry matter content of tubers. Total free sugar content of tubers ranged between 14 and 29 % of dry matter content. The study of Campos et al. (2012) showed that the amount of reducing sugars, sucrose and FOS of 35 yacon cultivars, which were cultivated in Peru, ranged between 19.7-75.9 %, 2-16.8% and 6.4-65% of dry matter content, respectively. Moreover, they determined that yacon tubers of different cultivars contained a different total phenolic content, that varied between 8.9 and 30.8 mg chlorogenic acid equivalent/g DM and possessed different antioxidant capacities that which ranged from 23.3 to 136.0 µmol trolox equivalent/g DM. In addition, variation in phenolic content of yacon leaves of various accessions has also been reported by Valentová et al. (2006). They noted that total phenolic content of leaves of five yacon accessions varied between 1.5 and 2.5 mg gallic acid equivalent/g dried leaves (Valentová et al., 2006).

The effect of environmental conditions during and after harvest may induce changes in the phyto/chemical properties of yacon tubers and leaves. In this regard, studies have been conducted to determine the influence that various factors such as agricultural practice and geographical aspect of location of harvest and storage conditions after harvest may have on the phyto/chemical content of yacon tubers. The effect of planting date (September, October, and November) and location of planting in New

Zealand (Pukekohe, Hamilton, Lincoln, and Mosgiel) has been studied by Douglas et al. (2007). The findings showed that both yield and quality of yacon tubers were affected by planting date, while planting in November resulted in lowest yield and lowest FOS contents in tubers in all locations. This study noted the importance of determining the proper planting date, cultivation duration and harvest date considering the location to ensure the maximum yield of both fresh weight and carbohydrates in tubers (Douglas et al., 2007). Another study investigated the effect of harvest date (March, April, May, and June) as well as genotype of yacon tubers which were cultivated in New Zealand on yield and fructan content. The findings revealed that the fructan contents varied significantly among the four studied genotypes from 240 to 320 mg/g DM. Time of harvest was a key aspect for having the highest yield (Douglas et al., 2002). Silva et al. (2018) showed that altitude of cultivation location also affects the quality of yacon tubers in combination with planting time. They showed that at altitudes of 837 m in South of Espirito Santa State (Brazil) the quality of tubers in terms of their soluble solids content and juice quality were independent of planting date. However, at altitudes of 113 m the tubers which were planted in April to July had a better quality as their texture had lower hardness and they had lighter juice (Silva et al., 2018).

2. 5. Phyto/chemical characteristics of yacon leaves and tubers under the influence of post-harvest handling and food processing conditions

The availability of fresh yacon is seasonal, and to maintain the supply of yacon products during the whole year processing and preservation of tubers and leaves is essential. In this regard, post-harvest handling of tubers plays an influential role on the quality of fresh yacon tubers. The effect of altitude during storage on FOS content of yacon tubers showed that during 6 days of storing yacon tubers in shade, tubers which were stored at higher altitude (2930 m a.s.l.) contained higher FOS in comparison to those which were stored at lower altitude (1990 m a.s.l.) in the Peruvian region. The authors explained that at lower altitude the higher day and night temperature might have partly contributed to higher enzymatic activity and conversion of FOS. Moreover, they discussed that although the lower temperature at higher altitude may suppress the enzymatic activity during short-term storage period (6 days), during 2 weeks of storage the conversion of FOS may be alike in both altitudes (Graefe, Hermann, Manrique, Golombek, & Buerkert, 2004). Additionally, the colour of freshly sliced yacon darkens rapidly when exposed to air due the fact that their phenolic compounds undergo enzymatic polymerization (Cao et al., 2018; Delgado et al., 2013). Hence, avoiding the darkening of yacon slices to maintain the physicochemical characteristics of them is another reason for which several studies have focused on the processing of yacon tubers. As for yacon leaves, drying of leaves after harvest under mild conditions has been performed to preserve the leaves to be used later in form of infusions and extracts. Due to the fact that food processing and preservation can influence the physicochemical quality of the final product to a great extent, the findings of recent studies towards the effect of food processing on the quality of products developed from yacon in terms of their phyto/chemical content are presented in the following parts of this thesis. In this regard, the extraction process of leaves has been of interest. The traditional way of preparing tea from yacon leaves is either by decoction or infusion which are two major conventional methods of extraction in terms of the academic classification of processing of dried leaves of plants. Regarding postharvest handling and processing of tubers, the focus has been given to storage duration and conditions after harvest and more profoundly the effects of drying processes on the tubers, because it is among the most promising processes that is currently used for the production of yacon products such as yacon chips and flours in the market (Gurung, 2018; Exporting yacón to Europe, 2016).

2. 5. 1. Extraction processes of yacon leaves

The beneficial health effects of yacon leaves extract suggest its potential to be used in food, pharmacological or nutraceutical products. Various conventional solid-liquid extraction methods can be applied to extract the bioactive compounds from plant materials. In general, the conventional extraction methods such as Soxhlet extraction, maceration, DE, and hydrodistillation take advantage of heat or mixing to extract the bioactive material from the plant material using proper solvents (Azmir et al., 2013; Brusotti, Cesari, Dentamaro, Caccialanza, & Massolini, 2014). Jirovsky et al. (2003) studied the effect of extraction process by DE and infusion using water as the solvent and soxhlet extraction method using methanol as the solvent on the extraction of individual phenolic compounds and their quantity from yacon leaves. The results of their study showed that gallic acid, feluic acid, protocatechuic acid, chlorogenic acid, and caffeic acid were extracted from yacon leaves when methanol and soxhlet extraction was used, while a lower amount of the three former phenolic compounds were present in the aqueous extract (Jirovský, Horáková, Kotouček, Valentová, & Ulrichová, 2003). investigation, ethanol, methanol, and ethylacetate were used as solvents for extraction of yacon leaves originating in Japan. The extractions by above mentioned solvents were done at room temperature for 10 minutes. Besides, DE of yacon leaves was performed for 45 minutes. The findings showed that DE resulted in highest TPC (279 ± 11 µg chlorogenic acid equivalent/ mg of extract) in the final extract and higher reducing power against antioxidants compared to extracts resulted by using other solvents (Sugahara et al., 2015). Moreover, extraction of yacon leaves grown in Brazil were performed by means of infusion and DE using water as the solvent and soxhlet extractions by means of methanol. The findings of this study demonstrated that when infusion and DE were used, higher yield of extraction and higher TPC (39.9 \pm 2.62, and 42.2 \pm 4.58 mg gallic acid equivalent/g dry weigh of leaves, respectively for infusion and DE) were achieved. In addition, gallic acid, caffeic acid, p-coumaric acid, ferullic acid, rutin, and myricetion were identified in the aqueous extracts while gallic acid, caffeic acid, rutin, myricetion, quercetin and kaempherol were identified in the methanolic extract (De Andrade et al., 2014). Furthermore, the TPC of leaves of five different yacon landraces extracted by means of water and DE method varied between 17.1 ± 0.9 and 43.2 ± 3.2 mg gallic acid equivalent/ g of extract. When extraction was performed by ethanol the TPC varied between 14.0 ± 2.4 and 37.4 ± 3.9 mg gallic acid equivalent/ g of extract. The antioxidant activity of yacon leaves extract that were measured according to DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and Ferric reducing antioxidant power (FRAP) under the influence of tested landraces and solvent was higher in aqueous extracts than ethanolic extracts for the same landrace (Russo et al., 2015).

Although solid-liquid extraction of yacon leaves has been the subject of investigation by several authors, the extraction of yacon leaves by means of novel methods has not been investigated previously. Several non-conventional methods have been introduced and developed aiming to use more environmental friendly techniques, reduce the use of solvent and extraction time as well as enhancing the yield and quality of extracts. Ultrasound, microwave, pulsed electric field, and ohmic assisted solid-phase extraction are among such novel techniques (Gavahian, Chu, & Sastry, 2018). In particular, ohmic-assisted extraction has been studied for extraction of bioactive compounds such as anthocyanins from black rice bran, polyphenols from grape pomace, and phyto/chemicals from coloured potatoes (El Darra, Grimi, Vorobiev, Louka, & Maroun, 2013; Loypimai, Moongngarm, Chottanom, & Moontree, 2015; Pereira et al., 2016). Ohmic-assisted extraction works according to the principles of volumetric heating. An alternating electrical current is passed during the heating process directly through the sample offering benefits such as lower processing time and energy consumption, higher extraction efficiency and lower operating costs (Sakr & Liu, 2014) (Fryer, De Alwis, Koury, Stapley, & Zhang, 1993).

2. 5. 2. Drying of yacon tubers

The effects of post-harvest and food processing on the FOS content of yacon tubers and food products have been the objective of several studies while fewer studies focused on phyto/chemical content and antioxidant activity of yacon tubers and food products (Delgado et al., 2013; Ojansivu et al., 2011). The quality of fresh yacon tubers in terms of their phyto/chemical content after the harvest relays on the condition of post-harvest handling. Sunning of tubers after harvest has been investigated. It was noted that sunning of tubers converts the FOS to simple sugars namely, fructose and glucose and sweetens the taste of tubers (Graefe et al., 2004). The influence of storing Bolivian ecotype of yacon cultivated under Czech Republic environmental conditions determined that the amount of inulin content nearly halved after 140 days of storage while the content of fructose, glucose and sucrose raised in tubers (Lachman, Havrland, Fernández, & Dudjak, 2004). Furthermore, in accordance to perishability and seasonality of agricultural products, various methods were developed since ancient times to prolong their shelf life and preserve them to be used later. Drying of yacon slices, pulp and juice are examples of the preservation methods that have been used to extend the availability of yacon products throughout the whole year (De Mendonça, Corrêa, De Jesus Junqueira, Pereira, & Vilela, 2016; Lago, Bernstein, Brandelli, & Noreña, 2012; Ojansivu et al., 2011; Reis, Lenzi, & Masson, 2012). In fact, drying is one of the oldest food processing methods that has been used for the preservation of plant food materials. It can be explained as a process during which moisture removal happens as the result of simultaneous heat and mass transfer (El-Sebaii & Shalaby, 2012). Dried plant food materials have longer shelf life according to their low moisture content, reduced weight and volume during transportation, and lower packaging costs (Chou

& Chua, 2001; El-Sebaii & Shalaby, 2012). Development of more than 500 types of dryers has been noted in literature while around 100 are commercialized (Mujumdar & Law, 2010). Drying methods such as CHAD, solar drying, vacuum drying, spray drying, spouted bed drying, fluidized bed dryings, heat pump drying, FD, microwave drying, infrared drying, etc., have been developed based on economic considerations, environmental impact of processing techniques, and quality of final product (Chou & Chua, 2001; El-Sebaii & Shalaby, 2012; Mujumdar & Law, 2010). In this respect, drying of yacon slices, pulp and juice has been the subject of several studies with the main focus on the effect of drying on chemical quality of the final product specifically in terms of their carbohydrate profile, and physical quality for example colour and texture. The effect of hot air drying at 50, 60, and 70 °C on FOS content of yacon chips was investigated and showed that the higher the temperature of drying the less the FOS content in the final yacon chips (Scher, De Oliveira Rios, & Noreña, 2009). Moreover, encapsulation of yacon juice by spray drying and hot air drying of yacon pulp at 50, 60, and 70 °C has been studies by Lago et al. (2012), and showed that the amount of sugars and inulin was higher in pulp than in juice. Furthermore, the inulin content of encapsulated vacon juice and dried pulp in 100 grams of dry matter was lower than that of yacon juice and pulp, while the amount of glucose and fructose of encapsulated juice and dried pulp increased significantly compared to those of juice and pulp which might suggest the hydrolysis of inulin during the drying process (Lago et a., 2012).

The influences of post-harvest handling and processing of yacon tubers and in particular the effect of drying on the phenolic compounds and antioxidant activity of their final product has been neglected so far. While the amount of phenolic compounds and antioxidant activity of yacon tubers have been studied by various researchers (Campos et al., 2012; Castro, Caballero, Herbas, & Carballo, 2012; Pereira et al., 2016), the effects of duration and storage conditions as well as drying and pre-treatments on phenolic constitutes and antioxidant activity of yacon final products have not been studied.

3. Publications

The present cumulative thesis includes three articles, which have been published or submitted to peer-reviewed international journals. Please use the references given below for citation of the three articles that are consistent with the Chapters I-III of this thesis.

Chapter I

Khajehei, F., Niakousari, M., Seidi Damyeh, M., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2017). Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction. *Molecules*, 22(12), 2043.

Chapter II

Khajehei, F., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2018). Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars. *Molecules*, 23(2), 278.

Chapter III

Khajehei, F., Hartung, J., & Graeff-Hönninger, S. (2018). Total Phenolic Content and Antioxidant Activity of Yacon (*Smallanthus Sonchifolius* Poepp. and Endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying. *Agriculture*, 8(12), 183.

4. Chapter I: Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction

Publication I:

Khajehei, F., Niakousari, M., Seidi Damyeh, M., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2017). Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction. *Molecules*, 22(12), 2043.

Studies have focused on the phyto/chemicals of yacon leaves and their biological activities such as hypoglycaemic, antioxidant, antifungal, and pesticide attributes. The phyto/chemical content of yacon leaves depends primary on the cultivar of yacon plant and the environmental conditions during the cultivation. Moreover, the amount of reported values for phyto/chemical content of yacon leaves may vary due to the method of extraction and solvent system used for extraction. That being so, chapter I investigated the differences in phyto/chemical content and antioxidant activity of leaves of two cultivars, namely, red and white, at two leaf maturity stages (young and old) which were grown under the environmental conditions of southwestern Germany. Water as non-toxic solvent was chosen for extraction of dried yacon leaves as it is also used when leaves are prepared for yacon tea as herbal remedy. Using water as solvent further minimizes the environmental impact of the extraction process. Moreover, application of OH-DE was examined to evaluate its potential for extraction of phyto/chemicals from yacon leaves in terms of quality as well as energy consumption.





Article

Impact of Ohmic-Assisted Decoction on Bioactive Components Extracted from Yacon (*Smallanthus sonchifolius* Poepp.) Leaves: Comparison with Conventional Decoction

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Abstract: Yacon (Smallanthus sonchifolius Poepp.) leaves are a potentially rich source of bioactive compounds, such as phenolic acids and flavonoids. In this study, the effect of the extraction method (ohmic-assisted decoction (OH-DE) and decoction (DE)), yacon cultivar (red and white), and leaf age (young and old) on the quality/quantity of extracted phytochemicals were investigated. Extraction yield, energy consumption, total phenolic content (TPC), total flavonoid content (TFC), ABTS and DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) were determined. Additionally, HPLC-DAD was used to identify the major individual phenolic and flavonoid compounds of yacon leaves. The results showed that a three-way interaction of process-variables (extraction method xyacon cultivar xage of leaves) influenced the extraction yield, TPC, TFC, ABTS, and DPPH radical scavenging activity, and FRAP, significantly (p < 0.05). However, energy consumption of the extraction process was only affected by method of extraction (p < 0.05) and was halved when OH-DE was applied as compared to DE alone. Additionally, the phytochemical quality of extracts was either improved or comparable when OH-DE was used for extraction. Also, it was shown that yacon leaves contained considerable amounts of caffeic acid, chlorogenic acid, ferrulic acid, myricetin, p-coumaric acid, and rutin, while leaves of the red cultivar had higher contents of each compound compared to leaves of the white cultivar.

Keywords: antioxidant activity; decoction; flavonoids; ohmic-assisted decoction; phenolic acids; *Smallanthus sonchifolius* Poepp.; yacon leaves

1. Introduction

The growing attention towards the health benefits of medicinal plants has originated from an increasing tendency of consumers towards substituting synthetic compounds in food and pharmaceutical products by their potential natural alternatives. Phenolic acid and flavonoid compounds are secondary metabolites of plants and, due to their antioxidant activity, one of the most important groups of bioactive constituents of medicinal plants. A huge number of studies has been conducted to determine the phytochemicals and in particular phenolic profiles of medicinal plants, their mechanism of action against certain diseases, health-enhancing effects, safety and their potential to be used in food products, nutraceuticals and pharmaceuticals [1,2].

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Yacon (Smallanthus sonchifolius Poepp.) is a tuber plant that is native to the Andean region. Yacon leaves have been prepared as a traditional medicinal tea that can be useful against chronic diseases, such as diabetes and renal disorder. For this reason, yacon leaves have recently gained attention as a natural remedy. In this regard, several studies has been focusing on hypoglycaemic activity of yacon leaves [3-6]. Furthermore, Investigations have shown that yacon leaves have antioxidant, antifungal, and pesticidal properties [6-8]. Therefore, determination of the phytochemicals in yacon leaves extract that might induce such biological activities has been the focus of several studies. Yacon leaves contain biologically active compounds such as catechol, phenolic acids, terpenes, and flavonoids, to which their antioxidant, antidiabetic, and antitumor properties may be attributed [3,6,8]. Phytochemical content and antioxidant activity have been reported in extracts of yacon leaves cultivated in Brazil using aqueous decoction and infusion methods and their methanolic extracts [9]. The influence of genotype and solvent on phytochemicals extracted from yacon leaves cultivated in the Czech Republic was indicated in study of Russo et al. [10]. Also, different amounts of phytochemicals have been reported in yacon leaves from various origins such as Ecuador and China [11,12]. Thus, the quantities of phytochemicals determined depends on various parameters, such as the origin of the yacon leaves, method and the solvent used for extraction. More specifically, chlorogenic acid, caffeic acid, ferulic acid, quercetin, rosmarinic acid, luteolin, gallic acid, rutin, and myricetin were among the phytochemicals that have been identified in various quantities in crude extracts of yacon

Several methods have been developed to perform extraction of phytochemicals and their effects on composition and functionality of extracts from plant materials have been studied [15,16]. Novel technologies, such as microwave-, ultrasound-, and ohmic-assisted extraction, have been applied to improve the efficiency of extraction of phytochemicals from food materials. These methods have attracted considerable interest from the scientific community [15]. Particularly, ohmic-assisted extraction is a process where an alternating electrical current is passed directly through the processed materials. The thermal energy required for the extraction process is generated internally as a result of this passage of electrical current through the materials [17]. Ohmic heating has been the topic of a considerable number of studies to improve the extraction process in terms of efficiency and quality of product. The effectiveness of ohmic-assisted extraction of polyphenols from red grape pomace, anthocyanins from black rice bran, and phytochemicals from colored potato has been previously reported in the literature [18–20]. When compared to conventional extraction methods, ohmic-assisted extraction has more merits. Ohmic-assisted extraction requires a shorter processing time due to the rapid generation of heat within the plant material, it consumes less energy, and the operating costs are much lower [21]. Besides, ohmic heating technology has been evaluated as a green technology, because it works with electricity, which can be produced using renewable energies (e.g., solar and wind energy) and results in lower carbon emissions [22,23].

To the best of our knowledge, there are no studies on total phenolic and flavonoid content, antioxidant activity, and quantity of main individual phenolic and flavonoid compounds of leaves of red and white yacon cultivars which were cultivated in Germany. Therefore, the main objectives of this work were to: (1) evaluate the influence of two extraction methods, namely, ohmic-assisted decoction (OH-DE) and decoction (DE), on biological characteristics of aqueous crude extracts from yacon leaves and; (2) determine the effect of cultivar and age of yacon leaves on the polyphenol profile and antioxidant activity.

2. Results and Discussion

2.1. Extraction Process: Extraction Yield and Energy Consumption

According to previous studies, extraction of yacon leaves using the DE method and water as an environmental friendly solvent resulted in extracts with higher amounts of extracted phytochemicals [9,10]. Therefore, in this study aqueous extraction of yacon leaves was investigated

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using the DE method with different heating mechanisms. The DE method consists of using a hot surface from which the thermal energy is transferred to food materials. DE was used as conventional method. During DE, heat transfer occurs according to the conduction and convection principles. OH-DE was performed taking advantage of volumetric heating principles as a novel technique.

Electrical conductivity is a key factor in ohmic heating. The addition of salt in an extraction medium can increase the electrical conductivity and ensure the generation of heat within the mixture [21,24]. Therefore, before OH-DE was used in this study, 0.3% w/v of NaCl was added to the mixture of leaves and water in order to improve the electrical conductivity of the extraction medium. According to Table 1, there were significant differences between the electrical conductivities of each sample with and without salt (p < 0.05). The electrical conductivity increased between 1.28 and 1.98 times the original sample when salt was added to the mixture, which can enhance the generation of thermal energy during OH-DE (Table 1).

Table 1. Electrical conductivity of dried yacon leaves.

Cultivar	Age of Leaves	Average Electrical Conductivity (s			
Cuitivar	Age of Leaves	With 0.3% w/v NaCl	Without NaCl		
white	young	$1.00~^{\mathrm{Aa}}\pm0.00$	$0.44~^{ m Ab} \pm 0.01$		
white	old	$0.99~^{ m ABa} \pm 0.00$	$0.39^{\text{ Cb}} \pm 0.01$		
red	young	$0.98^{\mathrm{\ Ba}}\pm0.01$	$0.41~^{\mathrm{ABb}}\pm0.00$		
red	old	$0.91~^{\text{Ca}}\pm0.01$	$0.31^{ ext{ Db}} \pm 0.01$		

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column and same lowercase letter in a row are not significantly different as indicated by Tukey's test (p < 0.05).

Table 2 shows the energy consumption and the corresponding extraction yield. The energy consumption was only influenced significantly by the extraction method (p < 0.0001) (Table 3). When OH-DE was applied, the heating-up time (28.20 \pm 8.02 min on average) was considerably lower than the heating-up time when DE was used (44.12 \pm 6.98 min on average). Consequently, the energy consumption was halved using OH-DE in comparison to DE (Table 2). Rapid heat generation inside the extraction medium by conversion of electrical energy into thermal energy when using OH-DE is seen as the main reason for significant reduction of energy in contrast to DE.

Table 2. Yield of extraction (%) and energy consumption.

Cultivar	Age of Leaves	Extraction Method	Yield of Extraction (%)	Energy Consumption (kWh)
white	young	OH-DE	$4.84~^{AB}\pm0.18$	$0.20~^{\mathrm{A}}\pm0.00$
white	young	DE	$4.37^{\text{ C}} \pm 0.33$	$0.43~^{ m B}\pm0.05$
white	old	OH-DE	$5.05~^{ m AB}\pm0.16$	$0.20~^{ m A}\pm0.00$
white	old	DE	$5.03~^{\mathrm{AB}}\pm0.22$	$0.43~^{ m B}\pm0.05$
red	young	OH-DE	$5.23~^{ m AB}\pm0.12$	$0.20~^{ m A}\pm0.00$
red	young	DE	$5.09 \ ^{\mathrm{AB}} \pm 0.28$	$0.50^{\ \mathrm{B}} \pm 0.10$
red	old	OH-DE	$5.33 ^{ ext{A}} \pm 0.11$	$0.20~^{ m A}\pm0.00$
red	old	DE	$5.09 ^{\mathrm{AB}} \pm 0.25$	$0.46~^{ m B}\pm0.11$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). OH-DE = Ohmic assisted decoction and DE= decoction.

In this study, extraction yield was assessed using the total dry matter of the extracts. Statistical analysis of data showed that the interaction between extraction method, cultivar, and age of yacon leaves influenced the yield of extraction significantly (p = 0.0339, Table 3). The extraction yield of leaves of the red cultivar was higher compared to that of the leaves of the white cultivar (Table 2). The extraction yield of old leaves of the red cultivar extracted by OH-DE (extraction yield (%) = 5.33 ± 0.11) was significantly higher than the yield of the other samples, while

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the lowest yield of extraction corresponded to the young leaves of the white cultivar extracted by DE (extraction yield (%) = 4.37 ± 0.33 , Table 2). No significant statistical difference was observed between the extraction yields when the same kind of leaves were extracted with different methods except for the young leaves of the white cultivar, which had significantly lower yields of extraction when extracted with DE in comparison to OH-DE (Table 2).

Table 3. ANOVA of results of energy consumption, yield of extraction, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS), and Ferric reducing antioxidant power (FRAP) as a function of extraction method (ohmic assisted decoction and decoction), cultivar (red and white) and age (young and old) of leaves.

Process-Variable	Energy Consumption	Yield of Extraction	TPC	TFC	DPPH	ABTS	FRAP
Extraction method	p < 0.0001	p = 0.0011	p = 0.0190	p = 0.029	p < 0.0001	p < 0.0001	p < 0.0001
Cultivar	p = 0.3322	p < 0.0001	p = 0.0002	p = 0.0016	p < 0.0001	p < 0.0001	p = 0.2236
Age of leaves	p = 0.7432	p = 0.0004	p = 0.1101	p = 0.0007	p < 0.0001	p < 0.0001	p = 0.1025
Extraction method * Cultivar	p = 0.3322	p = 0.6922	p = 0.0639	p = 0.1093	p = 0.1343	p = 0.1074	p = 0.0885
Extraction method * age of leaves	p = 0.7432	p = 0.1667	p = 0.6238	p = 0.5590	p = 0.0395	p = 0.0413	p = 0.2565
Cultivar * age of leaves	p = 0.7432	p = 0.0033	p = 0.8614	p = 0.4544	p = 0.0020	p = 0.0002	p = 0.4736
Extraction method * Cultivar * age of leaves	p = 0.7432	p = 0.0339	p = 0.0483	p = 0.0484	p = 0.0034	p = 0.0055	p = 0.0084

^{*} Interaction between process-variables.

In addition, the effects of processing on the physical structure of leaves were observed to better understand the differences between mechanisms of extraction by OH-DE compared to DE. Figure 1 illustrates the effect of processing on young leaves of the red cultivar as an example.

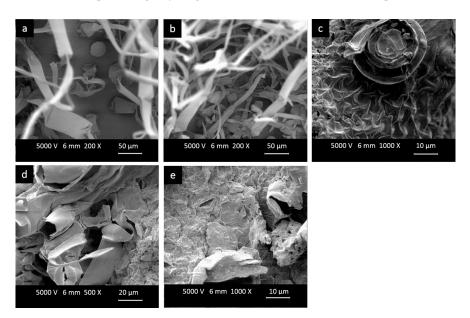


Figure 1. Scanned electron micrograph of young yacon leaves of the red cultivar (a) upper surface of fresh leaves- $200\times$; (b) lower surface of fresh leaves- $200\times$; (c) surface of dried leaves- $1000\times$; (d) surface of dried leaves after OH-DE- $500\times$ and (e) surface of dried leaves after DE- $1000\times$.

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Figure 1a,b show the glandular and non-glandular trichomes on the upper and lower surface of epidermis of a fresh leaf, respectively. Non-glandular trichomes are in the form of hairs and glandular trichomes are raised above the epidermis of the leaves. The glandular trichomes take part in the production and storage of compounds that are essential for plant adaptation and interaction with environmental conditions [6,25]. The epidermis of the leaves were intensely affected after drying, and shrinkage and collapse of leaf surface is the outcome of drying the leaves (Figure 1c). Collapse of cell structure due to drying has been previously reported [26]. Figure 1d,e can be used to distinguish the difference in effect of OH-DE and DE on the physical structure of leaves, respectively. The cell structure of leaves after OH-DE is more porous and damaged compared to the sample treated by DE (Figure 1d,e). Drastic destruction of cellular structure and consequently a release of compounds inside the cell are a result of applying ohmic heating, which has been reported by other researchers [19,22,23]. Ruptured cells are a consequence of the rapid heating mechanism of ohmic heating. Suddenly converting electrical energy into thermal energy causes heat stress to cells, which in turn reuslts in rupture in their structure, and enhances the diffusion of solutes in solvents during the extraction process. Besides, the dominant non-thermal effect of ohmic heating known as cell membrane electroporation could be another factor that can cause damage to cells which further assists in the extraction process. The breakage of cellular structure during DE extraction is less severe, because the heat transfers by means of conduction and convection takes place at a slower pace in contrast to volumetric heat transfer in the case of OH-DE [23,27]. Consequently, the average amount of solid diffused in the solvent is lower using DE in comparison to OH-DE (Table 2).

2.2. Total Phenolic Content (TPC)

Phenolic compounds are secondary metabolites in plants, which contribute to the sensorial (taste, flavor, color, etc.) and functional (antioxidant activity, antidiabetic, anticancer activity, etc.) characteristics of food products [2,28]. The amount of TPC in yacon leaves is shown in Table 4.

Table 4. Total phenolic content, total flavonoid content, ABTS radical scavenging activity, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) values of yacon leaves which were extracted with ohmic-assisted decoction (OH-DE) and decoction (D).

