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Nitrogen balance after a single oral consumption of Sacha Inchi (*Plukenetia volúbilis* L) protein compared to soy protein. A randomized study in humans

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Abstract

Sacha inchi is a nut produced in the Peruvian Amazonian and its oil is recognized by the lowering lipids effect in humans. The remaining material transformed to flour has a higher amount of protein, but, the nitrogen balance once ingested orally has not been studied. The present study was designed to evaluate the nitrogen balance after single consumption of 30 g of sachá inchi flour and compared with that obtained after consumption of 30 g soybean flour in adult men and women. This was a double-blind cohort study in 15 men and 15 women between 18-55 years old. Fifteen subjects received soy meal and fifteen subjects received sachá inchi meal. Group receiving sachá inchi flour has comparable initial parameters as those receiving soybean flour ($p > 0.05$). Blood samples at different times were obtained. Urine for 24 hours was collected to calculate nitrogen balance, $p < 0.05$ was considered significant. Plasma insulin levels increased post-prandial with a peak at 30 minutes. Thereafter, a reduction occurred. The magnitude of changes in insulin levels were similar in sachá inchi and soybean groups ($p < 0.05$). Lipid profile and inflammatory marker, C-reactive protein (CRP) and interleukin 6 (IL6) was not different at 0 or 24 h after sachá inchi or soy flour administration. The nitrogen balance was negative in the study but similar between both groups ($p > 0.05$). In conclusion, protein consumption of sachá inchi flour has the same nitrogen balance as soybean flour, shows acceptability for a single consumption and does not present serious adverse effects.

Keywords

Sacha inchi flour; soybean flour; nitrogen balance; acceptability; insulin

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Introduction

Protein-calorie malnutrition is still a problem of public health affecting many developing nations in the world (Nisar et al, 2016). Proteins are important in nutrition since they participate in growth and development and their amino acid are used to produce enzymes, hormones, neuropeptides, immunoglobulins, receptors, and transporters. Protein in the organism is important since contribute to muscle function (Loenneke et al, 2016; Sahni et al, 2015) and in general health status (Gregorio et al, 2014).

Protein in diet may be obtained from vegetable and animal sources. There are studies, which compare efficacy or risk of diseases by consumption of one or another source of protein. There is evidence that protein from plant origin may decrease the risk of cardiovascular disease (Richter et al, 2015).

Actually, there is a trend in the world to reduce red meat from diet and increasing the use of protein from vegetable source (van Vliet et al, 2015). Proteins from both sources have the same effect on appetite, satiety and ingestion of food in healthy adults (Douglas el al, 2015). Then, use of protein from plant source could be a good choice to replace protein from animal source.

In the search of vegetable foods as protein source was studied Canola/Rapeseed protein (Campbell et al, 2016), and soy (Mariotti et al, 1999; Fleddermann et al, 2012).

In Peru is cultivated an Amazonian plant producing seeds with high content of oil (54%) and protein (27%) named Sacha Inchi (*Plukenetia volúbilis*) and characterized by its high content in oleic, linoleic and alpha linolenic acids and vitamin E (tocopherols y tocotriols) (Sathe et al, 2002). This plant is recognized because its high content of alpha linolenic acid (45.2%) compared to values in others kind of seeds.

Sacha Inchi (*Plukenetia volúbilis*) is produced in high and low jungle in the Peruvian Amazonian. Initially wild, this plant is now cultivated, but it has high genetic variability which may produce confusion trying to identify the genus *Plukenetia* (Rodriguez et al, 2010). Actually, it is recognized the importance of oil of sacha inchi (also known as Inka peanut) because not only by its content of alpha linolenic acid (omega 3) but also by its content of triacylglycerols, polyphenols and tocopherols (Fanali et al, 2011). Oil of Sacha Inchi (*Plukenetia volúbilis*) is appreciated by its properties improving lipid profile (Huaman et al, 2008).

If seed of sacha inchi is known by its high content in linolenic acid (omega 3) in the oil, little or nothing is known in relations to the utility of the high protein content. Actually, some enterprises produce flour of sacha inchi with 55% protein content, gluten-free and containing omega 3. A protein content of more than 51% in sacha inchi cake is reported, with amino acid values higher than the requirement in preschoolers, school children and adults for isoleucine, leucine, methionine + cysteine, phenylalanine + tyrosine, threonine, valine and histidine (Hurtado, 2013). Then, this food may become in an important functional food and nutritional supplement for all ages.

The present study has been designed aimed to assess the nitrogen balance after an unique intake of flour of sachu inchi by humans and compare data with those obtained after unique ingestion of soy flour.

The hypothesis is that protein content in the flour of sachu inchi after a single orally intake is tolerated and does neither produce gastrointestinal discomfort, nor allergic reactions and produce a nitrogen balance similar to that observed after single intake of soy flour containing proteins.

Subjects and Methods

Design

This is a cohort study assessing the nitrogen balance after single oral consumption of flour of sachu inchi or flour of soy in men and women aged 18 and 55 years.

