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Antioxidative components in traditionally prepared seeds of *Plukenetia volubilis* L.

Master Thesis

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Declaration

I, Veronika Čamková, hereby declare that I have worked on this thesis entitled *Antioxidative components in traditionally prepared seeds of Plukenetia volubilis* L. independently, using only the sources listed in the bibliography.

Prague, 21th April, 2016

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Abstract

Sacha inchi (*Plukenetia volubilis* L.) seeds are rich in protein and oil, composed of 93% of unsaturated fatty acids. The effect of three types of boiling (boiled in water, boiled under pressure, boiled in water bath without touching water 'sous-vide') and three different types of roasting (roasted at 125°C, 190°C and coated in honey and roasted for 170°C) in different time (0-200 min.) was examined to describe and compare changes in antioxidant compounds with non-treated Sacha inchi (*Plukenetia volubis*) kernels. The samples were measured for content of α-, β + γ- , δ- tocopherols total phenolics and their radical scavenging activity using DPPH assay. All thermal treatments significantly affected the antioxidant activity on $p<0.05$. The overall influence of thermal treatments caused that total phenolic content increased (255.69%), β + γ- tocopherol level decreased (49.55%), δ- tocopherol level slightly decreased (0.87%), and radical scavenging capacity also decreased (7.97%) compared to the raw kernels. Pressure boiling and water bath boiling are the most suitable thermal treatment methods. Roasting had a significant negative effect on tocopherol content but not on the total phenolic content. Especially roasting at 190°C for 35°C min and honey roasting at 170°C for 30 min of Sacha inchi kernels has a positive effect of increasing TPC. The present work confirmed that appropriate thermal treatment and adequate time can significantly improve the antioxidant activity and thus nutritional values of *Plukenetia volubilis* kernels during food preparation.

Key words: Sacha inchi, Tocopherols, Phenolic compound, Antioxidant capacity, Thermal treatments

Abstrakt

Semena Sacha inchi (*Plukenetia volubilis* L.) jsou bohatá na obsah bílkovin a oleje, ve kterém je obsaženo až 93% nenasycených mastných kyselin. V této práci byl zkoumán vliv tří způsobů vaření (ve vodě, pod tlakem, ve vodní lázni 'sous-vide') a pražení (za nízké teploty 125°C, za vysoké teploty 190°C, pražení jader obalených v medu při 170°C) v různých časech (0-200 min.) na obsah vybraných antioxidantů v jádrech semen Sacha inchi. Bylo provedeno měření obsahu α-, β + γ- , δ- tokoferolů, celkových polyfenolů a celkové antioxidační kapacity metodou DPPH. Všechny tepelné úpravy statisticky významně ovlivnily obsah hodnocených parametrů na úrovni $p<0.05$. Celkově tepelné úpravy způsobily zvýšení celkové hladiny polyfenolů (o 255.69%), pokles β + γ- tokoferolů (o 49.55%). Hladina δ- tokoferolů mírně poklesla (o 0.87%) podobně jako celková antioxidační kapacita (o 7.97%) v porovnání se syrovými jádry. Pražení má výrazný negativní vliv na obsah α-, β + γ- , δ- tokoferolů, ale ne na celkový obsah tokoferolů. Především pražení při teplotě 190°C po dobu 35 min a pražení obalených jader v medu při teplotě 170°C po dobu 30 min má pozitivní efekt na vzrůstající obsah celkových tokoferolů. Jako nejšetrnější způsob tepelné úpravy pro jádra Sachi inchi se jeví metoda vaření pod tlakem a metoda vaření jader ve vodní lázni ´sous-vide´. Výsledky této práce potvrdily, že vhodný způsob tepelné úpravy po přiměřenou dobu může zvýšit antioxidační kapacitu v jádrech Sachi inchi a tím i jejich nutriční hodnotu.

Klíčová slova: Sacha inchi, Tokoferoly, Fenoly, Antioxidační kapacity, Tepelné úpravy

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List of Abbreviation

AGEs = Advanced Glycation End Products

ANUGA = General Food and Drink Trade Fair

ANOVA = The Analysis of Variation

CoL= Catalogue of Life Database

DPPH = 2,2-diphenyl-1-picrylhydrazyl radical

EFTA = The European Free Trade Association

FAO = The Food and Agriculture Organization of the United Nations

FDA = The Food and Drug Administration

GAE = Gallic Acid Equivalent

GBIF = Global Biodiversity Information Facility

GRAS = Generally recognized as safe

HR = Honey Roasting

HSD = Tukey´s Honest Significant Difference Test

HTR = High Temperature Roasting

LTR = Low Temperature Roasting

MRPs = Maillard Reaction Products

OB = Open Boiling

PB = Pressure Boiling

PUFA = Unsaturated Fatty Acids

RPM = Revolutions per Minute

RSC = Radical Scavenging Capacity

SD = Standard Deviation

SV = “Sous-vide” Bath Boiling

TE = Trolox Equivalent

TPC = Total Phenolic Content

WCSP = World Checklist of Selected Plant Families

WHO = World Health Organization

1. Foreword

Natural antioxidants has generally been attributed to have protective effects against number of cancers and cardiovascular diseases. Nowadays customers are seeking for food possess health benefits (Mazzeo et al., 2011). Recently, number of epidemiological and clinical studies have provided evidence that frequent nut consumption is associated with favorable plasma lipid profiles, reduce risk of chronical heart diseases and type of 2-diabetes and cancer (Alasalvar and Shahidi, 2009).

Sacha inchi (*Plukenetia volubilis* L.) is the most well-known and commercially valuable *Plukenetia* species. Seeds have high protein and level content and its oil is one of the richest plant source of the omega fatty acids, essential for human life (Hamaker et al., 1992; Cai et al., 2011; Cai et al., 2013). It found to be comparable in food and nutritional quality to the soybean and it can be used as a substitute for imported oil and high-protein meals (Hamaker at al., 1992). Owing these benefits, the manufacture of *Plukenetia volubilis* as a supplement and the demand for this plant has increased considerably (Guillén et al., 2003).

Raw seeds of Sacha inchi have strong bitter taste and without heat treatment it is almost impossible to consume it fresh (Gillepsie and Armbruster, 1997; Pereira de Souza 2013). At present, *Plukenetia volubilis* seeds are roasted or cooked for direct consumption as a snack in order to eliminated astringent off-flavor and possible anti-nutritional factors. Even though, Sacha inchi oil is extracted without prior roasting of the seeds (Cisneros et al., 2014).

It is known that cooking induces significant changes in chemical composition affecting the bioaccessibility and the concentration of the health-promoting compounds (Mazzeo et al., 2011). However, heat processing can cause damages of essential amino acids resulting in decreased content. Hence, it is important that after thermal seed processing there should be scientific evidence that nutritional and other useful properties are still of significant value and that anti-nutritional components have been reduced considerably (Arinola and Adesina, 2014; Udeonyia et al., 2014; Anyalogbu et al., 2015). It is, therefore, of great interest to assess how the thermal treatments effects the antioxidant status of *Plukenetia volubilis* thermal treated seeds. Thus, this thesis is focused on determination of antioxidant levels in Sacha inchi thermal treated kernels.

2. Literature review

2.1 *Plukenetia volubilis* L.

2.1.1 Taxonomy description

Euphorbiaceae is one of the five biggest flowering plant families. *Plukenetia* L. is unusual in the Euphorbiaceae for its 4-carpellate ovary and vine or liana habit (Gillepsie, 2007). Accorging to Webster classification (1974; 1975) five subfamilies are currently recognized (Gillepsie and Armbruster, 1997). *Plukenetia* belongs to the subfamily Acalyphoideae, which is the largest subfamily in the Euphorbiaceae family (Webster 1975; Webster, 1994; Gillepsie and Armbruster, 1997). Species characteristically lack latex, have petiolar glands and stamine flowers with valvate sepals (Gillepsie, 1993; Gillepsie and Armbruster, 1997).

The Plukenetieae tribe was for first time monographed by Pax (1890) and Pax and Hoffmann (1919; 1931). The pollen morphology was described by Gillapsie (1994). Tribe Plukenetieae is worldwide distributed in tropical and warm temperate regions. This tribe can be distinguished by its bisexual racemose or spicate inflorescence. It has typically entire, massive style with completely or basally connate shape. The tribe Plukenetieae comprises three subtribes. Subtribes Plukenetiinea lack urticating hairs and has usually lamilar glands on the leaf blade (Webster, 1974; Gillepsie and Armbruster, 1997). Genus *Plukenetia* is a pantropical genus of over 20 species (Cai et al., 2013). *Plukenetia* species are lianas or twining vines, monoecious or rarely dioecious, latex absent plants (Gillepsie and Armbruster, 1997; Gillepsie, 2007).

Taxonomic classification of *Plukenetia volubilis* L according to Global Biodiversity Information Facility databases (GBIF, 2015):

Kingdom: Plantae
Phyllum: Magnoliophyta
Class: Magnoliopsida
Order: Euphorbiales
Family: Euphorbiaceae
Subfamily: Acalyphoideae
Tribe: Plukenetieae
Genus: *Plukenetia*
Species: *Plukenetia volubilis* L.

2.1.2 Origin and distribution

Plukenetia volubilis L is native to the rain forest of the Andean region of South America (Hamaker et al., 1992; Sathe, 2002; Cai et al., 2011; Fanali et al., 2011; Cai et al., 2013; Pereira de Souza, 2013). *Plukenetia* is widespread in the Lesser Antilles and South America, where it is found primary in the northern and western regions and margins of the Amazon Basin in Surinam, Venezuela, Columbia, Ecuador, Peru Bolivia and Brazil (Gillepsie and Armbruster, 1997).

Plukenetia volubilis is mostly found from the sea level to less than 1000 m. According to Guillén (2003) it can be found in the range between 200 and 1500 m above the sea level. However, in Peru *Plukenetia* was found at altitudes from 1600-2100 m above the sea level (Gillepsie, 1993; Bussmann, 2009; Wang et al., 2012). The greatest degree of variation in *Plukenetia volubilis* is found in collections from the eastern slopes of the Andes bordering the Amazon basin in Peru (Gillepsie, 1993).

Fig. 1 Distribution of Sacha inchi (WCSP, 2015).

2.1.3 Common names and synonyms

Plukenetia volubilis is mostly commonly known as a Sacha inchi. Depanding on the concrete region in spanish speaking countries *Plukenetia volubilis* L. is also called Inca Inchic, Sacha Inchic, Maní del Monte, Sacha Maní, Maní del Inca, Maní Jibaro, Supua, Ticano, Yuchi (Bondioli et al., 2007; Bussmann et al., 2009; Pereira de Souza et al., 2013).

English vernacular names for *Plukenetia volubilis* are forest peanut, inca peanut, sacha peanut, wild peanut or mountain peanut (Guillén et al., 2003; Chirinos et al., 2013; Pereira de Souza et al., 2013).

According to Global Biodiversity Information Facility (GBIF), Catalogue of Life Database (CoL), Tropicos and World Checklist of Selected Plant Families (WCSP) databases (2016) we can find following synonyms: *Fragariopsis paxii* Pittier, *Plukenetia macrostyla* Ule, *Plukenetia peruviana* Müll. Arg., *Sajorium volubile* (L.) Baill.

2.1.4 Botanical description

Plukenetia volubilis is a monoecious perennial, oleaginous twining vine or slender liana (Gillepsie, 1997; Guillen et al., 2003).

Stems are glabrescent to pubescent. Leaves are alternate, petiole is 2.5-7.5 cm long, glabrous to sparsely pubescent. Blade is membranous, triangular-ovate 7- 13 (-18) cm long and 4-lo (-18) cm wide. Apex is long-acuminate, base is truncate to cordate, glabrescent below and 3-veined at base.

Flowers are bisexual. Inflorescence is axillary or terminal on short shoot, racemose, 5-18 cm long. Pistillate flower is 1 or rarely 2 at basal node, staminate flowers are white, numerous in condensed cymes above. Bracts are narrowly triangular, 1.5-2.5 mm long.

Fruit is capsule, 4-lobed 2.5-4 (-6) cm in diameter, dehiscent, glabrous, initially fleshy, becoming woody. Each carpel is lobed with central wing to 2 mm wide.

Seeds are lenticular, broadly oblong in outline, 1.8 x 0.8 x 1.6 cm. Seed has intense green color and becoming brown when are ripen with course dark brown markings (Bondioli and Della Bella, 2006; Gillepsie 1993; Gillepsie, 1997). The weight of each seed ranges between 0.8-1.4 g (fig. 2, fig.3; appendix 1)(Bondioli and Della Bella, 2006).