Cultivar	Age of Leaves	Extraction Method	Total Phenolic Content (mg GAE g DW ⁻¹)	Total Flavonoid Content (mg RE g DW ⁻¹)	ABTS Radical Scavenging Activity (mM TE g DW ⁻¹)	DPPH Radicals Scavenging Activity (mg AAE g DW ⁻¹)	FRAP (mM Fe ²⁺ g DW ⁻¹)
white	young	OH-DE	$53.39 ^{BC} \pm 1.94$	$138.16^{ \text{ B}} \pm 5.12$	1573.02 $^{\circ}$ \pm 97.87	102.77 $^{\text{C}}$ \pm 4.44	$825.03 ^{BC} \pm 22.62$
white	young	DE	$59.23 ^{\mathrm{ABC}} \pm 14.81$	$153.79 ^{AB} \pm 40.64$	$1529.84^{\circ} \pm 257.22$	99.71 $^{\circ}$ \pm 16.76	$838.14 ^{\mathrm{ABC}} \pm 185.46$
white	old	OH-DE	$55.50^{\mathrm{BC}}\pm10.10$	$135.78^{ \mathrm{B}} \pm 16.23$	$2008.80^{ \text{ B}} \pm 146.94$	$124.82 \text{ B} \pm 8.86$	$990.36 \text{ AB} \pm 76.61$
white	old	DE	$46.51^{\circ} \pm 0.79$	$110.34 \text{ B} \pm 4.68$	$1560.27^{\circ} \pm 87.30$	$94.81^{\circ} \pm 5.25$	$798.11^{\circ} \pm 52.23$
red	young	OH-DE	$76.67 \text{ A} \pm 21.67$	$199.29 \text{ A} \pm 58.75$	$1843.76^{ \text{ B}} \pm 141.16$	$120.11^{ B}\pm 9.33$	994.55 $^{ m A}$ \pm 83.38
red	young	DE	$59.42 ^{ABC} \pm 2.51$	$157.43^{AB} \pm 15.307$	$1432.31^{\circ} \pm 151.37^{\circ}$	$93.18^{\circ} \pm 9.88$	$771.17^{\circ} \pm 80.00$
red	old	OH-DE	$67.89 ^{AB} \pm 1.91$	$153.23 \text{ AB} \pm 7.56$	2378.89 ± 52.70	$143.148 \text{ A} \pm 2.86$	$976.90 \text{ AB} \pm 56.23$
red	old	DE	$59.66^{\ ABC} \pm 4.19$	$134.013^{\ B} \pm 12.99$	$2034.06^{ \text{ B}} \pm 96.17$	$121.28 ^{\text{B}} \pm 5.87$	$838.40 ^{ABC} \pm 75.46$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). GAE = galic acid equivalent, RE = rutin equivalent, TE = trolox equivalent and AAE = ascorbic acid equivalent.

Statistical analysis of data determined the significant effect of interactions between extraction methods, cultivar, and age of leaves on TPC (p=0.0483, Table 3). The average amount of TPC was higher in red cultivar leaves as compared to the white cultivar. This result is aligned with the higher average extraction yield for leaves of the red cultivar (Tables 2 and 4). The TPC that was extracted from leaves of red cultivar varied between 59.42 ± 2.51 and 76.67 ± 21.67 (mg GAE g DW $^{-1}$), while leaves of white cultivar contained between 46.51 ± 0.79 and 59.23 ± 14.81 (mg GAE g DW $^{-1}$) of TPC (Table 4). The TPC of yacon leaves has also been investigated by other researchers [9–11,13,14]. The amount of TPC in yacon leaves extracted by DE in a study of De Andrade et al. was 42.2 ± 4.58 (mg GAE g DW $^{-1}$) [9]. Also, the TPC in yacon aqueous extract obtained by DE from five yacon landraces varied between 17.10 ± 09 and 43.20 ± 3.20 (mg GAE g DW $^{-1}$) [10]. Variations in

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the TPC content of yacon leaves reported in previous studies and in our study could be due to a combination of several factors. The quality of plant material is an important factor that can affect the extraction yield and quality of biological compounds. The quality of plant material can be influenced by several factors, such as cultivar, environmental conditions, and handling and processing conditions of plant material after harvest. Extraction method, solvent type, the ratio of solvent to plant material (v/w), particle size of plant materials, and time and temperature of extraction are some of the technological parameters that can influence the extraction of biological compounds and lead to differences in the overall TPC [29–32].

Furthermore, no significant statistical difference was indicated between the TPC of the same type of leaves, which were extracted with DE and OH-DE (Table 4). This indicates that there was no adverse effect on the amount of TPC by OH-DE. In fact, the average amount of TPC extracted from old leaves of the white yacon cultivar, young and old leaves of the red cultivar using OH-DE was 19.32%, 29.03% and 13.79% higher compared to results of extracted TPC from the same type of leaves using DE, respectively. In the case of young leaves of the white cultivar, the average amounts of TPC extracted with OH-DE and DE were statistically comparable, but the amount of extracted TPC was 9.85% lower using OH-DE in comparison to extracted TPC by DE. Therefore, OH-DE can be an effective alternative method for extraction of phenolic compounds, which supports the results of other investigators [18–20].

2.3. Total Flavonoid Content (TFC)

Flavonoids are a vast group of polyphenolic compounds, which can be found particularly in the leaves of plants. They are responsible for taste and flavor of food materials, while having certain health benefits such as antidiabetic effects and antioxidant and anticancer activity [33,34]. Table 4 shows the results for the TFC of yacon leaves. The interaction between the main process variables, namely, cultivar of yacon, age of leaves, and method of extraction, on TFC was significant (p=0.0484, Table 3). Similar to the TPC outcomes, the average amount of TFC of leaves of red cultivars were higher than that of leaves of the white cultivar (Table 4). The average amount of TFC in leaves of the red cultivar ranged from 134.01 \pm 12.99 to 199.29 \pm 58.75 (mg RE g DW $^{-1}$), while it ranged from 110.34 \pm 4.68 to 153.79 \pm 40.64 (mg RE g DW $^{-1}$) in leaves of the white cultivar (Table 4). Although, no significant difference was determined between mean values of TFC in the same type of leaves extracted with different methods (Table 4), The average amount of TFC of leaves with the same age and cultivar extracted by OH-DE was higher than that of the same leaves extracted using DE. However, there were some exceptions in cases with young leaves from the white cultivar (Table 4).

TFC of yacon leaves in aqueous extracts obtained using DE was reported at 39.72 ± 1.37 (mg RE g DW $^{-1}$) in the study of De Andrade et al., which is considerably lower than our findings [9]. Similar to TPC, the amount of extracted TFC can be affected by various factors such as different ratios of solvent to plant material, extraction condition, origin, cultivar, quality, and particle size of the leaves. Moreover, in the present study, each sample was extracted two times, while in the study of De Andrade et al. each sample was extracted only once [9]. According to principles of mass transfer, renewal of solvent can keep the concentration gradient high and postpone reaching the equilibrium point. Therefore, adding fresh water to leaves for a second cycle of extraction as done in this study might be a contributing factor to higher extracted yields of flavonoid compounds.

2.4. Antioxidant Activity

2.4.1. ABTS Radical Scavenging Activity

ABTS radical scavenging activity is a fast and simple method to determine the total antioxidant capacity in food materials which is determined by measuring the reduction in blue-green color of the radical cation ABTS through donation of hydrogen or electron by the antioxidant compounds [35].

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The ABTS radical scavenging activity of yacon leaves are presented in Table 4. According to the statistical analysis of data, the interaction effect of the three process variables (extraction method, cultivar, and age of leaves) had a significant effect on the ABTS radical scavenging activity of leaves (p = 0.0055, Table 4). Higher ABTS radical scavenging activity of red yacon leaves in comparison with that of the white yacon leaves aligns with their higher TPC and TFC results. The ABTS radical scavenging activity of old leaves of the red cultivar that was extracted with OH-DE $(2378.89 \pm 52.70 \text{ (mM TE g DW}^{-1}))$ was significantly higher than that of all other samples (Table 4). ABTS radical scavenging activity of aqueous extracts of yacon leaves extracted by decoction was reported at 391.55 \pm 22.32 (μ M Trolox g DW $^{-1}$) in a study of De Andrade et al., which is significantly lower than the results obtained in this study [9]. Variation in cultivar of yacon leaves and differences in origin of them might be among contributing factors to different ABTS radical scavenging activity obtained in this study. Furthermore, as phenolic acid and flavonoid compounds may contribute to ABTS radical scavenging activity of yacon leaves, the higher amount of ABTS radical scavenging activity obtained in the present study is in agreement with higher amounts of TFC and TPC extracted compared to findings of De Andrade et al. [9]. ABTS radical scavenging activity of aqueous extracts of yacon leaves has been also studied using EC₅₀ methodology in study of Sugahara et al. which cannot be compared to the results of present study due to differences in analytical methods [36].

ABTS radical scavenging activity of yacon leaves indicated a moderate correlation with TPC (R = 0.452), while it did not significantly correlate with TFC (Table 5). Antioxidant activity of phenolic acid and flavonoid compounds and their mechanism of action in regards to free radicals are related to their structure. Therefore, differences in correlation among TFC and TPC with the result of ABTS radical scavenging activity might be under influence of the structure of phenolic and flavonoid compounds in yacon leaves extract [37,38].

Table 5. Correlation coefficient (*R*) between total phenolic content, total flavonoid content, ABTS radical scavenging activity, DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) values of yacon leaves which were extracted with ohmic-assisted decoction (OH-DE) and decoction (D) values of yacon leaves.

	Total Flavonoid Content	DPPH Radical Scavenging Activity	ABTS Radical Scavenging Activity	FRAP
DPPH radical scavenging activity	0.350 *			
ABTS radical scavenging activity	$0.226 ^{\mathrm{NS}}$	0.983 ***		
FRAP	0.542 ***	0.791 ***	0.715 ***	
Total phenolic content	0.943 ***	0.545 ***	0.452 **	0.627 ***

* p < 0.05, ** p < 0.01, *** p < 0.001, and NS not significant.

2.4.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity method is widely used to determine the ability of antioxidants in a sample to quench free radicals of DPPH by donating hydrogen. DPPH radicals have a purple color, which undergoes a color change upon neutralization when it receives hydrogen [35].

The result of evaluating the DPPH radical scavenging activity of yacon leaves is reported in Table 4. The statistical analysis showed that the interaction between the three independent process variables (extraction method, cultivar, and age of leaves) significantly influenced the DPPH radical scavenging activity of leaves (p = 0.0034) (Table 3). The red yacon leaves showed higher average values of DPPH radical scavenging activity in comparison to the leaves of the white cultivar (Table 4). This is in agreement with results previously reported in a study of Russo et al., which showed higher DPPH radical scavenging activity in yacon leaves that belonged to landraces that had tubers with purple-grey colored peels compared to landraces with red-purple and grey-orange colored tuber peels [10]. Likewise, the outcome of ABTS radical scavenging activity the old leaves of the red cultivar extracted with OH-DE showed a significantly higher DPPH radical scavenging activity than the other samples (Table 4). The result of Pearson correlation analysis showed that there was a moderately

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positive correlation between the DPPH radical scavenging activity and TPC (R = 0.545, Table 5). Also, a strong positive correlation was determined between ABTS radical scavenging activity and DPPH radical scavenging activity (R = 0.983, Table 5). In addition, there was a week positive correlation between DPPH radical scavenging activity and TFC (R = 0.350, Table 5). DPPH radical scavenging activity of yacon leaves extract was studied according to EC₅₀ methodology by other researches [9,36]. Due to differences in analytical methods applied in these investigations and the present study, the results cannot be compared. A positive correlation between TPC and TFC and DPPH radical scavenging activity of yacon leaves extract was reported in the study of De Andrade et al., which is supported by our findings [9]. This suggests that the antioxidant characteristics of yacon leaves are attributable to phenolic acids and flavonoid compounds.

2.4.3. FRAP Assay

FRAP assay is another method based on electron transfer for measuring the antioxidant characteristics of food materials. In this method, an antioxidant's power is determined under acidic conditions by reducing the ferric 2,4,6-tripyridyl-s-triazine complex to the ferrous complex. The later exhibits an intense blue color which can be measured spectrophotometrically. The FRAP assay results are reported as an equivalent concentration of ferrous ions (mM) [35].

The FRAP of yacon leaves is noted in Table 5. The statistical analysis of data showed that the FRAP of yacon leaves was significantly affected by the interaction between extraction method, cultivar, and age of leaves (p = 0.0084) (Table 3). In line with the DPPH and ABTS radical scavenging activity outcomes, the leaves of the red cultivar showed a higher average amount of FRAP in comparison to leaves of the white cultivar (Table 4). Young and old leaves of the red cultivar, which were extracted with OH-DE had the highest average amount of FRAP (994.55 \pm 83.38 and 976.90 ± 56.23 (mM Fe²⁺ g DW⁻¹), respectively), were statistically comparable to each other (Table 4). The results of present study are in agreement with results of the study of Russo et al. who reported higher FRAP values for the extract that was obtained from leaves of yacon samples with grey-purple tuber peel [10]. Moreover, a positive correlation (R between FRAP and TPC = 0.627, R between FRAP and TFC = 0.542, R between FRAP and DPPH radical scavenging activity = 0.791, R between FRAP and ABTS radical scavenging activity = 0.715) was determined between FRAP of yacon leaves and their TPC, TFC, DPPH radical scavenging activity, and ABTS radical scavenging activity (Table 5). The correlation between TPC or TFC and FRAP was stronger than the correlation between TPC or TFC and DPPH radical scavenging activity or ABTS radical scavenging activity (Table 5). This might suggest that: (1) the phenolic and flavonoid compounds in yacon leaves express better antioxidant activity in acidic conditions and (2) the mechanism of action for dominant antioxidant compounds in yacon leaves is not based on the radical quenching mechanism, but instead is in line with their ability to act as reductants.

2.5. Individual Phenolic acid and Flavonoid Compounds

In the present study, HPLC-DAD was used for quali-/quantification of phenolic compounds in yacon leaves. Table 6 reports the amount of six phenolic compounds, which were successfully identified and quantified using external standards, including four hydroxycinnamic acids, namely, ferrulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, and two flavonoid compounds, namely, myricetin and rutin. The presence of ferrulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, myricetin and rutin in aqueous extract of yacon leaves has been confirmed according to existing literature [5,9–11,13,14].

The outcomes (Table 6) showed that caffeic acid had the highest average amount, followed by chlorogenic acid, and p-coumaric acid, while ferrulic acid had the lowest average amount among the hydroxycinnamic acids in yacon leaves. Myricetin and rutin were the two flavonoid compounds which were detected in yacon leaves in this work. The average amount of myrecitin was much higher than the mean amount of rutin constitution of yacon leaves (Table 6). The findings showed that the

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average amount of each individual phenolic and flavonoid compound, which was screened in this study, was higher in the leaves of the red cultivar when compared to the leaves of the white cultivar. This aligns with the results of TPC and TFC and antioxidant activity measurements (Tables 4 and 6). Furthermore, these results are in agreement with the outcomes of the study of Russo et al., who reported high amounts of phenolic compounds in aqueous extracts of yacon leaves, while there was a difference between the profile of phenolic compounds and their quantity in extracts of leaves from different yacon landraces [10].

Table 6. Individual phenolic acid and flavonoid compounds of yacon leaves that were extracted with ohmic-assisted decoction (OH-DE) and decoction (D).

Cultivar	Age of Leaves	Extraction Method	Ferrulic Acid (mg g DW ⁻¹)	Caffeic Acid (mg g DW $^{-1}$)	Myricetin (mg g DW ⁻¹)	P-Coumaric Acid (mg g DW ⁻¹)	Rutin (mg g DW ⁻¹)	Chlorogenic Acid (mg g DW ⁻¹)
white	young	OH-DE	$0.44^{\circ} \pm 0.04$	$17.92^{\circ} \pm 0.77$	$24.53 ^{ ext{A}} \pm 3.35$	$3.35^{ B} \pm 0.14$	$0.15^{\text{ CD}} \pm 0.04$	$9.92^{EF} \pm 0.54$
white	young	DE	nd	$19.42^{\circ} \pm 0.28$	$16.24 \text{ A} \pm 11.30$	$2.68 \text{ B} \pm 1.55$	$0.14^{\text{ D}} \pm 0.04$	$15.73 ^{\mathrm{BC}} \pm 0.32$
white	old	OH-DE	nd	$16.81^{\circ} \pm 1.35^{\circ}$	$12.01 ^{A} \pm 0.32$	$3.37^{ B} \pm 0.32$	$0.25 ^{\text{BCD}} \pm 0.01$	$9.12 ^{\text{F}} \pm 0.71$
white	old	DE	nd	$19.94^{\circ} \pm 1.11$	$16.85 \text{ A} \pm 0.92$	$2.94 \text{ B} \pm 0.03$	$0.16^{\text{ CD}} \pm 0.006$	$12.45^{\text{ DE}} \pm 0.07$
red	young	OH-DE	$0.70^{ \mathrm{B}} \pm 0.04$	$28.95 \text{ A} \pm 0.21$	$25.98 \text{ A} \pm 6.31$	$6.18 ^{ ext{A}} \pm 0.05$	$0.52 \text{ A} \pm 0.01$	$22.33 \text{ A} \pm 0.68$
red	young	DE	$0.62^{BC} \pm 0.11$	$25.11^{B} \pm 0.69$	$15.03 ^{A} \pm 2.40$	$6.03^{\text{ A}} \pm 0.17$	$0.42^{AB} \pm 0.006$	$18.76^{\ B} \pm 0.14$
red	old	OH-DE	$0.90^{\text{ A}} \pm 0.09$	$24.33 \text{ B} \pm 3.85$	$24.35 \text{ A} \pm 7.32$	$4.01^{B} \pm 1.12$	$0.31 ^{\text{BCD}} \pm 0.19$	$13.96^{\text{ CD}} \pm 3.21$
red	old	DE	$0.67^{ B} \pm 0.09$	$19.56^{\circ} \pm 0.46^{\circ}$	$19.32 ^{A} \pm 2.23$	$2.99^{ B} \pm 0.08$	$0.30^{\mathrm{BCD}} \pm 0.01$	$11.11^{\text{ DEF}} \pm 0.94$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05).

In addition, the highest amount of each phenolic and flavonoid compound was obtained when young leaves of the red yacon cultivar were extracted by OH-DE. Old leaves of red yacon extracted with OH-DE ranked second with higher individual phenolic acids and flavonoids (Table 6). Also, the average amount of myricetin, p-coumaric acid, and rutin was higher in leaves of the white cultivar when OH-DE was applied for extraction (Table 6). However, the average amount of caffeic acid and chlorogenic acid extracted from leaves of the white cultivar was higher when the extraction was performed by DE (Table 6). Overall, the results showed no difference in profile of identified individual phenolic acids and flavonoid compounds which were extracted from yacon leaves with exception for ferrulic acid (Table 6). Ferullic acid was only extracted from young leaves of the white cultivar when OH-DE was applied for extraction (Table 6). Higher retention of phenolic compounds, when ohmic heating was applied, has been reported by other researchers in cases of extraction of polyphenols from red grape pomace, extraction of colorant from rice bran, and extraction of phytochemicals from potato [18–20]. Rapid heating, when ohmic heating is applied, reduces the overall processing time by reducing the heating up time. Shorter overall processing time might play a role in producing less destructive effects on heat sensitive phenolic compounds. Furthermore, rapid heating can cause structural damage to cellular structures through heat stress, as discussed in previous sections and enhance the release of phenolic compounds. In addition, electroporation effect of ohmic heating can be named as another contributing factor in release of higher amounts of biological compounds from cellular structures [21,39].

3. Materials and Methods

3.1. Chemicals

Ascorbic acid, Folin–Ciocalteu's reagent, FeCl₃, FeSO₄, NaOH, HCl, and NaNO₂, were purchased from Merck (Darmstadt, Germany). 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), were provided from Sigma (Darmstadt, Germany). Gallic acid (Scharlau, Barcelona, Spain), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CalBiochem, Darmstadt, Germany), AlCl₃ (Fluka, Seelze, Germany), Na₂CO₃ (AppliChem, Darmstadt, Germany), potassium persulfate (Bernd Kraft, Duisburg, Germany), Trolox (Cayman, Ann Arbor, MI, USA), were used. Caffeic acid, myricetin, *p*-coumaric acid, and quercetin (HPLC grade) were purchased from Sigma (Darmstadt, Germany). Ferrulic acid, gallic acid, kaempherol, and rutin

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(HPLC grade) were supplied from Carl Roth GmbH (Karlsruhe, Germany). Methanol and ethanol from Chemsolute (Hamburg, Germany), acetic acid (AppliChem, Darmstadt, Germany), and acetonitrile (J.T.Baker, Hamburg, Germany) purchased were HPLC grade.

3.2. Plant Material

Young and old yacon leaves of two different yacon cultivars (red and white) were collected from a field trial carried out at the organically operating Kleinhohenheim research station of the University of Hohenheim (Stuttgart, Germany) in October 2015 at harvest time. Cultivars of yacon were classified according to the color of their tuber peels. Yacon plants were grown at the same field under the same growing conditions and management to ensure *ceteris paribus*. Young leaves refer to smaller leaves on top of yacon stems, while old leaves imply big leaves collected from the lower part of stems. On both cultivars, the number of leaves was counted to ensure that the collected leaves had the same growth stage. Collected leaves were dried at 40 °C for 24 h. Afterwards, the leaves were kept in a dry and cool place for further analysis. Dried leaves were ground and passed through a sieve (40 mesh) to have homogenous samples, before initiating the extraction process.

3.3. Extraction Process

3.3.1. Decoction (DE)

Leaf powder was mixed with distilled water (ratio of leaves:water = 1:20 (w:v)). Then, the mixture was heated to boiling point under cooling reflux to avoid water loss with evaporation. The heating up time was recorded using a stopwatch. Afterwards, holding time at boiling point was 10 min. Then, the extract was cooled and filtered through Whatman No. 40 paper (Whatman, Buckinghamshire, UK). The residue was extracted under the same conditions for the second cycle of extraction. The extracts were mixed together and evaporated by means of a vacuum rotary evaporator at 35 °C (Rotavapor® R-100, Büchi, Essen, Germany) and freeze dried.

3.3.2. Ohmic-Assisted Decoction (OH-DE)

OH-DE was performed using an ohmic device (designed and built in the Transport Properties Laboratory at the Department of Food Science and Technology, Shiraz University, Iran) that consisted of a Teflon cylindrical chamber (7 cm internal diameter and 25 cm length), which has two titanium-coated 316 stainless steel electrodes. The device is automated so the voltage (0–350 V), current (0–16 A), and temperature could be monitored.

Prior to the extraction process with OH-DE, leaf powder was soaked for 10 min in salted water (0.3% w/v NaCl solution) (ratio of leaves:water = 1:20 (w:v)). OH-DE was performed using 150 V under a cooling reflux in order to avoid losing water through evaporation. Heating up time was also recorded. Then, OH-DE was maintained for 10 min holding time after reaching the boiling point. Afterwards, the extract was filtered through Whatman No. 40 paper (Whatman). The residue was extracted under the same conditions for a second cycle and extracts were mixed together, evaporated using a Rotavapor® R-100 vacuum rotary evaporator at 35 °C, and freeze dried.

3.4. Yield of Extraction

To evaluate yield of extraction, total solid contents of extracts were measured gravimetrically. Approximately five grams of extracts were dried at 60 ± 1 °C overnight and then cooled in a desiccator for an hour before weighing. Yield was calculated using Equation (1):

Yield % = ((weight of sample after dehydration)/(initial weight of sample)) \times 100 (1)

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3.5. Measurement of Electrical Conductivity

The electrical conductivity of plant materials was evaluated by an electrical conductivity meter (Mi180, Milwaukee, Szeged, Hungary). Average electrical conductivity in the ratio of 1:20 (leaves:water) at room temperature was recorded, while one sample contained 0.3%~w/v NaCl and another sample was without NaCl.

3.6. Energy Consumption

Energy consumption was evaluated using a digital single phase kWh meter with 0.01 kWh accuracy. The device was connected to the main power cable of the ohmic device and mantel. The total energy consumption was calculated by summing up the energy consumption of both stages of extraction for each extraction experiment.

3.7. Total Phenolic Content (TPC)

Briefly, 1 mL of the reconstituted yacon extract (1 mg mL $^{-1}$ of distilled water) was added to 1 mL of Folin–Ciocalteu's reagent. After 3 min, 1 mL of saturated Na₂CO₃ (35%) was added to the mixture. Then, the volume of mixture was made up to 10 mL with distilled water. Afterwards, the reaction mixture was left in darkness for 90 min. The absorbance was read at 725 nm using UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK). The calibration curve was generated with gallic acid solution (0.004–0.25 mg gallic acid mL $^{-1}$ distilled water) as a reference standard. TPC is expressed as gallic acid equivalent per gram of dried weight of leaves (mg GAE g DW $^{-1}$) [40].

3.8. Total Flavonoid Content (TFC)

To measure the TFC, $500~\mu L$ of the reconstituted yacon extract (1 mg mL $^{-1}$ of distilled water) was added to 1 mL of NaNO₂ (5%) and mixed well. After 6 min, 1 mL of 10% AlCl₃ and 10 mL of NaOH (1 M) were added to the mixture and the volume of mixture was adjusted to 25 mL with distilled water. Afterwards, the reaction mixture was left to stand for 15 min at room temperature before reading the absorbance at 510 nm by means of UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). Rutin (0.06–4 mg rutin mL $^{-1}$ 70% ethanol) was used as a reference standard to draw the standard curve. TFC was expressed as rutin equivalent per gram of dry weight of leaves (mg RE g DW $^{-1}$) [41].

3.9. Determination of Antioxidant Activity

3.9.1. ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid) Diammonium Salt) Radical Scavenging Assay

ABTS was dissolved in water (7 mM concentration). Then, potassium persulphate (2.45 mM) was added to ABTS solution (1:1, v/v) and the mixture was left to stand in the dark at room temperature for 12–16 h before being used to produce ABTS radical cations (ABTS $^{\bullet+}$). The ABTS $^{\bullet+}$ solution was diluted with distilled water to an absorbance of 0.70 \pm 0.02 at 734 nm. The %-inhibition of extract against ABTS $^{\bullet+}$ solution was performed as following [42]: 3.0 mL of diluted ABTS $^{\bullet+}$ solution was briefly added to 100 μ L of leaves extract (1 mg mL $^{-1}$). The reaction solution was kept in 30 °C after mixing for 10 min. The absorbance was read at 734 nm with UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). A blank was prepared using distilled water. The %-inhibition of leaves extract and Trolox solutions (0.02–0.2 Trolox (mM)) which were used as the reference standard for generation of standard curve was calculated using Equation (2):

Inhibition (%) =
$$((A_B - A_S)/A_B) \times 100$$
 (2)

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where A_B is the absorbance of the blank sample and A_S is the absorbance of samples. ABTS radical scavenging activity was expressed as Trolox equivalent per gram of dried weight of leaves (mM TE g DW⁻¹).

3.9.2. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging activity of yacon leaves extracts was measured as follows [42]: 0.1 mL of the reconstituted yacon extract (1 mg mL $^{-1}$ of distilled water) was added to 3 mL of freshly prepared 6×10^{-5} M methanolic DPPH $^{\bullet}$ solution. Then, the mixture was kept at 37 °C for 20 min. The absorbance was read at 515 nm using UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). Distilled water was used for preparation of the blank sample. The %-inhibition was calculated according to Equation (2). Calibration curve was drawn for %-inhibition of ascorbic acid solution (0.02–0.2 mg ascorbic acid mL $^{-1}$ distilled water) as a reference standard. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent per gram of dried weight of leaves (mg AAE g DW $^{-1}$).

3.9.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP working solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine) in HCl (10 mM), and 20 mM FeCl₃ solution in a 10:1:1 (v/v/v) ratio. 0.15 mL of the reconstituted yacon extract (1 mg mL⁻¹ of distilled water) was mixed with 2.85 mL FRAP solution and incubated at 37 °C for 30 min. The total antioxidant activity of the samples was evaluated by the absorbance of Fe²⁺-TPTZ at 593 nm using UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). The results of the FRAP assay were expressed in FeSO₄ (mM) equivalent per gram dry weight of leaves (mM Fe²⁺ g DW⁻¹) [43].