Subjects

The study was designed for a final sample of 30 adult healthy subjects (15 men and 15 non pregnant women) weighing from 44.6 Kg to 74.2 Kg in women and 50.3 Kg to 99.3 Kg in men. Sample size has been obtained with an error of 20% and a confidence level of 95% with a heterogeneity level of 50%. Fifteen subjects receive flour of sachu inchi and fifteen receive flour of soy. Randomization was stratified to obtain the participation of 15 men and 15 women.

The Institutional Review Board (IRB) at the Universidad Peruana Cayetano Heredia (Lima, Peru) approved the study (**CODE SIDISI: 67226**). All subjects participated voluntarily after been obtained detailed explanation of the study and signing their consent form. To be selected, 53 recruited men and women were asked to participate in a skin Prick test to discard allergic reactions and in an assessment of hematological, liver and kidney function tests. All recruited subjects filled the health-related quality of life (HRQL) test. Inclusion criteria comprised body fat percentage lower than 25% in men and lower 35% in women. Subjects were excluded of the study if they are chronic smokers, vegans (eat neither meat, egg nor dairy products), took medicine in the last month or if they have gastrointestinal, kidney or liver diseases, diabetes mellitus, metabolic syndrome, dyslipidemias, or diagnosed hypertension. Subjects were also excluded if they were positive to the skin Prick test or if they have a HRQL test score below 60.

After assessment of liver and kidney function, three other subjects were also discarded. The 30 volunteers recruited were randomly divided equally into two groups. Computer generated random numbers. The group receiving sachu inchi has eight women and seven men and the group receiving soy has seven women and eight men (Figure 1).

Blinding

The sachu inchi or soy flours are placed in envelopes containing 30 grams each without any label except the letters A or B. Selection of the label was performed for a third person no participating from the study. This person writes the definition of each label in a piece of

paper, covered with carbon paper, and closed in a sealed envelope. This envelope is kept closed in a cabinet until the end of the study.

Skin prick test

This cutaneous test is sensitive but not specific for the diagnosis of the allergic disease secondary to the release of IgE (hypersensitivity type I) (Soares-Weiser et al., 2014). Allergens (sacha inchi, soy or histamine) are placed in the anterior forearm. Reaction is assessed at 15 min and a positive response is considered when papule was >3 mm (Martínez et al, 2005). This test was performed in all volunteers previously to be accepted to participate in the study. A positive test indicates sensitization, but has not specificity for predicting clinical allergy (Chokshi & Sicherer, 2016).

Three subjects were excluded because the skin prick test (allergy test) was positive. No differences were observed between allergy to sacha inchi or soy ($p>0.05$). Although there is no evidence that these positive cases are allergic (Soares-Weiser et al., 2014), we decided to exclude them from the study.

Sacha inchi meal

Sacha inchi flour from *Plukenetia volubilis* were produced by Amazon Health Products (Lima, Peru). For this study is used sacha inchi flour Starseed® as approved by the General Direction of Environmental Health (DIGESA in Spanish). The product is prepared according the Peruvian Technical Regulation (NTP) 151.407:2015.

Sacha inchi meal extracted from the pressed seed cake contains proteins, carbohydrates, fiber and protein residues. This flour has 92.4% digestibility to pepsin. The flour contains 55.92% protein, 13.3% fat, 5.28% ash and 7.47% humidity.

Soybean meal

Texturized vegetable protein from soy was used for the comparison group. The soy flour Prosante® EXTM-7P was supplied by Dimerco Comercial SAC (Av. Las Camelias 790 Of 605, San Isidro, Lima, Peru). It contains 53.28% protein, 0.52% fat, 1.1% ash, and 10.24% humidity.

Soy and sacha inchi meals (30 g each) were dissolved in 200 ml water and orally ingested after overnight fasting.

Content of amino acids in the sacha inchi flour and soy flour is presented in Table 1. Tryptophan, arginine, and glycine contents are higher in sacha inchi flour. In Table 2 is observed the chemical composition of both flours. Protein content was >50% in sacha inchi meal and soy meal. Fat was 20 times higher in sacha inchi meal than in soy meal.

The true nitrogen digestibility using an *in vivo* rat assay were similar for sacha inchi ($89.21\pm1.52\%$, mean $\pm$ standard error of the mean) and for soy ($88.19\pm0.71\%$), but lower than that obtained with casein ($96.96\pm0.47\%$) (Gasco et al, personal communication). This assay used the same batched for sacha inchi and soy flour as in the present study.

Energy and nutrient intake

Nutrient and energy intake is calculated by using food consumption record by a trained nutritionist staff using the Peruvian table of food composition. For this, each participant fill a survey of food consumption record in the last 24 hours. This survey is applied at the time of selection to participate in the study and then, after a three-day period of consumption of a standardized diet. The following data was estimated:

- Total protein (Animal protein and vegetable protein)
- Fats
- Carbohydrates
- Energy.