Excellent field identification for the *Plukenetia* is the presence of distinct paired, elliptical or circular basilaminar glands on the adaxial surface of the leaf blade and 4-parted fruit (Gillepsie, 2007).

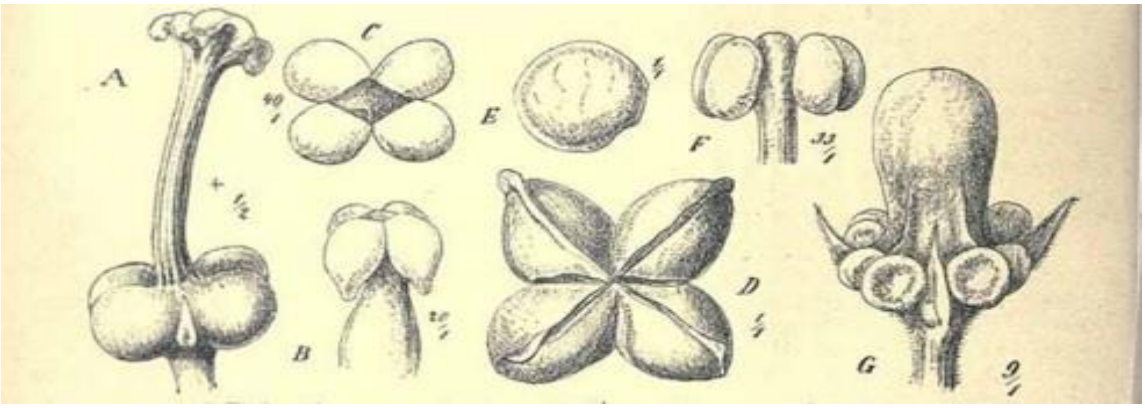

Fig. 2. Plukenetia volubilis botanical description 1: A- Flos, B-Stamen, C-Anthera, D-Capsula, E-Semen, G- P. verrucosa (Eagler, 1919).

Fig. 3. *Plukenetia volubilis* botanical description 2: A- Habitat, B- Male Flower, C-Female Flower (Mourré, 1967).

2.1.5 Relative species

The closest relative species for *Plukenetia volubilis* is *Plukenetia stipellata*. While considered as distinct species but closely related, they could be deemed as a second species complex. *Plukenetia stipellata* differs from the *P. volubilis*, by its stipellate leaf blade base, shorter styles and longer slender stamens. *P. stippellata* has five sepals instead of six. This species is widespread and moderately common in the Cental America, while *P. volubilis* a species is restricted to South America and the Lesser Antilles (Gillepsie, 1993). Even nowadays *P. stipellata* has never been reported in Peru (Dostert et al., 2014). *Plukenetia volubilis* has also morfological similarities and can be mixed with *Plukenetia polyadenia* Muell. and *Plukenetia loretensis* Ule and *Plukenetia brachybotrya* species (Gillepsie, 1993).

2.1.6 Uses

Plukenetia volubilis seeds are rich in oil and proteins, which composition of large number of unsaturated fatty acids make this plant special (Guillén et al., 2003; Follegatti-Romero et al., 2009). It is also low in the content of saturated fatty acids and contains heat-labile substances with bitter taste (Fanali et al., 2011; Kumar et al., 2014; Pereira de Souza 2013). Due to intrinsic characteristics *Plukenetia volubilis* seeds indicating to have numerous health benefits as an anti-atherogenic, hypercholesterolemic and anti-thrombogenic effects. Also seeds shells are low in the content of the saturated fatty acids. It found to be comparable in food and nutritional quality to the soybean and it can be used as a substitute for imported oil and high-protein meals (Hamaker et al., 1992). Thus, the incorporation of Sacha inchi in the human diet is promising for its health benefits, as well as the use of the shell in food processing (Pereira de Souza et al., 2013).

Sacha inchi is the important component for the Amazon native diet. *Plukenetia volubilis* has traditionally been consumed by the Indians of Peru. It was probably cultivated by the pre-Incas and the Incas for thousands of years because representation of this plant and of its fruit have been found in vessels in Inca tombs (Fanali et al., 2011; Guillén et al., 2003). Chancas Indians and other tribes groups of the region extract oil from the seeds, they obtain flour from the seeds, blender and cooked leaves of *Plukenetia volubilis*. These products are used in the preparation of different meals and beverages

(Chirinos et al., 2013; Fanali et al., 2011; Guillén et al., 2003). Seeds are mostly roasted or mixed and consumed with maize meals and peppers (*Capsiccum* spp.) (Hamaker et al., 1992; Sathe et al., 2002). Seeds of wild plants were found to be distasteful and inedible when eaten raw (Gillepsie and Armbruster, 1997). In folk medicine in the Amazonia region leaves are used for skin diseases, to treat rheumatic problems and aching muscles (Hamaker et al., 1992). The traditional reported uses for this oil are in cosmetics, in therapy and as nutraceutical for its high content in essential fatty acids (Bondioli and Della Bella, 2006). Sacha inchi oil is used for skin care, in order to maintain skin softness and for the treatment of wounds, insect bites and skin infections. Current research confirmed the ability of Sacha inchi oil to inhibit *Staphylococus aureus* adherence (Gonzales-Aspajo et al., 2015). *Plukenetia volubilis* leaves extracts possess antioxidant and antiproliferative properties (Nascimento et al., 2013).

Currently, Sacha inchi oil is obtained by cold pressing and commercialized as the crude oil (Follegatti-Romero et al., 2009). Sacha inchi oil is an excellent table oil with slight vegetal flavor. The oil and the protein of Sacha inchi have been awarded in 2004 in Paris at the international competition 'Oil of the World' and obtained the gold medal (Křivánková et al. 2012). In the 2005, *Plukenetia volubilis* oil was selected as one of the top innovation of ANUGA in Germany. Compared to other food seeds, the oil content in Sacha inchi seeds (33.4-37.6%) was superior to that reported for different soybean cultivars (16.5–17.5%), chia (26.7–35.0%) and safflower (27.5%) and it is within the range reported for flax seeds (33.6–44.8%). For comparison higher amount of oil content is reported for pistachio (50.4–58.0%) and macadamia kernel nut varieties (63.0–71.8%) (Yoshida et al., 2003; Arena et al., 2007; Bozan and Tenelli, 2008; Wall, 2010; Ixtaina et al., 2011; Ciftci et al., 2012; Chirinos et al., 2013).

In Peru was Sacha inchi cultivated as a poultry-feed supplement (Hamaker et al., 1992). It is considered as a promising resource for the oleochemical industry, including its use in biodiesel production (Zuleta et al., 2012). According to Kumar (2016) *Plukenetia volubilis* extracts contain the functional substances which can be used as an alternative source to conventional methods and to develop new green/eco-friendly technologies. Also *Plukenetia volubilis* shell biomass should be considered as an important source of phytochemicals for the synthesis and stabilization of silver nanoparticles. Utilization of these crops in industrial process for the production of high performance materials could be an additional source of revenue for farmers and also help

in agro-industry diversification by providing a non-food-based market for agro-wastes (Kumar et al., 2014).

2.1.7 Trade

In 2013, Sacha inchi oil entered the EU market as a food ingredient for the first time. Sacha inchi oil producer Agroindustrias Amazonias first novel food application was in 2005, which was followed by a long waiting process. This breakthrough was possible through the procedure within the framework of the Novel Food Regulation, thus making way for the integration of Sacha inchi oil into the European food industry. The Novel Food Regulation covers foodstuffs that are newly developed by industry, but also natural foodstuffs or ingredients which were not consumed to a significant degree within the EU before year 1997. Sacha inchi oil is appreciated by European markets as a gourmet oil and is also highly valued for its health benefits. Specialty oils are becoming more popular in Europe, creating niche opportunities for developing country exporters (CBI, 2015). Since 2014, Sacha inchi oil is formal recognition as being 'Generally Recognized as Safe' (GRAS) by the United States (US) Food and Drug Administration (FDA) (INTRACEN, 2014).

Although greater awareness and increasing demand led to cultivation of these nuts, wild collection still plays an important role. Traditionally, Sacha inchi nuts were obtained only through wild collection. Ingredients collected in the wild can be certified according to sustainable wild-collection and equitable sharing of benefits from biodiversity (according to the Nagoya Protocol) (SIPPO, 2012; CBI, 2014).

Peru is the only significant producer and exporter of Sacha inchi oil worldwide. Peru has more than 2,000 hectares dedicated to the production of Sacha inchi trees, in the provinces of San Martín, Junín, Huánuco, Ucayali, Loreto y Amazonas. According to Agencia Agraria de Noticias (2014) each hectare has a production yield of 3 t, approximately and exported approximately 224 t of Sacha inchi oil to various destinations worldwide in 2014. A quarter of these exports (approximately 46 t) was destined for the European (EU and EFTA) market. It is estimated that more than half of these exports was organic-certified Sacha inchi oil. The major importers of Peruvian Sacha inchi oil in 2014 were Spain and France, closely followed by Germany and the UK. Spain imported 13 t

of Sacha inchi oil from Peru, as compared with 11 t for France. Germany and the UK imported approximately 4 and 8 t, respectively (fig.4) (CBI, 2014).

Market channels Sacha inchi oil is speciality oil which is traded in much smaller volumes when compared to the large commodity oils such as palm and coconut oils, or even to other speciality oils such as sesame oil. Although Sacha inchi oil is not commonly used as an ingredient in the food industry, there might be future prospects in specific niche markets (omega-3 food products). At present, Sacha inchi oil is mostly used in an unprocessed state. Sacha inchi oil is mostly used in the food market and in the cosmetics industry (CBI, 2015; SIPPO, 2012).

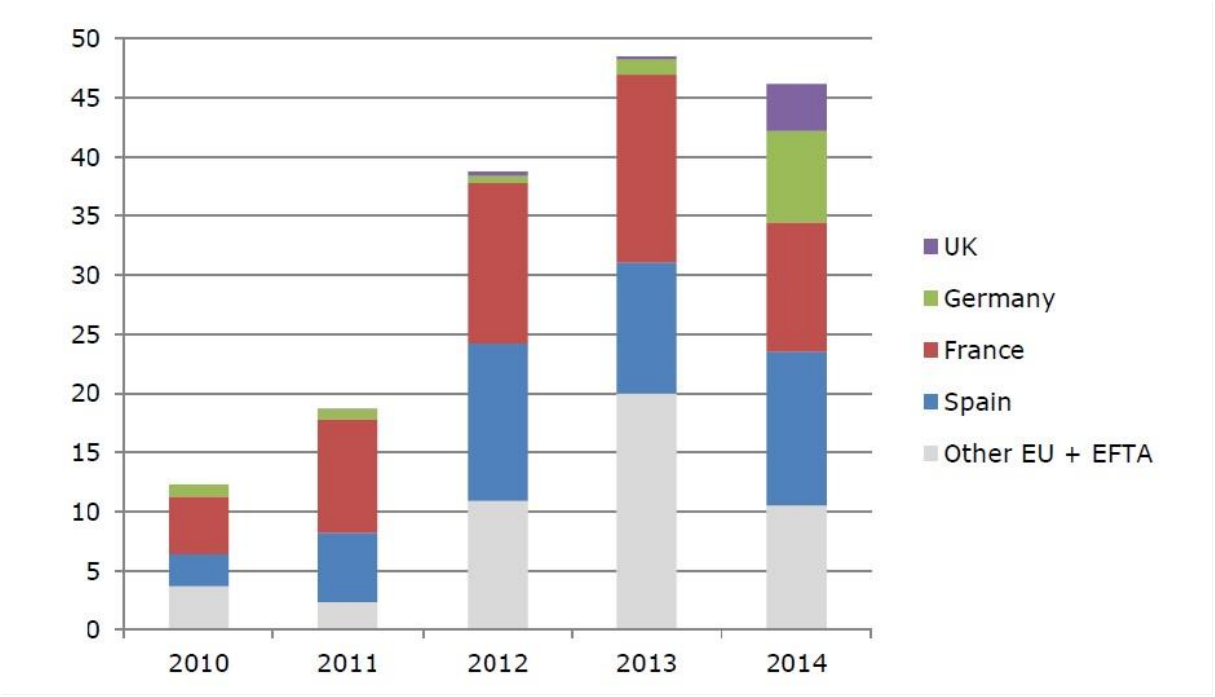

Fig. 4. Increasing Peruvian exports of Sacha inchi oil to the five largest EU markets

2.2 Plant husbandry

2.2.1 Ecology

Plukenetia volubilis grows in hot, humid climate and requiring a minimum temperature of 10°C and a maximum of 36°C. Higher temperatures are unfavourable causing the abort of flowering and the loss of leaves (Arévalo, 1996; Manco, 2006). Plant is well adapted to different environment. As a twining vine bush, it is highly adaptable, growing at both low and middle altitudes of the rainforest. It grows from 100 m.a.s.l. up to 1500 m.a.s.l. depending on the availability of water and good drainage (Gillepsie, 1993). Sacha inchi was successfully introduced from South America to China, Xishuangbanna area where 95% of the land is covered by mountains (Cai et al., 2011; Cai et al., 2012, Yang et al., 2014). According to Cai et al. (2012) lower altitudes (<900 m a.s.l.) could be the optimum zone for growth and yield of Sacha inchi plants whereas seeds collected from plants growing at higher altitudes (>900 m a.s.l.) and in the cool season appear to have better quality. Plant biomass and fruit production were highest at lower altitudes and dramatically decreased above 900 m a.s.l., which can primarily be attributed to a C source limitation. Sacha inchi could be useful for cropping in cooler regions because the reproductive growth is adapted to high elevations. The oil and protein content and linolenic and unsaturated fatty acid concentrations in seeds were highest in the cool season (Cai el al., 2012).