3.10. Measurement of Individual Phenolic Acid and Flavonoid Compounds by Means of HPLC

High performance liquid chromatography was used for screening phenolic in yacon aqueous extracts. A Merck-Hitachi HPLC system (HPLC, Darmstadt, Germany) operated using an L-7100 solvent delivery pump, an L-7200 auto-sampler, a Smartline column oven, an L-7612 solvent degasser, and DAD L-7450A detector. Separation of phenolic compounds was performed using a Kinetex 5 μ 00G-4601 E0 column (Phenomenex, Torrance, CA, USA)) while it was kept at a constant 25 °C. Data was analyzed using D-7000 HSM software (Merck-Hitachi, Darmstadt, Germany). Mobile phase, consisting of A (acetic acid (2%)) and B (acetic acid (0.5%)–acetonitrile (50:50, v:v)), was eluted gradiently as follows for a total time of 65 min: 0 min (82% A + 18% B); 25 min (75% A + 25% B); 55 min (45% A + 55% B); 56 min (0% A + 100% B); and 62 min (82% A + 18% B). An injection volume of 50.0 (μ L) and flow rate of 1 (mL/min) was applied. The detector used wavelengths between 220 and 600 nm for detection. The calculated wavelength was 256 nm. The phenolic compounds in yacon extracts were identified and quantified by means of comparing the retention times and peak area equivalent standards. The following standards were used: caffeic acid, ferrulic acid, gallic acid, kaempherol, myricetin, p-coumaric acid, rutin, and quercetin.

3.11. Scanning Electron Microscopy (SEM)

SEM images of dried leaves were obtained from fresh leaves, leaves after the drying and grinding process, and extracted leaves. Dried leaves were fixed on an aluminum sample holder and spattered with 20% gold and 80% palladium for 8 min. Then, the samples were scanned using a scanning electron microscope (SEM) (DSM-940, Zeiss, München, Germany) under high-vacuum conditions with an accelerating voltage of 5.0 kV.

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3.12. Statistical Analysis

Extraction experiments were performed in triplicate. Chemical analysis (TPC, TFC, ABTS and DPPH radical scavenging activity and FRAP) of extracts were done in duplicate in the laboratory. For HPLC analysis, one independent extract powder from each combination of variables (extraction method, leaves cultivar, and leaves age) was chosen randomly, two times independently reconstituted and two independent injections were applied. Results were reported as mean value \pm standard deviation and subjected to three-way analysis of variance (ANOVA) and the mean differences between evaluated parameters were established by performing Tukey's test at 5% significance level. Correlations between TPC, TFC, DPPH radical scavenging activity, ABTS radical scavenging activity, and FRAP results were examined using Pearson's correlation coefficient (r). Statistical analysis of data was performed using SAS Software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

4. Conclusions

The outcome of this work showed that utilization of OH-DE for the extraction of phytochemicals from yacon leaves offers certain benefits over DE. The average amount of extracted total phenolic and flavonoid compounds as well as antioxidant characteristics of leaves, which were processed by OH-DE, was either comparable or higher than the results obtained when the DE method was used. The energy consumption of OH-DE was also significantly lower than that of DE. Furthermore, leaves of the red yacon cultivar possessed higher levels of phytochemicals than the leaves of the white cultivar. Young leaves of red cultivar had the highest average amount of caffeic acid, myricetin, *p*-coumaric acid, rutin, and chloregic acid. Moreover, the old leaves of the red cultivar possessed the highest antioxidant activity level, contained higher average amounts of ferrulic acid, and comparable amounts of myricetin, TPC and TFC compared with young leaves of the red cultivar extracted with OH-DE. Therefore, extraction of both young and old leaves of the red cultivar by OH-DE can be suggested to achieve higher extraction of phytochemicals with lower energy consumption.

Further studies with regard to the optimization of the OH-DE process using various holding times and various voltages can be suggested. In addition, fractionation of phenolic acids and flavonoid compounds extracted from yacon leaves to determine their mechanism of action as antioxidant and/or antidiabetic compounds are required. Also, encapsulation of yacon leaves extract to maintain its health promoting effects and optimizing its application in food products, nutraceuticals and pharmaceuticals would be of interest.

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Author Contributions: F.K. and M.S.D. conceived, designed and carried out the extraction experiments. F.K. performed the chemical analysis, analyzed the data and wrote the manuscript. M.N. supervised the extraction experiments and contributed materials and extraction tools. N.M. performed the HPLC analysis. S.G.-H. advised and supervised the study. M.N., N.M., M.S.D., S.G.-H., and W.C. revised the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the yacon leaves are available from the authors.



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5. Chapter II: Yacon (Smallanthus sonchifolius Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars

Publication II:

Khajehei, F., Merkt, N., Claupein, W., & Graeff-Hoenninger, S. (2018). Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars. *Molecules*, 23(2), 278.

Several researchers carried on studies focusing on the phyto/chemicals of yacon tubers particularly emphasizing on the FOS, inulin and sugar content. Furthermore, some studies have been investigating the TPC and their antioxidants, which showed that yacon tubers are a remarkable source of such compounds as well. In addition, the results presented in chapter I noted the variation in phyto/chmical content of yacon leaves of different cultivars. The study confirmed that the phyto/chemical content of yacon leaves depends primarily on the cultivar. Moreover, there is a gap in literature with regard to differentiating between phyto/chemical content of flesh, peel and whole tubers of yacon. For that reason, chapter II examined the differences in phytochemical content and antioxidant activity of different parts of yacon tubers (flesh, peel and whole tuber) of seven cultivars, namely, Cajamarca, Cusco, Early White, Late Red, Morado, New Zealand, and Quinault, which were grown under the environmental conditions of southwestern Germany.





Article

Yacon (Smallanthus sonchifolius Poepp. & Endl.) as a Novel Source of Health Promoting Compounds: Antioxidant Activity, Phytochemicals and Sugar Content in Flesh, Peel, and Whole Tubers of Seven Cultivars

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Abstract: The aim of this study was to evaluate the quality characteristics of seven yacon (Smallanthus sonchifolius Poepp. and Endl.) cultivars (Cajamarca, Cusco, Early White, Late Red, Morado, New Zealand and Quinault) cultivated in the southwest of Germany. The following phyto/chemical traits were investigated in different yacon tuber parts (flesh, peel, and whole tubers): total dry matter, sugar content (fructose, glucose, and sucrose content), total phenolic content (TPC), total flavonoid content (TFC), 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and Ferric reducing antioxidant power (FRAP). The results indicated a significant interaction between cultivar and tuber part on all of the examined traits (p < 0.0001). Of flesh and whole tuber, cv. Late Red, cv. Morado, and cv. Cajamarca had the highest TPC, TFC, DPPH radical scavenging activity, and FRAP. They also had relatively higher total sugar content. Cv. New Zealand had the lowest amount of sugars, TPC, TFC, DPPH radical scavenging activity, and FRAP, but the highest ABTS radical scavenging activity content in its flesh and whole tuber. Moreover, the results indicated that the peel of yacon tubers contained considerably high amounts of phytochemicals while possessing low sugar contents. Overall, this study provides a broad insight into the phyto/chemical content of yacon tubers from different cultivars, which can be used for further breeding programs, and the selection of proper cultivars for specific food product development.

Keywords: yacon; *Smallanthus sonchifolius* Poepp. and Endl.; sugar; total phenolic content; total flavonoid content; ABTS radical scavenging activity; DPPH radical scavenging activity; Ferric reducing antioxidant power

1. Introduction

Consumption of fruits and vegetables is recommended as part of the human diet not only as a source of energy, but also as a source of health promoting compounds. Epidemiological researchers showed a favorable relationship between the consumption of fruits and vegetables and a reduction in risk of diseases such as cancer, cardiovascular diseases, etc. [1,2]. Phenolic compounds are secondary metabolites of plants and one of the most important groups of bioactive constitutes of fruits and vegetables due to their antioxidant activity [3,4]. Therefore, due to the phenolic profile of plant foods, their mechanism of action against certain diseases, health enhancing effects, safety, and potential in food products, plant-based nutraceuticals and pharmaceuticals are of interest and have been extensively investigated by researchers [5,6].

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Yacon (Smallanthus sonchifolius Poepp. and Endl.) is a root crop native to the Andean region, but has also been cultivated in other parts of the world for example in Brazil, Czech Republic, Ecuador, Germany, Japan, and New Zealand [7]. Yacon tubers are crunchy and juicy with a relatively sweet taste and are traditionally consumed as fresh fruit [7]. 70-80% of the total dry matter content of yacon tubers consists of saccharides. They contain fructose, glucose, and sucrose as sugars while fructooligosaccharides (FOS) serve as their dominant saccharide [8-10]. FOS are prebiotic non-digestible carbohydrates, therefore yacon tubers have gained attention due to their potential not only as a part of a diet for those who are suffering from digestive disorders such as diabetes and obesity, but also as a health promoting food for dieters [11,12]. In the recent decade, several investigations have evaluated the amount of FOS in fresh yacon tubers or processed yacon products as well as their health benefits [11,13–16]. Besides having health promoting carbohydrates, yacon tubers contain bioactive compounds (e.g., phenolic compounds and antioxidants); accordingly, yacon is considered as a multifunctional food [7]. The total phenolic content (TPC) and antioxidant capacity of flesh of thirty-five accessions of yacon tubers, which were grown under Peruvian environmental conditions, have been investigated in a study of Campos et al. (2012) [17]. Their results showed that the TPC in the flesh of yacon tubers varied within a wide range of 7.9 ± 0.8 to 30.8 ± 0.1 (mg chlorogenic acid equivalent $\rm g^{-1}$ DW) and their antioxidant capacity ranged between 23.3 ± 2.5 and 136.0 ± 6.1 (µmol trolox equivalent g^{-1} DW) according to ABTS radical scavenging activity [17]. The average amount of TPC, DPPH radical scavenging activity, ABTS radical scavenging activity, and Ferric reducing antioxidant power (FRAP) of yacon flesh provided from three regional markets in Peru were 93.2 (mg gallic acid equivalent g^{-1} DW), 56.6 ± 0.4 (µmol trolox equivalent $\rm g^{-1}$ DW), 61.6 \pm 0.8 (µmol trolox equivalent $\rm g^{-1}$ DW), and 134.0 \pm 7.2 (µmol trolox equivalent g⁻¹ DW), respectivly [18]. Sousa et al. (2015) reported the total antioxidant capacity of sterilized flour of yacon flesh grown in Brazil using ABTS radical scavenging activity at 222 ± 2 mg (ascorbic acid equivalent 100 g $^{-1}$ DW) and its TPC at 275 \pm 3 (mg gallic acid equivalent 100 g $^{-1}$ DW) [19]. Yacon chips produced from yacon flesh grown in Bolivia were reported to have 9.7 \pm 0.2 (mg gallic acid equivalent 100 g^{-1} FW) of TPC [20]. Therefore, the results of the previous investigations showed that yacon tubers and their processed food products contain considerable amounts of phenolic compounds and antioxidants, which can significantly vary according to cultivar, environmental conditions during cultivation, post-harvest, and processing conditions.

Similar to other fruits and vegetables, the availability of fresh yacon is seasonal [7]. Moreover, food processing such as drying, evaporation, and fermentation can be used to develop food products such as yacon chips, flour, syrup, vinegar, etc. to extend the shelf life of yacon tubers [7]. One of the major by-products of such food processing are the peels. Utilization of fruit peels as a source of valuable phyto/chemicals in nutraceuticals, value-added food products, pharmaceuticals, and cosmetic products has been introduced as an efficient and green strategy to reduce the waste in fruit production and consumption systems [21]. That being the case, the recovery of valuable nutritional compounds in the peels of various fruits has been suggested by several studies as they are considered to be a good source of phenolic and antioxidant compounds [22-25]. In respect to novel food product developments using yacon tubers, the flesh of tubers has been the focus of several recent studies, but yacon peels and recovery of their valuable compounds for potential applications has not been considered in detail yet [19,26-28]. A study of Pereira et al. (2016) investigated the phytochemical content in the peels and flesh of one yellow yacon cultivar cultivated in Brazil [29]. It was reported that these yacon peels had a TPC and ABTS radical scavenging activity of 2500.0 ± 23.1 (mg gallic acid equivalent kg⁻¹) and 372.5 ± 15.9 (µmole trolox equivalent g^{-1} DW) [29]. Thus, differentiation between phyto/chemical content of flesh, peel, and whole yacon tuber is required to facilitate the selection of suitable raw material for specific food products to insure the aimed phyto/chemical quality of the final product.

Hence, the main objectives of this study were to evaluate the phytochemical content (TPC, total flavonoid content (TFC), ABTS radical scavenging activity, DPPH radical scavenging activity and FRAP in flesh, peel, and whole yacon tubers from seven cultivars—namely, Cajamarca, Cusco, Early White,

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Late Red, Morado, New Zealand, and Quinault grown under the same environmental conditions in Southwestern Germany. In addition, the sugar content (fructose, glucose, and sucrose) in the flesh, peel, and whole yacon tubers was investigated because it plays an important role in the sweetness of tubers and their resulting glycemic index.

2. Results and Discussion

2.1. Total Dry Matter Content

The statistical analysis of data indicated a significant interaction between cultivar and tuber part on total dry matter content of yacon tubers (p < 0.0001) (Table 1). The results for total dry matter content of different parts of yacon tubers are reported in Table 2.

The total dry matter content of flesh of yacon tubers ranged between 9.38 \pm 0.40 to 15.13 \pm 0.41 (g 100 g FW $^{-1}$) and followed a decreasing order of cv. Morado > cv. Late Red > cv. New Zealand > cv. Early White > cv. Quinault > cv. Cusco > cv. Cajamarca (Table 2). Comparing the total dry matter content of whole tubers, the lowest values were determined for cv. Cajamarca (10.21 \pm 0.41 (g 100 g $^{-1}$ FW)) and cv. Cusco (10.17 \pm 0.63 (g 100 g $^{-1}$ FW)) while the highest significant values belonged to cv. Morado (16.93 \pm 1.08 (g 100 g $^{-1}$ FW)) (Table 3). Of the peels, the total dry matter content varied between 9.39 \pm 0.84 (g 100 g $^{-1}$ FW) for cv. Quinault and 15.68 \pm 0.24 (g 100 g $^{-1}$ FW) for cv. Morado (Table 2).

Total dry matter content of edible parts of plants is an important factor for determination of yield of crops as well as being a quality parameter which is related to the nutrient content of crops [30,31]. Total dry matter content in yacon tubers has been reported to range between 15 and 30 (g $100 \, \text{g}^{-1} \, \text{FW}$) [32]. The results of total dry matter in this study were in agreement with previous investigations noting the total dry matter of 13.7 (g $100 \, \text{g}^{-1} \, \text{FW}$) in yacon tubers cultivated in Japan [10], 7.5–19.1 (g $100 \, \text{g}^{-1} \, \text{FW}$) in flesh of 35 accessions of yacon tubers cultivated in Peru [17], and 9.8–13.6 (g $100 \, \text{g}^{-1} \, \text{FW}$) in yacon tubers of 10 accessions cultivated in Ecuador [33].

2.2. Glucose, Fructose and Sucrose Content

The statistical analysis of data showed that the interaction of cultivar and tuber part had a significant influence on glucose, fructose, and sucrose content in flesh, peel, and whole yacon tuber (p < 0.0001, p < 0.0001 and p < 0.0001, respectively) (Table 1). Table 3 reports the fructose, glucose, and sucrose content in different parts of yacon tubers.

The amount of fructose in the flesh of yacon tubers varied between 1.63 \pm 0.30 (g 100 g⁻¹ DW) for cv. Morado and 10.83 ± 0.37 (g 100 g⁻¹ DW) for cv. Quinault (Table 3). The fructose content of whole tuber ranged between 0.17 ± 0.15 and 21.55 ± 0.74 (g 100 g $^{-1}$ DW) for cv. Morado and cv. Quinault, respectively (Table 3). The peels of cv. Late Red and cv. Morado had the lowest amount of fructose at 0.04 ± 0.00 (g $100~\text{g}^{-1}$ DW) while the peels of cv. Quinault contained the highest fructose content of 3.09 ± 0.14 (g 100 g $^{-1}$ DW) (Table 3). The glucose content of flesh of yacon tubers ranged between 0.18 ± 0.00 (g 100 g $^{-1}$ DW) for cv. Late Red and 9.35 ± 0.36 (g 100 g $^{-1}$ DW) for cv. Cajamarca (Table 3). The whole tuber of cv. Late Red and cv. Cajamarca contained the lowest and highest glucose content $(0.29 \pm 0.12 \text{ and } 8.40 \pm 0.25 \text{ (g } 100 \text{ g}^{-1} \text{ DW})$, respectively) (Table 3). The glucose content in peels varied between 0.17 ± 0.02 and 1.04 ± 0.06 (g 100 g^{-1} DW) for cv. Late Red and cv. New Zealand, respectively (Table 3). The results for sucrose content showed that the sucrose content in flesh of yacon tubers ranged between 21.00 ± 1.81 (g 100 g $^{-1}$ DW) for cv. New Zealand and 53.54 ± 0.98 (g 100 g^{-1} DW) for cv. Early White (Table 3). The sucrose content in the whole tubers was lowest for cv. New Zealand (29.81 \pm 0.47 (g 100 g $^{-1}$ DW)). The highest sucrose content was found in the whole tubers of cv. Early White (58.08 \pm 1.21 (g 100 g $^{-1}$ DW)) (Table 3). The range of sucrose content in peels of yacon tubers was between 0.21 ± 1.26 and 29.89 ± 1.35 (g 100 g⁻¹ DW) (for cv. Cusco and cv. Cajamarca, respectively) (Table 3).

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6-sulfonic acid) (ABTS) radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and Ferric reducing antioxidant power as a function of the cultivar and different parts (flesh, peel, and whole tuber) of the yacon tubers. Table 1. ANOVA results of total dry matter, fructose, glucose and sucrose content, total phenolic content, total flavonoid content, 2,20-azino-bis(3-ethylbenzothiazoline-

	Total Dry	Fructose	Glucose	Sucrose	Total Phenolic	Total Flavonoid	ABTS Radical	DPPH Radical	Ferric Reducing
	Matter	Content	Content	Content	Content	Content	Scavenging Activity	Scavenging Activity	Antioxidant Power
Tuber part	p = 0.0003	p < 0.0001	p < 0.0001	p < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001
Cultivar	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001
Cultivar.Tuber part	p < 0.0001	p < 0.0001	p < 0.0001	p < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001	<i>p</i> < 0.0001

Table 2. Total dry matter content (g 100 g⁻¹ FW) of the flesh, peel, and whole tuber of different yacon cultivars.

Cultivas	Total Dry I	Total Dry Matter Content (g $100~\mathrm{g}^{-1}\mathrm{FW})$	$0\mathrm{g}^{-1}\mathrm{FW})$
Cuiuvai	Flesh	Peel	Whole Tuber
Cajamarca	$9.83^{ ext{ Db}}\pm0.40$	$11.77^{\text{ BCa}} \pm 0.11$	$10.21^{\mathrm{Eb}} \pm 0.41$
Cusco	$10.29^{\text{ Db}} \pm 0.32$	12.51 Ba ± 0.44	$10.17^{\rm \; Eb}\pm0.63$
Early White	$12.77^{\mathrm{BCa}} \pm 0.62$	$10.20~^{\mathrm{DEc}}\pm0.12$	$11.67^{\mathrm{Db}} \pm 0.20$
Late Red	$14.13~^{\mathrm{ABa}} \pm 0.62$	$12.58^{\ \mathrm{Bb}} \pm 0.67$	$14.69^{\mathrm{Ba}} \pm 0.27$
Morado	$15.13^{\text{ Ab}} \pm 0.41$	$15.68~^{\mathrm{Aab}}\pm0.24$	$16.93^{\text{ Aa}} \pm 1.08$
New Zealand	$12.94^{\ BCa} \pm 0.61$	$10.80^{ ext{CDb}} \pm 0.26$	$13.30^{\circ} = 0.64$
Quinault	$11.84^{\mathrm{~Ca}} \pm 0.86$	$9.39~^{\mathrm{Eb}}\pm0.84$	11.30 $^{\mathrm{DEa}} \pm 0.21$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). Mean values with the same small letter in a row are not significantly different as indicated by Tukey's test (p < 0.05).

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Overall, the outcomes of examining the sugar content of flesh and whole tubers indicated that the amount of sugars in a decreasing order was as following: sucrose > fructose > glucose, with exception for cv. Cajamarca and cv. New Zealand for which the amount of sugars in a decreasing order was: sucrose > glucose > fructose (Table 3). The sugar content results for the peel showed that the sucrose content was higher than the fructose content while glucose content in the peel had the lowest amount in cv. Cajamarca, cv. Cusco, cv. Early White and cv. Quinault (Table 3). However, the order of sugar content in the peels of cv. Late Red, cv. Morado and cv. New Zealand was sucrose > glucose > fructose. Furthermore, in this study the sugar content of the yacon peel was reported for the first time. The outcomes showed that within each cultivar the sugar content of the peel was lower than that of the flesh and the whole tuber (Table 3). The low sugar content found in the yacon peel suggests its potential to be used in products with low sugar content.

A study of Graefe et al. (2004) reported sucrose, fructose, and glucose contents of 16.7-17.1, 10.5-15.2 and 1.1-3.3 (g 100 g⁻¹ DW), respectively, in yacon tubers with different peel colors (purple, white, yellow) harvested in Peru [34]. The sugar content in the flesh of yacon tubers in their study had a decreasing order of: sucrose > fructose > glucose which is in agreement with the outcomes of this study [34]. A study of Lachmann et al., (2007) showed a different decreasing order of: fructose > glucose > sucrose content (19.5–21.7, 7.95–10.3 and 2.22–3.4 (g $100 \text{ g}^{-1} \text{ DW}$) for yacon tubers from five different cultivars, cultivated in Czech Republic [35]. The fructose and glucose content of yacon tubers bought from a local market in Brazil were reported with 50.68 ± 0.1 and 26.93 ± 0.03 (g 100 g⁻¹ DW), respectively [36]. A broader variation regarding the sum of reducing sugars and sucrose content (22.3–88.7 (g 100 g⁻¹ DW)) in vacon tubers of thirty-five accessions cultivated in Peru was noted in a study of Campos et al. (2012) [17]. Fructose content and total sugar content (fructose + glucose + sucrose) of yacon tubers cultivated in Japan were reported at 35 and 58 (g $100 \text{ g}^{-1} \text{ DW}$), respectively [10]. Such variations in sugar content may be due to the cultivar, environmental conditions during growth of yacon, and especially the post-harvest conditions as well as a possible combination of these factors [11]. In particular, the postharvest handling of yacon tubers contributes significantly to the simple sugar content and sweetness of tubers or products derived from them at consumption time [34]. For example, sunning of tubers is used as curing process for increasing the sweetness of tubers which causes the breakdown of FOS to FOS with lower degrees of polymerization and/or free fructose and glucose [34]. Food processes such as drying may also change the profile of carbohydrate content of yacon tubers [36]. As our tubers were not exposed to any post-harvest curing process, it is evident that no breakdown of FOS or sucrose had taken place yet leading to the determined order of sugars.

2.3. TPC

The statistical analysis showed that the amount of TPC in yacon tubers was significantly affected by an interaction of cultivar and tuber part (p < 0.0001) (Table 1). The amount of TPC in flesh, peel, and whole tubers of seven yacon cultivars is reported in Table 4.

Of the flesh, TPC of cv. Late Red was highest (3307.51 \pm 21.84 mg GAE 100 g $^{-1}$ DW) followed by cv. Cajamarca and cv. Morado containing 2710.39 \pm 69.07 and 2571.95 \pm 31.95 mg GAE 100 g $^{-1}$ DW), respectively. The lowest TPC of 1855.89 \pm 3.55 (mg GAE 100 g $^{-1}$ DW) was measured in flesh of cv. New Zealand (Table 4). The TPC in whole tubers ranged between 2845.05 \pm 0.87 and 3656.37 \pm 74.60 (mg GAE 100 g $^{-1}$ DW) for cv. New Zealand and cv. Morado (Table 4). A greater variation of TPC was found in the peels of yacon tubers from different cultivars when compared with the flesh and the whole tuber. TPC was lowest with 5602.23 \pm 46.87 (mg GAE 100 g $^{-1}$ DW) and highest with 14,144.53 \pm 45.34 (mg GAE 100 g $^{-1}$ DW) for cv. Late Red and cv. New Zealand, respectively (Table 4).

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Table 3. Fructose, glucose, and sucrose content $(g 100 g^{-1} DW)$ in the flesh, peel, and whole tuber of different yacon cultivars.

111111111111111111111111111111111111111	Fru	Fructose (g 100 g^{-1} DW)	W)	Glu	Glucose (g 100 g ⁻¹ DW)	(M)	nS.	Sucrose (g 100 g ⁻¹ DW)	W)
Cultival	Flesh	Peel	Whole Tuber	Flesh	Peel	Whole Tuber	Flesh	Peel	Whole Tuber
Cajamarca	$8.54~^{\mathrm{Ba}}\pm0.42$	$0.37^{\circ} \text{Cc} \pm 0.13$	$7.35^{\circ} \pm 0.24$	$9.35^{\text{ Aa}} \pm 0.36$	$0.18\mathrm{Cc}\pm0.00$	$8.40^{\text{ Ab}} \pm 0.25$	$44.59^{\mathrm{Ba}} \pm 0.74$	$29.89~^{\mathrm{Ab}}\pm1.35$	$43.12^{\text{ Ca}} \pm 1.05$
Cusco	$10.64^{\text{ Ab}} \pm 0.42^{\text{ Ab}}$	$0.50^{\circ} \text{Cc} \pm 0.13$	$16.99^{\mathrm{\ Ba}} \pm 0.60$	$6.65^{\circ} = 0.29$	$0.47^{\ \mathrm{Bb}} \pm 0.02$	$6.88^{\text{ Ba}} \pm 0.25$	$45.62^{-8a} \pm 0.98$	$21.00^{\text{ Cb}} \pm 1.26$	$48.31^{\text{ Ba}} \pm 3.10^{\text{ Ba}}$
Early White	$2.14^{\;\mathrm{Db}}\pm0.00$	$0.83 \text{ Bc} \pm 0.00$	$3.41^{\mathrm{Ea}} \pm 0.24$	$1.06~^{\mathrm{Eb}}\pm0.00$	$0.29^{\circ} \text{Cc} \pm 0.12$	$2.07~^{\mathrm{Da}} \pm 0.12$	$53.54^{\text{ Ab}} \pm 0.98^{\text{ Ab}}$	$24.45^{\text{ Bc}} \pm 0.00$	$58.74^{\mathrm{Aa}} \pm 2.01$
Late Red	$4.01^{\circ} = 0.91^{\circ}$	$0.04^{\mathrm{Dc}}\pm0.00$	$1.24~^{\mathrm{Fb}}\pm0.15$	$0.18^{~\mathrm{Fa}}\pm0.00$	$0.17^{\text{ Ca}} \pm 0.02$	$0.29~^{\mathrm{Ea}} \pm 0.12$	$52.79^{\text{ Ab}} \pm 3.92$	$24.09^{ Bc} \pm 0.42$	$58.08^{\text{ Aa}} \pm 1.21$
Morado	$1.63~^{\mathrm{Da}}\pm0.30$	$0.04^{ ext{ Db}} \pm 0.00$	$0.17\mathrm{Gb}\pm0.15$	$0.51^{\mathrm{Fa}} \pm 0.12$	$0.24^{\text{ Ca}} \pm 0.00$	$0.45^{\mathrm{\ Ea}}\pm0.00$	$45.85^{\text{ Ba}} \pm 0.84$	$18.26^{\mathrm{Dc}} \pm 0.87$	$31.00^{\mathrm{Db}} \pm 1.05$
New Zealand	$3.69^{\text{ Cb}}\pm0.12$	0.30 Cc ± 0.00	$4.39^{\mathrm{Da}} \pm 0.15$	$5.33^{\mathrm{Da}} \pm 0.24$	$1.04~^{\mathrm{Ac}} \pm 0.06$	$4.73^{\circ} \pm 0.13$	$21.00^{\mathrm{Da}} \pm 1.81$	$6.00^{ ext{ Eb}} \pm 0.77$	$19.81^{\text{ Ea}} \pm 0.47$
Quinault	$10.83~^{\mathrm{Ab}}\pm0.37$	$3.09^{\mathrm{Ac}}\pm0.14$	$21.55~^{\mathrm{Aa}}\pm0.74$	$8.32~^{\mathrm{Ba}}\pm0.17$	$0.18~\text{Cc}~\pm~0.00$	$5.09~^{\mathrm{Cb}}\pm0.23$	$27.15^{\mathrm{~Ca}} \pm 0.93$	$8.10~^{\mathrm{Fc}}\pm0.78$	$22.58^{~\mathrm{Eb}} \pm 1.14$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). Mean values with the same small letter in a row for each measured trait are not significantly different as indicated by Tukey's test (p < 0.05).