Body composition

To obtain data on body composition anthropometry and bio-impedancemetry assessment was performed after voiding bowels and bladder in overnight fasting conditions. For bio-impedancemetry a Tanita body composition analyzer BF 350 (Tanita Corporation. Tokio, Japan) is used. The following anthropometric measurements were performed: height, weight, abdominal circumference, hip circumference, triceps and subscapular skinfolds. Body mass index is calculated from height and weight data as weight Kg/height² (m).

Body weight (Kg) was measured while the participants were wearing light clothing and no shoes with TANITA Body Composition Analyzer BF 350. Height was determined with a stadiometer to the nearest of 0.1 cm.

Biochemical parameters

Blood samples were taken for routine hematology and biochemistry screens: hemoglobin, hematocrit, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma glutamyl transferase, serum albumin, serum total proteins, serum creatinine, and serum uric acid levels. These markers were assessed as part of the criteria for selection.

Health-Related quality of Life

A generic health-related quality of life (HRQL) questionnaire was applied to each volunteer. The HRQL test used for this study was the Short Form 20 (SF-20). This SF-20 questionnaire is based in a Spanish version of a validated previous HRQL survey (SF-36) which contains 36 questions (Vilagut y col, 2005). Each item examined is scored on a scale of 0 to 100 in which higher score indicates better HRQL. Maximal value for overall test is 2000 points and it is equivalent in percent to 100. This questionnaire (SF-20) has been previously validated for populations in Peru (Gonzales et al, 2013).

Standardized diets

Three days before the study, subjects from both groups (soy meal and sachu inchi meal) were asked to receive a standardized diet, which includes 1509.25±43.35 calories per day.

Subjects during the three previous days with standardized diet and the day of the study were asked to no perform intense physical exercises nor endurance training.

Study protocol

The day of the study, subjects received 30 grams of soy flour or 30 grams of sachu inchi flour in water in a final volume of 200 ml. Each subject took the solution between 7 and 9 am. During the next 12 hours after flour intake, the volunteers received two standardized diets with low protein content (12.5 g), but containing carbohydrates and fats consisting of fruits, vegetables, rice bread (gluten-free), butter and jam. Duplicate portions of the dietary packages were freeze dried and submitted to a specialized laboratory for analysis of the nutritional composition.

On the day of the intervention, a fasting blood sample was obtained. Postprandial period lasted 8 hours. After 8 hours a meal is offered to each participant. Venous blood samples were obtained at 0, 30, 60, 90, 120, 480 minutes and 24 hours after protein intake. Blood samples were collected using BD Vacutainer (BD Franklin Lakes, NJ, USA). In each sample is measured blood glucose and plasma insulin (Mariotti et al, 1999).

Urine samples are collected in over a period of 24 hours. In the morning, the first specimen is flushed. Then, all specimens must be saved, stored, and kept cold, for the next 24 hours. In urine were measured compounds containing nitrogen as creatinine, uric acid, and urea.

Sitting arterial blood pressure (BP)

Sitting arterial BP (mm Hg) was measured in the left arm using an aneroid sphygmomanometer. Systolic (SBP) and diastolic (DBP) blood pressure were obtained from each subject at the beginning and at the end of the study.

Biochemical assays

At 0 and after 24 hours of consumption of soy meal or sachu inchi meal, serum samples were used for measurements of the ultrasensible C-reactive protein (CRP), IL-6, levels of total plasma cholesterol, LDL-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and plasma triacylglycerols (TAG) and markers of kidney functions under fasting conditions.

C-reactive protein (CRP), an inflammatory marker, was assessed using the Nycocard^R CRP single test system using Nycocard READER II (Axis-Shield PoC AS, Oslo, Norway). The test is a solid phase immunometric assay. Intra-assay CV was <7%. The reference range was 5 mg/L.

Serum creatinine, urea and uric acid levels were determined using commercial kits (Cypres diagnostics, Langdorp, Belgium). Measurements were done using spectrophotometer at 505 nm and 520 nm, respectively. Reference values for serum creatinine are 0.7-1.4 mg/dL for males and 0.6-1.1 mg/dL in females, whereas for uric acid were 3.6-7.7 mg/dL in males and 2.5-6.8 mg/dL in females. Limit of detection was 0.11 mg/dL for creatinine and 0.03 mg/dL for uric acid. Intra-assay CV was 1.99% and 0.63% for creatinine and uric acid, respectively. Between-assay CV was 1.68% and 1.58% for creatinine and uric acid, respectively.

Serum triacylglycerol, total cholesterol, and HDL-cholesterol levels were determined using commercially available enzymatic colorimetric assays (Cypres diagnostics, Langdorp, Belgium) on spectrophotometer at 505 nm. Normal range for triacylglycerol was 40-160 mg/dL in men and 35-135 mg/dL in females; for serum total cholesterol was less than 200 mg/dL and for HDL-cholesterol were 45-65 mg/dL in women and 35-55 mg/dL in men.