Minimum precipitation per year is 850-1000 mm, equally distributed throughout the year. Longer periods of dry weather or lower temperatures cause growth retardation. The excess of water increase the infestation by diseases. Pests and diseases are various but the early attack *Meloidogyne* species and *Aphelenchus* species can cause big damages. Big losses are also cause by fungi *Fusarium, Stagonospora, Leptospaeira* and other species (Arévalo, 1996; Manco, 2006). When the plants grow in relative humidity 78% and an average temperature 25 °C, they are practically disease free (Dostert et al., 2014).

Sacha inchi grows in a variety of soils, it prefers acidic clay soils (pH 5.5-7.8) with high content of aluminium and requires low light intensity but constant water supply with appropriate drainage. The crop has also shown to be resilient to conditions of low fertility. Fertilizers are not needed for optimal growth in the first few years.

Although Sacha inchi requires low light intensity abundant light is advantageous. The more light plant receive growth and development of branches, inflorescence and pods

is more intense. On the other hand, too much shading diminish the flowering and production decreases (Arévalo, 1996; Manco, 2006). Sacha inchi regenerates in the shade of an overstory canopy, but for commercial production, it is normally cultivated on cleared land devoid of overhead shade. *Plukenetia volubilis* is high-light demanding species. The shade delay initial flowering date and decreased flower and fruit parts (Cai et al., 2011).

2.2.2 Propagation and phenology

The propagation of *P. volubilis* is most commonly done by seeds; both directly in field or in the nursery. Good quality of seeds is very important. Before sowing the pesticides and insecticides should be used to avoid fungi diseases. Vegetative propagation may be done by cuttings (Manco, 2006). *Plukenetia volubilis* plants do not exhibit winter dormancy. They grow continuously in tropical regions and therefore flower and fruit almost continuously throughout the year (Yang et al., 2014).

Germination normally occurs in two weeks after sowing. The stem and second green leaf appear in another week. Flowering is initiated from 3 months to 5 after being planted. First male flowers appear immediately followed by female flowers. The floral differentiation of both male and female flowers is completed in 7 to 19 days. An earlier initiation of flowering is associated with higher flower biomass and plant total biomass (Cai et al., 2011).

Formation and development of the fruits start afterwards and in 4 months after flowering is terminated. Consequently, maturing process of fruits start and when the green capsules turn dark brown or almost black fruits are ready to be harvested. The maturing process of fruits take 15–20 days, initiates approximately after 7.5 months after sowing (Arévalo, 1996).

2.2.3 Cultivation

Sacha inchi sowing in the Peruvian Amazon is conditioned by the rainfall regime. Seeds are directly planted in dry conditions at the beginning of the rainy season which is between November and December in order to guarantee good germination. When land is irrigated, it can be planted any time during the year (Manco, 2006). For direct sowing 1.0-1.5 kg of seeds in needed. The distance between rows should be 2.5-3 m and seeds should be sow in deep 2-3 cm (Arévalo, 1996).

According to Yang et al. (2014) use of NPK fertilizers do not affect seed size and phenological development. However the level of fertilizers and plant density significantly affected seed oil content. Total seed and oil yields increased continuously with the increasing the level of fertilizers use. Approximately 4.445 plants/ha is required to ensure maximum yield in the field (Yang et al., 2014).

The end of dry season can be significantly improves by irrigation. Irrigation during the dry period can enhance plant growth affecting overall yields in the year and possibly in the subsequence years. Dry-season irrigation increases adaxial stomatal density, fine-root volume, leaf area index, total biomass and fruit biomass. Plants grow under natural drought conditions have less flowers and fruits per plant and higher percentage of fruit abortion (Jiao et al., 2012).

Sacha inchi can be associated with annual, biennial, and/or permanent crops in their natural habitat. Farmers have associated it with almost all regional crops: cotton, banana, beans, corn, cassava, fruits, forest species, etc. Some experiences have shown cultivating it with medium and determined growth legumes or species with short growing season (like cow peas or pigeon peas) is preferable when employing a trellis system, it can be associated with short cycle crops, such as peanuts, beans, upland cotton, and other low growing crops, planting them between rows (Manco, 2006).

The species has also desirable features for reforestration and slope protection and it is appointed as an alternative to the recovery of degraded areas, especially destroyed by swidden system which is traditionally use in the Amazon areas but it is not recommended. Cutting of vegetation and burning destroying the soil nutrients, stopping the decomposition of organic matter, resulting to the heavy compact soil which cannot absorb water (Cordoso et al., 2015; Manco, 2006).

2.2.4 Harvest

First harvest can be done 6.5-8 month after planting. *Plukenetia volubilis* is harvested every 20-25 days. Plants are most productive in the months of November to May. Between June and October the productivity rapidly decrease because of the period with low precipitation. Mature fruits are harvested by hands. Because of contamination possibility fruit on the ground are not collected. Plants can be productive approximately 10 years.

Manco (2006) indicated that in the first year average yields are obtained of 0.7-2.0 t/ha. The yield increasing first 3 years, then gradually decreasing. According to Yang (2014) study the total seed yield over growing season range from 1.340-2.786 kg/ha and total oil yield over growing season can be in range from 501-899 kg/ha.

2.2.5 Post-harvest operations

After harvesting the capsules are transported in 25–30 kg polypropylene, jute or net sacks for drying and threshing. Post-harvest drying can be accomplished naturally or artificially, according to heat source. Natural drying is under direct sunlight, scattering capsules over a cement surface. Drying time depends on ecotype or variety since some capsules are thicker and less dehiscent than others, making the threshing process more difficult. Artificial methods employ dryers of different energy sources: solar, wood burning, oil, etc. Not many farmers use that method and only when very large fields are cultivated. Farmers prefer to wait until summer to dry their crops, or they harvest more capsules while waiting, postponing the drying and threshing until summer. Artificial and solar dryers used to dry e.g. annatto, cacao, coffee, corn and other products can be also used for Sacha inchi. In general, it is recommended to use just natural drying since dryers can heat capsules too much and alter oil quality in the seeds. When the capsules are dry most of the capsules crack and open because of their dehiscent nature. Some of the threshers have been adapted to separate capsules from the seeds and even to separate shells from nut. This process results in roughly 55% dry seed and 45% capsule remains. Seeds can be stored in 50–70 kg jute sacks in dry places. It is advisable not to mix old and new harvests because some seeds can be dry and other fresh, causing the rotting (Dostert et al., 2014; CIED, 2007).

2.3 Chemical composition of the seeds

2.3.1 Fatty acids

Chemical composition of seeds is very interesting because of its nutritional value. The fatty acid composition of *Plukenetia volubilis* makes it special because it contains a large amount of unsaturated fatty acids, about 93% in total (Chirinos et al., 2013; Fanali et al., 2011)

Linoleic and linolenic are known as essential fatty acids because humans cannot produce them themselves, they are intermediate in the biosynthesis of important compounds in human body and they must be obtained in their diet. Linoleic (18:2 n-6) and α-linolenic (18:3 n-3) acids are the main essential unsaturated fatty acids (PUFA) obtained from vegetable oils (Pereira de Souza et al., 2013).

High content of linoleic ω-6 and linolenic ω-3 representing more than 80% of total fatty acids. The composition of oil is particular rich in linoleic ω-6 approximately 35% (12.4–14.1 g/100 g seed) and linolenic ω-3 acid approximately 45% (12.8–16.0 g/100 g seed) (Chirinos et al., 2013). Content of palmitic, stearic, oleic acids and the other constituents is in lower concentration (Follegatti-Romeo et al., 2009; Fanali et al., 2011; Liu et al., 2014).

The health and nutritional importance of the n-3 polyunsaturated acyl group present in fish lipids or the α-linolenic acyl group present in some vegetable oils is known. These kinds of acyl groups provide protection against pathogenesis, rheumatoid arthritis, cancer and prevention of coronary heart diseases and hypertension and besides showing a hypocholesterolemic effect (Simopoulos, 2002; Guillén et al., 2003; Lorgeril and Salen, 2004; Gebauer et al., 2006; Follegatti-Romeo et al., 2009; Wendel and Heller, 2009; Fanali et al., 2011).

It is also worth noting that the balance between ω3, ω6, and ω9 fatty acids is an important nutritional property of plant oils. Growing evidence shows how a proper balance in diet between ω3 and ω6 fatty acids plays a key role in in the prevention of chronic diseases. The optimal ratio between linoleic acid and α-linolenic acid in the diet ranging between 4:1 to 5:1, without exceeding 10:1. These values are very often shifted to the advantage of linoleic acid in the common western diet, reaching values as high as 20:1. Such a disproportion is considered one of the main risk factors leading to the

development of chronic diseases such as obesity, cancer, and cardiovascular disease (King et al., 2007; Fanali et al., 2011; Simopoulos, 2011; Chirinos et al., 2013; Liu et al., 2014). The optimal dietary intake of α-linolenic seems to be about 2 g/day or 0.6- 1.0 % of the total energy intake (Follegatti-Romeo et al., 2009). The ratio between ω 6 and ω 3 for *Plukenetia volubilis* is near the ideal value of 1:1 (Chirinos et al., 2013; Pereira de Souza et al., 2013). For this reason, *Plukenetia volubilis* oil that are rich in polyunsaturated fatty acids can be of great interest for a balanced diet and represent a good alternative to fish-based foods and supplements that so far represent the main food sources of ω3 fatty acids (Fanali et al., 2011). However, today Western diets have a ratio of 10:1 to 20–25:1, indicating that Western diets are deficient in ω3 fatty acids (Simopoulos et al., 2011; Chirinos et al., 2013).

In fact, *Plukenetia volubilis* provides more useful among plant oils, only linseed oil (*Linum usitatissimum*) contains a percentage of linolenic acid comparable to that of Sacha inchi oil, together with low percentages of saturated fatty acids such as palmitic and stearic (tab.1). Among edible plant oils, linseed oil, because of its predominant composition in ω3 fatty acids, has been widely employed as a food supplement showing a particularly favorable nutritional profile (Guillén et al., 2003; Fanali et al., 2011).

Sacha inchi seeds presented values of polyunsaturated fatty acids, monounsaturated and saturated fatty acids within the 78.0–81.1%; 10.8–13.2% and, 7.9–9.1% ranges, respectively (Chirinos et al., 2013).

Maurer et al. (2012) showed that the following vegetable oils exhibit increasing levels of saturated fatty acids: canola < sunflower < flaxseed < corn < olive < cotton, ranging from 8.5% to 25.2%. These saturated fatty acids values are higher than those presented by Sacha inchi oil analyses.

Tab. 1. The fatty acid composition of Sacha inchi oil (Hamaker et al., 1992).

Fatty acid profile of Inca peanut oil compared to other oilseed oil[a]

Fatty acid	Inca peanut	Soybean	Peanut	Cottonseed	Sunflower
Total oil	54	19	45	16	48
Saturated					
$C_{14:0}$, Myristic	0.0	0.0	0.0	0.0	0.0
$C_{16:0}$, Palmitic	4.5	10.5	12.0	18.7	7.5
$C_{18:0}$, Stearic	3.2	3.2	2.2	2.4	5.3
Unsaturated					
$C_{16:0}$, Palmitoleic	0.0	0.0	0.3	0.6	0.0
$C_{18}:_0$ Oleic	9.6	22.3	41.3	18.7	29.3
$C_{18:2}$ Linoleic	36.8	54.5	36.8	57.5	57.9
$C_{18:3}$Linolenic	45.2	8.3	0.0	0.5	0.0
$C_{20:1}$ Gadoleic	0.0	0.0	1.1	0.0	0.0

[a] All values shown are percents. Values for soybean, peanut, cottonseed, and sunflower are taken from Bodwell and Hopkins (1985).