Table 4. Total phenolic content (mg GAE $100 \, \mathrm{g}^{-1} \, \mathrm{DW}$) and total flavonoid content (mg RE $100 \, \mathrm{g}^{-1} \, \mathrm{DW}$) in the flesh, peel, and whole tuber of different yacon cultivars.

<u> </u>	Total Pheno	Total Phenolic Content (mg GAE 100 g ⁻¹ DW)	$00 \mathrm{g}^{-1} \mathrm{DW})$	Total Flav	Total Flavonoid Content (mg RE 100 g ⁻¹ DW)	10 g ⁻¹ DW)
Cultival	Flesh	Peel	Whole Tuber	Flesh	Peel	Whole Tuber
Cajamarca	$2710.39^{\text{ Bb}} \pm 69.06$	8402.94 Ba ± 221.45	2964.93 CDb ± 95.77	$2726.80^{\ \mathrm{Bb}} \pm 120.81$	$16,494.91^{\mathrm{Ba}} \pm 324.01$	3304.86 ^{CDb} ± 353.80
Cusco	$2477.75^{\mathrm{Cb}} \pm 60.79$	$7007.29^{\circ} = 475.86$	$3608.79^{\mathrm{Ab}} \pm 72.98$	$2247.73^{\mathrm{BCc}} \pm 221.44$	$12,959.57^{\mathrm{Ca}} \pm 214.57$	$4645.10^{\mathrm{ABb}} \pm 126.29$
Early White	$2213.73^{\mathrm{Dc}} \pm 38.59$	$6720.50^{\mathrm{Ca}} \pm 200.95$	$3397.55^{\mathrm{ABb}} \pm 61.64$	$2303.99 \text{ Bc} \pm 559.50$	$11,541.16^{\text{ CDa}} \pm 276.43$	$4147.11 ^{ABCDb} \pm 70.96$
Late Red	$3307.51^{\text{ Ab}} \pm 21.84$	$5602.23^{\mathrm{Da}} \pm 46.87$	$3181.52^{\mathrm{BCb}} \pm 83.97$	$4142.02^{\text{ Ab}} \pm 471.98$	$9814.18^{\text{ Da}} \pm 1096.11$	$4365.77 ^{ABCb} \pm 312.05$
Morado	$2571.95 \text{ BCc} \pm 31.95$	$6260.79^{\mathrm{CDa}} \pm 75.74$	$3656.37^{\text{ Ab}} \pm 74.60$	$2621.66^{\mathrm{Bc}} \pm 264.06$	$9670.46^{\mathrm{Da}} \pm 454.18$	$4959.37 \text{ Ab} \pm 340.51$
New Zealand	$1855.89^{\;\mathrm{Ec}} \pm 3.55$	14,144.53 $^{\mathrm{Aa}}\pm45.34$	$2845.05^{\mathrm{Db}} \pm 0.87$	$1041.69^{\circ} = 25.36$	25,488.31 $^{\mathrm{Aa}}\pm554.02$	$3221.47^{\text{ Db}} \pm 354.17$
Quinault	$2470.21^{\circ} \pm 9.29$	$8439.17^{-\mathrm{Ba}} \pm 159.98$	$3005.57^{\circ} \pm 83.59^{\circ}$	$2886.14^{ Bc} \pm 124.93$	$12,627.69^{\text{ Ca}} \pm 183.34$	$3593.64 ^{\text{BCDb}} \pm 142.03$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). Mean values with the same small letter in a row for each measured trait are not significantly different as indicated by Tukey's test (p < 0.05).

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Variation in the TPC of tubers from different yacon cultivars was in agreement with variation in the TPC content of biomass [35] and flesh [17], as well as yacon flour [19]. Chlorogenic acid has been reported as a predominant phenolic compound in yacon roots while ferulic acid, coumaric acid, caffeic acid and its derivatives were identified in yacon root extracts [11,19,37]. Furthermore, the outcomes showed that the TPC was significantly higher in the peel of yacon tubers followed by the whole tuber and the flesh (Table 4). The effect of a cultivar on the TPC of plants or resulting food products as well as higher phenolic content in peels of other fruits and vegetables such as apples [23,38], bananas [25], potatos [24], and exotic fruits [22], has been reported by other researchers. Higher TPC in peel of fruits and vegetables might be in accordance to defense systems of plants. Phenolic compounds exhibit antioxidant and antimicrobial properties and their accumulation in the outer part of fruits and vegetables protects them against potential pathogens and harmful effects of the environment.

2.4. TFC

Results of ANOVA showed that the amount of TFC in the flesh, peel, and whole yacon tubers was significantly affected by the interaction of the cultivar and tuber part (p < 0.0001) (Table 1). Table 4 shows the TFC in the flesh, peel, and whole tuber of seven yacon cultivars.

The TFC of the flesh of yacon tubers varied between 1041.69 ± 25.36 and 4142.02 ± 471.981 (mg RE $100~g^{-1}$ DW) for cv. New Zealand and cv. Late Red, respectively, which is in agreement with results of TPC in the flesh being lowest and highest for the same cultivars (Table 4). Of the whole tubers, cv. Morado had the highest TFC of 4959.37 ± 340.51 (mg RE $100~g^{-1}$ DW), followed by similar values for cv. Cusco, cv. Late Red and cv. Early White at 4645.10 ± 126.29 , 4365.77 ± 312.05 and 4147.11 ± 70.96 (mg RE $100~g^{-1}$ DW), respectively (Table 4). The lowest TFC in whole tubers was noted for cv. New Zealand (3221.47 ± 354.17 (mg RE $100~g^{-1}$ DW)) (Table 4). Similar to results of TPC, the peels of cv. New Zealand had the highest TFC ($25,488.31 \pm 554.02$ (mg RE $100~g^{-1}$ DW)) and peels of cv. Late Red and cv. Morado were similar to each other according to the TFC. They contained the lowest TFC (9814.18 ± 1096.11 and 9670.46 ± 454.18 (mg RE $100~g^{-1}$ DW), respectively) (Table 4).

Flavonoids are polyphenolic compounds that contribute to sensorial properties of plant food products (e.g., taste and flavor). Further, they are considered to have antioxidant effects, anticancer activities, and antidiabetic effects [39]. To the best of our knowledge, this is the first study reporting the TFC of different parts of yacon tubers from various cultivars. Like TPC, the amount of flavonoids were influenced by cultivar which may suggest the influence of genotype on biosynthesis of flavonoid compounds in yacon plants. Of the flesh of yacon tubers, the amount of TFC was in the same range or higher than TPC in all cultivars except for cv. Cusco and cv. New Zealand. The latter two had respectively 9.28% and 43.87% lower TFC than TPC in the flesh of their tubers. The TFC in the flesh of cv. Late Red that had the highest TPC among investigated cultivars was 25.23% higher than its TPC. Moreover, for each cultivar the TFC, which were stored in the peel were higher than that of the whole tuber while the lowest TFC was measured in the flesh of tubers. This is in agreement with results of peels containing higher amounts of TPC (Table 4). Furthermore, these results are in agreement with results of TFC and their distribution among the peel and the flesh of apples [38,40], pears (peeled and unpeeled) [40], and potatoes [41]. In addition, it was noted that the TFC of whole tubers and peels was higher than their TPC content in all of the studied cultivars. More precisely, the TFC of whole tubers were higher than their TPC between 11.46% in case of cv. Cajamarca and 37.22% in case of cv. Late Red. Comparing the TFC and TPC in peel of tubers it was noted that TFC was profoundly higher than TPC within a range of 49.63% to 96.29% for peels of cv. Quinault and cv. Cajamarca, respectively. The results of previous studies regarding the individual flavonoids determined the presence of kaempherol, myricetin, quercetin and rutin in yacon leaves in various quantities depending on yacon cultivar, environmental conditions during cultivation, method of extraction and solvent used for extraction [42,43]. Simonovska et al. (2003) investigated the individual flavonoids in vacon leaves and tubers and suggested that vacon tubers might contain quercetin and other flavonoids, which remained unknown in their study [37]. Flavonoids are natural occurring Molecules **2018**, 23, 278 8 of 19

antioxidants, which can be used to improve the health condition of consumers especially those who are suffering from diseases associated with oxidative stress such as diabetes [44]. Investigations showed a positive association between consumption of yacon tubers and improvement in health of diabetes, because of their hypoglycemic effect, which has been related to their FOS content [9]. The high TFC determined in this study might suggest that the consumption of yacon tubers might help to further improve the health conditions of consumers. Therefore, identification of individual flavonoid compounds in yacon tubers and their mechanism of action alone and in association with FOS, in vitro and in vivo are suggested.

2.5. Antioxidant Activity

2.5.1. ABTS Radical Scavenging Activity

Statistical analysis of data showed that the interaction of the cultivar and tuber part had a significant effect on ABTS radical scavenging activity of yacon tubers (p < 0.0001) (Table 1). The ABTS radical scavenging activity of the flesh, peel, and whole tuber of seven yacon cultivars is presented in Table 5.

The ABTS radical scavenging activity of flesh was lowest for cv. Late Red at 366.81 \pm 0.74 (mM TE 100 g $^{-1}$ DW) and highest for cv. New Zealand at 407.62 \pm 2.77 (mM TE 100 g $^{-1}$ DW) (Table 5). Among the whole tuber of various cultivars, the lowest ABTS radical scavenging activity belonged to cv. Cusco (356.16 \pm 0.52 (mM TE 100 g $^{-1}$ DW)) while cv. New Zealand had the highest ABTS radical scavenging activity of 377.23 \pm 0.43 (Table 5). The ABTS radical scavenging activity of peels was lowest and highest for cv. Morado and cv. Late Red at 261.98 \pm 1.25 and 293.58 \pm 0.98 (mM TE 100 g $^{-1}$ DW), respectively (Table 5).

ABTS radical scavenging activity of the flesh, peel, and whole tubers was significantly affected by the cultivar (p < 0.0001) (Table 1). Furthermore, the results showed that the ABTS radical scavenging activity in the flesh of yacon tubers was higher than that of the whole tubers'. ABTS radical scavenging activity of the peels indicated the opposite trend compared to the results of TPC and TFC. Antioxidant activity of bioactive compounds and their mechanism of action with regard to free radicals are related to their structure. Therefore, the difference between TFC and TPC in different parts of the yacon tubers and their ABTS radical scavenging activity might be due to the structure of present phenolic and flavonoid compounds and their distribution in different parts of tubers [45,46]. ABTS radical scavenging activity in the flesh of yacon tubers of 35 yacon accessions was reported to range between 23 and 136 (μM TE 100 g^{-1} DW), which was lower than our findings [17]. Sousa et al. (2015) reported the ABTS radical scavenging activity of flour of flesh of yacon tubers with 222 \pm 2 (mg ascorbic acid equivalent 100 g⁻¹ DW) [19]. However, the results of the present study cannot be exactly compared to the results of the aforementioned studies, because of the differences in analytical methods for performing the measurement of ABTS radical scavenging activity. Furthermore, differences in solvents and extraction methods existed. Moreover, the environmental conditions during the cultivation may influence the phytochemical quality of plant food products leading to variations in their biological activity [47,48].

2.5.2. DPPH Radical Scavenging Activity

Statistical analysis of data demonstrated that the DPPH radical scavenging activity of yacon tubers was significantly influenced by the interaction of the cultivar and tuber part (p < 0.0001) (Table 1). The outcomes of the DPPH radical scavenging activity of the flesh, peel, and whole tubers of seven yacon cultivars are presented in Table 5.

The DPPH radical scavenging activity of yacon tubers slightly varied when comparing different cultivars and tuber parts (Table 5). Of the flesh, the DPPH radical scavenging activity varied between 976.98 \pm 103.18 and 1526.70 \pm 2.22 (mg AAE 100 g $^{-1}$ DW) for cv. New Zealand and cv. Morado, respectively. There was no significant difference between cv. Morado, cv. Late Red, cv. Quinault,

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cv. Cusco, cv. Cajamarca and cv. Early White according to their flesh's DPPH radical scavenging activity (Table 5). The DPPH radical scavenging of whole tubers ranged between 1473.92 \pm 16.20 and 1540.98 \pm 13.76 (mg AAE 100 g⁻¹ DW) for cv. Cajamarca and cv. Late Red (Table 5). The peels of yacon tubers had a DPPH radical scavenging activity between 1503.97 \pm 10.55 and 1541.74 \pm 7.97 for cv. Late Red and cv. Cusco (mg AAE 100 g⁻¹ DW), respectively (Table 5).

Results of this study showed that no significant difference between DPPH radical scavenging activities of the different parts of yacon tubers of cv. Cajamarca, cv. Early White, cv. Late Red and cv. Quinault existed (Table 5). This might indicate an even distribution of antioxidant compounds in tubers, which can effectively scavenge free DPPH radicals in the peel and flesh of yacon tubers. However, the outcomes of DPPH radical scavenging activity are not in agreement with ABTS radical scavenging activity. The outcomes of the ABTS radical scavenging activity showed differences between the peel and flesh for all cultivars. The peel had a lower ABTS scavenging activity when compared to the flesh (Table 5). These results may indicate the variation in mechanism of action and effectiveness of bioactive compounds in yacon tubers against free radicals. The DPPH radical scavenging activity of yacon flesh has been studied by Yan et al. (1999) who separated and identified chlorogenic acid and L-tryptophan as two antioxidants in yacon tubers [49]. Additionally, it was determined that chlorogenic acid was much more effective than L-tryptophan in the scavenging of free DPPH radicals [49]. Moreover, Simonovska et al. (2003) noted the presence of an unknown non-polar compound which expressed high DPPH radical scavenging activity [37]. On that account, isolation and identification of bioactive compounds from the peel and flesh of yacon tubers can be suggested to determine the main antioxidant compounds and their mechanism action against oxidative stresses in vitro and in vivo.

2.5.3. FRAP

The statistical analysis revealed a significant interaction between cultivar and tuber part on FRAP of yacon tubers (p < 0.0001) (Table 1). The results of FRAP of the flesh, peel, and whole tubers of seven yacon cultivars are noted in Table 5.

The FRAP of yacon tubers showed a great variation when comparing cultivar and different tuber parts. In the flesh of yacon tubers FRAP ranged between 6343.02 \pm 74.17 and 24,393.48 \pm 141.37 (mM Fe²+ 100 g $^{-1}$ DW) for cv. New Zealand and cv. Late Red, respectively (Table 5). There was a significant difference between FRAP of whole tubers of all cultivars. The FRAP of whole tubers ranged between 17,020.23 \pm 60.65 (mM Fe²+ 100 g $^{-1}$ DW) for cv. Quinault and highest values of 25,418.22 \pm 78.64 for cv. Morado (Table 5). The FRAP value of the peel was higher than that of the whole tubers and flesh and ranged between 27,959.12 \pm 137.14 and 28,450.95 \pm 35.15 (mM Fe²+ 100 g $^{-1}$ DW) for cv. Morado and cv. Early White, respectively (Table 5).

To our knowledge, the present study is the first study that investigated the antioxidant activity of different parts of yacon tubers from various cultivars according to their reducing power. The findings showed that the ranking of tuber parts according to their FRAP values in a descending order is as follows: peel > whole tuber > flesh, except in the case of cv. Late Red which had the following decreasing order of: peel > flesh > whole tuber. This is consistent with the results of TPC and TFC (Tables 4 and 5). Therefore, it might be suggested that flavonoid and phenolic compounds in different parts of yacon tubers are responsible for FRAP. Furthermore, higher FRAP values in the peel of yacon tubers was in agreement with higher FRAP in the peels of common fruits such as guava, white pomegranate, mango, kiwifruit etc. [50], as well as exotic fruits from Colombia such as coastal sapote, algarrobo, Borojo, and cassabanana [22].

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Table 5. ABTS radical scavenging activity (mM TE 100 $\rm g^{-1}$ DW), DPPH radical scavenging activity (mg AAE 100 $\rm g^{-1}$ DW), and FRAP (mM Fe²⁺ 100 $\rm g^{-1}$ DW) of the flesh, peel, and whole tuber of different yacon cultivars.

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	ABTS Radicals S	cavenging Activity (ABTS Radicals Scavenging Activity (mM TE 100 g ⁻¹ DW)	DPPH Radical Scar	DPPH Radical Scavenging Activity (mg AAE 100 g ⁻¹ DW)	$AAE100 g^{-1} DW$	FR	FRAP (mM Fe ²⁺ 100 g^{-1} DW)	(A)
Cuitiva	Flesh	Peel	Whole Tuber	Flesh	Peel	Whole Tuber	Flesh	Peel	Whole Tuber
Cajamarca	$376.40^{\mathrm{Da}} \pm 0.79$	$376.40^{\mathrm{Da}} \pm 0.79 \qquad 262.29^{\mathrm{Cb}} \pm 0.14$	$371.48^{ABCa} \pm 3.50$	$1498.64^{\text{ Aa}} \pm 3.06$	$1513.45^{\mathrm{Aa}} \pm 9.88$	$1473.92^{\text{ Ba}} \pm 16.20$	$17,495.62$ Bc ± 178.98	28,222.52 ABa ± 73.17	19,762.35 Eb ± 55.30
Cusco	$384.81^{\circ} \pm 0.13$	$266.19^{ Bc} \pm 0.40$	$356.16^{\text{ Db}} \pm 0.52$	$1498.85^{\text{ Ab}} \pm 3.88$	$1541.74^{\text{Aa}} \pm 7.97$	$1507.47^{\text{ ABb}} \pm 0.12$	$15,521.66$ Cc ± 59.38	$28,450.95^{\text{Aa}} \pm 35.15^{\text{Aa}}$	$24,508.18^{Bb} \pm 184.62$
Early White	$397.30^{\mathrm{Ba}} \pm 1.84$	$264.68^{\mathrm{BCc}} \pm 0.04$	$369.43^{\mathrm{BCb}} \pm 1.27$	$1498.28^{\text{ Aa}} \pm 28.81$	$1509.07^{\mathrm{Aa}} \pm 3.47$	$1511.84^{\text{ABa}} \pm 2.35^{\text{ABa}}$	$10,745.21 \text{ Ec} \pm 85.78$	$28,271.57$ ABa ± 97.07	$21,967.91$ Db ± 92.47
Late Red	$366.81^{\text{ Eb}} \pm 0.74$	$293.58 \text{ Ac} \pm 0.98$	$374.68^{ABa} \pm 1.55$	$1520.51^{-0.0} \pm 8.54^{-0.0}$	$1503.97^{\text{ Aa}} \pm 10.55^{\text{ Aa}}$	$1540.98^{Aa} \pm 13.76$	$24,393.48$ Ab ± 141.37	$28,040.22$ Ba ± 116.32	$22,852.36$ Cc ± 34.32
Morado	$392.25^{\mathrm{Ba}} \pm 1.27$	$261.98 \text{ Cc} \pm 1.25$	$372.28 ^{ABCb} \pm 1.78$	$1526.70^{\mathrm{Aab}} \pm 2.22$	1507.48 Ab \pm 5.29	$1540.91^{\text{Aa}} \pm 11.34$	$15,137.52$ Cc ± 19.02	$27,959.12^{-8a} \pm 137.14$	25,418.22 $^{\mathrm{Ab}}$ \pm 78.64
New Zealand	$407.62^{\text{ Aa}} \pm 2.77$	$262.18^{\text{ Cc}} \pm 1.82$	$377.23^{\text{Ab}} \pm 0.43$	$976.98^{ \text{ Bb}} \pm 103.18$	$1515.62^{Aa} \pm 1.22$	1513.12 $^{ABa} \pm 1.96$	$6343.02 \text{ Fc} \pm 74.17$	$28,122.04$ ABa ± 89.46	$17,020.23 \text{ Gb} \pm 60.65$
Quinault	$393.00^{\mathrm{Ba}} \pm 2.53$	$263.34^{\mathrm{BCc}} \pm 0.03$	$367.05^{\circ} \pm 0.83^{\circ}$	1510.69 $^{\mathrm{Aa}} \pm 47.79$	1507.32 $^{\text{Aa}} \pm 25.72$	$1529.75^{\mathrm{Aa}} \pm 9.86$	$14,145.80 \text{ Dc} \pm 73.92$	$28,231.91$ ABa ± 40.48	$18,959.50^{\text{ Fb}} \pm 50.17$

Reported values are presented as mean values \pm standard deviation. Mean values with the same capital letter in a column are not significantly different as indicated by Tukey's test (p < 0.05). Mean values with the same small letter in a row for each measured trait are not significantly different as indicated by Tukey's test (p < 0.05).

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2.6. Classification of Yacon Cultivars according to Their TPC and Total Sugar Content

Classification of various cultivars and breeding lines of plants is an important factor for breeding programs and food product development. For instance, potato tubers have been investigated in line with their phytochemical content in support of further breeding programs [51]. Moreover, potato tubers have been classified in compliance with their processing competence [52]. Such studies showed the importance of considering both nutritional factors and suitability of cultivars for specific food products with regard to final product characteristics for the selection of a cultivar for a certain purpose or for developing new cultivars.

On the one hand, classification of yacon tubers as specified by their TPC can contribute to breeding programs that develop new cultivars containing beneficial nutritional traits. On the other hand, the sugar composition of yacon tubers is a deciding factor that determines their potential usage for development of trail-made food products for diabetics. In this regard, the cultivars with lower reducing sugar and sucrose content are more suitable as they lead to a lower glycemic index after consumption. Therefore, yacon cultivars might be classified in accordance to their sugar content or further selective breeding approaches to meet the specific dietary requirements of the target group of consumers, too. On that basis, the investigated yacon cultivars in the present study were categorized based on their TPC and total sugar content in the peel and whole tubers (Figures 1 and 2).

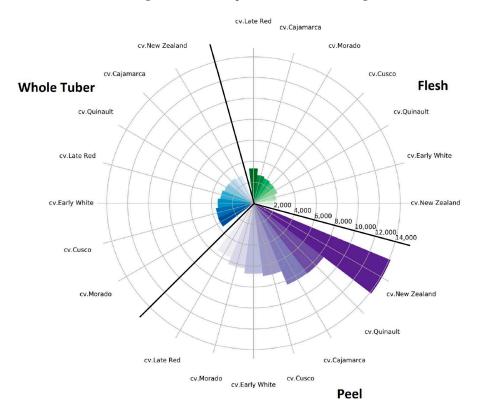


Figure 1. Classification of different parts (flesh, whole tuber, and peel) of the yacon tuber according to total phenolic content (mg GAE 100 g^{-1} DW).

As it is illustrated in Figures 1 and 2, cv. Late Red and cv. Morado had the highest amounts of TPC in their flesh, while the total sugar content of their flesh was in a medium range. Consequently, they may be recommended for cultivation under European climatic conditions aiming for final food products with high health promoting compounds. The lowest total sugar content, TPC in flesh,

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and whole tuber was determined for cv. New Zealand while this cultivar also had the highest amounts of TPC and lowest amounts of total sugar in its peel (Figures 1 and 2). That being the case, cultivation of cv. New Zealand under European environmental conditions might be of interest for the development of food products for diabetics.

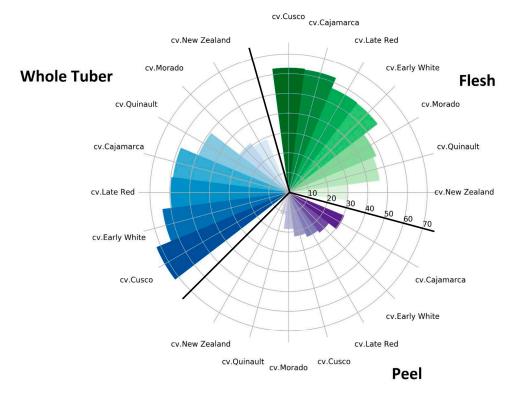


Figure 2. Classification of different parts (flesh, peel, and whole tuber) of the yacon tuber according to total sugar (fructose + glucose + sucrose) content (g 100 g^{-1} DW).

3. Materials and Methods

3.1. Chemicals

Ascorbic acid, Folin–Ciocalteu's reagent, FeCl₃, FeSO₄, HCl, NaNO₂, NaOH, fructose, glucose, and sucrose were provided from Merck (Darmstadt, Germany). 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) and 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), were purchased from Sigma (Darmstadt, Germany). AlCl₃ (Fluka, Seelze, Germany). In addition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CalBiochem, Darmstadt, Germany), Gallic acid (Scharlau, Barcelona, Spain), Na₂CO₃ (AppliChem, Darmstadt, Germany), potassium persulfate (Bernd Kraft, Duisburg, Germany), and Trolox (Cayman, Ann Arbor, MI, USA) were used. Methanol and ethanol were purchased from Chemsolute (Hamburg, Germany) and were HPLC grade.

3.2. Plant Material

Individual tubers from seven cultivars, which are presented in Table 6, were collected in October 2016 at harvest time from a field trial carried out at the research station Ihinger Hof of the University of Hohenheim (Stuttgart, Germany). Yacon rhizomes of all cultivars were purchased from Cultivariable (Moclips, WA, USA). Plantlets were cultivated in the greenhouse for 6 weeks and

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planted at the end of May 2016 in the field in hills (50 cm \times 60 cm). The field was fertilized with 40 kg of nitrogen (ENTEC 26) before planting.

Table 6. Color of the peel and	the flesh of va-	con tubers from	different cultivars.
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Cultivar	Peel Color	Flesh Color
Cajamarca	tan	white
Cusco	tan	white
Early White	tan	white
Late Red	red, tan	orange, yellow
Morado	purple	white
New Zealand	purple, tan	white
Quinault	white, tan	white

At harvest, tubers were washed with tab water and left in the open air to dry. Sample collection was done as follows: Tubers of one plant were cut in half and randomly divided into two portions. One portion was taken to be peeled manually with a hand peeler. Samples from flesh were collected by cutting flesh without peel into small cubic pieces (1 \times 1 \times 1 cm). Another portion of tubers was cut into small cubic pieces without being peeled and collected as a sample of a whole tuber. All samples were immediately frozen with liquid nitrogen and kept in a frozen state (-18 °C) before freeze drying. Afterwards, samples were freeze dried and milled.

3.3. Total Dry Matter Content

Total dry matter content of flesh, peel, and whole tuber samples was measured gravimetrically. The weight of samples was recorded before and after freeze drying and the total dry matter content was calculated using Equation (1).

Total dry matter content =
$$\frac{weight\ of\ samples\ after\ freeze\ drying}{weight\ of\ samples\ before\ freeze\ drying} \times 100$$
 (1)

3.4. Determination of Glucose, Fructose, and Sucrose Content

Extraction of simple sugars was done according to the method used by Kolb et al. (2001) with slight modification [53]. Briefly, 0.1 g of sample powder was placed in a 250 mL Erlenmeyer flask and 50 mL ethanol (70%) was added to it. Then, the mixture was sonicated at 60 °C for 30 min. Afterwards, the mixture was allowed to cool down at room temperature. The extract was filtered using 0.45 μm nylon filters attached to a syringe.

High performance liquid chromatography (HPLC) was performed for determination of fructose, glucose, and sucrose content using a Dionex BioLC HPLC system (HPLC, Darmstadt, Germany). The device operated using a GS50 gradient pump, an AS 50 auto-sampler, an AS 50 Column oven, and DAD ED 50 Electrochemical Detector. Separation of sugars was done using Dionex CarboPac TM PA1 4 \times 250 mm column and Dionex CarboPac PA1 40 mm pre-column at 25 °C. The mobile phase consisted of A (sodium hydroxide (150 mM)) and B (water) and C (sodium hydroxide (150 mM) + sodium acetate (500 mM)). It was eluted gradiently as follows for a total time of 20 min: 0 min (20% A + 80% B + 0% C); 10 min (20% A + 80% B + 0% C); 15 min (0% A + 0% B + 100% C); 18 min (0% A + 0% B + 100% C); and 20 min (0% A + 0% B + 100% C). An injection volume of 10.0 (μ L) and a flow rate of 1 (mL/min) was applied. The fructose, glucose, and sucrose content in yacon extracts were determined using a standard curve drawn by injecting fructose, glucose, and sucrose (0–1 mg mL $^{-1}$).