LDL-cholesterol was calculated according to the Friedewald formula: $\text{LDL-cholesterol} = [\text{Total cholesterol} - \text{VLDL cholesterol} - \text{HDL cholesterol}]$. VLDL cholesterol is calculated as triacylglycerol/5.

Serum albumin and total proteins were measured by colorimetric test (Cypres diagnostics, Langdorp, Belgium). Albumin was measured at 630 nm and normal values range from 3.5 to 5.0 g/dL. Serum total protein was measured at 546 nm and normal range varies from 6.6 to 8.3 g/dL.

Glycemia and insulin

For glucose measurement, a portable glucometer Accu-Chek (ROCHE, USA) was used. This method is based in the activity of the glucose oxidase enzyme. Data are referred as mg/dL. Glucose was measured in whole venous blood at each time of study. Normal values range 70-110 mg/dl.

Insulin was measured in plasma by a Enzyme-Linked ImmuneSorbent Assay method (DRG, USA). Normal values for insulin were <29.4 mIU/L; sensitivity was 1.2 mIU/L, intra-assay coefficient variation was 3.3–12.7% and inter-assay coefficient of variation was 6.6-12.4%.

Acceptability and adverse effects

Acceptability and adverse effects were monitored at the end of the study and it was based in a Likert test.

Nitrogen Balance

The Nitrogen (N) balance (mg nitrogen/Kg BW*day) was calculated as follows:

$$\text{N Balance} = \text{N intake} - (\text{urine N} + \text{fecal N} + \text{skin N})$$

N intake was estimated from food intake. Intake of 1 g nitrogen is equivalent to 6.25 mg of protein.

N output was expressed as 24-h N excretion through urine and feces and N loss by skin and others.

Urine N was calculated from data on urea, uric acid, and creatinine in a 24 h urine collection. The fecal N excretion is considered 10% of the N intake (Fledderman et al, 2013). Nitrogen loss by skin and others is represented as 5 mg N/Kg body weight/day (Campbell et al, 2008).

Statistical analysis

Data are entered in an Excel database. Data is presented as means and standard error of the mean if are normally distributed or medians and percentile if they are no normally distributed. To determine homogeneity of variances, the Bartlett test was used.

In case of normal distribution, data was assessed using parametric statistics. If variances are non-homogeneous then, non-parametric statistics were used. For categorical variables, the chi square test was used. A statistical significance was determined when p was below 0.05.

Results

Baseline characteristics

Sociodemographic, anthropometrical and biochemical characteristics were similar between groups of comparison (Tables 3-5). Serum levels of liver function markers, kidney markers and hematological markers were similar in both groups. Similarly, age was comparable in the group receiving sachu inchi meal (34 ± 3.25 years) and in the group receiving soy meal (32.8 ± 2.28 years, $p>0.05$).

Diet composition

Diet compositions at the time of recruitment were similar for the group of sachu inchi flour and the group with soy flour. The group of sachu inchi flour consumed 1946.7 ± 117.6 Kcal/day and the group with soy flour flour consumed 1879.92 ± 87.79 Kcal/day ($p>0.05$). Sachu inchi group consumed 69.18 ± 5.88 g protein/day and the soy group consumed 61.72 ± 3.25 g protein/day ($p>0.05$).

These values were reduced after the three-day of standardized diet to 1497.36 ± 66.24 Kcal/day in the group with sachu inchi flour ($p<0.01$ respect to values at recruitment) and to 1521.15 ± 62.70 Kcal/day in the group with soy flour ($p<0.01$ with respect to value at recruitment). Protein was 39.66 ± 2.02 g in sachu inchi group and 40.57 ± 0.90 g/day in soy group ($p>0.05$). In the day of the experiment calories and protein were further reduced (Table 6). However, no difference was observed in diet composition in the group treated with sachu inchi meal or soy meal ($p>0.05$) except for protein intake, in which the sachu inchi group received 0.6 grams more than in the soy group ($p=0.024$) and this represents 0.1 g more N in the sachu inchi group ($p=0.024$). Fiber was higher in the soy group ($p=0.0001$).

Biochemical measurements and inflammatory markers after sachu inchi and soy meals

Serum urea, uric acid and creatinine were similar at 0 and 24 hours after intake of sachu inchi meal ($p>0.05$) or soy meal ($p>0.05$). Similarly, markers of lipid profile were not different at 0 and 24 h of the intake of sachu inchi ($p>0.05$) or soy ($p>0.05$). Serum CRP or IL-6 were not different at 0 or at 24 h after sachu inchi or soy meal consumption (Table 7).

Glycaemia increases modestly with highest value at 120 minutes. A reduction is then observed at 480 minutes without further change at 24 hours of the flour administration. No differences were observed in glycaemia levels between groups of intervention at any of the times studied (Figure 2).

Plasma insulin levels increase significantly after 30 minutes of flour intake in both groups of intervention. Thereafter, a reduction was observed such as values at 24 h were similar to basal values at time 0. Plasma insulin at 480 minutes was lower than basal values ($p>0.05$) and then returned to basal values at 24 hours (Figure 3). No differences were observed in plasma insulin between groups of intervention at any of the times studied ($p>0.05$).