2.3.2 Amino acids

Great attention has been so far devoted to food supplements containing essential fatty acids and to the proportion between ω3 and ω6 fatty acids, but the potential use of Sacha inchi products as ingredients for food supplements is not only related to the fatty acid profile but also to the amino acid composition of the seeds (Yada et al., 2011). Sacha inchi seeds contain 40-60% oil and around 27 – 30% proteins. The quality of nutritional status of a protein in defined by its amino acid composition. An albumin protein, representing 31% of the total *Plukenetia volubilis* seed proteins, was also isolated and characterized. It is a glycoprotein containing all of the essential amino acids in adequate amounts (Sathe et al., 2002; Fanali et al., 2011; Pereira de Souza et al., 2013; Udeonyia et al., 2014). Sacha inchi contains all of the essential amino acids in adequate amounts when compared with the FAO/WHO recommended amino acid pattern. *Plukenetia volubilis* is therefore a complete protein with respect to the amino acid requirements of adult humans (Sathe et al., 2002).

Sacha inchi proteins are rich in level of cysteine, tyrosine, threonine, and tryptophan (Hamaker et al., 1992; Guillén et al., 2003; Follegatti-Romeo et al., 2009; Fanali et al, 2011; Chirinos et al., 2013). *Plukenetia volubilis* approximately contains the same amount of proteins as the other oilseed found in the Andean region. The amino acids content was comparable and in some cases higher than for other oilseeds (tab. 2). Level of leucine and lysine is lower than those in of soybean, although equal or better that the levels in peanut, cottonseed or sunflower. The sulfur-containing amino acids methionine

and cysteine, tyrosine, threonine, and tryptophan are presented in higher amount than in other oilseeds. Tryptophan is over twice and cysteine nearly twice the levels in other proteins. Total essential amino acids are comparable to the other protein sources (Chirinos et al., 2013; Hamaker et al., 1992).

Tab. 2. Amino acid profile of Sacha inchi (Hamaker et al., 1992).

Amino Acid Profile of Inca Peanut Protein Compared to Other Oilseed Protein[a,b]

Amino Acid	Inca Peanut	Soybean	Peanut	Cottonseed	Sunflower	FAO/WHO/UNU Scoring Pattern[c]
Total protein, %	27	28	23	33	24	
Essential						
His	26	25	24	27	23	19
Ile	50	45	34	33	43	28
Leu	64	78	64	59	64	66
Lys	43	64	35	44	36	58
Met	12	13	12	13	19	...
Cys	25	13	13	16	15	...
Met + Cys	37	26	25	29	34	25
Phe	24	49	50	52	45	...
Tyr	55	31	39	29	19	...
Phe + Tyr	79	80	89	81	64	63
Thr	43	39	26	33	37	34
Trp	29	13	10	13	14	11
Val	40	48	42	46	51	35
Nonessential						
Ala	36	43	39	41	42	...
Arg	55	72	112	112	80	...
Asp	111	117	114	94	93	...
Glu	133	187	183	200	218	...
Gly	118	42	56	42	54	...
Pro	48	55	44	38	45	...
Ser	64	51	48	44	43	...
TEAA[d]	411	418	349	365	366	...
TAA[e]	976	985	945	936	941	...
TEAA as percent of TAA	42	42	37	39	39	...

[a] Values for soybean, peanut, cottonseed, and sunflower were taken from Bodwell and Hopkins (1985).
[b] Values shown are milligrams/gram of protein, unless otherwise noted ($N \times 6.25$).
[c] Recommended level for children of preschool age (2–5 years), although recently recommended for evaluation of dietary protein quality for all age groups except infants (Joint FAO/WHO Expert Consultation 1990).
[d] TEAA = total essential amino acids.
[e] TAA = total amino acids.

2.3.3 Tocopherols

Tocopherols and are fat-soluble compounds and fractions of vitamin E, which are identified by the prefixes α-, β-, γ- and δ-. They are compounds with different activities of vitamin E (Ryan et al., 2007). The isomer α- tocopherol is the most biologically active and it is related to the protection of unsaturated lipids present in the biological systems, since it is a lipophilic substance. These compounds are found only in the plants (Taipina et al., 2009; Yada et al., 2011; Pereira de Souza et al., 2013).

The numerous health benefits have been attributed to antioxidant compounds such as tocopherols. The presence of tocopherols in nut may reduce the risk of heart disease, type 2 diabetes, decrease the risk of certain types of cancer and also can reduce blood

cholesterol (Knekt et al., 1994; Kushi et al., 1996; Köksal et al., 2006; Yang et al., 2009; Pereira de Souza et al., 2013).

The most active antioxidants in *Plukenetia volubilis* lipids are γ-tocopherol and δ-tocopherol. Decreased in the order: γ > δ > β > α - tocopherol. As already stated, the greater amounts of γ- and δ- tocopherols with respect to α- could be attributed to their greater antioxidant capability. This makes the oil more stable to oxidation. From a nutritional point of view, intestinal absorption of γ- tocopherol is similar to that of α-tocopherol, and it could play a specific role in preventive side effects of some radicals like peroxynitrite and NO_x (Fanali et al., 2011).

Tocopherols α-, β-, γ- and δ- were detected in Sacha inchi seeds. The total tocopherol content fluctuated within the range 78.60-137.00 mg/100g seeds. γ - and δ - tocopherol were the predominant and the most important isomers in all examined cultivars of Sacha inchi representing 57.40–68.20% (56.80–81.40 mg/100 g of seed) and 30.90–40.30% (29.20–47.60 mg/100 g of seed) of the total tocopherol content, respectively and making it stable to oxidation. α - and β -tocopherols were within the range 1.13-1.25 and, 0.75–0.95 mg/100 g seed, respectively, representing less than 3 % of total tocopherols (Follegatti- Romeo et al., 2009; Chirinos et al., 2013; Pereira de Souza et al., 2013). However, the α- tocopherol is considered the most representative antioxidant in olive oil, while γ- and δ- tocopherols are found principally in seed oils like soybean and sunflower, and in particular, a large amount of γ-tocopherol is present in soybean oil (Fanali et al., 2011).

The total tocopherol content was higher than other oilseeds (Pereira de Souza et al., 2013). For comparison the total tocopherol content for flax is 9.3-14.3 mg/100g seeds. Also lower tocopherol β- (15.2 mg/100g seed) γ- (7.4 mg/100 g seed) and δ- (0.59 mg/100 g seed) have been reported for the Brazil nut (Costa et al., 2013). Sacha inchi seeds displayed higher contents of total tocopherols, β, δ and γ- tocopherols than other highly consumed nuts and cashew, hazel nut, peanut, pecans and pistachios (Kornsteiner et al., 2006; Chirinos et al., 2013).

2.3.4 Total phenolic content

Phenolics are a major phytochemical (all plant-derived chemicals) class and include the broad term 'polyphenols', meaning the molecule with one or more phenolic groups (Boilling et al., 2011). Polyphenols comprise over 8000 already identified substances and they can be divided into groups according to their chemical structure, such

as phenolic acids, stilbenes, coumarins, lignans and flavonoids (Ross and Kasum, 2002; Faller and Fialho, 2009). These plants compounds are considered to promote human health, since they are responsible for critical biological functions (Chirinos et al., 2013). The quality and quantity of the polyphenols present in plant food can vary significantly due to different factors, such as plant genetics and cultivars, soil composition and growing conditions, mature state, post-harvest conditions and others (Jaffery et al., 2003; Faller and Fialho, 2009). They can act as antioxidants in various ways. Thanks to its antioxidant properties, the polyphenolic content ensures the oxidative stability of polyunsaturated fatty acids imparting the characteristic flavor to the oil. Moreover, the effect of many of them on some common diseases such as hypertension, atherosclerosis, prevention of certain cancers, and modification of immune and inflammatory responses is notorious (Fanali et al., 2011)

The concentration of phenolics in *Plukenetia volubilis* was 6.20 mg/100 g of oil expressed as GAEs (Fanali et al., 2011). The level of phenolic compound for raw seeds was within the 64.60–80.00 mg GAE/100 g seed range. Lower values of TPC were reported for almonds, macadamias and pine nuts (32–47 mg GAE/100 g) compared to Sacha inchi seeds but higher values were reported for Brazilian nuts, cashews, hazelnuts, peanuts, pecans, pistachios and walnuts (from 112 to 1625 mg GAE/100 g) as well as for flax and safflower seeds (383 and 559 mg GAE/100 g (Kornsteiner et al., 2006; Bozan and Tenelli, 2008; John and Shahidi, 2010; Fanali et al., 2011)

2.3.5 Antioxidant capacity

In the case of foods it is necessary to determine the efficacy of natural antioxidants for food protection against oxidative damage, to avoid deleterious changes and loss of nutritional and commercial value (Halliwell et al., 1995; Halliwell, 1997; Sanchez-Moreno, 2002). In addition, measuring of radical scavenging activity can be used to select and determine to make a selection appropriate varieties among different species, maturation degree and culture conditions, in order to obtain high content of natural antioxidants in foods (Fogliano et al., 1999; Leonardi et al., 2000). Therefore, total antioxidant capacity of food product could be a parameter to evaluate its quality (Arnao et al., 1998).

In the case of biological systems, oxidative stress, an imbalance between reactive oxygen species and defence and repair antioxidant systems, has been shown to be

involved in the development of degenerative diseases. Hence, determination of the antioxidant status in biological systems could contribute to prevention and evaluation of diseases connected to aging. Moreover, to organism protection, the intake of dietary antioxidants and evaluation of the real contribution of foods to antioxidant status in biological systems must be evaluated (Namiki, 1990; Sánchez-Moreno, 2002).

Many different substrates, system compositions and analytical methods are employed in screening tests to evaluate the effectiveness of antioxidants, evidence that many different methods are necessary to evaluate different antioxidant effects. The methodology for evaluating natural antioxidants must be carefully interpreted according to the system and to the analytical method used to determine the extent and end-point of oxidation (Arnao et al., 1998; Fogliano et al., 1999; Frankel and Meyer, 2000).

Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate. After the substrate is oxidized under standard conditions, the extent of oxidation is measured by instrumental, chemical or sensory methods (Sánches-Moreno, 2002).

Antioxidant in foods and biological systems can be classifieed into those assays used to evaluate lipid peroxidation, in which a lipid or lipoprotein substrate under standard conditions is used and the degree of oxidation inhibition is measured and those assays used to measure free radical scavenging capacity (Sánchez-Moreno, 2002).

2.4 Thermal processing

2.4.1 Maillard reaction

Maillard reaction, also called glycation was for first time described in 1912 (Coghe, 2004). This reaction take a place in food stuff, within the thermal treatment and storage. Maillard reaction is a complex of non-enzymatic reactions subsequent to the reaction between reducing sugar (simple monosaccharides glucose, fructose and the disaccharide maltose) and amino compounds (predominantly the ε-amino group of lysine and the guanidine group of arginine) without catalytic actions of enzymes (Obšil and Pavliček, 1997; Coghe, 2004). The products of the Maillard reaction (MRPs) differ considerably. MRP can reduced solubility of proteins, darkening of colour, development of bitter flavors and reduced nutritional availability of certain amino acids such as lysine (Eskin., 1990). The rate of this reaction is influenced by the water activity, temperature and pH of the food product (Coghe, 2004).

Maillard reaction is subdivided into three main stages: early, intermediate, and late. In the early stage, glucose (or other reducing sugars such as fructose, pentoses, galactose, mannose, xylulose) react with a free amino group to form the Shiff base, unstable compounds. Then through acid-base catalysis, this labile compound undergoes a rearrangement to a more stable early glycation product known as Amadori product. Within the intermediate stage, Amordi products *via* oxidation, dehydratation, oxidation and other chemical reactions, degrades to a variety of reactive dicarbonyl compounds (such as glyoxal, methylglyoxal and deoxyglucosones). Dicarbonyl compounds are much more reactive than the initial sugars. This compounds act as propagators of the reaction, where again reacting with free amino groups of biomolecules. In the late stage irreversible compounds, the advanced glycation products (AGEs) are formed, through oxidation, dehydratation and cyclization reactions. The AGEs are yellow-brown, often fluorescent and insoluble adducts that accumulate on long-lived proteins and affecting their physiological functions (Obšil and Pavliček, 1997).