3.5. Extraction of Phytochemicals

The extraction procedure was performed by adding 5 mL of methanol to 0.25 g of dried powder of yacon flesh, peel, and whole tuber. Then, the mixture was shaken (100 rpm) for 30 min at room

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temperature. Afterwards, the mixture was centrifuged (5810R, Eppendorf, Hamburg, Germany) at 4000 rpm for 10 min (20 $^{\circ}$ C) to separate the supernatant from the solid residuals. The methanol extracts were used for performing the following analysis:

3.5.1. TPC

The TPC was determined following Folin–Ciocaltue methodology [54]. Briefly, $0.5\,\mathrm{mL}$ of prepared extract was mixed with 30 mL of distilled water in a 50 mL volumetric flask. After 6 min, $7.5\,\mathrm{mL}$ of sodium carbonate solution (20%) was added and the final volume was adjusted to 50 mL. The mixtures were left at room temperature for 2 h before reading the absorbance at 760 nm by means of a UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK). The standard curve was drawn using a gallic acid solution (0.3–3 mg gallic acid/mL distilled water) as a reference standard. TPC was expressed as gallic acid equivalent per 100 grams of dry weight (mg GAE $100\,\mathrm{g}^{-1}\,\mathrm{DW}$).

3.5.2. TFC

TFC was measured as follows: $0.5 \, \text{mL}$ of extract was well mixed with 1 mL sodium nitrite solution (5%). After 6 min, 1 mL of AlCl₃ (10%) and 10 mL of sodium hydroxide (1 M) was added to the mixture. The final volume of the mixture was adjusted to 25 mL by distilled water. Then, the mixture was kept for 15 min at room temperature. Finally, the absorbance was read at 510 nm using UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). Rutin ($0.0625-4 \, \text{mg}$ rutin/mL 70% ethanol) was prepared to generate the standard curve. TFC was expressed as rutin equivalent per 100 grams of dry weight (mg RE $100 \, \text{g}^{-1}$ DW) [55].

3.5.3. Determination of Antioxidant Activity

ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid) Diammonium Salt) Radical Scavenging Activity

ABTS radical scavenging activity was measured following the method used by Dudonne et al. (2009) [56]. In order to produce ABTS radical cations (ABTS^{+•}) potassium persulfate (2.45 mM) and ABTS solution (7 Mm) were mixed together and left to stand in the dark at room temperature for 12–16 h before use. The ABTS^{+•} solution was diluted to an absorbance of 0.700 ± 0.02 at 734 nm before being used. 3.0 mL of diluted ABTS^{+•} solution was added to 0.1 mL of extract. The reaction solution was maintained at 30 °C after mixing for 10 min. Then, the absorbance was read at 734 nm with UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). The standard curve was generated using trolox solution (0.02–0.2 (mM)). ABTS radical scavenging activity was expressed as trolox equivalent per 100 grams of dry weight (mM TE 100 g⁻¹ DW).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radicals Scavenging Activity

The DPPH radical scavenging activity was measured as follows [56]: 0.1 mL of the extract was mixed to 3 mL of freshly prepared 6×10^{-5} mol/L DPPH $^{\bullet}$ solution in methanol. Afterwards, the reaction mixture was kept at 37 °C for 20 min before reading the absorbance at 515 nm using UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). Ascorbic acid solution (0.02–0.2 mg ascorbic acid/mL distilled water) was used as a reference standard to draw the standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent per 100 grams of dry weight (mg AAE 100 g $^{-1}$ DW).

FRAP

FRAP assay was performed as follows [57]: Fresh FRAP working solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine) in HCl (10 mM) and 20 mM FeCl₃ solution in a 10:1:1 (v/v/v) ratio. 0.15 mL of the extract was mixed with 2.85 mL

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of the FRAP solution and incubated at 37 $^{\circ}$ C for 30 min. The FRAP of the samples was evaluated by measuring the absorbance of Fe²⁺-TPTZ at 593 nm with UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). The results of the FRAP assay were reported as Fe²⁺ (mM) equivalent per 100 grams of dry weight (mM Fe²⁺ 100 g⁻¹ DW).

3.6. Statistical Analysis Of Data

Sample preparation and analysis were performed in duplicate and the results are reported as mean value \pm standard deviation. For HPLC analysis, two extractions were performed for each sample and for each sample two injections were applied. The results were subjected to a two-way analysis of variance (ANOVA) (cultivar.tuber part) and the mean differences between evaluated parameters were established by performing Tukey's test at 5% significance level. Statistical analysis of data was performed using SAS Software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Figures were generated using matplot library from Python version 3.6.4 (Python Software Foundation, Wilmington, DE, USA).

4. Conclusions

The results of this study showed that the cultivar and yacon tuber part had a significant effect on the total dry matter content, sugars, TPC, TFC, and antioxidant activity of yacon tubers.

The ranking of the studied cultivars in decreasing order according to the total dry matter content of their flesh and whole tuber is as follows: cv. Morado > cv. Late Red > cv. New Zealand > cv. Early White > cv. Quinault > cv. Cusco > cv. Cajamarca. The total sugar content varied between cultivars. The lowest sugar content was noted for cv. New Zealand in the flesh, peel, and whole tuber. With regard to TPC, TFC, DPPH radical scavenging activity and FRAP of flesh and whole tubers, cv. Late Red, cv. Cajamarca, and cv. Morado were the three top cultivars while cv. New Zealand contained the lowest TPC and TFC when grown under European environmental conditions. However, the highest ABTS radical scavenging activity of the flesh and whole tubers was determined in cv. New Zealand and was the lowest for cv. Late Red which points to the importance of further investigations to determine the individual bioactive compounds.

Moreover, total dry matter content and phyto/chemical content of the peels of yacon tubers showed that the peels of yacon tubers are a good source of phytochemicals and exhibit considerable antioxidant activity while having low content of sugar. It was noted that the TPC, TFC and antioxidant activity of the peel of yacon tubers was higher than their flesh and even higher than those of whole tubers. The opposite trend was noticed for sugar content which was lowest in the peel of tubers. Therefore, it can be suggested that for minimizing the waste of food processing of yacon tubers the peels could use in other food and/or feed products, nutraceuticals, pharmaceuticals, and cosmetic products. However, more detailed investigations of the characterization of yacon peels is necessary to ensure their safety when being utilized as an ingredient in other products.

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Sample Availability: Samples of the compounds are not available from the authors.



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6. Chapter III: Total Phenolic Content and Antioxidant Activity of yacon (*Smallanthus sonchifolius* Poepp. and Endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying

Publication III:

Khajehei, F., Hartung, J., & Graeff-Hönninger, S. (2018). Total Phenolic Content and Antioxidant Activity of Yacon (*Smallanthus Sonchifolius* Poepp. and Endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying. *Agriculture*, 8(12), 183.

The importance of food processing and preservation is due to the fact that agricultural products are perishable and their fresh supply is seasonal. Drying of fruits is one of the oldest methods for preservation of fruits and several researchers carried out studies focusing on the effect of drying on the phyto/chemicals of different dried products derived from yacon tubers. In particular, the focus of such studies has been on the FOS, inulin and sugar content of the final yacon products. Hereinafter, the results of Chapters II focused on the phyto/chemicals of fresh yacon tubers, while the aim of Chapter III was to evaluate the effect of curing, pretreatment with diluted lime juice, and the drying method and conditions on TPC and antioxidants in yacon chips produced from two cultivars (red and white). The overall aim of Chapter III was to provide information regarding the proper time after harvest for processing of yacon tubers and to examine the effect of pre-treatment and drying method on retention of TPC and antioxidant activity of yacon chips.





Article

Total Phenolic Content and Antioxidant Activity of Yacon (Smallanthus Sonchifolius Poepp. and Endl.) Chips: Effect of Cultivar, Pre-Treatment and Drying

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Abstract: Recent studies have associated the consumption of yacon root as a functional plant food with reduced glycemic index and, due to its considerable phenolic acid levels, a protection of cell membranes against free radical damage. This study examined the effect of four different treatments including: (1) storage duration after harvest (one and three weeks after harvest); (2) pre-treatment before drying (untreated, pre-treatment with diluted lime juice); (3) drying method (freeze drying (FD) and convective hot air drying (CHAD)); and (4) cultivar (white and red), on the quality of yacon (*Smallanthus sonchifolius* Poepp. and Endl.) chips in terms of their total phenolic content (TPC) and antioxidant activity (AA) (ABTS (2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) Diammonium Salt) radical scavenging activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and ferric reducing antioxidant power (FRAP)). Overall, the chips that were produced using pre-treatment with diluted lime juice and FD had the highest amounts of TPC and AA. Regarding the chips produced by means of CHAD, retention of higher TPC and AA was possible with lime-juice pre-treatment and use of higher hot air temperatures. Moreover, chips produced from the white cultivar had higher TPC and AA than chips produced from the red cultivar.

Keywords: functional foods; yacon; *Smallanthus sonchifolius* Poepp. and Endl.; convective hot air drying; freeze drying; total phenolic content; ABTS radical scavenging activity; DPPH radical scavenging activity; ferric-reducing antioxidant power

1. Introduction

Consumption of plant food, particularly fruits, has been shown to be directly or indirectly positively associated in reducing the risk of chronic diseases such as cancer, coronary heart disease, stroke, type 2 diabetes, obesity, hypertension, etc. owing to their nutrients and non-nutritive constitutes [1]. The beneficial effects of fruit consumption have been associated with their high content of bioactive compounds (e.g., phenols) which can act as antioxidants and protect the body from oxidative stress, lower energy content and dietary fibre content [1–4]. Accordingly, the phenolic content of plant foods, their antioxidant activity, health-promoting effects, and prospective potential for the development of functional food products, plant-based nutraceuticals and pharmaceuticals have been the focus of ample investigations [3,5]. Moreover, there is growing interest in the neglected plant foods for reasons such as their favourable nutritional characteristics, their potential to diversify the diet and enhance the status of food security, and optimistic prospects in some of the rising mega trends in food supply chain (e.g., "food for beauty and health", "food that meets our expectations", "more food from less resources", and "food for a healthy planet") [6–8].

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Yacon (*Smallanthus sonchifolius* Poepp. and Endl.) belongs to the plant family Asteraceae and is an underutilized tuberous crop. Although it has its origin in the Andean region, it has been cultivated in other regions such as Brazil, Czech Republic, Ecuador, Germany, Japan, and New Zealand. Yacon tubers taste relatively sweet, have a crunchy juicy texture and are consumed as fresh fruits. They received recognition during the past two decades due to their nutritional, functional constitutes, and health beneficial effect. In particular, yacon tubers have high contents of bioactive compounds (e.g., phenolic compounds), fructooligosaccharides (FOS) and low sugar content [9,10]. On that account, yacon has gained attention as an interesting crop for production of functional foods with low sugar content [7,11]. Like other fruits, the availability of fresh yacon is seasonal. Therefore, food processing such as drying, evaporation, and fermentation can be used to develop food products such as yacon chips, flour, syrup, vinegar, etc. to extend the shelf life of yacon tubers [9].

Drying of fruits is one of the oldest preservation methods that has been widely used to produce durable food commodities such as final products like fruit chips. Dried fruits can be considered as a concentrated form of fruit, which allows for considerable extension of their shelf life by removal of water as well as easing transport and packaging [12]. Several factors including the quality of raw material, pre-treatment of raw material before drying and the method of drying can have an impact on the physicochemical quality of the final product [13,14]. In this regard, the physicochemical characteristics of fresh fruits depend on cultivar, environmental conditions during cultivation and conditions of post-harvest handling. Moreover, pre-treatments such as sulphur-fumigation, osmotic treatment and blanching used before drying can also affect the physicochemical quality of the final dried fruit products [14]. With regard to the drying method, various drying techniques have been investigated and developed including sun drying, convective hot air drying (CHAD), vacuum drying, hybrid drying techniques such as microwave and ultrasound assisted drying, fluidized bed, spouted bed, freeze drying (FD) etc. [15,16]. However, the final choice of the drying technique depends on the nature of the product, desired quality of the final product, energy consumption as well as the availability of the desired drying technology [13]. One of the most common drying techniques that has the advantage of technological simplicity is CHAD which operates according to the application of heat based on the principles of a convection mechanism for evaporation of water and removal of vapor by forced air [13]. FD is a technique in which water in food materials is removed by sublimation under a vacuum, and owing to the low temperature used, this technology results in products with premium quality. However, its application is contested as a consequence of the high cost of its operating system [17].

In particular, preservation of yacon using drying has been investigated before, and the production of certain dried products from yacon tubers including yacon chips, whole yacon flour, yacon juice powder, yacon peel flour and yacon pulp flour have been studied [18-22]. Most of these studies have evaluated the effects of various drying processes on the quality of final dried yacon products regarding their sugar and FOS content [19,22,23]. In addition, the focus of some studies has been on the determination of bioactive compounds such as phenolic compounds and antioxidant activity of fresh yacon tubers [11,24,25]. It has been noted that fresh yacon tubers possess high amounts of phenolic compounds including chlorogenic acid, ferulic acid, coumaric acid, quercetin, caffeic acid and its derivatives [24,26,27]. However, few studies have investigated the effect of drying on bioactive constitutes and antioxidant activity yacon tubers. Castro et al. showed that drying of yacon slices at 50 °C in a cabinet dryer results in yacon chips with a higher total phenolic content (TPC) and antioxidant activity compared to those which were dried at 40 °C and 60 °C [18]. Moreover, vacon slices dried at 55 °C in a forced air oven with and without soaking of them in a solution of sodium hypochlrorite (20 mg L^{-1}) and 0.1% sodium disulfide to produce yacon flour, showed that retention of TPC and individual phenolic compounds namely, chlorogenic acid, ferulic acid and caffeic acid, were better when pre-treatment was applied before drying [20]. On the other hand, the quality of yacon dried products can be influenced by the quality of the fresh yacon tubers. The quality of fresh tubers in terms of their chemical composition depends on the cultivar, conditions during the cultivation, as well Agriculture 2018, 8, 183 3 of 18

as conditions during the storage of tubers [28–30]. In particular, storage conditions and duration of storage after the harvest of tubers before procesing them may change their phytochemical quality [28]. For example, sunning tubers after the harvest has been used traditionally to yield a sweeter taste in the tubers which is due to the conversion of FOS to simple sugars [30,31]. Phenolic compounds of yacon tubers may also undergo changes during storage and food processing. Specifically, yacon tubers darken during storage, cutting and processing. Enzymatic oxidation of phenolic compounds (e.g., chlorogenic and caffeic acids) catalyzed by native polyphenol oxidase may occur during storage and food processing as a result of changes in cellular integrity, which leads to browning of products [32–34]. Moreover, heterogeneity in the profile of phenolic compounds and polyphenol oxidase of different cultivars of the same species may affect such alternations as well as the intensity of such changes in composition under the influence of storage and food processing [2,35].

To the best of our knowledge, there is a gap in literature regarding the effect of post-harvest handling conditions of yacon tubers on phenolic compounds and antioxidant activity of yacon chips from different yacon cultivars. Therefore, the main objective of this work was to determine the effect of storage duration after harvest, pre-treatment before drying, and the drying of yacon slices using FD techniques and various drying temperatures during CHAD on the TPC and antioxidant activity of yacon chips produced from two cultivars grown in the environmental conditions of south-west Germany.

2. Materials and Methods

2.1. Chemicals

Ascorbic acid, Folin–Ciocalteu's reagent, FeCl₃, FeSO₄, HCl, and NaOH were purchased from Merck (Darmstadt, Germany). 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) and 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), were provided by Sigma (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CalBiochem, Darmstadt, Germany), Gallic acid (Scharlau, Barcelona, Spain), Na₂CO₃ (AppliChem, Darmstadt, Germany), potassium persulfate (Bernd Kraft, Duisburg, Germany), and Trolox (Cayman, Ann Arbor, MI, USA) were used. Methanol was purchased from Chemsolute (Hamburg, Germany).

2.2. Plant Material

The yacon tubers used in the present work were taken from a field trial carried out in 2015 at the organically operating research station of Kleinhohenheim of the University of Hohenheim (Stuttgart, Germany). The tubers of two yacon cultivars were specified in accordance to the color of the tubers' peels: red and white. Yacon tubers, which were cultivated at the same field under the same growing conditions and management to ensure *ceteris paribus*, were bulked across replicates, sorted, washed with tap water, and left in the open air to drain for two hours one day after harvest. Afterwards, the tubers from each cultivar were divided into two batches. The first batch was kept at ambient temperature in a closed dark box before drying for one week after harvest. The second batch was stored at ambient temperature in a closed dark box for three weeks after harvest before drying.

2.3. Drying of Yacon Slices

Table 1 shows the process variables for drying trials.

Drying of yacon slices was carried out on two dates within a two-week interval. The first batch of yacon tubers was dried one week after harvest and considered as tubers that had undergone no curing and the second batch which was stored at ambient temperature for three weeks after harvest was considered to as cured tubers (Table 1). Sample preparation at each drying trial date was done as follows: tubers of yacon from each cultivar were peeled using manual peelers. Yacon slices with a thickness of 3 ± 0.5 mm were prepared using manual slicers (Graef, Gebr. Graef GmbH & Co. KG, Arnsberg, Germany). Afterwards, yacon slices were divided into two parts: a first part that had

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undergone no pre-treatment before drying and was regarded as untreated. Yacon slices belonging to this group were put into perforated plastic bags immediately after slicing in a single layer where each plastic bag contained approximately 80--100~g of yacon slices. The second part of the yacon slices were dipped in a diluted lime juice (pH = 2.50 ± 0.05 , $3.3\pm0.2\,^{\circ}$ Brix) for 10 min, then left to drain at room temperature for 10 min, which was regarded as pre-treated with diluted lime juice, and then were arranged in a single layer in perforated plastic bags. Plastic bags containing these yacon slices were placed in a convective hot air dryer (Vötsch Industrietechnik, Reiskirchen, Germany) operating at $40\,^{\circ}$ C, $50\,^{\circ}$ C and $60\,^{\circ}$ C and drying was carried out for 20 h. After the drying process, samples were left to cool down before being ground. In the case of FD, yacon slices from each treatment were placed in plastic bottles, and were frozen immediately by means of liquid nitrogen. Afterwards, the frozen samples were kept at $-18\,^{\circ}$ C before FD. FD was performed using a freeze dryer (Dieter Piatkowski, Munich, Germany) with the maximum temperature inside the chamber of the freeze drier reaching to $30\,^{\circ}$ C. Therefore, the following samples were produced:

- Yacon chips produced from white (W) or red (R) cultivar without curing without pre-treatment in diluted lime juice by means of FD or CHAD using hot air drying at 40 °C, 50 °C and 60 °C (hot air dried at 40 °C (HA40), HA50, and HA60, respectively) including: white, freeze dried (WFD), WHA40, WHA50, WHA60, red, freeze dried (RFD), RHA40, RHA50, and RHA60.
- Yacon chips produced from white (W) or red (R) cultivar without curing with pre-treatment in diluted lime juice (L) by means of FD or CHAD using hot air drying at 40 °C, 50 °C and 60 °C (HA40, HA50, and HA60, respectively) including: white, freeze dried, pre-treatment in diluted lime juice (WFDL), WHA40L, WHA50L, WHA60L, red, freeze dried, pre-treatment in diluted lime juice (RFDL), RHA40L, RHA50L, and RHA60L.
- Yacon chips produced from white (W) or red (R) cultivar with curing without pre-treatment
 in diluted lime juice by means of FD or CHAD using hot air drying at 40 °C, 50 °C and 60 °C
 (HA40, HA50, and HA60, respectively) including: white, freeze dried, cured (WFDC), WHA40C,
 WHA50C, WHA60C, red, freeze dried, cured (RFDC), RHA40C, RHA50C, and RHA60C.
- Yacon chips produced from white (W) or red (R) cultivar with curing and pre-treatment in diluted lime juice by means of FD or CHAD using hot air drying at 40 °C, 50 °C and 60 °C (HA40, HA50, and HA60, respectively) including: white, freeze dried, cured, pre-treatment in diluted lime juice (WFDCL), WHA40CL, WHA50CL, WHA60CL, red, freeze dried, cured, pre-treatment in diluted lime juice (RFDCL), RHA40CL, RHA50CL, and RHA60CL.

			Process Variables		
	Row Mat	terial	Pre-Treatment Before Drying	Drying Method	
			Pre-treatment with diluted lime juice	CHAD	40 °C 50 °C 60 °C
		No curing		FD	
			No pre-treatment	CHAD	40 ° 0 50 ° 0 60 ° 0
C Iv	Red			FD	
Cultivar	White		Pre-treatment with diluted lime juice	CHAD	40 °C 50 °C 60 °C
		Curing		FD	
			No pre-treatment	CHAD	40 °C 50 °C 60 °C

Table 1. Process variables for drying of vacon slices

CHAD = convective hot air drying, and FD = freeze drying.

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Dried samples were grounded by means a laboratory mill (Retsche GM 200, Haan, Germany) and yacon powders were kept in closed plastic bottles in a cool dark place until the chemical analysis was carried out.

The initial and final dry matter content of yacon slices were determined gravimetrically. The previously ground samples were dried at 105 ± 1 °C for 5 h and the weight of samples was recorded before and after drying. Equation (1) was used to calculate the total dry matter content.

Total dry matter content =
$$\left(\frac{\text{weight of samples after drying}}{\text{weight of samples before drying}}\right) \times 100$$
 (1)

2.4. Extraction of Phytochemicals

The extraction was carried out by adding 5 mL of methanol to 0.25 g of dried yacon chip powder. This was followed by shaking the mixture for 30 min at room temperature. Afterwards, the mixture was centrifuged (5810R, Eppendorf AG, Humburg, Germany) at 4000 rpm for 10 min (20 $^{\circ}$ C) to separate the supernatant from the solid residuals. The methanolic extracts were used for the following analysis.

2.4.1. Total Phenolic Content (TPC)

The TPC was conducted according to the Folin-Ciocaltue methodology [36]. Briefly, 0.5 mL of prepared extract was added to 30 of distilled water in a 50 mL volumetric flask. Then 2.5 mL of Folin-Ciocalteu's reagent was added to the flask and mixed with it. After 6 min, 7.5 mL of sodium carbonate solution (20%) was added to the mixture and the final volume was adjusted to 50 mL. The mixtures were left at room temperature for 2 h. Then, the absorbance at 760 nm was measured by a ultraviolet (UV)/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience, Buckinghamshire, UK). Gallic acid solution (0.3-3 mg gallic acid /mL distilled water) was used to draw the standard curve. Finally, TPC was expressed as gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} DW).

2.4.2. Determination of Antioxidant Activity

2.4.2.1. ABTS (2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) Diammonium Salt) Radical Scavenging Assay

The ABTS radical scavenging activity was determined in accordance with the method used by Dudonne et al. [37]. Firstly, potassium persulfate (2.45 mM) and ABTS solution (7 mM) was mixed and left to stand in the dark at room temperature for 12–16 h to produce the ABTS radical cations (ABTS*+). The ABTS*+ solution was diluted so the absorbance of the solution was 0.700 ± 0.02 at 734 nm before the analysis. For the analysis, 3.0 mL of diluted ABTS*+ solution was mixed to 0.1 mL of extract and the reaction solution was maintained at 30 °C for 10 min. Then, the absorbance was measured at 734 nm with a UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience). The standard curve was drawn with trolox solution (60–200 (μ M)) as the reference substance. ABTS radical scavenging activity was expressed as trolox equivalent per 100 g of dry weight (μ M TE 100 g⁻¹ DW).

2.4.2.2. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity

To determine the DPPH radical scavenging activity, 0.1 mL of the extract was added and mixed with 3 mL of freshly prepared 6×10^{-5} mol/L DPPH $^{\bullet}$ solution in methanol. Then, the reaction mixture was maintained at 37 °C for 20 min before measuring the absorbance at 515 nm using a UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience) [37]. Ascorbic acid solution (0.02–0.2 mg ascorbic acid/mL distilled water) was used to draw the standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent per 100 g of dry weight (mg AAE 100 g^{-1} DW).

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2.4.2.3. Ferric-Reducing Antioxidant Power (FRAP)

The FRAP assay was carried out as follows: Fresh FRAP solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine) in HCl (10 mM) and 20 mM FeCl₃ solution in a 10:1:1 (v/v/v) ratio. Then, 0.15 mL of the extract was mixed with 2.85 mL of the FRAP solution. The mixture was incubated at 37 °C for 30 min before the the absorbance of Fe²⁺-TPTZ was measured at 593 nm with a UV/Visible spectrophotometer (Ultrospec 3100 Pro, Amersham Bioscience) [38]. The results of the FRAP assay was reported as Fe²⁺ (mM) equivalent per 100 g of dry weight (mM Fe²⁺ 100 g⁻¹ DW).

2.5. Statistical Analysis of Data

Data was analysed using a mixed model approach. The model can be described as:

$$y_{ijk} = \mu + \tau_i + d_j + b_k + e_{ijk} \tag{2}$$

where μ is the general effect, τ_i is the ith treatment effect, d_j is the random effect of the jth day and b_k is the random effect of the kth bag within the CHAD/FD. e_{ijk} is the error associated with observation y_{ijk} . Treatments were further split in a 2 × 2 × 2 × 4 factorial design with two cultivars, two pre-treatments, two storage durations and four drying methods (three temperatures in case of CHAD and FD). In this case, τ_i is modelled as:

$$\tau_i = c_n \times p_m \times s_o \times t_p \tag{3}$$

where c_n , p_m , s_o and t_p are the main effects for cultivar n, pre-treatment m, storage duration o and drying method p. The crossing operator \times between two main effects indicates that both main effects and their interactions are included. Residuals were checked graphically for homogeneous variances and normal distribution. Furthermore, the fitting of drying method specific variances were checked by comparing the Akaike Information Criteria (AIC) with and without this heterogeneous variance [39]. Note, that due to the sampling procedure, there is no true replicate for cultivar-by-storage-combinations, as all material from one cultivar and one storage duration were treated together. Thus, care should be taken in interpreting the significance of these effects, as their true error variances can be underestimated by the estimated repeated measures error. After finding significant differences via the F test, a simple multiple t test (the Least Significant Difference (LSD) test) and a letter display were performed. Additionally, to simplify interpretation, especially for traits with several significant three-way-interactions, means and their standard error for the four-way-interactions were presented. Note that results are presented separate for each cultivar, as there is no true replicated sample of cultivars.

For calculating the correlation between traits, a bivariate model was fitted [40]. The model in (1) can be extended for a bivariate model as follows:

$$y_{ijkl} = \mu_l + \tau_{il} + d_{jl} + b_{kl} + e_{ijkl} \tag{4}$$

where $\mu_l = \begin{pmatrix} \mu_t \\ \mu_f \end{pmatrix}$ is a vector of two fixed general effects for traits t and t. τ_{il} is a vector of length 64 with elements for all treatments at both traits. A Kronecker product of an ID matrix for the 32 treatments and an unstructured variance covariance structure for two traits were fitted. This allows a trait specific variance of treatment effects and a covariance between treatment effects. A similar variance—covariance matrix was fitted to day, bag, and error effects, respectively.

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3. Results and Discussion

3.1. Total Dry Matter Content

The statistical analysis of data determined that the total dry matter of yacon chips was under the significant effect of four-way interactions of studied variables (storage duration after harvest (curing or no curing), pre-treatment before drying, drying temperature, and cultivar) (p = 0.018) (Table 2). The total dry matter of yacon white and red yacon slices one week after harvest was 10.91 ± 0.65 and $15.65\pm0.89\%$ respectively, while it ranged between 11.58 ± 0.26 and $16.84\pm0.47\%$ for white and red cultivar, respectively, three weeks after harvest. The total dry matter content of yacon chips produced one week after harvest from the white cultivar ranged between 91.20 ± 0.32 and 92.80 \pm 0.32% for WHA40L and WHA40, respectively, while it ranged between 91.47 \pm 0.45 and 92.92 \pm 0.45% for WHA40CL and WHA40C, for white yacon chips which were produced three weeks after harvest (Table 3). Table 4 reports the total dry matter content of yacon chips produced from the red cultivar one week after harvest which was between 90.64 \pm 0.32 and 92.08 \pm 0.32% for RHA40L and RFD, respectively. The total dry matter of red yacon chips produced three weeks after harvest ranged between 91.85 \pm 0.45 and 92.56 \pm 0.45% for RHA40CL and RHA50C, respectively (Table 4). Higher dry matter contents were achieved when yacon slices were dried by FD or by means of CHAD at lower hot air temperatures. This could be due to the fact that at higher hot air drying temperatures, case hardening might occur [41]. As a consequence, the external surface becomes rigid which may reduce the rate of drying and result in lower dry matter content in product at the end of the drying process.