Nitrogen balance

Consumption of nitrogen on the experimental day resulted in a negative N balance (range: -0.47 to -13.44 g N/d). The difference between N balance for sachachi meal and soy meal failed to reach statistical significance (Table 8).

Acceptability and adverse effects

Acceptability (in accordance and totally in accordance with taste) was observed in 10/15 taking sachachi meal and 7/15 subjects taking soy meal. No differences were observed between groups of intervention ($p>0.05$).

Dislike of the taste of the products (in discordance and completely in discordance) was observed in 27% of subjects consuming sachachi meal and 27% of subjects consuming soy meal ($p>0.05$).

No difference between groups of intervention were also observed in relation to acceptability of the smell of the product ($p>0.05$).

15 subjects consuming sachachi meal and 13 subjects consuming soy meal were in discordance or completely in discordance that they have digestive discomfort without differences between groups ($p>0.05$).

12 subjects consuming sachachi meal and 15 subjects consuming soy meal were in discordance and total in discordance, that flour produced adverse effects. No difference was observed between interventions ($p>0.05$).

Blinding

Analysis of participants' response to the question of whether they thought they had received sachachi flour or soy flour indicated that participants were blinded to study assignment. Participants receiving the flours believed they had received sachachi meal or soy meal in a similar proportion. There was no difference in blinding between the intervention groups (Chi square test = 0.53; $p = 0.462$).

Discussion

Protein requirement is defined as the low value of protein intake with diet needed to maintain the nitrogen loss in healthy persons with modest physical activity in a zero balance (Rand et al, 2003).

The estimated requirement (median) and the recommended nitrogen intake (97.5th percentile) is 105 mg N/Kg/day (0.66 g protein of high quality/Kg/day) and 132.05 mg N/Kg/day (0.83 g protein of high quality/Kg/day), respectively (Rand et al, 2003).

In the present study, the protein intake was 0.48 g/Kg BW/day in the sachu inchi group and 0.44 g/Kg BW/day in the soy meal, whereas N intake was 76 mg/Kg BW/day for sachu inchi meal and 71 mg/Kg/day for the soy meal. These values for protein represents for sachu inchi meal, 73% of the estimated protein requirement or 58% of the recommended protein intake (97.5th percentile) and for nitrogen estimated and recommended requirements represents 72.4% and 57.6% respectively. These values are below the protein requirement and recommended protein intake.

With these values of N intake in the present study, we have calculated a N balance using the square root model recommended by Rand & Young (1999), resulting in N balance of - 6.38 g/day for sachu inchi meal and a N balance of -6.45 g/day for soy meal. These values are almost similar to those observed in our study suggesting that sachu inchi meal is adequate as source of protein of high quality is the right amount of protein required is consumed.

One of the methods used to determine protein quality is the measurement of true protein digestibility in an *in vivo* rat assay (Abdel-Aziz et al., 1997). Assessment of true protein digestibility and true biological value in the same products used in the present study showed that these were similar with sachu inchi meal and with texturized soy flour (Gasco, et al, personal communication).

Soy flour has 86% true digestibility in humans (Protein quality evaluation in human diets, 1991). This is in accordance with data obtained for the texturized soy flour used in this study (89%). Sachu inchi flour as used in the present study has the same true digestibility as that obtained with soy flour.

According to our analysis, proteins from sachu inchi flour and texturized soy flour are well balanced in essential amino acids (EAA). Sachu inchi flour in respect to soy flour has higher content of tryptophan, arginine, and glycine.

Arginine as precursor of nitric oxide synthesis has come into prominence clinically because of the role in cardiovascular physiology and pathophysiology (Baker, 2007). Moreover, lysine and arginine are amino acids linked to growth hormone release in young children via the somatotrophic axis (Uauy et al., 2015).

Tryptophan is an essential amino acid, and recently, it has been reported that an increase in the plasma tryptophan concentration was significantly associated with a decreased risk of CVD (Yu et al., 2017).

A report shows that plasma total amino acids increases 30 min after soy milk consumption. From these, alanine and arginine are involved in the hyperinsulinaemia after soy meals (Sun et al., 2016). Both, amino acids are present in the sachu inchi flour, and may explain the increase on plasma insulin after sachu inchi meal.

Lysine is an indispensable amino acid since cannot be synthesized by mammals. The main role of lysine is to participate in protein synthesis and muscle function. The catabolism of lysine is principally located in the liver. Lysine released from digested protein undergoes a significant first-pass metabolism of approximately 30 to 42% in humans (Tomé and Bos, 2007). Lysine is a limiting amino acid in cereals. WHO (2007) recommends a requirement value of 30 mg lysine/Kg/day. For a mean weight of 66.82 Kg observed in the group, consuming sachu inchi flour a value of 2004.6 mg lysine/day is required. This is accomplished with 67.95 g of sachu inchi flour. Lysine is arguably the most deficient amino acid in the food supply of countries where poverty exists (Baker, 2007).