Maillard reaction was earlier investigated only for food browning associated with thermal treated and stored products, now take a part also as a problem in glycation of Western diet and connection with ability to prevent AGEs formation in medicine (Obšil and Pavliček, 1997; Lee et al., 2008). Glycation of proteins can distort molecular formations, change enzymatic activity, reducing degradation capacity, and interfering

with receptor recognition. Some plants important compounds such as phenolics, oligosaccharides and polysaccharides, carotenoids and unsaturated fatty acids possess anti-glycation activity (Lee et al., 2008). For example green coffee and tea consumption which is rich in phenolic compounds has high anti-glycation activity (Huang et al., 2008).

For food industry inhibition of non-enzymatic browning is a major problem. There are many ways to inhibit glycation, depending on the particular type of food. Refrigeration is effective, since browning has a high-temperature coefficient, the rate increasing 3-6 times with a 10°C rise in temperature. Most foods will not brown below -10°C during normal storage. Dehydration can be also effective in prevention of Maillard reaction browning. However, the rate of browning often reach a maximum with a moisture content of 5-30% (Coghe, 2004). Lowering the pH and ascorbic acid application will help to prevent initiation of non-enzymatic browning by its oxidation. Sulphur dioxide application, aspartic and glutamic acids application, oxygen excluding by packing under inert gas are also often used methods to prevent browning (Sapers et al., 2002).

Recently a big attention was given to the anti-glycation capacity of numerous medicinal herbs and dietary plants compounds. Except polyphenols which constitute a major group of plant derived compounds with anti-glycation activity, some amino acids, triterpens, saponins and polysacharids and oligosacharides were shown to decrease the AGEs activity (Huang et al., 2008). Plant compounds with anti-glycation activity have positive influence to many pathogenic reactions in many human metabolic disorders. The anti-glycation activity correlates with the phenolic content of the plant extract. The plant anti-glycation compounds are promising candidates not only for anti-browning food non-enzymatic action, but also they are considered by new mode of treatment of such diseases as: diabetes mellitus, other metabolic diseases (Schamberger and Labuza, 2006). It is reported that MRPs are benefit to increase the antioxidant activity of nuts oil and play an important role in improving oxidative stability (Durmaz et al., 2010; Cai et al., 2013).

2.4.2 Thermal processed seeds

Food materials are usually processed in order to improve palatability and reduce toxicity and as a means of preservation. Processing methods such as thermal processing, refrigeration, freezing, and fermentation have been applied to various food materials to achieve these purposes. Thermal or heat processing is one of the most important methods. During thermal processing anti-nutritional components and possible astringent off-flavours are reduced or eliminated. Thermal processing also has the major effect on the antioxidant activity (Lima et al., 2009). Heat processing such as roasting and boiling have dramatic effect on phenolic content of food stuff and thus on antioxidant activity (Turkmen et al., 2005; Lima et al., 2009; Ee et al., 2011). One of the main desired outcomes of roasting process in the increase in antioxidant activity that occurs mainly due to the formation of Maillard reaction products (Durmaz and Gökmen, 2011). However, heat processing also has a detrimental effect on the nutritional and functional properties of foods. It is reported that heat processing can increase or decrease level of amino acids (Arinola and Adesina, 2014; Udeonyia et al., 2014; Anyalogbu et al., 2015; Lin et al., 2016). Heat processing can cause damages of essential amino acids resulting in decreased content. It is therefore important that after processing there should be scientific evidence that nutritional and other useful properties are still of significant value and that anti-nutritional components have been reduced considerably (Arinola and Adesina, 2014; Udeonyia et al., 2014; Anyalogbu et al., 2015).

Recently, Cisteros (2014) published the study focused on chemical composition and antioxidant capacity of oil extracted from roasted seeds of *Plukenetia volubilis.* Some desired changes may bring benefit to the roasted oil. In food processing, roasting prior to oil extraction has been reported to improve the oxidative stability of a variety of oils, including mustard seeds, safflower, pistachio nuts, and rice germs (Kim et al., 2002; Lee et al. 2004; Wijesundera et al., 2008; Durmaz and Gökmen, 2011; Mariod et al., 2012; Cai et al., 2013). Sacha inchi oil is extracted commercially in Peru without previous roasting of seeds, however the roasting of safflower, sesame and canola seeds has been shown to increase the oxidative stability of the extracted oil. Roasting resulting in changes of kernels color and taste and the typical astringent taste of raw kernels disappeared. The results showed that roasting partially increased oil oxidation, but its antioxidant capacity

was increased. Roasting favored production of phenolic compounds and thus potent free radical scavenging abilities. The increasing roasting temperature increase the formation of phenolic compounds. Only γ- and δ- tocopherols were detected in Sacha inchi oil. Only γ- tocopherol content slightly decreased by roasting and no further decreases in content were detected by increasing roasting intensity. Roasting did not affect the oil fatty acid profile (Cisteros et al., 2014).

Another species belonging to the genus *Plukenetia* was examined after heat processing. *Plukenetia conophora* nuts are rich source of fat and proteins, as well as *Plukenetia volubilis*. Increase in boiling time progressively decreased the crude protein and most essential amino acids contents of African walnut (*Plukenetia conophora*) (Odoemelam, 2003; Arinola and Adesina, 2014; Anayalogbu et al., 2015). The effect of boiling and roasting reduce 30% of the protein content compared to the raw seeds. The reduction of protein content during the heat treatments may be due to denaturation or solubilisation of some nitrogenous compounds (Ijeh et al., 2010; Arinola and Adesina, 2014). Boiling also significantly reduced the antinutritional components which are known to reduce the bioavailability of nutrients in the body. This effect can be explain partly due to leaching into the cooking medium, degradation by heat and formation of insoluble complexes between other components (e.g. phytates, proteins, minerals). On the other hand, roasting generally led to increase the level of anti-nutritional components in the seeds (Arinola and Adesina, 2014). In case of African walnut boiling increased the antioxidant significantly, but the roasting cause decrease of antioxidant capacity (Arinola and Adesina, 2014).

Pine nuts (*Pinus gerandiana*), an international trade commodity, is another example of after heat-treatment changes in bioactive components in kernels and its oil. Pine nuts are great source of unsaturated fatty acids, which promote health benefits in human diet. Generally, pine nut kernels are consumed after being roasted to add flavor, change physical, chemical, and nutritional properties of the nuts. It was showed that oils obtained from roasted nuts are more resistant to oxidative deterioration. But excessive thermal processing will also bring about off flavor and deeply browning in nut oils. It this study they indicated the increase of antioxidant activity in first 30 min of roasting. After that, with increasing time that antioxidant activity decreased. The increase during first 30 min of roasting may be cause by increased phenolic compounds, remaining bioactive compounds (such tocopherols), formation of MRPs (Cai et al., 2013). Four types of

tocopherols were found in Pine nuts. γ- tocopherol was the major tocopherol isomer and α- tocopherol was the secondary isomer. During the roasting the tocopherol content slightly decreased during the increasing time of roasting. Results showed that total tocopherol content decrease about 6% after 60 min. of roasting compared to the raw pine nut kernels. Roasted nut oil showed 166-230% increase in total phenolic compounds, which was significantly higher than for raw kernels (Cai el al., 2013).

Pistacia (*Pistacia terebinthus*) in known to contain high level of phenolic compounds and tocopherols as well as high level of unsaturated fatty acids. After 5 min. of roasting (180°C) level of α- tocopherol clearly decreased compared to the unroasted oil sample. Within roasting time prolongation the level of α- tocopherol did not change significantly. γ- tocopherol was found to be secondary tocopherol isomer in pistacia oil. Higher roasting time (after 10 min.) significantly increase the level of γ- tocopherol. Furthermore, roasting cause clear increase in antioxidant capacity. Pistachio oil antioxidant activity gradually increased during roasting, reaching maximum in 20 min. The similar trend was observed for level of total phenolic content (Durmaz and Gökmen, 2011).

According to Schlörmann (2015), who tested the influence of roasting conditions for hazelnuts, almonds, macadamia nuts, pistachios and for walnuts, the lower or medium roasting (120-140°C) of nuts may be favourable, providing a balance between health-promoting and potentially harmful nut compounds.

3. Aim of the Thesis

According to the previous literature review there were no data about *Plukenetia volubilis* L. heat-treated seeds. However, heat processing is necessary for the seeds consumption. Based on this, the objective of my research was to determine the antioxidant capacity in six different types of traditionally thermal preparations of *Plukenetia volubilis* L. seeds. Levels of radical scavenging activity, total phenolic content and α-, β-, γ- , δ-tocopherols were measured for three types of boiling (boiled in water, boiled under pressure, boiled in water bath without touching water) and three different types of roasting (roasted at 125°C, 190°C and coated in honey and roasted for 170 °C) to describe and compare changes in antioxidant compounds with non-treated kernels during the different time of preparation.

4. Materials and Methods

4.1 Plant Material

Sacha inchi seeds were acquired from local producers on the market in Tarapoto region San Martín in Peruvian Amazon. Seeds were stored in a cool and dark place until further use. Seeds were shelled manually, using small hammer. Subsequently, after visual control decayed or damaged kernels were excluded. The peeled kernels were stored in plastic bags for maximum five days in a refrigerator (4°C) until thermal processing.

4.2 Thermal Processing

Three different roasting conditions and three different boiling conditions were used for thermal processing of Sacha inchi kernels. All processes were carried out in similar way of preparation, as it is commonly used by local consumers (personal communication). The whole duration of processing was in all variants long enough to clear the astringent off-flavour. Firstly, for each heat-treatment type the test preparation was done to set up the sustainable time intervals to avoid undesirable damages caused by high temperature over-exposure. In order to follow the changes in antioxidant properties samples were collected during the process (where possible) in intervals ranging from 5 min. to 200 min., depending on the concrete type of preparation. Collected samples were homogenized to fine powder, using standard laboratory grinder and stored in sealed colourless plastic bags at -24 °C until analysis.

Open boiling (OB). Whole seeds were boiled in excessive amount of distilled water (120 g of seeds in 1 l of water salted with 4 g of NaCl). The boiling system was open under atmospheric pressure that responds to the temperature of boiling water 100 °C. Samples were collected after 15, 30, 45, 60, 90, 120, and 135 min. For this type of preparation the water samples were also collected at the same intervals as seeds.

Pressure boiling (PB). Whole seeds were boiled in excessive amount of distilled water (120 g of seeds in 1 l of water salted with 4 g of NaCl) in pressure caserol constructed according to ČSN EN ISO 9001. The boiling system was airtight at 1.91 bar that responds to the temperature of water 119°C. The samples were collected at 0 time

and then after 120 min. For this type of preparation the water samples were also collected at the same intervals as seeds.

"Sous-vide" – water bath boiling (SV). Grounded seeds were boiled in open water bath at constant mixing. No direct contact with water was allowed. Samples were collected after 15, 30, 45, 60, 90, 120, and 135 min.

Low temperature roasting (LTR). Whole seeds were roasted at 125 °C in a dry oven for 200 min. Samples were collected at following intervals 10, 20, 40, 80, 120, 160, and 200 min.

High temperature roasting (HTR). Whole seeds were roasted on open tray at 190°C in a dry oven for 35 min. Samples were collected after 5, 10, 15, 20, 25, 30, and 35 min.

Honey roasting (HR). Whole seeds were roasted with honey based on method from US patent no. US4161545A, but simplified. Briefly, dry seeds were coated with 80% honey diluted in distilled water. Seeds were immersed in 80% honey for 5 min., afterwards seeds were retained for 5 min. on strainer. The average weight of coating was 23 g of honey solution per 100 g of seeds. Coated seeds were roasted in the dry oven at 170°C. Samples were collected after 5, 10, 15, 20, 25, 30, and 35 min.

4.3 Chemical Analysis

Tocopherol content

Samples were extracted by hexan after discontinuing alkaline extraction according to Delgado-Zamarreño et al. (2001). 5 ml of aqueous 10% ascorbic acid, 10 ml of 80% KOH, 25 ml of EtOH, 4.5 ml of distilled water was add to 2g of sample and protected from light by aluminium foil. The samples were shaking for 2.5 hour in water bath (40°C). The analytes were extracted with hexan (2 × 20 ml). Samples were manually shaken after each extraction. The hexan extracts were connected and evaporated using vacuum rotary evaporator set up for 50 °C to remove the organic phase. Afterwards, 1 ml MeOH was added to analytes residues and mixed on vortex. Standards of α- , γ-, δ- tocopherols in

concentration 35 mg/ml were diluted with MeOH to 1 mg/ml. Subsequently the 1 mg/ml was diluted to 500, 250, 125, 62.5, 12.5 μg/ml for calibration line. The tocopherols were determined using HPLC separation on Waters 2695 Separations Module Alliance (Waters, USA) on reverse phase (Lichrospher C18 250×4@4μm, Merck GmBH, Germany) and detected with fluorescence detector Waters 474 (λ (excitation) = 285 nm, λ (emission) = 330 nm). The octadecyl silica used as a stationary phase in chromatographic separation is not able to separate β- and γ- isomer. Thus the tocopherol content is present as α-tocopherol and δ-tocopherol separately whereas the content of β-tocopherol and γ-tocopherol is presented as a sum of the two and expressed in γ-tocopherol equivalents (γ-tE).