Table 2. Analysis of variance (ANOVA) of results of total dry matter content, total phenolic content, ABTS radical scavenging activity, DPPH radical scavenging activity, and ferric-reducing antioxidant power as a function of the process variables of the yacon chips.

Effect	Total Dry Matter Content	Total Phenolic Content	ABTS Radical Scavenging Activity	DPPH Radical Scavenging Activity	Ferric-Reducing Antioxidant Power
Cultivar	0.0065	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Curing	0.0054	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Drying temperature	0.7668	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pre-treatment	0.0001	< 0.0001	< 0.0001	0.7390	< 0.0001
Cultivar \times curing	0.0770	0.1957	0.0016	0.0035	0.0358
Drying Temperature × cultivar	0.0213	0.2298	< 0.0001	< 0.0001	0.0003
Pre-treatment \times cultivar	0.4215	0.4629	0.1261	0.2297	0.4920
Drying Temperature × curing	0.1895	< 0.0001	< 0.0001	0.0152	0.0008
Drying temperature × Pre-treatment	< 0.0001	< 0.0001	< 0.0001	0.0018	0.1602
Pre-treatment × curing	0.1424	0.0079	0.9512	< 0.0001	0.0001
Pre-treatment \times Drying temperature \times curing	0.6443	0.0753	< 0.0001	0.0390	0.3708
Drying Temperature \times curing \times cultivar	0.3803	< 0.0001	< 0.0001	< 0.0001	0.0002
Pre-treatment \times curing \times cultivar	0.9251	< 0.0001	< 0.0001	0.0148	0.0337
Pre-treatment × Drying temperature × cultivar	0.0485	0.0002	0.2016	0.3993	0.0006
$Pre\text{-treatment} \times Drying \ temperature \times cultivar \times curing$	0.0189	0.1652	< 0.0001	< 0.0001	0.5791

Table 3. The total dry matter content, total phenolic content, ABTS radical scavenging activity, DPPH radical scavenging activity, and ferric-reducing antioxidant power of the yacon chips produced from white yacon cultivar.

Sample	Total Dry Matter Content (%)	Total Phenolic Content (mg GAE 100 g ⁻¹ DW)	ABTS Radical Scavenging Activity (µM TE 100 g ⁻¹ DW)	DPPH Radical Scavenging Activity (mg AAE 100 g ⁻¹ DW)	Ferric-Reducing Antioxidant Power (mM Fe ²⁺ 100 g ⁻¹ DW)
WFD	$92.38~^{abcd*} \pm 0.32$	$1434.81^{\ b} \pm 29.36$	$4,603,298^{\text{ c}} \pm 198,865$	$1473.36^{a} \pm 34.99$	17,264.45 ab ± 523.53
WHA40	$92.80^{\ a}\pm0.32$	$336.83^{k} \pm 29.36$	$2,478,116^{i} \pm 198,865$	$879.13 \text{ g} \pm 34.99$	$8924.44 \text{ g} \pm 523.53$
WHA50	$91.55^{\text{ cdef}} \pm 0.32$	$559.77^{j} \pm 29.36$	$1,869,012^{j} \pm 198,865$	$1068.07 \stackrel{\text{de}}{=} 34.99$	$10,371.17 \text{ g} \pm 523.53$
WHA60	$91.72^{\text{ bcdef}} \pm 0.32$	$703.40^{i} \pm 29.36$	$2,696,786^{\text{hi}} \pm 198,865$	$1291.05^{\text{ b}} \pm 34.99$	9751.33 g \pm 523.53
WFDL	$92.08~^{abcde}\pm0.32$	$1655.34\ ^{a}\pm 29.36$	8,564,803 a \pm 198,865	$1442.56\ ^a\pm 34.99$	$17,941.17^{ab} \pm 523.53$

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Table 3. Cont.

Sample	Total Dry Matter Content (%)	Total Phenolic Content (mg GAE 100 g ⁻¹ DW)	ABTS Radical Scavenging Activity (µM TE 100 g ⁻¹ DW)	DPPH Radical Scavenging Activity (mg AAE 100 g ⁻¹ DW)	Ferric-Reducing Antioxidant Power (mM Fe ²⁺ 100 g ⁻¹ DW)
WHA40L	$91.20^{\text{ f}} \pm 0.32$	$791.84 \text{ gh} \pm 29.36$	$3,567,287^{ m ef}\pm198,865$	$874.72 \text{ g} \pm 34.99$	$13,067.62^{\text{ ef}} \pm 523.53$
WHA50L	$91.40^{ m \ ef} \pm 0.32$	$1033.50^{ ext{ ef}} \pm 29.36$	$2,963,810^{ m ghi}\pm198,865$	$1139.00 \text{ cd} \pm 34.99$	$14,344.79^{\mathrm{de}} \pm 523.53$
WHA60L	$91.76^{\text{ bcdef}} \pm 0.32$	$963.56^{ \text{ f}} \pm 29.36$	$3,031,599$ ghi \pm 198,865	$1074.84^{\mathrm{de}} \pm 34.99$	$14,276.69$ de ± 523.53
WFDC	92.84 $^{\rm a}\pm0.45$	$1182.67^{\text{ cd}} \pm 29.36$	$4,594,690^{\text{ c}} \pm 198,865$	$1460.17^{a} \pm 34.99$	$15,726.19$ ^{cd} \pm 523.53
WHA40C	92.92 $^{\rm a}\pm 0.45$	$714.34^{\text{ hi}} \pm 29.36$	$2,989,000 \text{ ghi} \pm 198865$	$950.48 \text{ fg} \pm 34.99$	$12,067.90^{\circ} \pm 523.53$
WHA50C	$91.48^{ m def} \pm 0.45$	$864.06 \text{ g} \pm 29.36$	$3,446,631$ fg \pm 198,865	1212.28 bc ± 34.99	$12,518.07^{\text{ f}} \pm 523.53$
WHA60C	$92.38 \text{ abc} \pm 0.45$	$1010.13^{\text{ f}} \pm 29.36$	$3,188,069$ fgh \pm 198,865	$1211.72^{\text{ bc}} \pm 34.99$	$12,784.27^{\text{ f}} \pm 523.53$
WFDCL	$91.74^{ m \ bcdef} \pm 0.45$	$1477.23^{\text{ b}} \pm 29.36$	$5,480,063^{\text{ b}} \pm 198,865$	$1435.27^{\ a} \pm 34.99$	$18,171.17^{ab} \pm 523.53$
WHA40CL	$91.47^{ m \ def} \pm 0.45$	$1112.70^{\mathrm{de}} \pm 29.36$	$3,975,272^{\mathrm{de}} \pm 198,865$	$980.84^{\text{ ef}} \pm 34.99$	$16,437.41$ bc ± 523.53
WHA50CL	$92.89^{a} \pm 0.45$	$1222.92^{\ c} \pm 29.36$	$423,6791$ ^{cd} \pm 198,865	1237.05 bc ± 34.99	$17,458.59$ ab ± 523.53
WHA60CL	$92.85 \text{ a} \pm 0.45$	$1131.69 \text{ d} \pm 29.36$	$4,197,870^{\text{ cd}} \pm 198,865$	1209.25 bc ± 34.99	$17,072.86$ abc \pm 523.53

Reported values are presented as mean values \pm standard deviation (n = 4, for each sample two bags of sample were dried and measurements of each parameter in laboratory were carried out twice.). * Mean values with the same small letter in a column are not significantly different (p < 0.05). (W = white, FD = freeze dried, HA40 = hot air dried at 40 °C, HA50 = hot air dried at 50 °C, HA60 = hot air dried at 60 °C, L = pre-treated in diluted lime juice, C = cured).

Table 4. The total dry matter content, total phenolic content, ABTS radical scavenging activity, DPPH radical scavenging activity, and ferric-reducing antioxidant power of the yacon chips produced from red yacon cultivar.

Sample	Total Dry Matter Content (%)	Total Phenolic Content (mg GAE 100 g ⁻¹ DW)	ABTS Radical Scavenging Activity (μM TE 100 g ⁻¹ DW)	DPPH Radical Scavenging Activity (mg AAE 100 g ⁻¹ DW)	Ferric-Reducing Antioxidant Power (mM Fe ²⁺ 100 g ⁻¹ DW)
RFD	$92.08 \text{ a}^* \pm 0.32$	$731.82 \text{ d} \pm 29.36$	$3,286,318$ ^{cd} \pm 198,865	$1402.22^{ab} \pm 34.99$	12,396.13 ^{cd} ± 523.53
RHA40	$91.77^{ m abc} \pm 0.32$	$236.47^{\text{ h}} \pm 29.36$	$2,179,701$ fgh \pm 198,865	$820.73^{\mathrm{de}} \pm 34.99$	$7180.02^{i} \pm 523.53$
RHA50	$91.93^{ab} \pm 0.32$	$321.90 \text{ g} \pm 29.36$	$1,816,080^{\text{ h}} \pm 198,865$	$828.63 \text{ d} \pm 34.99$	$7560.53^{\text{ hi}} \pm 523.53$
RHA60	$91.07^{\text{ cd}} \pm 0.32$	$328.07 \text{ g} \pm 29.36$	$2,000,191$ gh \pm 198,865	$722.08 \text{ fgh} \pm 34.99$	$7259.57^{\text{ hi}} \pm 523.53$
RFDL	$90.66^{d} \pm 0.32$	978.97 $^{\mathrm{b}}$ \pm 29.36	$4,650,806^{\text{ b}} \pm 198,865$	$1306.14^{\ b} \pm 34.99$	$16,130.51^{\text{ b}} \pm 523.53$
RHA40L	$90.64 \text{ d} \pm 0.32$	$471.15^{\text{ f}} \pm 29.36$	$2,568,722$ ef \pm 198,865	$681.28 ^{\mathrm{gh}} \pm 34.99$	$9549.82 \text{ fg} \pm 523.53$
RHA50L	$91.77^{ m abc} \pm 0.32$	$560.25^{\text{ e}} \pm 29.36$	$2,418,077^{ m efg}\pm198,865$	$750.08 \text{ defg} \pm 34.99$	$9605.81 \text{ fg} \pm 523.53$
RHA60L	$91.21^{\text{ bcd}} \pm 0.32$	$494.71^{ m ef}\pm29.36$	$2,532,742^{ m ef}\pm198,865$	$729.60^{ m efgh} \pm 34.99$	$10,258.89 \text{ ef } \pm 523.53$
RFDC	$92.41 \text{ a} \pm 0.45$	908.45 bc ± 29.36	$3,728,362$ c \pm $198,865$	$1447.94 \text{ a} \pm 34.99$	$12,911.06$ ^{cd} \pm 523.53
RHA40C	92.20 $^{\rm a}\pm0.45$	$205.51^{\text{ h}} \pm 29.36$	$2,161,129$ fgh \pm 198,865	$644.75^{\text{ h}} \pm 34.99$	$6641.70^{\mathrm{i}} \pm 523.53$
RHA50C	$92.56 \text{ a} \pm 0.45$	$361.26~\mathrm{g} \pm 29.36$	$2,159,616$ fgh \pm 198,865	$810.27^{ m def} \pm 34.99$	$6580.29^{i} \pm 523.53$
RHA60C	$92.42~^{a}\pm0.45$	$526.28^{ ext{ ef}} \pm 29.36$	$2,748,104^{ ext{ de}} \pm 198,865$	$1044.84 \text{ c} \pm 34.99$	$8704.07 \mathrm{gh} \pm 523.53$
RFDCL	$91.93^{ m abc} \pm 0.47$	$1369.73 \text{ a} \pm 29.36$	$5,823,222 \text{ a} \pm 198,865$	$1470.97^{\mathrm{\ ab}}\pm34.99$	$18,366.23 \text{ a} \pm 523.53$
RHA40CL	$91.85^{ m abc} \pm 0.45$	$521.66^{\text{ ef}} \pm 29.36$	$3,622,481^{\text{ c}}\pm198,865$	$948.23^{\ c} \pm 34.99$	$11,442.73^{\mathrm{de}} \pm 523.53$
RHA50CL	$92.19^{a} \pm 0.45$	$841.21^{\ c}\pm 29.36$	$3,492,729^{\text{ c}} \pm 198,865$	$951.27^{\text{ c}} \pm 34.99$	$12,312.45$ ^{cd} \pm 523.53
RHA60CL	92.19 $^{\rm a}\pm 0.45$	$842.40\ ^{c}\pm 29.36$	3,490,626 $^{\rm c} \pm 198,865$	970.48 $^{\rm c} \pm 34.99$	12971.19 $^{\rm c} \pm 523.53$

Reported values are presented as mean values \pm standard deviation (n = 4, for each sample two bags of sample were dried and measurements of each parameter in laboratory were carried out twice.). * Mean values with the same small letter in a column are not significantly different (p < 0.05). (R = red, FD = freeze dried, HA40 = hot air dried at 40 °C, HA50 = hot air dried at 50 °C, HA60 = hot air dried at 60 °C, L = pre-treated in diluted lime juice, C = cured).

3.2. TPC

Phenolic compounds are secondary metabolites in plants which contribute to sensorial (taste, flavor, color etc.) and functional (antioxidant activity, antidiabetic, anticancer activity etc.) characteristics of food products [5,42]. The amount of TPC in fresh fruits can be influenced by several factors such as cultivar, geographical origin, and environmental factors during the cultivation period, or by the harvest date of the plant. Storage duration and conditions of fruits between harvest and processing also affects the TPC of raw material at the time of processing [43].

The TPC of yacon chips was analyzed to attain an overall understanding of the changes in phenolic constitutes of yacon after harvest and under the influence of drying processes. In accordance with the statistical analysis, the TPC of yacon chips was not significantly influenced by the four-way-interactions while three of the three-way interactions had a significant effect on it (Table 2).

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The TPC of yacon chips produced from the white cultivar is presented in Table 3. The TPC of chips produced with FD were significantly higher in contrast to those which were processed by means of CHAD (Table 3). Comparing to the chips produced from the white cultivar at the same time after harvest using the same temperature of drying, with chips that were treated by dipping in diluted lime juice, the lime juice treated chips indicated higher TPC compared to those that had no pre-treatment before drying. The TPC of chips produced from the white cultivar one week after harvest ranged between 336.83 \pm 29.36 (mg GAE 100 g $^{-1}$ DW) and 1655.34 \pm 29.36 (mg GAE 100 g $^{-1}$ DW) for WHA40 and WFDL, respectively (Table 3). TPC of chips that were produced by CHAD one week after harvest, pre-treated with diluted lime juice and processed at 50 and 60 $^{\circ}$ C was at 1033.50 \pm 29.36 (mg GAE 100 g $^{-1}$ DW) and 963.56 \pm 29.36 (mg GAE 100 g $^{-1}$ DW), respectively. They were statistically non-significantly different from each other (Table 3). The TPC of yacon chips produced three weeks after harvest varied from 714.34 \pm 29.36 (mg GAE 100 g $^{-1}$ DW) to 1477.23 \pm 29.36 (mg GAE 100 g $^{-1}$ DW) for WHA40C and WFDCL, respectively. Among chips produced using the white cultivar three weeks after harvest and dried using CHAD, the two highest TPCs were noted for WHA50CL and WHA60CL at 1222.92 \pm 29.36 (mg GAE 100 g⁻¹ DW) and 1131.69 \pm 29.36 (mg GAE 100 g⁻¹ DW), respectively (Table 3).

Table 4 reports the TPC of yacon chips produced from the red yacon cultivar. Likewise, the results from white cultivar, a generally higher TPC was indicated in yacon chips from the red cultivar which were produced by FD compared to those that were produced using CHAD (Tables 3 and 4). Moreover, among the chips that were processed under the same conditions with regard to the drying temperature and at the same time after harvest, those which had undergone the pre-treatment with diluted lime juice before drying had higher TPC in comparison to chips that were produced without any pre-treatment (Table 4). The yacon chips from the red cultivar, which were dried one week after harvest, contained TPC in the range of 236.47 \pm 29.36 (mg GAE 100 g⁻¹ DW) to 978.97 \pm 29.36 (mg GAE 100 g⁻¹ DW) (Table 4). The results showed that the highest TPC in the chips came from the red cultivar that was produced one week after harvest by means of CHAD and was noted at 560.25 ± 29.36 (mg GAE $100~{\rm g}^{-1}$ DW) and 494.71 ± 29.36 (mg GAE $100~{\rm g}^{-1}$ DW) for RHA50L and RHA60L, respectively. There was no significant difference between them (Table 4). The yacon chips produced from the red cultivar three weeks after harvest had a TPC ranging between 205.51 ± 29.36 (mg GAE 100 g^{-1} DW) and 1369.73 ± 29.36 (mg GAE $100 \, \mathrm{g}^{-1}$ DW) for RHA40C and RFDCL, respectively (Table 4). There was no significant difference between the TPC of RHA50CL and RHA60CL at 841.21 \pm 29.36 (mg GAE $100 \text{ g}^{-1} \text{ DW}$) and 842.40 ± 29.36 (mg GAE $100 \text{ g}^{-1} \text{ DW}$), respectively (Table 4).

The results of the present study noted higher TPC in chips produced by white cultivar compared to the TPC of chips developed from the red cultivar (Tables 3 and 4). These results indicate the effect of cultivar on the TPC of yacon chips and are in agreement with the results of previous studies which reported the variation in TPC of different yacon cultivars as well as other fruits and vegetables such as apple, beetroot, figs, and potatoes [25,44-47]. Moreover, the TPC content of sterilized yacon tuber flour produced from tubers grown in Brazil extracted in boiling water with ratio of 8.9% (w/v) of yacon, were reported equal to 275 ± 3 (mg GAE 100 g^{-1} DW) which is lower than the findings of this study (Tables 3 and 4) [24]. However, the TPC of yacon chips produced from tubers grown in Bolivia, pre-treated with solutions of lemon juice and water before drying in a cabinet dryer at 40 °C, 50 °C and 60 °C were reported at around 710, 1010, and 680 (mg GAE 100 g⁻¹ DW), respectively, which is in the agreement range of TPC of the vacon chips in the present work [18]. Such variation in amount of TPC may be influenced by other factors such as differences in post-harvest handling of yacon tubers, processing of yacon tubers, extraction methods and solvents used for extraction of phenolic compounds and analytical methods. Moreover, it was noticed that the amount of TPC in white yacon chips decreased with increasing storage time after harvest before processing with FD, while TPC content in red yacon chips increased with increasing storage time after harvest before processing by means of FD (Tables 3 and 4). The observed different trend regarding the change in amount of TPC between the two investigated cultivars could point to a variation in the nature and

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structure of their phytochemical content and enzymes that are active and catalysing the biosynthesis or biodegradation of such compounds in tubers during storage. Variation in the expression of polyphenol oxidase activity with regard to optimal conditions (e.g., pH, and temperature) and substrate specificity has been noted between species and even within the same species [2,35]. For instance, inconsistency in substrate specificity between different cultivars of the same species have been noted in previous studies [2,48]. In addition, biosynthesis of phenolic compounds such as anthocyanins during storage of potato tubers has been reported in the study of Lewis et al. (1999) [49]. They proposed that a rise in sugar as substrates for biosynthesis of anthocyanins contributed to biosynthesis of anthocyanins in potato tubers during storage time [49]. It has been noted that during storage the FOS of yacon tubers may convert to sugars [31]. Therefore, the increase in TPC of yacon chips produced by the red cultivar might be due to conversion of FOS to sugars, which in turn may lead to biosynthesis of phenolic compounds. In addition, higher retention of TPC in samples processed by FD in comparison to samples dried by means of CHAD is in agreement with previous investigations with regard to drying of mango peels and kernels, mango cubes and sour cherries [50-52]. Formation of ice crystals in cellular structure plants during freezing will cause rupture of cellular structure and hence release of compounds during extraction [53]. Besides, operational conditions during FD including low temperature, absence of oxygen, and absence of liquid water plays a role in preventing the destruction of phenolic compounds thermally or enzymatically [54]. Moreover, among the chips produced in CHAD without pre-treatment, the higher TPC was noted for the chips, which were dried at 60 °C. This is in line with the results of previous studies which showed higher TPC in fruit chips produced at higher hot air temperatures due to the formation of phenolic compounds according to the presence of precursors of phenolic compounds, which may undergo non-enzymatic interconversion reactions [55,56]. Moreover, chips that were produced by applying pre-treatment in diluted lime juice, had higher TPC compared to those that were produced under the same conditions without pre-treatment. Various physical (e.g., heating) and chemical (e.g., vitamin C) methods have been investigated to avoid the browning of plant tissue during storage and food processing by influencing the enzymes or substrates or the products involved in browning reactions [34,57]. In this regard, when using acidic pre-treatments like diluted lime juice, low pH and the presence of vitamin C as a chemical inhibitor may have played a role in protection of the phenolic compounds from oxidation and the final products from enzymatic browning.

3.3. Antioxidant Activity

3.3.1. ABTS Radical Scavenging Activity

ABTS radical scavenging activity is a fast and simple method to determine the total antioxidant capacity in food materials. ABTS radical scavenging activity is determined by measuring the reduction in blue-green color of the radical cation ABTS at 734 nm through the donation of hydrogen or electrons by the antioxidant compounds [58]. In the present study, the findings of ABTS radical scavenging activity of yacon chips were reported as μ mol equivalent of trolox per 100 g dry weight of yacon chips.

The statistical analysis of the results indicated that the ABTS radical scavenging activity of yacon chips was significantly affected by four-way interactions between cultivar, duration of storage after harvest (curing or no curing), pre-treatment and drying temperature (p < 0.0001) (Table 2).

The ABTS radical scavenging activity of yacon chip products from the white cultivar under various conditions is noted in Table 3. The ABTS radical scavenging activity of chips produced by means of FD was higher than those which had undergone the same processing conditions with regard to duration of storage and pre-treatment but were dried by means of CHAD. In addition, the samples pre-treated by diluted lime juice had a higher ABTS radical scavenging activity than chips which were dried untreated. The ABTS radical scavenging activity of chips produced from the white cultivar one week after harvest varied between 1,869,012 \pm 198,865 μ mol TE 100 g $^{-1}$ DW and 8,564,803 \pm 198,865 $(\mu$ mol TE 100 g $^{-1}$ DW) for WHA50 and WFDL, respectively (Table 3). Among the chips that were produced

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by means of CHAD one week after harvest, the highest ABTS radical scavenging activity was noted at 3,567,287 \pm 198,865 (µmol TE 100 g $^{-1}$ DW) for WHA40L. Chips produced from the white cultivar three weeks after harvest indicated an ABTS between 2,989,000 \pm 198,865 and 5,480,063 \pm 198,865 (µmol TE 100 g $^{-1}$ DW) for WHA40C and WFDCL, respectively (Table 3). The highest amount of ABTS radical scavenging activity among chips from the white cultivar, which were processed using CHAD three weeks after harvest, was noted for WHA50CL and WHA60CL at 4,236,791 \pm 198,865 and 4,197,870 \pm 198,865 (µmol TE 100 g $^{-1}$ DW), respectively (Table 3).

Table 4 presents the ABTS radical scavenging activity of yacon chips that were produced under different conditions from the red cultivar. Likewise, the results of ABTS radical scavenging activity of yacon chips from the white cultivar the ABTS radical scavenging activity of chips produced from the red cultivar was higher when they were processed by means of FD. Moreover, yacon chips from the red cultivar that were pre-treated by diluted lime juice had higher ABTS radical scavenging activity when compared to those which were produced at the same time after harvest at the same drying temperature. As it can be noted in Table 4, ABTS radical scavenging activity of chips of red cultivar which were dried one week after harvest ranged from 1,816,080 \pm 198,865 to 4,650,806 \pm 198,865 (μ mol TE 100 g⁻¹ DW) for RHA50 and RFDL, respectively. With respect to the chips produced from the red cultivar one week after harvest using CHAD at 40 °C, 50 °C and 60 °C higher ABTS radical activity belonged to chips that were pre-treated with diluted lime juice. However, results were not significantly different from each other (2,568,722 \pm 198,865, 2,418,077 \pm 198,865 and 2,532,742 \pm 198865 (μ mol TE 100 g^{-1} DW) for RHA40L, RHA50L and RHA60L, respectively (Table 4). The ABTS radical scavenging activity of chips of the red cultivar that were produced three weeks after harvest ranged between 2,159,616 \pm 198,865 (μ mol TE 100 g⁻¹ DW) and 5,823,222 \pm 198,865 (μ mol TE 100 g⁻¹ DW) for RHA50C and RFDCL, respectively (Table 4). The chips produced from the red cultivar using CHAD at 40 °C, 50 °C and 60 °C three weeks after harvest those which had been pre-treated with diluted lime juice had higher ABTS radical scavenging activity 3,622,481 \pm 198,865, 3,492,729 \pm 198,865 and 3,490,626 \pm 198,865 (μ mol TE $100\ g^{-1}$ DW) for RHA40CL, RHA50CL and RHA60CL, respectively, which were statistically different from each other.

The results of ABTS radical scavenging activity of yacon chips that were produced from tubers of the white cultivar were higher than those of chips that were produced from tubers of red cultivars which is in line with the results of the TPC (Tables 3 and 4). This may suggest that the TPC of yacon chips may contribute to their ABTS radical scavenging activity. In addition, individual phenolic compounds could be influential on the ABTS radical scavenging activity of yacon chips. Therefore, further studies with regard to the identification of individual phenolic compounds of yacon chips are required. Moreover, the results of previous studies showed a significant effect of cultivar on the ABTS radical scavenging activity of yacon tubers that is in line with differences between ABTS radical scavenging activity of yacon chips from the two cultivars in this study [11,24,25]. It has been noted that the ABTS radical scavenging activity of the yacon tubers of 35 yacon accessions grown in Peru varied between a reported range between 23 and 136 (μ M TE g⁻¹ DW), which was lower than our findings [25]. Sousa et al. reported the ABTS radical scavenging activity of sterilized yacon flour produced from tubers grown in Brazil at 222 ± 2 (mg ascorbic acid equivalent 100 g^{-1} DW) [24]. In agreement with the outcomes of TPC, the ABTS radical scavenging activity of yacon chips which were produced by means of FD was higher than those which were produced by means of CHAD (Tables 3 and 4). Higher ABTS radical scavenging activity in dried jujubes, saskatoon berries, sour cherries, and strawberries, was also noted by other investigations when FD was applied in comparison to hot air drying [52,59-61]. The ABTS radical scavenging activity of chips that were processed by means of CHAD were highest when a higher drying temperature (60 °C) was used in combination with pre-treatment with diluted lime juice for both processing times after harvest. Higher ABTS radical scavenging activity was also reported in dried apple pomace, citrus fruit peels, and pineapple by applying higher temperatures in CHAD [55,62,63]. At higher drying temperatures, production of antioxidant compounds as a result of enzymatic and non-enzymatic browning may contribute to higher antioxidant activity. Other studies

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also reported the effect of drying temperature on ABTS radical scavenging of sour cherry and jujube fruits that showed higher ABTS radical scavenging activity when lower hot air drying temperatures were used [52,64]. Such differences in various investigations may be due to differences in native bioactive compounds in different fruits and their behavior and sensibility to drying conditions such as duration and temperature. Moreover, pre-treatment with diluted lime juice may protect the antioxidant compounds responsible for ABTS radical scavenging activity which are present at the cut-surface of yacon slices against degradation by oxidizing. The residual lime juice on the surface of yacon slices could be another factor that contributes to the ABTS radical scavenging activity of yacon chips.

3.3.2. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity method is widely used to determine the ability of antioxidants in a sample to quench free radicals of DPPH by donating hydrogen. DPPH radicals have a purple color, which undergo a color change upon neutralization by receiving hydrogen. Therefore, the intensity of discoloration, which is evaluated at 517 nm, is a measure for antioxidant ability. The results of DPPH radical scavenging activity can be expressed as equivalent of a reference substance (e.g., trolox and ascorbic acid etc.) [58]. In this study, ascorbic acid was used as the reference substance and, therefore, the results were expressed as mg equivalents of ascorbic acid per 100 g dry weight of yacon chips.