Both, sachu inchi and soy increase glycemia in a same proportion suggesting that high amount of fat in the sachu inchi meal does not delayed gastric emptying. Moreover, sachu inchi is characterized by its high content in linolenic acid (Gonzales et al., 2013). The content of fats in sachu inchi flour could be useful to reduce hyperlipidemia as it has been demonstrated for the sachu inchi oil (Huaman et al., 2008).

Conclusions

In conclusion, the nitrogen balance after the consumption of sachu inchi flour was similar to that observed after soy flour intake in healthy adult men and women. The hyperinsulinemia after consumption of sachu inchi and soy occurred early with a peak at 30 minutes suggesting that amino acids after protein meals consumption has increased early and in similar proportion with sachu inchi and soy. In the search of new sources of proteins mainly of vegetable origin, we can suggest that sachu inchi protein is of importance for nutrition.

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References

- Abdel-Aziz S, Hussein L, Esmail S, et al. In vivo rat assay for true digestibility and protein quality of beef and meat products extended with soy protein. *Int J Food Sci Nutr*. 1997; 48(1):51–6. [PubMed: 9093549]
- Baker DH. Lysine, arginine, and related amino acids: an introduction to the 6th amino acid assessment workshop. *J Nutr*. 2007; 137(6 Suppl 2):1599S–1601S. [PubMed: 17513434]
- Campbell W, Johnson C, McCabe G, Carnell N. Dietary protein requirements of younger and older adults. *Am J Clin Nutr*. 2008; (88):1322–9. [PubMed: 18996869]
- Campbell L, Rempel CB, Wanasundara JP. Canola/Rapeseed Protein: Future Opportunities and Directions-Workshop Proceedings of IRC 2015. *Plants (Basel)*. 2016; 5(2) pii: E17. doi: 10.3390/plants5020017
- Chokshi NY, Sicherer SH. Interpreting IgE sensitization tests in food allergy. *Expert Rev Clin Immunol*. 2016; 12(4):389–403. [PubMed: 26666347]
- Douglas SM, Lasley TR, Leidy HJ. Consuming Beef vs. Soy Protein Has Little Effect on Appetite, Satiety, and Food Intake in Healthy Adults. *J Nutr*. 2015; 145(5):1010–6. [PubMed: 25809680]
- Fanali C, Dugo L, Cacciola F, et al. Chemical characterization of Sachu Inchi (*Plukenetia volubilis* L.) oil. *J Agric Food Chem*. 2011; 59:13043–9. [PubMed: 22053706]