Folin assay

Total phenolic content (TPC) was determined spectrophotometrically with the Folin-Ciocalteau reagent. 2 g of sample were extracted with 20 ml of 80% methanol (MeOH) in a centrifugation tube. Tubes were protected from sunlight by aluminium foil and put on the laboratory shaker for 60 minutes. Afterwards, 5 min on the laboratory centrifuge, speed 5000 RPM. Metabolic extract was separated. Remained pallet was homogenized using vortex, and all action were carried out again (10 ml 80% methanol, 1 hour shaking, 5 min. centrifuged). Second metabolic extract (supernatant) was transferred and connected with the first collected supernatant. 0.5 ml of the extract was pipetted into 50 ml volumetric flask and diluted with distilled water. Then 2.5 ml Folin-Ciocalteau reagent (PENTA, Czech Republic) and 7.5 ml 20% sodium carbonate solution was added after agitation. After 2 h standing in dark at laboratory temperature, absorbance at wave length λ = 765 nm on the spectrophotometer Genesys 10UV (Thermo Scientific, USA) was measured against blank. 500, 250, 100, 50, 25, 10, 5, 1 μg/ml gallic acid concentration were used as a standards for calibration. Results were quantified using gallic acid (Merck, Germany) and expressed as gallic acid equivalents (GAE).

DPPH Assay

Free radical scavenging assay. The radical scavenging capacity (RSC) was determined on microtiter plates in methanol extracts using the stable radical DPPH·(2,2-diphenyl-1-picrylhydrazyl). 20 ml of methanol was added to 1 g of sample and shaked for 90 minutes (200 RPM) while protected from light by aluminium foil. Afterwards, the samples were filtrated through laboratory filter papers. 0.025 g DPPH was diluted with 100 ml MeOH. For DPPH solubility the ultrasound was used. Subsequently the basic DPPH solution was diluted 10 times. The sample and DPPH transformation on the microtiters has to be done in short intervals. On microtiters 20 μl of extract reacted for 10 min with 150 μl DPPH solution with initial absorbance A = 0.6 at 550 nm. The absorbance balance 0.6 was set up by adding MeOH or by adding basic DPPH solution. The reaction occurred in the dark, and the absorbance at 550 nm was read afterwards using a spectrophotometer (Sunrise absorbance reader, Tecan, Switzerland). The ability to scavenge the DPPH radical was determined using the standard curve obtained with Trolox (Sigma Aldrich, Germany) in the range from 0.0 to 0.2 mmol/l. The results were expressed as Trolox equivalent (TE) antioxidant capacity. The ascorbic acid was used for detoxication solution.

4.4 Statistical Analysis

All the experiments were performed in triplicate repetitions. The results are expressed as mean value ± SD (standard deviation). All statistical analyses were carried out using software Statistica 12.0 CZ. In order to investigate the significant differences in levels of antioxidant capacities and total phenolic contents and levels of tocopherols between examined samples was applied factorial analysis ANOVA (the analysis of variance) and subsequently Tukey´s Honest Significant Difference (HSD) test was performed at $p \leq 0.05$. Correlation analysis was applied between measured parameters (DPPH, α- , β- , γ- , δ- tocopherols), type of processing (OB, PB, SV, LTR, HTR and HR) and time of processing. For graphical expression (graphs, tables) of results was used software Microsoft Excel 2013.

5. Results

Six different type of heat processed seeds (OB, PB, SV, LTR, HTR, HR) were evaluated for its total radical scavenging activity, total phenolic content and for α-, β-, γ-, δ- tocopherol levels. All final changes in antioxidant levels are shown in tab. 3. Because of different time of collecting the samples during the heat process, the results are expressed as mean values ± SD. Therefore, the process flow of the changes in antioxidant levels for diverse time are shown in fig.5, fig. 6, fig.7, fig.8.

Thermal treatments influenced sensorial characteristic of the Sacha inchi kernels. The varying degree of temperature and time of exposure resulting in the changes kernels colour and taste. After roasting and boiling procedure the undesirable astringent bitter taste was completely eliminated and the colour vary from creamy yellow/light brown to dark brown (Appendix 1).

Within open boiled and pressure boiled method the water samples were also collected and measured for its antioxidant properties. The water after pressure boiling with Sacha inchi kernels does not gain significant antioxidant properties. However in the case of water (open boiled) we detected increased in the level of radical scavenging activity and TPC after 90 min. of processing. The level of TPC and RSC was increasing by time, in 135 min. the level of RSC and TPC reached 91.5 mmol TE/100 g and 21.2 mg/100 g GAE (fig. 5, fig. 6, fig. 7, fig. 8.)

Tocopherols

Four types of tocopherols are found in *Plukenetia volubilis* thermal treated seeds. The major tocopherols isomers are β + γ- tocopherols with the concentration of 41.40 ± 3.3 mg γ-tE/100 g for raw seeds. β + γ- tocopherols were thermal sensitive. All types of thermal processing cause decline of β + γ- tocopherols, however slight fluctuation within the sample collecting time was observed (fig. 5). The level of β + γ- tocopherols in thermally processed kernels is ranging from 9.04 ± 0.64 to 28.99 ± 2.19 mg γ-tE/100 g. The biggest losses in β + γ- tocopherol contents were observed for low temperature roasted (125 °C) and for high temperature roasted (190 °C) kernels compared to the raw kernels. The levels decreased to 9.04 ± 0.64 mg/100 g (78.16 %) and 9.33 ± 2.77 mg /100 g (77.46 %), respectively. The pressure boiled method caused the lowest losses in the β + γ- tocopherols content, 31.20 ± 1.60 mg γ-tE/100 g (24.64 %). Followed by sous-vide

(water bath boiling) and open boiled method, 28.99 ± 2.19 mg γ-tE/100 g (28.99 %) and 26.47 ± 1.45 (36.06 %), respectively.

Raw kernels with honey contain 40.60 ± 0.00 mg γ-tE/100 g. After roasting (170°C), β + γ- tocopherols decreased to 20.28 ± 0.89 mg γ-tE/100g, which means 50.05% loss in the level of β + γ- tocopherols.

It is observed that the overall influence of thermal processing for β + γ- tocopherols decrease about 49.55 % in compared to the raw kernels.

The δ- tocopherols was found to be the secondary tocopherol isomer in *Plukenetia volubilis* seeds. δ- isomer is significantly less thermal sensitive compared to the β + γ- tocopherols. The overall influence of processing showed that the δ- tocopherol level just in 0.87 % decline. Raw kernels contain δ - tocopherols with the concentration of 25.80 ± 0.80 mg δ-tE/100 g of seeds. Among low temperature roasted (125 °C), high temperature roasted (190 °C) and Sous-vide (water bath boiling) procedures the significant losses were showed compared to the raw seeds. The levels decreased to 17.99 ± 0.92 mg δ-tE/100 g (30.27 %), 18.32 ± 0.27 mg/100 g (28.99 %), 22.52 ± 0.72 mg δ-tE/100 g (12.71 %), respectively. Pressure boiled and open boiled procedure cause increase in level of δ-tocopherols with values 29.67 ± 1.88 mg δ-tE/100 g (15.00 %), 27.18 ± 0.38 mg δ-tE/100 g (5.35 %), respectively.

Raw kernels with honey contain 21.66 ± 3.90 mg δ-tE/100 g. After roasting (170 °C), δ- tocopherols decreased to 19.70 ± 0.44 mg/100g, which means 9.05 % loss in the level of δ- tocopherols. Change of δ– tocopherol on Sacha inchi kernels during different processing are shown in fig.6.

α- tocopherol was detected in the concentration less than 5 mg/100 g in raw seeds in each sample and measurement.

Total phenolic content

The effect of thermal processing methods on polyphenol content is shown in tab. 3.

It is observed that the overall influence of processing significantly increased the level of TPC. The TPC concentration of raw kernels 16.70 ± 2.70 mg/100 g GAE increased for thermally processed kernels to 42.57 ± 31.52 mg/100 g GAE (255.69%). The most significant TPC change occurred in honey roasted seeds. Raw kernels with honey contain 21.50 ± 0.00 mg/100 g GAE. After roasting (170°C), TPC level increased

to 102.70 ± 9.64 mg/100g, which means 477.67 % increase in the level of TPC. Noticeable change in level of TPC occurred after 20 min. of roasting (170°C). The levels of TPC highly increased till 30 min. of roasting. The TPC level fluctuations within time are not so distincted compared to these in tocopherols levels (fig. 7).

High temperature roasted (190°C) was the second method with the highest increase of TPC level with concentration 55.73 ± 7.90 mg/100 g GAE (333.71 %) compared to the raw kernels. Significant change in TPC was also observed through PB procedure. The TPC level increased to concentration 40.87 ± 1.97 mg/100 g GAE (144.73 %). Open boiling procedure slightly decreased the level of TPC 16.00 ± 0.35 mg/100 g GAE (4.19 %). Change of TPC on Sacha inchi kernels during different processing are shown in fig.7.

The radical scavenging capacity

The DPPH radical scavenging activity concentration for raw kernels is 241.30 ± 16.50 mmol TE/100 g. According to the overall influence of processing values the heat treatment negatively influenced the antioxidant capacity of *Plukenetia volubilis*. The overall antioxidant capacity concentration is 222.08 ± 4.65 mmol TE/100 g (7.97 %) compared to the raw kernels.

However, sous-vide (water bath boiling) and pressure boiling slightly increased the level of antioxidant capacity with concentrations 256.60 ± 4.83 mmol TE/100 g (6.34 %) and 241.67 ± 17.10 mmol TE/100 g (0.15 %). The most significant decline was observed through low temperature roasted (125 °C) procedure. The antioxidant capacity decreased to 186.40 ± 15.50 mmol TE/100 g (22.75 %). The high temperature roasting (190 °C) and open boiled procedure cause slightly decrease of antioxidant levels in concentrations 217.63 ± 7.18 mmol TE/100 g (9.81 %) and 217.70 ± 17.29 mmol TE/100 g (9.78 %), respectively. Raw kernels with honey contain 243.20 ± 0.00 mmol TE/100 g. After roasting (170 °C), antioxidant capacity decreased to 212.47 ± 31.64 mmol TE/100 g, which means 9.28 % loss in the antioxidant capacity. Change of RSC on Sacha inchi kernels during different processing are shown in fig.8.

Tab.3. Effect of thermal different thermal processing on TPC, β + γ + δ- tocopherols, RSC on Sacha inchi kernels

		TPC (mg/100 g GAE)	Gain/ loss of TPC (%)	α- tocopherol (mg/100g)	β + γ- tocopherol (mg γ-tE/100g)	Gain/ loss of β + γ- tocopherol (%)	δ- tocopherol (mg/100g)	Gain/ loss of δ- tocopherol (%)	RSC (mmol TE/100g)	Gain/ loss of RSC (%)
Unprocessed kernels*	Raw kernels	16.70 ± 2.70a		< 5	41.40 ± 3.3d		25.80 ± 0.80b		241.30 ± 16.50b	
	Raw kernels with honey	21.50 ± 0.00a		< 5	40.60 ± 0.00d		21.66 ± 3.90b		243.20 ± 0.00b	
Thermally processed kernels*	Open boiled	16.00 ± 0.35a	-4.19	< 5	26.47 ± 1.45bc	-63.94	27.18 ± 0.38bc	+5.35	217.70 ± 17.29ab	-9.78
	Pressure boiled	40.87 ± 1.97b	+144.73	< 5	31.20 ± 1.60c	-24.64	29.67 ± 1.88c	+15.00	241.67 ± 17.10b	+0.15
	Sous-vide (water bath boiling)	17.43 ± 1.01a	+4.37	< 5	28.99 ± 2.19c	-29.98	22.52 ± 0.72b	-12.71	256.60 ± 4.83b	+6.34
	Low temperature roasted (125 °C)	22.70 ± 0.40 a	+35.73	< 5	9.04 ± 0.64a	-78.16	17.99 ± 0.92a	-30.27	186.40 ± 15.50a	-22.75
	High temperature roasted (190 °C)	55.73 ± 7.90c	+333.71	< 5	9.33 ± 2.77a	-77.46	18.32 ± 0.27a	-28.99	217.63 ± 7.18ab	-9.81
	Honey roasted (170°C)	102.70 ± 9.64b	+477.67	< 5	20.28 ± 0.89b	-50.05	19.70 ± 0.44a	-9.05	212. 47 ± 31.64ab	-9.28
Overall influence of processing*		16.70 ± 2.70a		< 5	41.40 ± 3.30d		25.80 ± 0.80b		241.30 ± 16.50b	
		42.57 ± 31.52 b	+255.69	< 5	20.89 ± 9.29a	-49.55	22.57 ± 4.65a	-0.87	222.08 ± 27.39ab	-7.97