According to statistical analysis of data, the DPPH radical scavenging activity of yacon chips in the present study was significantly influenced by a four-way interaction between the studied parameters (p < 0.0001) (Table 2). Table 3 presents the DPPH radical scavenging activity of yacon chips produced from the white cultivar under various conditions. The DPPH radical scavenging activity of chips from the white cultivar that were dried one week after harvest was between 874.72 \pm 34.99 (mg AA 100 g $^{-1}$ DW) and 1473.36 \pm 34.99 (mg AA 100 g $^{-1}$ DW) for WHA40L and WFD, respectively (Table 3). The DPPH radical scavenging activity of WHA60 was the highest (1291.05 \pm 34.99 mg AA 100 g $^{-1}$ DW) among those of chips from white cultivar which were dried by CHAD. The yacon chips produced from the white cultivar three weeks after harvest had DPPH radical scavenging activity between 950.48 \pm 34.99 and 1460.17 \pm 34.99 (mg AA 100 g $^{-1}$ DW) for WHA40C and WFDC, respectively (Table 3).

The DPPH radical scavenging activity of yacon chips produced from the red cultivar is noted in Table 4. According to Table 4, the DPPH radical scavenging activity of yacon chips of red cultivars produced one week after harvest varied between 681.28 \pm 34.99 (mg AA 100 g $^{-1}$ DW) and 1402.22 \pm 34.99 (mg AA 100 g $^{-1}$ DW) for RHA40L and RFD, respectively. The highest amount of DPPH radical scavenging activity among the chips produced from the red cultivar one week after harvest using CHAD belonged to RHA40 and RHA50 at 820.73 \pm 34.99 (mg AA 100 g $^{-1}$ DW) and 828.63 \pm 34.99 (mg AA 100 g $^{-1}$ DW), respectively, while their DPPH radical scavenging activity was statistically not significantly different from each other (Table 4). As noted in Table 4, the DPPH radical scavenging activity of yacon chips produced from the red cultivar three weeks after harvest ranged between 644.75 \pm 34.99 (mg AA 100 g $^{-1}$ DW) and 1447.94 \pm 34.99 (mg AA 100 g $^{-1}$ DW) for RHA40C and RFDC, respectively. The highest amount of DPPH radical scavenging activity among the chips produced from the red cultivar three weeks after harvest in CHAD belonged to RHA60C at 1044.84 \pm 34.99 (mg AA 100 g $^{-1}$ DW) while it was not significantly different from those of RHA40CL, RHA50CL and RHA60CL (948.23 \pm 34.99, 951.27 \pm 34.99 and 970.48 \pm 34.99 (mg AA 100 g $^{-1}$ DW), respectively) (Table 4).

The higher DPPH radical scavenging activity of yacon chips produced from the white cultivar in comparison to chips produced from the red cultivar was consistent with results of TPC and ABTS radical scavenging activity (Tables 3 and 4). The outcomes of previous investigations are in agreement with the results of the present study, confirming the variation in DPPH radical scavenging activity of yacon tubers from various cultivars [11]. In addition, the DPPH radical scavenging activity of yacon tubers has been investigated by Yan et al. (1999) who reported chlorogenic acid and L-tryptophan

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as two antioxidants in yacon tubers [65]. Furthermore, higher DPPH radical scavenging activity of yacon chips when FD was used compared to CHAD was determined. Higher DPPH radical scavenging activity was reported in mango cubes, strawberries, and saskatoon berries when they were dried by means of FD in comparison to CHAD which is in agreement with the findings of the present study [51,60,61]. Moreover, higher DPPH radical scavenging activity in freeze-dried yacon chips is well aligned with the results of their TPC which may suggest that the phenolic compounds are responsible for DPPH radical scavenging activity of yacon chips. Additionally, yacon contains relatively low amounts of vitamin C and it has been noted that in the case of fruits which have low amounts of vitamin C, their antioxidant activity may generally be determined by their phenolic compounds [66-68]. Generally, the DPPH radical scavenging activity of yacon chips processed in CHAD was higher when higher temperatures were applied. This can be due to the formation of antioxidant compounds as a result of reactions like the Maillard [68]. The findings of investigations into the effect of hot air temperature during convective drying on DPPH radical scavenging activity of blueberries, citrus fruit peel, and golden berry also showed greater DPPH radical scavenging activity in fruits dried at higher hot air temperatures [55,69,70]. However, the red yacon chips processed in CHAD at 40 °C and 50 °C one week after harvest without pre-treatment had statistically comparable DPPH radical scavenging activity to each other. Overall, the DPPH of these samples was higher than those of the chips that were processed using CHAD at 60 °C without pre-treatment and with pre-treatment at 40 °C, 50 °C and 60 °C one week after harvest (Table 4). These results are contrary to the results of the TPC as the red yacon chips which were processed in CHAD at 40 °C and 50 °C one week after harvest without pre-treatment had the lowest TPC. This might be explained by taking into account the possibility that at lower temperatures of drying the native polyphenols oxidase of red yacon might still be active and in the presence of oxygen leading to the formation of antioxidant compounds. It has been noted that the polyphenols have higher antioxidant activity in an intermediate stage of oxidation [71].

3.3.3. FRAP

The FRAP assay is another method based on electron transfer for measuring antioxidant characteristics of food materials. In this method, the antioxidants power is determined in an acidic condition by reducing the ferric 2,4,6-tripyridyl-s-triazine complex to the ferrous complex. The later exhibits an intense blue color and its absorbance can be measured at 593 nm. The results of the FRAP assay are reported as equivalents of concentration of ferrous ions (mM) [58].

The statistical analysis of FRAP results of yacon chips showed that the four-way interactions were not significant while three of the three-way interactions were significant (Table 2).

Table 3 notes the FRAP of yacon chips from the white cultivar. The FRAP of yacon chips of the white cultivar which were produced one week after harvest varied between 8924.44 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) and 17,941.17 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) for WHA40 and WFDL, respectively. The highest FRAP among the yacon chips produced from the white cultivar one week after harvest by means of CHAD belonged to WHA50L and WHA60L at 14,344.79 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) and 14,276.69 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW), respectively (Table 3). Yacon chips from the white cultivar that were produced three weeks after harvest had a FRAP that ranged between 12,067.90 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) and 18,171.17 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) for WHA40C and WFDCL, respectively (Table 3). The highest FRAP value belonged to WHA50CL and WHA60CL among the yacon chips from the white cultivar that were dried by CHAD three weeks after harvest at 17,458.59 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW) and 17,072.86 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW), respectively. Their FRAP values were not significantly different from that of WFDCL (18,171.17 \pm 523.53 (mM Fe $^{+2}$ 100 g $^{-1}$ DW)) (Table 3).

The results of FRAP measurements of yacon chips from the red cultivar are presented in Table 4. The yacon chips of the red cultivar which were dried one week after harvest varied between 7180.02 ± 523.53 (mM Fe⁺² 100 g⁻¹ DW) and $16,130.51 \pm 523.53$ (mM Fe⁺² 100 g⁻¹ DW) for RHA40 and RFDL, respectively (Table 4). Among the yacon chips from the red cultivar which were dried one

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week after harvest in CHAD, the highest FRAP was indicated for RHA60L at 10,258.89 \pm 523.53 (mM Fe⁺² 100 g $^{-1}$ DW) (Table 4). The FRAP of yacon chips from red cultivars which were produced three weeks after harvest ranged between 6641.70 \pm 523.53 (mM Fe⁺² 100 g $^{-1}$ DW) and 18,366.23 \pm 523.53 (mM Fe⁺² 100 g $^{-1}$ DW) for RHA40C and RFDCL, respectively (Table 4). The FRAP of yacon chips from the red cultivar dried by CHAD three weeks after harvest was highest at 12,312.45 \pm 523.53 (mM Fe⁺² 100 g $^{-1}$ DW) and 12,971.19 \pm 523.53 (mM Fe⁺² 100 g $^{-1}$ DW) and was not significantly different from each other for RHA50CL and RHA60CL, respectively (Table 4).

Parallel to the results of TPC, ABTS and DPPH radical scavenging activity, the findings showed that the FRAP of white yacon chips was higher than those of red yacon chips (Tables 3 and 4). These results are in agreement with a study of Khajehei et al. who determined various FRAP for yacon tubers from different cultivars [11]. Higher FRAP was also reported for dried mango powder, strawberries, and sour cherries when they were dried by mean of FD in comparison to CHAD which is in line with the outcomes of the present work [51,52,61]. A drying process using CHAD can result in a decrease in antioxidant compounds of raw material as the process exposes the raw material to thermal treatment for a relatively long time in the presence of oxygen. Degradation of some of the antioxidants may occur under such conditions as they might be unstable or they could undergo enzymatic oxidation. Moreover, there was no significant difference between the FRAP value of yacon chips, which were processed by means of CHAD at the same time and with the same pre-treatment using various hot air temperatures when white yacon was used (Table 3). However, FRAP values were significantly higher in the case of red yacon chips which were produced by means of CHAD under the same pre-treatment conditions and at same time after harvest when higher hot air temperatures were used (Table 4). These results may suggest the difference between antioxidant compounds in two investigated cultivars which are responsible for FRAP and their behavior in response to different temperatures in the drying process.

4. Conclusions

In conclusion, the results of this study determined that the bioactivity of yacon chips is better preserved when pre-treatments with diluted lime juice were used and dried by means of FD. Moreover, when comparing the samples dried by means of CHAD, the findings of this study suggested that drying in higher hot air temperature and application of pre-treatment using diluted lime juice results in yacon chips with higher TPC and antioxidant activity. The outcomes of the present work suggest that for the production of yacon chips with higher phytochemical content from white cultivar, it is better to carry the drying processing out one week after harvest by FD, while for the red cultivar it is better to process the tubers three weeks after harvest using FD. Moreover, the results showed that in general the yacon chips from the white cultivar had higher TPC and antioxidant activity than the yacon chips produced from the red cultivar. These results reveal a considerable influence of genotype of yacon on phytochemical quality of yacon tubers and changes in their content of bioactive compounds during storage. Further studies into the association between the determination of bioactive compounds of yacon tubers, such as individual phenolic compounds, and their biological effects, such as antioxidant activity, are needed.

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7. General discussion

The main objectives of the present thesis were to explore the options for the development of a local supply chain for yacon and its derived food products in the EU. The specific focus was on the phyto/chemical characteristics of leaves and tubers of different cultivars which have been grown under the environmental conditions of southwest Germany. In addition, the thesis aimed at, identifying the effects of post-harvest and food processing on the phyto/chemical characteristics of yacon. More precisely, the emphasis was given on the phenolic content and antioxidant activity of yacon leaves and tubers as well as on simple sugar content of tubers. Chapter I, focused on the phenolic content and antioxidant activity of leaves of red and white vacon cultivars which were extracted by means of OH-DE and conventional DE. In chapters II and III, the main emphasis was given to the phenolic content and antioxidant activity of the tubers of various yacon cultivars. While chapter II investigated the variation of phyto/chemicals between seven cultivars, chapter III, focused on the impact of food processing and post-harvest handling on quality of yacon tubers in terms of total phenolic content and antioxidant activity. The general discussion will give a deeper insight into the findings of chapters I-III, draw different perspective and evaluate the potential for establishing a green local supply chain of yacon and its products in the EU. In addition, the obtained results in this work will be disputed in the framework of the changes which are in progress in the whole food supply chain under the impact of various challenges such as environmental conditions, food security, general public health concerns and alternations in consumers' attitude and diets.

7. 1. The potential of yacon leaves and tubers as functional and health promoting food

Functional and health promoting food products are those which have moved beyond the basic criteria of food for nutrition including assuring survival, satisfying hunger and eliminating the negative impacts of food consumption. In a wider perspective food consumption now has to serve the enhancement and improvement of the state of well-being and health while decreasing the risk of diseases (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). In fact, functional foods are reported as one of the top trends in the food industry since the 1990s (Verbeke, 2005). Other mega trends in food production such as ''food for beauty and health '', '' food that meets our expectations '', ''more food from less resources '', and '' food for a healthy planet '' are reflecting the demand for health promoting food products, which are produced in greener way (Augustin et al., 2016). The growing market of functional and health promoting food products has a multidimensional foundation. A continuously rising level of the overall public awareness towards personal health decay especially in developed centuries is among the greatest of contributing factors that has empowered the market of functional and health promoting foods (Granato et al., 2010).

However, there are other aspects at play which orchestrated the growing attention towards certain food products such as marketing of underutilized crops under functional and health promoting food products.

Respectively, the consequences of the green revolution - specifically the environmental concerns related to the use of fertilizers, pesticides, and loss of crop diversity - directed the attention of various sectors of the food supply chain to underutilized crops (Shiva, 2016). Moreover, in the face of the given global challenges like climate change underutilized crops may be able to offer new and viable solutions (Howden et al., 2007). Focusing on reviving forgotten or underutilized crops may contribute to eliminating the concerns over developing genetically modified organisms which are adaptable to environmental conditions but do not have the history of being safe to use, neither for the environment nor for the health of consumers.

Yacon as an underutilized Peruvian plant food, can be named as one of those promising plants offering functional and health promoting food products. It is distinguished in accordance with its high content of FOS and relatively low sugar content in its tubers compared to other fruits as well as being a remarkable source of phenolic compounds both in its leaves and tubers. The FOS being the main storage carbohydrate in the yacon tubers and their low sugar content marked them as a low calorie and low sugar plant food source as well as stamping them as health promoting due to the potential beneficial effects of FOS consumption. Besides, it can serve as a good natural source for sugar replacement. Moreover, yacon leaves and tubers are a remarkable source of phenolic compounds and antioxidants. Consequently, vacon is gaining attention as it has the potential to address the demand of food products for those suffering from chronic diseases such as diabetes and obesity, but also to realize the general consumers demand for health promoting food products. In Europe, an optimistic and growing market with significant sales of functional and health promoting foods specifically exists in Germany, France, and the Netherlands (Annunziata & Vecchio, 2011; Gray, Armstrong, & Farley, 2003). Yacon and its products, particularly yacon chips, flour and syrup have been imported into the EU from Peru and Colombia (Exporting yacón to Europe, 2016). As a growing market for yacon and its products is anticipated, the demand for local production of yacon in the EU and consequently the demand for research towards its feasibility is to be reasoned according to the points to be debated in the following chapters.

7. 2. Local food chain for yacon and its products

On the subject of future changes in the food supply chain, certain aspects influence the need for a local production of yacon in the EU. During the past decade, consumers' interest towards locally produced foods has increased (Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009). It has been noted that the positive perception of consumers of local foods is linked to their attitude towards enhancing the local economy and improving environmental welfare (Zepeda & Leviten-Reid, 2004). Moreover, consumers demand a greater transparency in food production and supply chains due to the adverse impacts of globalization, past food scandals and high levels of food processing. Larger distances between the place of production and consumption of food products may contribute to lower transparency of the food supply chain, so a growing number of consumers redirected their food purchases to locally

produced foods (Feldmann & Hamm, 2015). The rising attention and appeal of local foods among consumers suggests the potential of such products in food markets and consequently rising opportunities for the establishment of new local businesses for promoting them. On this account and according to health prompting effects of yacon as a plant food source as well as its potential to be used as sugar replacement, an optimistic future for its local production in the EU may be foreseen. Its products can be promoted under various rising trends in current food markets such as functional foods, health promoting foods, specialized low sugar products, and alternative low-calorie sweeteners. In this regard, studies on the production of ice cream, chocolate cake and fruit yogurt using yacon have evidenced the potential of yacon for the development of functional food products (Moscatto et al., 2006). Investigating the optimal formulation for the production of chocolate cake containing yacon flour showed that replacing 40% of wheat flour with yacon flour resulted in greater amounts of FOS in the cake while not changing the physical attributes of the cake significantly (texture parameters and specific volume) of the cake when compared to the control formulation (Moscatto et al., 2006). In addition, Parussolo et al. 2017 examined the production of symbiotic ice cream using Lactobacillus acidophilus and yacon flour. The results of their study showed that ice cream formulation containing 3% yacon flour with 0.06% or 0.13% lactic culture resulted in a product with significantly higher mineral content and greater counts of viable probiotics in comparison to the formulation that contained no or only 1.5% yacon flour. Overall, they concluded that ice cream, which contains yacon flour had acceptable sensory characteristics (Parussolo et al., 2017). Furthermore, production of low calorie yogurt has been investigated by adding 1.58, 2.56, 3.00 and 3.86 % yacon flour to light yogurt. It was shown that addition of yacon flour to yogurt resulted in low fat and low caloric products and that the addition of more than 2.65% yacon flour may result in prebiotic yogurt in accordance with Brazilian regulation (Mileib Vasconcelos, Rodrigues Minim, & Paes Chaves, 2012). Taking into account the scientific evidence that proves the potential of yacon for the development of functional food products, and the fact that phyto/chemical quality of yacon tubers and leaves as fresh food materials may differ when cultivated under the environmental conditions of southwestern Germany, the necessity to provide such information to establish a base line for proper product development from yacon tubers and leaves is obvious.

7. 3. The Phyto/chemical characteristics of yacon leaves and their extraction

Leaves of yacon plants have been used as a traditional remedy due to their potential health benefits for those suffering from diabetics and renal disorder (Honoré et al., 2015). The result of this study which is presented in chapter I, was in line with the finding of previous studies showing that yacon leaves contain significant amounts of phenolic compounds including ferulic acid, caffeic acid, Myricetin, P-Coumaric acid, Rutin and chlorogenic acid (De Andrade et al., 2014). Various studies have confirmed the positive effects of yacon leaves extract in diabetic rats (Aybar et al., 2001; Honoré et al., 2012). It has been reported that the flavonoid and phenolic compounds of yacon leaves related to chlorogenic acids are common constitutes of plants and have a low level of toxicity while exerting different biological activities such as antidiabetic, antilipidemic, and antioxidant activity (De Oliveira et al., 2011;

Zhao & Moghadasian, 2010). Moreover, this thesis noted that the leaves of a red cultivar contained higher and large amounts of TPC, TFC, and antioxidant activity determined by three methods (ABTS radical scavenging activity, DPPH radical scavenging activity, and FRAP) when compared to the leaves of a white yacon cultivar. In addition, the average amount of TPC, and TFC extracted by means of OHDE were respectively, 12.93 % and 30.05 % higher for younger leaves of the red cultivar when compared to its old leaves. However, it is worth keeping in mind that at the time of harvest the yield of young leaves may be significantly lower than the yield of old leaves. Therefore, it might be recommended that the separation of young and old leaves of yacon is not necessary at the harvest time for preparation of yacon leaves extract.

Furthermore, the transforming food supply chain and industry must respond to global environmental challenges including climate change and depletion of fossil fuels. In this sense, energy consumption can act as one measure of the environmental footprint of food processing technologies (Dutilh & Kramer, 2000). Consequently, reducing energy consumption of food processing while maintaining the quality of the product has been among the urgent challenges to be addressed. On that account, various novel methods have been developed to not only lower the energy consumption of food processing but also to enhance the quality of final products. Ohmic heating is among such novel volumetric heating methods, which according to previous studies offers several merits over the conventional heating methods including lowering the processing time, reducing the energy consumption, optimal investment due to higher efficiency and low capital cost, and reduction in the cost of maintenance according to lack of moving parts (Varghese, Pandey, Radhakrishna, & Bawa, 2014). The results of this work confirmed that OH-DE significantly lowers the time and energy consumption of extraction while the quality of extracted phyto/chemicals from yacon leaves are not negatively affected neither in terms of TPC, TFC, and individual phenolics and flavonoids nor in terms of antioxidant activity. Here, it should be mentioned, that ohmic heating is among the emerging technologies and more detailed investigations with regard to its effects on the safety of final food products have to be carried out. For example, the thermal and electrically induced alternations in phyto/chemicals under the influence of ohmic heating may be examined for instance for allergenicity of the final food product (Jaeger et al., 2016). Still, it should be mentioned that investigating other novel methods such as microwaves, pulse electric field, and ultrasound for extraction of phyto/chemicals from yacon leaves may be recommended as they might offer further improvements and other processing options.

Nevertheless, it is noted here that yacon leaves contain sesquiterpene lactones that are considered as allergic compounds (Schorr & Da Costa, 2005; Schmidt, 1999). De Oliveira et al. (2011), examined the toxicity of yacon leaf extract consumption for a prolonged period of 90 days in Wistar rats. They observed renal damage was associated with the presence of sesquiterpene lactones in the extracts. Therefore, the oral application of yacon leaves for treatment of diabetes was not recommended (De Oliveira et al., 2011). On that account, further and more in detail studies towards the potential toxicity of yacon leaves and their extracts are strongly required and suggested.

7. 4. Phyto/chemical characteristics of yacon tubers

In line with the findings of this work presented in chapters I and II, the phyto/chemical quality of yacon tubers and leaves were affected by cultivar. The findings of the present work that are reported in chapter II were in agreement with the results of previous studies that have investigated vacon tubers of various landraces and cultivars revealing huge differences in the amount of their TPC (Campos et al., 2012; Russo et al., 2015). Phenolic compounds may lower the risk of certain diseases such as chronic cardiovascular diseases, chronic artery diseases, cancers, inflammation and digestive diseases (Chandrasekara & Josheph Kumar, 2016; Prior & Cao. 2000; World Health Organization, 2003). Consequently, the focus of an ample number of studies during the past two decades was on the determination of chemical constitutes of plant foods, which play a role in their biological activity, and their mechanism of action against degenerative and chronic diseases (Kaur & Kapoor, 2001; Liu, 2013). In this regard, scientific evidence provided by previous studies have identified chlorogenic acid, caffeic acid and its derivatives, coumaric acid, and ferulic acid as individual phenolic compounds in yacon tubers. Specifically, chologenic acid, caffeic acid and its derivative have been identified as the dominant phenolic compounds in vacon roots (Takenaka et al., 2003; Yan et al., 1999). The scientific evidence supported by results of various studies suggest that these phenolic compounds are the most abundant in plant foods and exert biological activities such as anticarcinogenic, antidiabetic, antilipidemic, and antibacterial activities (Heleno, Martins, Queiroz, & Ferreira, 2015; Meng, Cao, Feng, Peng, & Hu, 2013; Oboh, Agunloye, Adefegha, Akinyemi, & Ademiluyi, 2015; Ong, Hsu, & Tan, 2013). Therefore, it might be suggested that the health beneficial effect of consumption of yacon and its products is due to its richness in such phenolic compounds.

Moreover, this study has provided information regarding the antioxidant activity of yacon tubers of mentioned cultivars in chapters II and III. The findings of in vitro measurement of antioxidant activity of yacon tubers (whole tuber, flesh of tubers) using three methods, namely, ABTS radical scavenging assay, DPPH radicals scavenging activity, and FRAP showed that yacon tubers can be considered significant sources of antioxidants. Comparing the results of antioxidant activity of vacon tubers based on these methods, it can be confirmed that cultivars with higher TPC contain higher antioxidant activities as well. However, differences in the antioxidant activity of flesh, peel and whole tubers evaluated by different methods were noticed and reported in chapter II. It has been reported that the antioxidant activity of phenolic and flavonoid compounds and their mechanism of action against free radicals depends on their structure (Rice-Evans, Miller, & Paganga, 1996). The FRAP of flesh, peel and whole tubers of various cultivars were in line with the results of TPC and TFC, which means it was higher in peels, followed by whole tubers and lower in their flesh for the studied cultivars, namely, Cajamarca, Cusco, Early White, Morado, New Zealand, and Quinault, except Late Red. In the case of the later, the FRAP of peel, flesh and whole tuber was in a descending order (chapter II). As all other studied cultivars had a white coloured flesh while cv. Late Red had an orange/yellow flesh, the colourful pigments in the flesh of cv. Late Red, may have contributed to the antioxidants with more profound FRAP. Yet, the TPC and TFC in peel of tubers were remarkably higher than the flesh and whole tubers for all the studied cultivars, while the ABTS radical scavenging activity of peels were significantly lower than that of flesh and whole tubers. With respect to the DPPH radicals scavenging activity, this trait was not significantly different in flesh, peel and whole tubers of some of the studied cultivars, namely, Cajamarca, Early White, Late Red, and Quinault, while it was lower for the flesh and whole tuber of cv. Morado compared to its peels, higher in peels of cv. Cusco in comparison to its flesh and whole tubers, and higher in peels and whole tubers of cv. New Zealand than its flesh. This may suggest the presence of differences in structure of individual phenolic and flavonoid compounds stored in peel and flesh of tubers. In addition, L-tryptophan has also been identified in vacon tubers which has a potent antioxidant activity (Yan et al., 1999). Hence, the possibility of variation in amount of L-tryptophan in peels and flesh of single cultivars as well as differences in the amount of L-tryptophan in different cultivars, may lead to differences in their antioxidant activity. On top of all that, the measurement of antioxidant activity by means of ABTS radical scavenging activity enables the evaluation of antioxidant compounds of food samples with both lipophilic or hydrophilic while the DPPH radicals scavenging activity allows the measurement of compounds which are only soluble in organic media (Kim, Lee, Lee, & Lee, 2002). Therefore, the difference in antioxidant activity of flesh, peel and whole tubers measured with the aforementioned two methods and the TPC and TFC may also be the results of solvents used and the method of measurement.

In addition, the concerns over the high sugar (sucrose and fructose) content in the modern diet are associated with its contribution to the rising worldwide level of obesity, type II diabetes and cardiovascular diseases (Bray, 2010; Raben & Richelsen, 2012). That being the case, to keep away from the high calories intake through high sugar consumption and its adverse impact on health, replacement of sucrose with non-calorie or low calorie alternative sweeteners is reasonable and consequently artificial sweeteners have gained attention (Raben & Richelsen, 2012). However, the application of artificial sweeteners in food product development has been contested as there is doubt towards their safety and health hazards in their long-term consumption (Bearth, Cousin, & Siegrist, 2014). Also, the application of most artificial sweeteners (e.g. aspartame, sucralose, and saccharin) in food products is challenging because they are more intensely sweeter than sucrose. Hence product development has to focus on the examination of intensity of sweetness and physical characteristics of the resulting product (Chattopadhyay et al., 2014). Moreover, consumers are demanding natural food products and have a negative perception towards artificial food additives including sweeteners (Dijksterhuis, 2016; Sylvetsky, Greenberg, Zhao, & Rother, 2014). Hence, yacon as a natural plant source of sweetener may be more appealing to consumers as an alternative to artificial sweeteners. The results of this study that are reported in chapter II, confirmed the fact that the simple sugar (fructose, glucose, and sucrose) content in flesh of different studied cultivars differed. This outlines the importance of cultivar selection and of raw material especially when it comes down to relevant nutritional requirements of targeted consumer groups. For example, according to the results presented in chapter II, product development with cv. New Zealand may be suggested for suitable low calorie/reduced sugar content food products for those suffering from type II diabetes as it had the lowest simple sugar content among other studied cultivars. Although, this study has provided the physicochemical characteristics of various yacon cultivars, other important aspects of food product development should not be neglected to ensure the success of newly launched food products in the market. The study of Dijksterhuis (2016) noted some of the most important factors, which should be taken into account. New food products should be developed on the consumer-led basis rather than market-led ones and innovations should address the delivery of the benefits that consumers are seeking in a new product (Dijksterhuis, 2016). Hence, studies that determine the perception and expectations of consumers towards yacon based products after transparently informing them about its potential are strongly recommended to ensure the success of such products.

Hereinafter, it is worth addressing the overall changes actually happening in the food supply chain. According to the derivatives of changes in the food supply chain, it may be pointed out that the aspect of waste of food production and consumption must be added into the equation. During the last two decades, there have been several policies and initiatives in Europe such as integrated product policy and resource efficiency. Innovations for sustainable growth are fostering the reduction of food waste and encouraging the strategies targeting at "zero waste" in the food production and consumption system (Mirabella, Castellani, & Sala, 2014; European Commission, 2003, 2010, 2012). Moreover, in accordance with the rising land use competition between food and feedstock crops, more attention in the scientific sector has been paid to the application of residues as raw material in other sectors. Respectively, the development and establishment of closed systems is the foundation of industrial symbiosis in which waste of food processing is used as a raw material containing valuable compounds for development of other products in food industries or other sectors such as nutraceuticals, pharmacological, cosmetic, textiles, etc. This has attracted remarkable attention as these practices benefit both the economy and the environment (Mirabella et al., 2014). In this regard, the first stage of waste valorisation is to determine the valuable characteristics of residues in terms of quality and quantity (Rosentrater, 2004). Taking the example of apple peels as residual of apple processing, it was shown that apple peel powder can be produced by drying and milling, which has been proven as a valuable ingredient because of its phyto/chemical content (Wolfe & Liu, 2003). In this work the phyto/chemical characteristics of dried vacon peels were evaluated and reported in chapter II. The findings provide an insight into possible future applications of vacon peel powder as an ingredient rich in phenolic compounds with significant antioxidant activity. The product would be competitive with the comparable products such as peel powder of apple, banana, pomegranate (Gullon, Pintado, Pérez-Álvarez, & Viuda-Martos, 2016; Rebello et al., 2014; Wolfe & Liu, 2003).