- Fleddermann M, Fechner A, Rößler A, et al. Nutritional evaluation of rapeseed protein compared to soy protein for quality, plasma amino acids, and nitrogen balance—a randomized cross-over intervention study in humans. *Clin Nutr*. 2013; 32(4):519–26. DOI: 10.1016/j.clnu.2012.11.005 [PubMed: 23260747]
- Gonzales GF, Gonzales C, Villegas L. Exposure of fatty acids after a single oral administration of sachá inchi (*Plukenetia volubilis* L.) and sunflower oil in human adult subjects. *Toxicol Mech Methods*. 2014; 24(1):60–9. [PubMed: 24090048]
- Gonzales GF, Rubio J, Gasco M. Chronic mountain sickness score was related with health status score but not with hemoglobin levels at high altitudes. *Respir Physiol Neurobiol*. 2013; 188(2):152–60. [PubMed: 23770310]
- Gregorio L, Brindisi J, Kleppinger A, et al. Adequate dietary protein is associated with better physical performance among post-menopausal women 60–90 years. *J Nutr Health Aging*. 2014; 18(2):155–60. [PubMed: 24522467]
- Huaman J, Chavez K, Castañeda E, et al. Efecto de la *Plukenetia volubilis* Linneo (sachá inchi) en la trigliceridemia postprandial. *An Fac Med*. 2008; 69:263–6.
- Hurtado, Z. Master Degree Thesis. Colombia: 2013. Análisis Composicional de la Torta y Aceite de semillas de Sachá Inchi (*Plukenetia Volubilis*) cultivada en Colombia. Universidad Nacional de Colombia.
- Loenneke JP, Loprinzi PD, Murphy CH, et al. Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance. *Clin Nutr*. 2016; pii: S0261-5614(16)30018-8. doi: 10.1016/j.clnu.2016.04.002
- Mariotti F, Mahé S, Banamouzig R, et al. Nutritional value of [15N]-soy protein isolated assessed from ileal digestibility and postprandial protein utilization in humans. *J Nutr*. 1999; 129:1992–7. [PubMed: 10539774]
- Martínez J, Méndez C, Talesnik E, et al. Skin prick test of immediate hypersensitivity in a selected Chilean pediatric population sample. *Rev Med Chil*. 2005; 133(2):195–201. [PubMed: 15824830]
- Nisar MU, Anwar Ul Haq MM, et al. Feeding Patterns and Predictors of Malnutrition in Infants from Poor Socioeconomic Areas in Pakistan: A Cross-sectional Survey. *Cureus*. 2016; 8(1):e452.doi: 10.7759/cureus.452 [PubMed: 26918220]
- Protein quality evaluation in human diets. Rome, Food and Agriculture Organization of the United Nations. 1991. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper No. 51
- Rand WM, Young VR. Statistical analysis of nitrogen balance data with reference to the lysine requirement in adults. *J Nutr*. 1999; 129:1920–6. [PubMed: 10498769]
- Rand WM, Pellett PL, Young VR. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr*. 2003; 77(1):109–27. [PubMed: 12499330]
- Richter CK, Skulas-Ray AC, Champagne CM, et al. Plant protein and animal proteins: do they differentially affect cardiovascular disease risk? *Adv Nutr*. 2015; 6(6):712–28. [PubMed: 26567196]
- Rodriguez A, Corazón-Guivin M, Cachique D, et al. Diferenciación morfológica y por ISSR (Inter simple sequence repeats) de especies del género *Plukenetia* (Euphorbiaceae) de la Amazonía peruana: propuesta de una nueva especie. *Rev Per Biol* 2010. 2010; 17:325–30.
- Sahni S, Mangano KM, Hannan MT, et al. Higher Protein Intake Is Associated with Higher Lean Mass and Quadriceps Muscle Strength in Adult Men and Women. *J Nutr*. 2015; 145(7):1569–75. DOI: 10.3945/jn.114.204925 [PubMed: 26019246]
- Sathe SK, Hamaker BR, Sze-Tao KWC, et al. Isolation, purification and biochemical characterization of a novel water soluble protein from Inca peanut (*Plukenetia volubilis*). *J Agric Food Chem*. 2002; 50:4906–8. [PubMed: 12166980]
- Soares-Weiser K, Takwoingi Y, Panesar SS, et al. EAACI Food Allergy and Anaphylaxis Guidelines Group. The diagnosis of food allergy: a systematic review and meta-analysis. *Allergy*. 2014; 69(1): 76–86. [PubMed: 24329961]
- Sun L, Tan KW, Slow PC, et al. Soya milk exerts different effects on plasma amino acid responses and incretin hormone secretion compared with cow's milk in healthy, young men. *Br J Nutr*. 2016; 116(7):1216–21. [PubMed: 27609509]

- Tomé D, Bos C. Lysine requirement through the human life cycle. *J Nutr.* 2007; 137(6 Suppl 2): 1642S–5S. [PubMed: 17513440]
- Uauy R, Kurpad A, Tano-Debrah K, et al. Role of Protein and Amino Acids in Infant and Young Child Nutrition: Protein and Amino Acid Needs and Relationship with Child Growth. *J Nutr Sci Vitaminol (Tokyo).* 2015; 61(Suppl):S192–4. [PubMed: 26598853]
- van Vliet S, Burd NA, van Loon LJ. The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *J Nutr.* 2015; 145(9):1981–91. [PubMed: 26224750]
- Vilagut G, Ferrer M, Rajmil L, et al. The Spanish version of the Short Form 36 Health Survey: a decade of experience and new developments. *Gac Sanit.* 2005; 19(2):135–50. Review Spanish. [PubMed: 15860162]
- World Health Organization. Protein and amino acid requirements in human nutrition. WHO/FAO/UNU Expert Consultation. WHO Technical Report Series N°. 2007; 935:1–284.
- Yu E, Ruiz-Canela M, Guasch-Ferré M, et al. Increases in Plasma Tryptophan Are Inversely Associated with Incident Cardiovascular Disease in the Prevención con Dieta Mediterránea (PREDIMED) Study. *J Nutr.* 2017; 147(3):314–22. [PubMed: 28179491]

Figure 1.
Flow diagram following the recruitment, enrollment, and number of subjects that finished treatment of the clinical trial

Figure 2.

Blood glucose levels under basal and post-prandial conditions in humans receiving 30 g flour of sacha inchi (n=15) or 30 g flour of soy (n=15). Values are mean±standard error of the mean. $a p < 0.05$ with respect to basal values in the Sacha inchi group. Red line: soy flour group. Blue: Sacha inchi flour group.

Figure 3. Plasma insulin (uIU/ml) levels under basal and post-prandial conditions in humans receiving 30 g flour of sacha inchi (n=15) or 30 g flour of soy (n=15). Values are mean $\pm$ standard error of the mean. *a* p <0.05 with respect to basal values in the Sacha inchi group. *b* p <0.05 with respect to basal values in the Soy group. Red line: soy flour group. Blue: Sacha inchi flour group.

Table 1

Amino acid composition in flour of sachu inchi and texturized flour of soy.