*Different letters in columns denote statistically significant difference (as defined by ANOVA test and HSD post hoc test at $p \geq 0.05$). Overall influence of processing is evaluated separately. Gains are indicated with +, losses are indicated with -

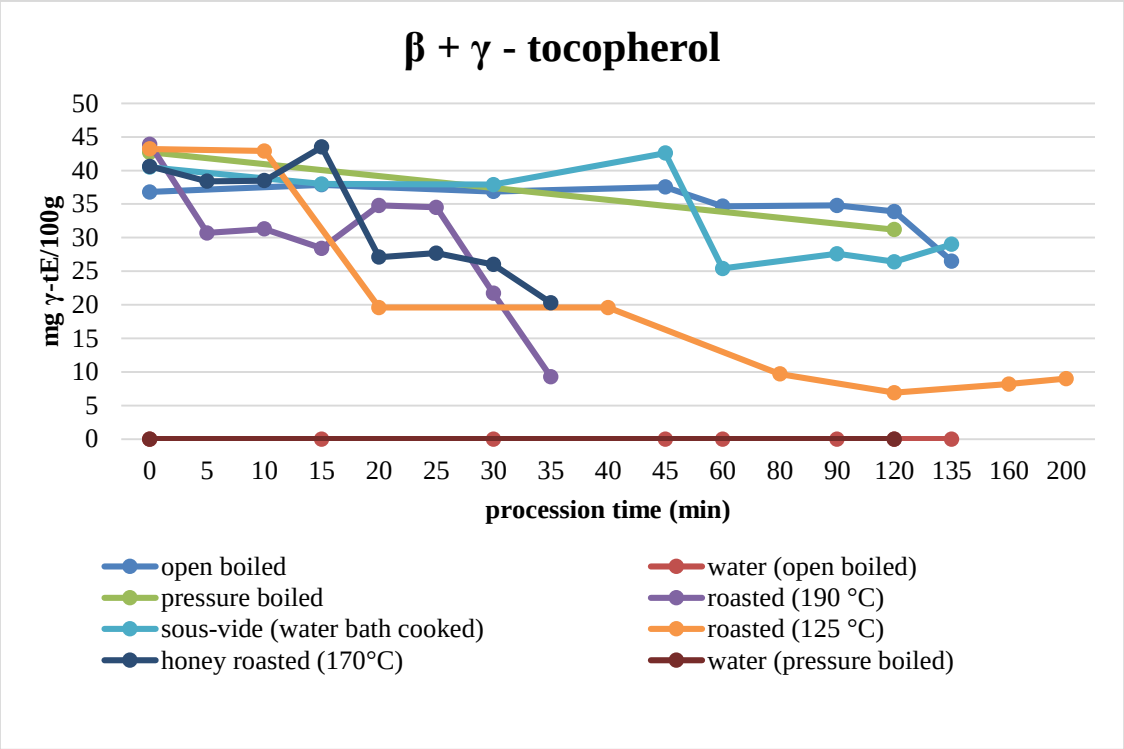

Fig. 5. Change of β + γ – tocopherol on Sacha inchi kernels during different processing

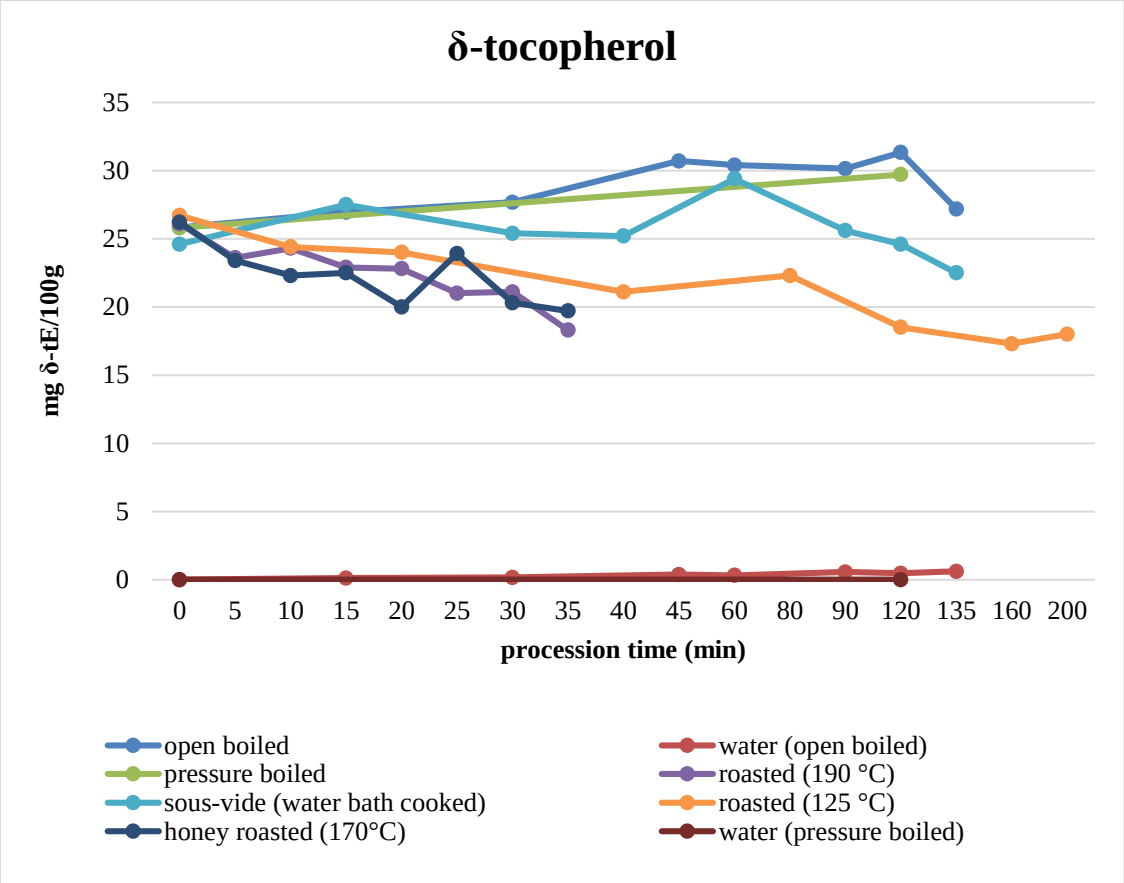

Fig. 6. Change of δ– tocopherol on Sacha inchi kernels during different processing.

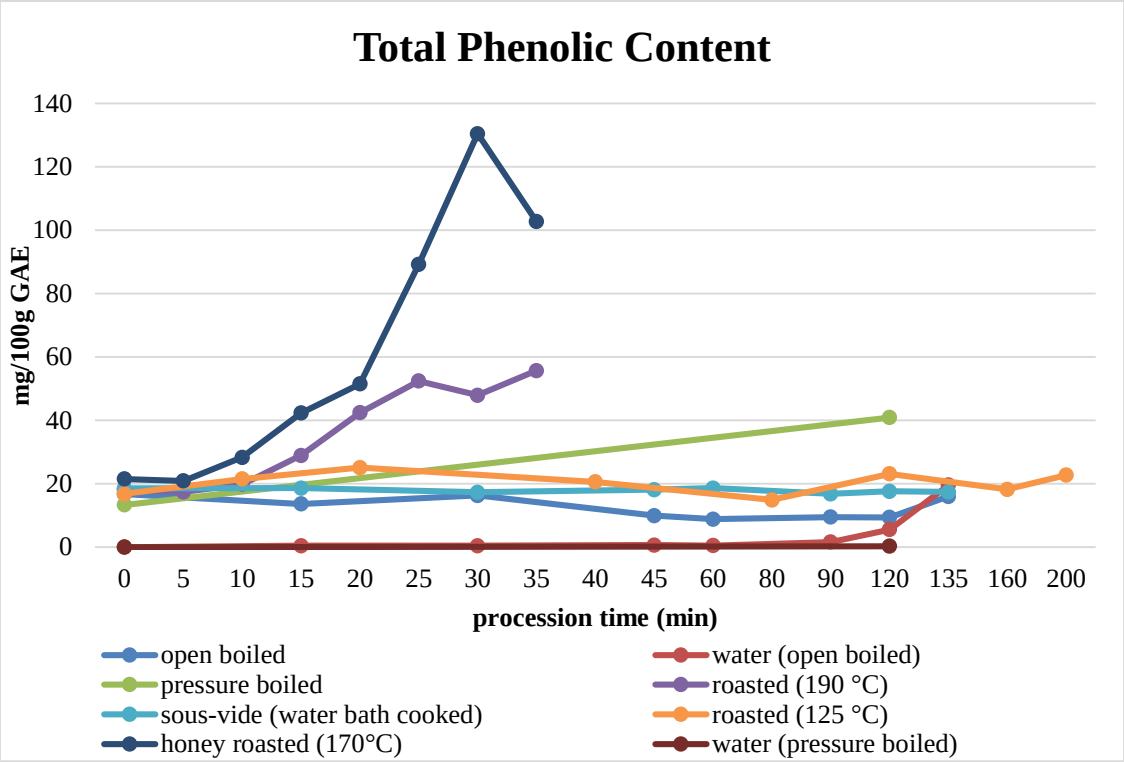

Fig. 7. Change of TPC on Sacha inchi kernels during different processing

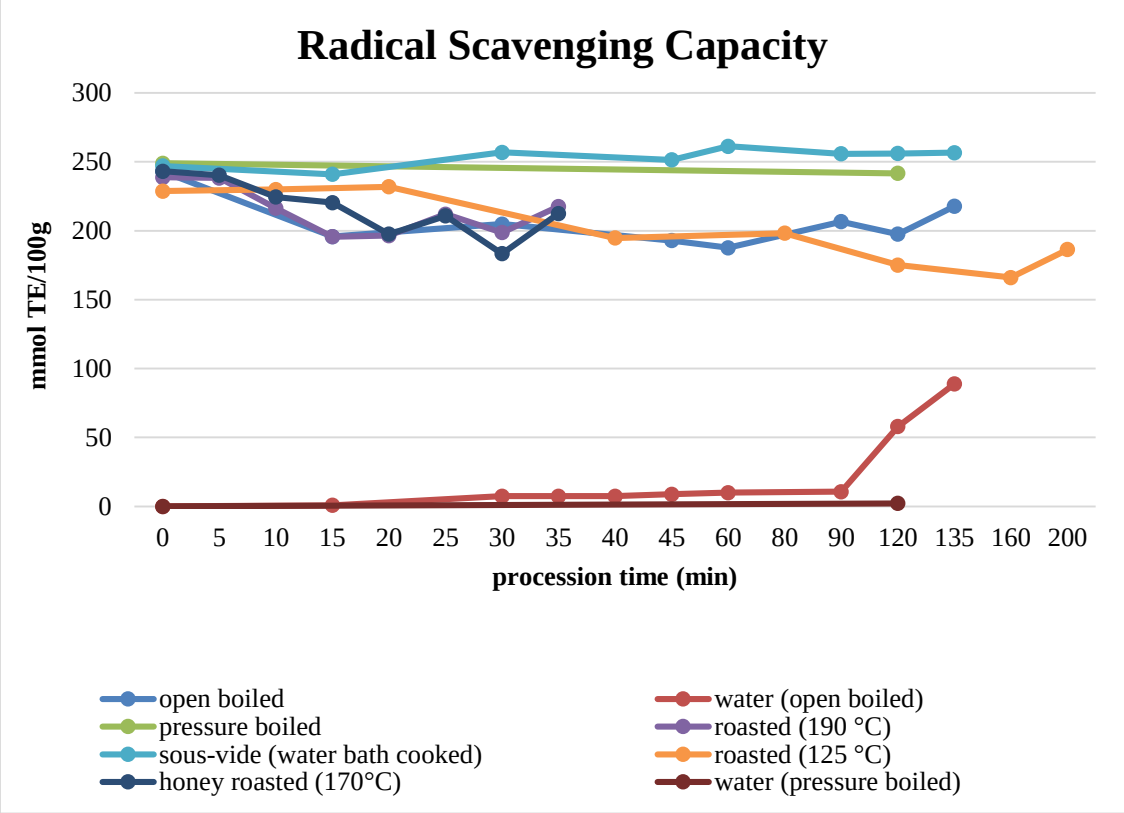

Fig. 8. Change of RSC on Sacha inchi kernels during different processing

6. Discussion

Previous reported studies about *Plukenetia volubilis* have concentrated on the chemical and functional characterization of the oil and much less on the entire seed. According to the previous literature review, focus on oil extracted from oil seeds in much more higher than for raw seeds in general.