To persist with the stages of waste valorisation, exploring the opposite technologies including conventional or emerging techniques for processing and refining the valuable components of residues may be explored (Galanakis, 2012). Designing and developing of such processes may be in accordance with their efficiency, energy consumption, cost, quality, functionality and safety of recovered ingredients, and impact on the environment (Galanakis, 2012; Vandermeersch, Alvarenga, Ragaert, &

Dewulf, 2014). In this regard, novel technologies have been in the spotlight for downstream processing of valuable compounds due to plus points they have against the conventional methods (e.g. lower energy consumption and better quality of recovered compounds in terms of their functionality) (Galanakis, 2012). Consequently, extraction and recovery of valuable phytochemicals such as phenolic compounds of yacon peel with novel technologies such as microwave, ohmic, pulse electric field, ultrasound, etc. may be of interest for future studies to design an optimal extraction process in terms of quality of recovered phenolic compounds and energy consumption. Moreover, choice of solvent system in extraction processes are another defining factor in the quantity and quality and profile of individual phenolic compounds, which may be extracted from yacon peels. Over and above all that, toxicological studies have to be carried out to ensure the safety the yacon peel powder as an ingredient in other products particularly for their application in the food, feed, nutraceutical, and pharmacological industry.

7. 5. Phyto/chemical characteristics of yacon chips

Like with all fruits, the availability of fresh yacon tubers is seasonal. Moreover, the phyto/chemical quality of tubers is under influence of storage duration and condition, just as it was reported in the literature review chapter of this thesis. In line with the fact that yacon tubers have become well-known due to their high FOS content in the first place, previous studies have examined the effect of storage duration and condition and processing on their FOS content and not their TPC and antioxidant activity. Therefore, in this work, the effect of drying of yacon slices on their phyto/chemical quality was examined in two time intervals, namely, one week after their harvest, and three weeks after their harvest (cured tubers). The results were reported in chapter III. Tubers were simply stored in a barn at ambient temperatures in a dark box. The drying process was chosen as it is one of the oldest methods used for preservation and extension of their arability during the whole year long. Drying offers several advantages like stabilizing and enhancing the microbial and chemical safety of dried fruits by reducing their water content, reducing the costs of packaging, storage and shipping. According to the findings of this studies that were presented in chapter III, it was shown that it is better to produce yacon chips by FD one week after harvest if the tested white cultivar is used. However, when the red cultivar is used it is better to produce the vacon chips by means of FD three weeks after harvest to improve the phyto/chemical characteristics in the chips. It was discussed that differences in enzymes and phenolic constitute of these two cultivars might be the reason for differences in changes in phyto/chemical characteristics of tubers during storage. These results further confirm the fact that for each cultivar an optimal post-harvest management has to be investigated to ensure the maintenance of the phyto/chemical quality of the final product. In addition, due to the endogenous enzymes of vacon (e.g. peroxidase and polyphenol oxidase) yacon slices darken shortly after cutting (Lago & Noreña, 2014). Thus, in our study, dipping of slices before drying in diluted lime juice was applied to avoid browning of slices after cutting and during drying. The findings showed that dipping in juice may be a practical option to produce yacon chips with better phyto/chemical characteristics for both cultivars. Physical methods such as heating, or chemical methods like sulphur dioxide may be named as possible pretreatment of slices to avoid browning of yacon slices if permitted as food additives. However, it is worth mentioning that the use of diluted lime juice as a pre-treatment may offer merits compared to such pretreatments. For example, heating of vacon slices in the context of blanching before drying may induce adverse effects on yacon phyto/chemicals through leaching or thermal degradation of them. The usage of sulphur dioxide has been debated due the concerns over health hazards, because it may induce allergenic reaction or asthmatic attacks (Güneş, & Bayindirli, 1993; Krokida, Maroulis, & Saravacos, 2001; Lago, & Noreña, 2014; Toniolo, Pizzariello, Susmel, Dossi, & Bontempelli, 2010). Moreover, lime juice contains considerable amounts of antioxidants, which can protect the yacon slices against oxidation. Plus, lime juice contains citric acid, which can significantly suppress the activity of polyphenol oxidase by chelating the copper in active sites of polyphenol oxidase as well as lowering the pH (McEvily, Iyengar, & Otwell, 1992). It has been reported that addition of 1% (w/v) citric acid to yacon juice reduced the activity of its native peroxidase, polyphenol oxidase and inulinase in yacon juice by 94%, 97% and 86% (Lago & Noreña, 2014). Although, in the present study the amount of FOS in yacon chips has not been monitored, based on this information it may be suggested that dipping of slices in the diluted lime juice before drying might protect the breakdown of FOS content of yacon slices during drying to some degree since it can reduce the activity of inulinase as well. Hence, more in detail studies towards changes in enzymatic activity of yacon slices under influence of pre-treatment with diluted lime juice and its mechanism of action associated with its possible protective effects on phenolic content and antioxidant activity of chips is required. Furthermore, use of pre-treatment by dipping in diluted lime juice is in line with the demand of consumers for food products produced with natural ingredients rather than artificial food additives. With regard to the drying method, it comes as no surprise that FD resulted in significantly better yacon chips in terms of their phyto/chemical quality. Low temperatures during FD and vacuum conditions, minimize the degradation of phytochemicals which may happen during CHAD due to both thermal and oxidative degradation. However, FD is an expensive method in terms of operating capital and operating costs while the process is usually operational in batches and is very long. Thus, it is applied mainly to the production of high value products for example in the pharmaceutical industry (Mujumdar & Law, 2010). CHAD of yacon slices was also evaluated in this study. According to the presented results in chapter III, drying of yacon slices in convective hot air dryers operating with 50 or 60 °C resulted in better phyto/chemical qualities compared to CHAD at 40 °C. One of the main reasons for these results might be that when drying took place at 40 °C enzymes were still active and dehydration took place at slower pace. Hence the conditions were more favourable for oxidative degradation of phyto/chemicals. Application of novel technologies such as microwave, ultrasound, pulse electric field, etc. in combination with CHAD as hybrid techniques or as pre-treatments prior to CHAD of yacon slices may be recommended to further study and optimize the drying process of yacon slices. Furthermore, it is worth pointing out that the examination of other possible food processing techniques for preservation of yacon tubers is crucial for establishing a local food supply chain for yacon in the EU.

In this study one of the various aspects, which has been consistently considered and was a main driver of the study, was the alternations in the whole food supply chain. A noticeable change in the food supply chain is the emergence of alternative food networks alongside the well-established industrial food network. The rising of alternative food networks has its roots in the demand of consumers, who are addressing their morality and ethical values in their food consumption or those who are favouring a more direct relationship with food producers in opposition to the dominant industrial food producing systems, perhaps seeking a more transparent, sustainable and traditional way of food production (Maye & Kirwan, 2010; Whatmore & Clark, 2006). Therefore, the alternative food networks may be instantiated with the rising in market share of organic, local and regional, fair trade, and creation of alternative outlets, such as famers markets, in opposition to supermarket chains (Maye & Kirwan, 2010; Goodman & Goodman, 2009). Among various drying technologies, which have been developed (e.g. freeze spray drying, heat pump drying, spouted bed drying and different hybrid drying systems), CHAD was studied in the view of the fact that it can be practically integrated into the possible supply chain of yacon in alternative food networks as well as being well established in the industrial food network. Farmers can produce yacon chips by establishing a simple drying process using convective hot air driers at the farm site and offering the chips in farmers' markets. Hence, the results of drying of yacon slices can be beneficial both to improve and enhance small businesses in the context of alternative food networks and in the industrial food network.

8. Summary

There is a growing demand for plant-based health promoting and functional food products due to a combination of factors including food and nutrition security as well as concerns over land and water utilization. In addition, environmental issues such as climate change, alternation in consumers' values for their food choices, the establishment of different diets, the growing level of awareness in consumers towards the connection between health and diets are contributing to the rising demand for plant-based food products. Moreover, the upward trends of certain chronic diseases in the population, such as obesity, type II diabetes, coronary heart diseases, etc. have drawn further attention towards a healthy nutrition. In particular, one of the most profound characteristics of modern dominant diets in developed countries is the high intake of caloric sweeteners in food and beverages. Studies have associated the high intake of sucrose as the main caloric sweetener with the higher risk of certain chronic diseases such as obesity and type II diabetes. Consequently, several strategies have been attempted with the aim to reduce the overall sucrose consumption of the population (e.g. taxation of sugar containing food and beverage products, campaigning to increase the public awareness for sugar in food products, etc.). In this regard, looking for alternative sugar replacements to provide the taste and sweetness in large groups of foods and beverages, has led to the emergence of alternative non-nutritive and/or artificial sweeteners (e.g. stevia, aspartame, acesulphame-K, saccharin, etc.). But, use of artificial sweeteners has been contested by consumers, because they have concerns over food additives and their possible effects on their health. Thus, the use of natural non-nutritive or low caloric sweeteners has attracted considerable attention not only to reduce the sugar consumption of the general population, but also to enhance the status of food and nutrition security for those suffering from diseases such as diabetes and to improve the variety of food products in the market of foods for them.

Yacon (Smallanthus sonchifolius Poepp. & Endl.) as an underutilized crop, native to the Andean region, has attracted growing attention. The tuberous roots of yacon have been advertised as an alternative low caloric plant source for replacing sucrose. In fact, yacon has gained recognition based on the fact that its sweet tasting tuberous roots and its leaves have a favourable phytochemical content to be included in a range of functional food products. The leaves on the one hand are a significant source of health promoting phenolic compounds (e.g. chlorogenic acid, caffeic acid, ferulic acid, quercetin, rosmarinic acid, gallic acid, rutin, myricetin, etc.) and their extract exerts certain biological activities such as antioxidant activity and hyperglycemic effects. The tubers on the other hand consist of carbohydrates including simple sugars, namely, fructose, glucose, sucrose and fructooligosaccharides (FOS). The FOS - representing the dominant polysaccharide in the tubers - are sweet tasting, prebiotic, and non-digestible oligosaccharides. Therefore, their consumption imposes several health benefits such as lowering the energy intake while enhancing the beneficial microflora of the colon. It is noted that 60-70 % of the dry matter content of yacon tubers is composed of FOS. Besides, yacon tubers are a remarkable source of

biological components such as phenolic compounds (e.g. chlorogenic acid, ferulic acid, coumaric acid, caffeic acid and its derivatives). Thus, yacon is considered as multifunctional plant food.

As a result of its beneficial attributes, yacon and its products are available in the EU food market being imported from Peru. In this respect, establishing a local supply chain for the production of vacon and its products would lead to a sustainable food supply chain of them in the EU. On that account, this thesis took a holistic approach for establishing a local supply chain of yacon and its products in the EU Market. The cultivation of vacon in the EU region may be named as first step for establishing its production in the EU. Moreover, further product development using yacon may be the next step that can contribute to establish its local food supply chain. In this regard, the quality of yacon leaves and tubers in terms of their chemical, physical, nutritional and sensory attributes is influenced by the chosen cultivar and the environmental conditions under which the yacon plants are cultivated. Furthermore, extending the shelf life of yacon leaves and tubers, storage condition and choice of food processing are of great importance for enhancing the quality of yacon products as well as their sustainable production. Therefore, the main objectives of this thesis were to 1) differentiate between the quality of young and old yacon leaves of two cultivars (red and white) in terms of their total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity when using ohmic-assisted decoction (OH-DE) and decoction (DE) extraction processes as well as energy consumption of extraction process, 2) differentiate between various parts of yacon tubers (flesh, peel and whole tuber) of seven cultivars in terms of their simple sugar (fructose, glucose and sucrose) content, TPC, TFC and antioxidant activity, 3) examine the TPC and antioxidant activity of yacon tubers of two cultivars (red and white) one week and three weeks after the harvest and under the influence of different pre-treatments before drying, and 4) determine the effect of drying on quality of yacon chips produced from two cultivars (red and white) at two time intervals after harvest. To achieve these objectives yacon was cultivated during the cultivation period of 2015 and 2016 at the experimental stations of Hohenheim University, namely, Kleinhohenheim and Ihinger Hof in southwestern Germany (Stuttgart, Germany).

According to the obtained results from the tested extraction processes of young and old leaves of red and white yacon cultivars, OH-DE resulted in extracts with higher or comparable amounts of the TPC and TFC to those extracted by DE. Moreover, OH-DE reduced the energy consumption of extraction up to 50% compared to DE. Therefore, OH-DE as novel method for extraction of phytochemicals from yacon leaves might be recommended. Furthermore, there was a significant difference in phytochemical content of young and old leaves of red and white yacon cultivars. More specifically, leaves of the red cultivar contained higher amounts of phytochemicals and had a higher antioxidant activity.

Furthermore, the finding of this work showed that yacon tubers of different cultivars had varying amounts of simple sugars, TPC, TFC, and antioxidant activity in their flesh, peels and the whole tuber. These findings can be used to determine the proper cultivar for production of certain food products for a specific group of consumers or for further breeding programs to develop new cultivars with desired phytochemical contents. Additionally, peels of yacon tubers represent the main waste of their

processing. Therefore, determination of the phytochemical content of the peels is among the most essential steps to administer the potential local food supply chain for yacon products in the direction of achieving a "zero waste strategy". In this regard, the results of the present work showed that peels had the highest amount of phenolic compounds and antioxidant activity while containing lowest sugar contents compared to flesh and whole tubers. Although, these findings favour the use of yacon peels for value added product development further studies are required in relation to the safety of their utilization in other products.

In addition, the availability of vacon tubers are seasonal and their preservation is necessary. Therefore, this work focused on drying as a method to extend the shelf life of yacon tubers. Drying of yacon slices offers the chance to produce yacon chips, which can be used as an end product in the food market in the range of dried fruits or can be further processed into an ingredient such as yacon flour for further food product development. In this respect, yacon tubers were kept at ambient temperature one and three weeks after harvest to determine the effect of curing on their TPC and antioxidant activity before processing. Moreover, dipping of vacon slices in diluted lime juice was chosen as pre-treatment before drying to enhance the quality of yacon chips as a natural treatment. Finally drying of yacon slices was carried out using freeze drying (FD) and convective hot air drying (CHAD). Overall, the outcomes showed that FD resulted in yacon chips with better quality in terms of their TPC and antioxidant content, compared to CHAD. The finding revealed that, production of vacon chips from white cultivars by FD one week after harvest resulted in chips with higher TPC and antioxidant activity. The production of vacon chips from the red cultivar three weeks after harvest of tubers by means of FD resulted in chips with higher TPC and antioxidant activity. In addition, the results of this study showed that using hot air at 50 or 60 °C compared to 40 °C in the convective hot ait dryers resulted in yacon chips with higher TPC and antioxidant activity and may be recommended as an easy preservation method.

Overall, this thesis provided a broad dataset und information with regard to phytochemical contents of yacon leaves and tubers of different cultivars grown under the environmental conditions of southwestern Germany. However, to ensure the success of local food supply chains for yacon and its products in the EU, further studies with regard to the determination of individual functional constitutes of leaves and tubers of yacon, their mechanism of action and effectiveness in promoting the health benefits, and their safety is essential. Moreover, with regard to novel product development from yacon leaves and tubers, further studies are strongly suggested to ensure the sustainability of final food products by optimizing energy consumption and environmental impacts of the whole food supply chain for such products as well as their quality.

9. Zusammenfassung

Die Nachfrage nach pflanzlichen, gesundheitsfördernden und funktionalen Nahrungsmitteln steigt aufgrund einer Kombination verschiedener Faktoren wie der Gewährleistung der Ernährungssicherheit und der Bedenken hinsichtlich der Land- und Wassernutzung. Darüber hinaus tragen Aspekte des Klimawandels oder auch ein Wertewechsel der Verbraucher bei der Auswahl ihrer Lebensmittel, die Etablierung unterschiedlicher Ernährungsweisen und das wachsende Bewusstsein der Verbraucher für den Zusammenhang zwischen Gesundheit und Ernährung zu einer steigenden Nachfrage nach pflanzlichen Lebensmitteln bei. Hinzu kommt, dass der Anstieg bestimmter chronischer Erkrankungen in der Bevölkerung, wie der Fettleibigkeit, Typ-II-Diabetes; koronare Herzkrankheiten usw., darüber hinaus zusätzliche Aufmerksamkeit auf eine gesunde Ernährung gelenkt hat. Die Ernährungsweise in Industrieländern ist vielfältig geprägt durch eine hohe Aufnahme kalorienhaltiger Süßstoffe in Lebensmitteln und Getränken. Studien haben speziell eine hohe Einnahme von Saccharose als Hauptsüßstoff mit einem erhöhten Risiko für bestimmte chronische Krankheiten, wie der Fettleibigkeit und Typ-II-Diabetis in Zusammenhang gebracht. Infolgedessen wurden verschiedene Versuche unternommen, mit dem Ziel, den Saccharoseverbrauch der Bevölkerung im allgemeinen zu reduzieren (z.B. Besteuerung von zuckerhaltigen Nahrungsmittel- und Getränkeprodukten, Kampagnen zur Sensibilisierung der Öffentlichkeit für Zucker in Nahrungsmitteln usw.). Die Suche nach alternativen Zuckerquellen, um den Geschmack und die Süße unterschiedlicher Lebensmittel- und Getränkeprodukte zu gewährleisten, hat zur Entwicklung alternativer, künstlicher Süßstoffe geführt (z. B. Stevia, Aspartam, Acesulpham-K, Saccharin usw.). Die Verwendung künstlicher Süßungsmittel ist unter den Verbrauchern umstritten, da sie Bedenken hinsichtlich der Lebensmittelzusatzstoffe und ihrer möglichen Auswirkungen auf ihre Gesundheit haben. Daher hat die Verwendung von natürlichen, kalorienarmen Süßungsmitteln enorme Aufmerksamkeit auf sich gezogen, um nicht nur den Zuckerkonsum der Bevölkerung zu reduzieren, sondern auch um den Ernährungsstatus und die Ernährungssicherheit für Menschen, die an Krankheiten wie Diabetes leiden, zu steigern und um die Vielfalt von Lebensmittelnprodukten auf dem Markt zu verbessern. Entsprechend dieser Entwicklungen hat Yacon (Smallanthus sonchifolius Poepp. & Endl.), eine Knollenfrucht, die in der Andenregion heimisch ist, wachsende Aufmerksamkeit auf sich gezogen, da die Knollen als alternative, pflanzliche Zuckerquelle beworben wurden. Neben den Knollen stellen die Blätter eine bedeutende Quelle für gesundheitsfördernde phenolische Verbindungen (z. B. Chlorogensäure, Kaffeesäure, Ferulasäure, Quercetin, Rosmarinsäure, Gallussäure, Rutin, Myricetin usw.) dar und ihr Extrakt weist bestimmte biologische Aktivitäten, wie ein hohes antioxidatives Potenzial und hyperglykämische Wirkung, auf. Die Yaconknollen bestehen aus Kohlenhydraten einschließlich der Einfachzucker Fructose, Glucose, Saccharose und Fructooligosacchariden (FOS). Die FOS gelten als süß schmeckende, präbiotische Oligosaccharide. Ihr Verzehr führt zu einigen gesundheitlichen Vorteilen, wie der Verringerung der Energiezufuhr, während gleichzeitig die vorteilhafte Mikroflora des Dickdarms verbessert wird. Es wurde festgestellt, dass 60-70 % des Trockensubstanzgehalts der Yaconknolle aus FOS besteht. Außerdem sind Yaconknollen eine bemerkenswerte Quelle für biologische Komponenten wie etwa den phenolischen Verbindungen (z. B. Chlorogensäure, Ferulasäure, Cumarsäure, Kaffeesäure und ihre Derivate). Yacon gilt somit als multifunktionales pflanzliches Nahrungsmittel, welches derzeit in erster Linie aus Peru importiert wird und somit auf dem EU-Lebensmittelmarkt erhältlich ist.

In dieser Hinsicht würde die Etablierung einer lokalen Wertschöpfungskette von Yacon zu einer Produktdiversifizierung in der EU führen. Die vorliegende Dissertation verfolgt daher einen ganzheitlichen Ansatz zur Etablierung einer lokalen Wertschöpfungskette von Yacon in der EU. Der Anbau von Yacon in der europäischen Region kann als erster erforderlicher Schritt zur Etablierung einer entsprechenden Produktion in der EU gesehen werden. Darüber hinaus stellt die Produktentwicklung aus dem Rohstoff Yacon den nächsten Schritt dar, um lokale/ regionale Lebensmittelprodukte aus Yacon zu entwickeln. Die Zusammensetzung der zu verwendenden Yaconblätter und Knollen hinsichtlich ihrer chemischen, physikalischen und ernährungsphysiologischen Eigenschaften wird durch die gewählte Sorte und die Umweltbedingungen, unter denen die Yaconpflanzen kultiviert werden, beeinflusst. Darüber hinaus ist die Haltbarkeitsdauer der Yaconblätter und Knollen, die Lagerungsbedingungen und die Wahl der Weiterverarbeitungstechnologie von großer Bedeutung für die resultierende Qualität der Yaconprodukte. Daher bestand das Hauptziele dieser Dissertation darin: 1) die Qualität junger und alter Yaconblätter zweier Sorten (rot und weiß) hinsichtlich ihres Gesamtphenolgehaltes (TPC), des Gesamtflavonoidgehalts (TFC) und des antioxidativen Potenzials mit Hilfe der Verwendung verschiedener Extraktionsverfahren sowie dem Energieverbrauch des Extraktionsprozesses zu unterscheiden, 2) verschiedene Teile der Yaconknollen (Fruchtfleisch, Schale und ganze Knolle) hinsichtlich ihres Gehalts an Einfachzucker (Fructose, Glucose und Saccharose), TPC, TFC und der antioxidativen Aktivität zu unterscheiden, 3) die TPC- und Antioxidationsaktivität von zweier Sorten (rot und weiß) eine Woche und drei Wochen nach der Ernte und unter dem Einfluss verschiedener Vorbehandlungen vor der Trocknung zu untersuchen und, 4) die Auswirkung der Trocknung auf die Qualität von Yaconchips von zwei Sorten (rote und weiß) in zwei Zeitabständen nach der Ernte zu bestimmen. Um diese Ziele zu erreichen, wurde Yacon in dem Anbauzeitraum 2015 und 2016 auf der Versuchsstation "Kleinhohenheim" der Universität Hohenheim (Stuttgart) und der Versuchsstation Ihinger Hof angebaut. Anhand der Ergebnisse der getesteten Extraktionsprozesse von jungen und alten Blättern roter und weißer Yaconsorten führte die Variante OH-DE (ohmic-assisted decoction) zu Extrakten mit höheren oder vergleichbaren Mengen an TPC und TFC im Vergleich zu denen die durch Dekoktion (wässrige Extraktion, DE) extrahiert wurden. Darüber hinaus reduzierte das OH-DE Verfahren den Energieverbrauch der Extraktion um bis zu 50 % im Vergleich zu DE. Daher kann OH-DE als neuartige Methode zur Extraktion sekundärer Pflanzenstoffen aus Yaconblättern empfohlen werden. Darüber hinaus wurde ein signifikanter Unterschied im phytochemischen Gehalt von jungen und alten Blättern der roten und weißen Yaconsorte festgestellt. Blätter der roten Sorte enthielten höhere Mengen an sekundären Pflanzenstoffen und hatten eine höhere antioxidative Aktivität. Die Ergebnisse dieser Arbeit zeigten außerdem, dass Yaconknollen verschiedener Sorten unterschiedliche Mengen an Einfachzucker, TPC, TFC und Antioxidationsaktivität im Fruchtfleisch, in der Schale und in der gesamten Knolle aufwiesen. Die Erkenntnisse können dazu verwendet werden, Sorten für die Produktion bestimmter Lebensmittelprodukte zu bestimmen oder um weitere Züchtungsprogramme zur Entwicklung neuer Sorten mit gewünschten phytochemischen Inhalten zu entwickeln. Darüber hinaus, stellen die Schalen der Yaconknollen das Hauptabfallprodukt ihrer Verarbeitung dar. Daher gehört die Bestimmung des phytochemischen Wertes der Schalen, zu den wichtigsten Schritten, um eine Wertschöpfungskette mit Fokus auf einer "zero-waste" Strategie zu entwickeln. In dieser Hinsicht zeigten die Ergebnisse der vorliegenden Arbeit, dass die Schalen die höchste Menge an phenolischen Verbindungen und antioxidativer Aktivität aufwiesen, während sie im Vergleich zum Fruchtfleisch und ganzen Knollen den geringsten Zuckergehalt enthielten. Obwohl diese Ergebnisse die Verwendung von Yaconschalen in einzelnen Lebensmittelprodukten favorisieren, sind weitere Studien in Bezug auf die Sicherheit der Verwendung der Schalen der Yaconknollen von entscheidender Bedeutung.

Darüber hinaus sind die Yaconknollen nur saisonal verfügbar was ihre Konsevierung notwendig macht. Das Trocknen von Yacon bietet die Chance, Yaconchips herzustellen, die als Endprodukt auf dem Lebensmittelmarktanalog zur Produktsparte getrockneter Früchte verwendet werden können oder durch Weiterverarbeitung zu Mehl in anderen Lebensmittelprodukten eingesetzt werden können. Im Hinblick darauf, wurden die Yaconknollen für eine bzw. drei Wochen nach der Ernte bei Umgebungstemperatur aufbewahrt, um den Effekt der Lagerung auf TPC und die antioxidative Aktivität vor der Verarbeitung zu bestimmen. Darüber hinaus wurde das Eintauchen von Yaconscheiben in verdünnten Limettensaft (natürliche Behandlung) als Vorbehandlung vor dem Trocknen gewählt, um die Qualität der Yaconchips zu verbessern. Schließlich wurde das Trocknen der Yaconscheiben unter Verwendung einer Gefriertrocknung und einer konvektiven Heißlufttrocknung durchgeführt. Insgesamt zeigten die Ergebnisse, dass die Gefriertrocknung im Vergleich zur konvektiven Heißlufttrocknung in höheren TPC-Gehalten und einem höheren antioxidativen Potenzial resultierten. - Die Ergebnisse zeigten, dass die Produktion von Yaconchips aus der weißen Sorte eine Woche nach der Ernte zu höheren TPC-Gehalten und höheren antioxidativen Aktivitäten führte. Im Vergleich dazu führte die Produktion von Yaconchips aus der roten Sorte drei Wochen nach der Ernte zu Chips mit höheren TPC-Gehalten und einem gesteigerten antioxidativen Potenzial. Die Ergebnisse dieser Studie zeigten außerdem, dass die Verwendung von Heißluft bei 50 oder 60 C in konvektiven Heißlufttrocknern zu Yaconchips mit höheren TPC-Gehalten und einem gesteigerten antioxidativen Potenzial führte und daher als einfache Konservierungsmethode empfohlen werden kann.

Die vorliegende Dissertation bietet daher eine umfassende Datengrundlage zu den phyotchemischen Gehalten verschiedener Inhaltsstoffe in Yaconblättern und –knollen, die unter den gegebenen Umweltbedingungen in Südwestdeutschland erzeugt wurden. Um jedoch eine lokale Wertschöpfungskette für Yacon und die daraus möglichen Lebensmittelprodukte in Deutschland zu gewährleisten, sind weitere Studien zur Bestimmung der Zusammensetzung der Blätter und Knollen von Yacon, ihres Wirkmechanismus und ihrer Wirksamkeit im Hinblick auf ihre ernährungsphysiologischen Vorteile sowie die finale Produktsicherheit von wesentlicher Bedeutung. Im Hinblick auf die

Entwicklung neuer Lebensmittelprodukte aus Yaconblättern und Knollen werden weitere Studien empfohlen, um die Nachhaltigkeit der Endprodukte zu gewährleisten und den Energieverbrauch und die Umweltauswirkungen der gesamten Wertschöpfungskette zu optimiere.

10. References

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Conference proceedings

Khajehei, F., Piatti, C., & Graeff-Hoenninger, S. (2018). Re-emerging raw materials: reviving diversity and improving food security. 5th International ISEKI_Food Conference,3-5 July, University of Hohenheim, Stuttgart, Germany.

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Stuttgart-Hohenheim, Septembre 2019 Forough Khajehei
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