Plukenetia volubilis seeds are an excellent source of macronutrients and bioactive compounds. One of the major problem associated with *Plukenetia volubilis* L. is beany taste and its potential oxidative instability due to the highly unsaturated nature of its oil (Cisneros et al., 2014). It is reported that thermal treatment may remove the unpleasant taste and change visual appearance (Lima et al., 2009). According to Cisneros et al. (2014) results, the typical astringent flavour disappeared after roasting of Sacha inchi kernels. The colour changed from cream to light/dark brown. This results corresponding with our observation. Worth to mention that Gonzales (2014) reported, Sacha inchi oil consumption is safe for oral consumption, without side effects. However, according to Buso et al. (2010) Sacha inchi can cause allergy reaction for susceptible person.

Many factors can affect differences between similar studies. Phytochemical content is environmental stress, infection, UV light can affect results. Some particular phytochemicals can be affected by processing (e.g. heat treatment as roasting and boiling) and storage (duration and temperature) (Bolling et al., 2011; Cisteros et al., 2014). Differences can be also attributed to different subspecies, geographical distribution, climate and growing conditions, maturity state, soil composition, harvest time, agricultural practices and quantitative method of the analysis (Faller and Fialho, 2009; Cai et al., 2011; Cai et al., 2012; Chirinos et al., 2013).

Before the heat treatment, the level of total phenolic content, α, β, γ and δ–tocopherols and radical scavenging capacity for raw kernels was evaluated. Our results are generally in agreement with similar studies. According to Chirinos et al. (2013), the most important tocopherols contained in raw kernels of *Plukenetia volubilis* were γ- and δ– tocopherols. The level of γ- tocopherol range was 56.8-81.4 mg/100 g seeds, for δ–tocopherol the range was lower, 29.20-47.60 mg/100 g seeds. The level of α- tocopherol was in range 1.13-1.25 mg/100 g. β- tocopherol was detected in the in the lowest level, 0.75-0.95 mg/100 g seeds. Folligatti-Romero et al. (2009) evaluated γ- and δ- tocopherols levels, 61.90 mg/100 g seeds and 67.80 mg/100 g seeds, respectively. Compared to our

results, where β + γ- tocopherols (41.40 mg/100g seeds) were the predominant isomers, followed by δ– tocopherol (25.80 mg/100 g seeds). The antioxidant activity of tocopherols in lipid follows this order: γ > δ > α > β, thus *Plukenetia volubilis* possesses antioxidant protection factors for seeds and oils rich in unsaturated fatty acids. Particularly, α-linolenic fatty acid could be associated with γ-, δ- tocopherol synthesis as a defense mechanism against oxidative processes (Bozan and Tenelli, 2008; Chirinos et al., 2013). All of thermal treatment of *Plukenetia volubilis* kernels caused significantly losses in level of β + γ- tocopherols. The low temperature roasting caused the biggest losses (78.16%), closely followed by high temperature roasting (77.45%), which indicated that increasing of temperature (125 °C – 190 °C) do not increase the level of β + γ- tocopherols losses. The pressure boiled treatment caused just 24.66% losses compared to the raw kernels. δ- tocopherol is significantly less thermal sensitive compared to the β + γ- tocopherols. Within the roasting methods were indicated losses ranging from 9.05% to 30.27%. Within two boiling methods (open boiled and pressure boiled) the level of δ- tocopherol increased. Cisneros et al. (2014) detected only γ- and δ- tocopherol in the Sacha inchi oil, where only γ- tocopherol was slightly affected by roasting, the level of δ- tocopherol was not affected. Cai et al. (2013) tested roasted pine nut seeds oil (*Pinus gerardiana*), oilseed with characteristics similar to the Sacha inchi. Also for pine nuts γ- tocopherol is the major isomer, followed by α- tocopherol. The level of tocopherols decreased during the roasting. γ- tocopherol showed slight fluctuation and the high stability which is attributed to its higher bond dissociation energy for O-H, compared to the γ- tocopherol. Decreasing of tocopherol levels within roasting with different temperatures was also reported in pistachio nut oil (Durmaz et al., 2011), argan kernels (Harhbar et al., 2011), sesame seeds (Yoshida and Takagi, 1997), hazelnut, walnut, almonds (Schlörmann et al., 2015) and for apricot kernels (Durmaz et al., 2010). Generally, the losses in tocopherols levels are attributed to the thermal degradation at high temperature or microstructural changes in kernels. On the other hand increase in the tocopherols levels was reported for safflower oil (Lee et al., 2004) and rice germ oil (Kim et al., 2002). Those different results imply that roasting may affects tocopherol in different ways, depending on degree of temperature, type of roasting, time of roasting, seed variety etc. Increasing of tocopherol is generally attributed to the increase in extractability of tocopherols by the thermal degradation of cellular structures (Kim et al., 2002; Durmaz and Gökmen, 2011; Cai et al., 2013).

Total phenolic content was significantly affected by thermal treatments. According to Cisneros et al. (2014), total phenol values in Sacha inchi kernels increased with roasting intensity, which corresponding with our results. The level of TPC slightly decreased within open boiled treatment, however within others boiling and roasting treatments the levels of TPCs significantly increase to 255.69% in average compared to the raw Sacha inchi kernels. In our study statistical significant differences were indicated between individual treatments. Within SV method the level of TPC increased slightly, only 4.37%, followed by pressure boiled method 144.73%. The increased of TPC level was much more dramatic within roasting methods. In HR and HTR the level of TPC increased for 477.67% and 337.71%, respectively. On the other hand within LTR the level of TPC increased only for 35% compared to the raw kernels. Worth to notice that combination kernels coated with honey showed the biggest increased in TPC after 20 min. of roasting in 170°C. We assumed that the increasing temperature can increase the level of TPC and roasting method in general, considerably increased the level of TPC compared to the boiling methods. For comparison within the pine nut oil roasting the level of TPC progressively increased to 166% and 230%. Also the similar trend was observed for pistachio and pumpkin seeds (Durmaz, Gökmen, 2011; Cai et al., 2013). Ee et al. (2011) reported the remarkable increase of TPC within roasting in time for wattle (*Acacia victoriae* Bentham). The TPC after 30 min. of roasting was 10 times higher than in raw seeds. It can be explain by partially destroyed cell structures of the kernels, resulting in the release of some of the phenolics bounds to the cell walls. The thermal treatment induces the Maillard reaction, resulting to the production of many compounds which can exhibit antioxidant activity (Ee et al., 2011). Different result for hazelnut (*Corylus avellana* L.) was reposted by Pelvan et al. (2012) and Schmitzer et al. (2011). In this case the skin was removed before roasting, which cause the decreasing trend in TPC level. The decrease of TPC could be also due to the thermal degradation of phenolic compounds at high temperature and long-time exposure of heat (Cai et al., 2013).

The correlation between antioxidant capacity and phenolic content is observed (Dent et al., 2013). Total antioxidant capacity reflects presence of naturally occurring and neo-formed antioxidants. Cisneros et al. (2014) reported that roasting increase the antioxidant capacity with roasting intensity which can be cause due to the formation of phenolic compound with more potent antioxidant capacity. However, MRPs which are created during thermal treatment also possess antioxidant activity. These results differ from our

results, which showed decrease in radical scavenging activity, 7.97% in average. Durmaz and Gökmen (2011) correlated with results Cisneros et al. (2014). A clear increased in antioxidant activity was detected. Consideration of all the thermal treatments of *Plukenetia volubilis*, only within pressure boiled and sous vide boiled the level of radical scavenging activity slightly increased for 0.15% and 6.34% compared to the raw kernels. Antioxidant with different chemical characteristics may act synergistically with one another in the antioxidant network (Alasalvar and Shahidi, 2009). The increasing of antioxidant capacity can be due to neo-formed MRPs and the remaining bioactive components, such as tocopherols. MRPs acting as antioxidants in oil, can also improve the thermal stability of tocopherols. Phenolic compounds were reported to pass into the oil phase better if roasting lead to the damage of cell structures and increase the antioxidant capacity (Cai et al., 2013). However, within LTR of *Plukenetia volubilis* kernels the biggest losses (22.75%) were noticed.

Changes in time

It is also interesting to observe how the levels of antioxidant compounds were changing in the length of exposure in different time. In the case of water (open boiled) we detected increased in the level of RSC and TPC after 90 min. of processing. The level of TPC and RSC was increasing by time, in 135 min. Correlation between TPC and RCA was previously reported (Deng et al., 2013). We can assumed that 90 min. of boiling was enough to disrupt the cell structures and the antioxidants compounds leached to the water. Our final time for open boiling procedure was set up for 135 min., but according to the increasing tendency of TPC and RSC we can estimate further increasing in mentioned levels and possible reach of the significant values compared to the treated kernels.

The open water boiled kernels level of TPC and RSC slightly decrease within time, without big fluctuations. Open boiling method increased the level of δ- tocopherol, especially after 120 min. of treatment. Through pressure boiling, the level of TPC, RSC and δ- tocopherol enhanced within 120 min. Thermal sensitive β + γ- tocopherol content slightly decreased. Within SV treatment the β + γ- tocopherol level reached maximum in 45 min., after that we noticed steep decrease in level of β + γ- tocopherols. Level of δ- tocopherol reached the maximum in 45 min as well, after that the similar trend in decreasing was observed. SV method slightly increased the level of TPC and RSC without significant fluctuations in time. It can be assumed that 45 min. of SV exposed treatment

can enhanced TPC, RSA, β + γ and δ- tocopherols levels. After 45 min. we losses in tocopherols were observed.

Within LTR and HTR the level of TCP was increasing with increasing of time. Especially HTR enhanced the level of TPC significantly with reaching the maximum level in 35 min. We suppose that the higher temperature enhanced the formation of MRPs and thus the level of TPC. HTR cause big losses in level of β + γ- tocopherols immediately, after 5 min. of roasting. The similar trend was observed for level of δ-tocopherols within LTR, in 10 min. of roasting. In case of δ- tocopherols the losses were not so dramatic compared to the β + γ- tocopherols. Within LTR and HTR we observed the decrease in RSA. This results are not in agreement with the statement that TPC correlate with RSA.

In general, we can observe that the honey enhanced the level of TPC and RSC compared to the raw kernels. HR reached the maximum level of TPC in 30 min of roasting. Worth to notice that in 30 min. the level of TPC increase in 478%. After that the level of TPC started to decrease. The level of β + γ- tocopherols increasing in first 15 min. of roasting, then steeply decreased. In δ- tocopherols we observed increasing and decreasing fluctuations in time with decreasing tendency. Compared to other thermal treatment the honey decreased the losses in β + γ- and δ- tocopherol. The similar trend was observed for level of RSA within honey roasting.

7. Conclusion

As summarized, roasting treatments cause significantly losses in β + γ- and δ- tocopherol and RSA, however significantly increase the TPC level. Combination kernel with honey enhanced the TPC, moreover decreased the tocopherol losses within thermal treatments. Within the roasting methods, the antioxidants level was affected in short time and only TPC was significantly increased.

The boiling method are gentle to the changes of *Plukenetia volubilis* antioxidant properties compared to antioxidant levels in the raw kernels and roasting treatments. Open boiling treatments do not cause big losses in antioxidant levels and no significant increasing in antioxidant levels was observed. Although the astringent taste was removed. Pressure boiling treatment enhance the level of TPC, RSA and δ- tocopherol. Sous-vide, 25 min. of treatment enhanced TPC, RSA, β + γ- and δ- tocopherol and we can recommend it as the gentlest method for the Sacha inchi kernels compared to other thermal treatments. We consider pressure boiling and sous-vide method as the most suitable methods for thermal treatment of Sacha inchi to improve nutritional values.

The results of this study indicate that appropriate thermal treatment and suitable time can improve the antioxidant activity and thus nutritional values of *Plukenetia volubilis* kernels.

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9. Appendices

9.1 Photographic illustration of Sacha inchi

Sacha inchi plant

Sacha inchi leafs

Sacha inchi 4-lobed harvested fruit


Sacha inchi seeds

Sacha inchi kernels

Sacha inchi kernels after HTR in 0, 5, 10, 15, 20, 25, 30, 35 min.