Amino acids (%)	Sacha Inchi flour	Soy flour
Tryptophan	2.25	0.76
Cystin	1.35	0.79
Methionine	0.68	0.76
Alanine	2.04	2.17
Arginine	6.13	3.70
Aspartic acid	6.83	6.07
Glutamic acid	7.71	9.77
Glycine	6.31	2.19
Histidine	1.17	1.35
Isoleucine	2.73	2.38
Leucine	4.03	4.00
Phenylalanine	1.42	2.62
Proline	2.29	2.58
Serine	3.48	2.65
Threonine	2.65	2.09
Total lysine	2.95	3.27
Tyrosine	2.87	1.70
Valine	3.46	2.53

Table 2

Chemical composition of sacha inchi flour and soy flour (fat and protein are given as percent of dry matter).

Composition	Sacha inchi flour	Soy flour
Dry matter (%)	97.69	99.01
Ash (%)	1.02	1.1
Fat (%Dry matter)	10.17	0.52
Protein (Nx6.25)(%, Dry matter)	56.53	53.28
Carbohydrates (g/100 g)	19.17	29.26
Fiber (g/100 g)	10.80	14.86

Table 3

Sociodemographic and physiological baseline characteristics of the subjects consuming 30 g sachá inchi flour and those consuming soy flour

Characteristics	Sachá Inchi (15)	Soy (15)	<i>p</i> Value
Sex (male/female)	7/8	8/7	>0.05
Systolic blood pressure (mm Hg)	103.33±3.33	97.33±3.00	>0.05
Diastolic blood pressure (mm Hg)	66.20±2.54	65.33±2.36	>0.05
SpO ₂ %	97.33±0.27	97.86±0.27	>0.05
Heart rate (pulse/min)	73.80±2.52	74.20±1.99	>0.05
HR-QL score	1623±43	1621±32	>0.05
Personal history of disease (yes)	4/15	5/15	>0.05
Family history of disease (yes)	9/15	8/15	>0.05
Smoke (yes)	2/15	1/15	>0.05
Drink frequently alcohol (yes)	1/15	0/15	>0.05

Data are mean±SEM. *p*>0.05 between groups. Differences for sexual proportion were calculated with chi square test. The number of subjects are between parentheses.

Table 4

Baseline anthropometric characteristics of the subjects consuming 30 g sachá inchi flour and those consuming 30 g soy flour.

Baseline characteristics	Sacha Inchi	Soy	<i>p</i> Value
Weight (Kg)	66.82±4.23	64.21±2.34	>0.05
Height (m)	1.78 ±3.02	1.64±0.02	>0.05
BMI (Kg/m ²)	24.5±0.56	22.75±1.24	>0.05
Abdominal circumference (cm)	91.25±3.17	87.92±2.00	>0.05
Hip circumference (cm)	99.38±2.85	96.67±2.16	>0.05
Triceps skinfold (mm)	18.26±1.54	17.13±2.14	>0.05
Subscapular skinfold (mm)	21.73±2.06	19.26±2.12	>0.05

Data are mean±SEM; between parentheses are the number of subjects

Table 5

Basal biochemical characteristics of the subjects consuming 30 g sachá inchi flour and those consuming 30 g soy flour

Characteristics	Sachá Inchi (15)	Soy (15)	<i>p</i> Value
GPT (IU/l)	24.6±4.8	25.±4.74	>0.05
GOT(IU/l)	20.7±1.7	22.93±2.36	>0.05
GGT (IU/l)	23.4±2.27	26.13±4.02	>0.05
ALkaline Phosphatase (IU/l)	191.86±11.38	183.26±14.01	>0.05
Total Protein (mg/dl)	7.6±0.13	7.57±0.12	>0.05
Uric acid (mg/dl)	4.74±0.39	4.41±0.27	>0.05
Creatinine (mg/dl)	0.84±0.03	0.80±0.03	>0.05
Urea (mg/dl)	28.26±1.83	25.4±1.52	>0.05
Hemoglobin (g/dl)	13.82±0.34	14.34±0.41	>0.05
Leucocytes/mm ³	6,486.6±407.6	6,206.6±282.20	>0.05
Platelets/mm ³	249,600±15,381.4	257,133.33±16,137.44	>0.05

Data are mean±SEM. Between parentheses are the number of subjects

Table 6

Diet composition according group of study during the day of experiment including flours at 8 am, a meal at 4 pm and a last meal at 8 pm.

	Sacha Inchi (15)	Soy (15)	<i>P value</i>
Calories (Kcal/day)	963.25±24.94	988.78±10.31	0.35
Protein (g/day)	29.28±0.15	28.68±0.20	0.024
Protein nitrogen (% protein)	12.29±0.38	11.61±0.06	0.08
Carbohydrates (g/day)	104.15±1.76	108.14±2.20	0.16
Fiber (g/day)	4.57±0.16	6.23±0.17	0.0001
Total Nitrogen intake (g/day)	4.69±0.02	4.59±0.03	0.024
Fat (g/day)	47.73±2.53	49.06±0.11	0.60
Vegetable fat (g/day)	14.33±0.26	13.85±0.12	0.10
Animal fat (g/day)	33.39±2.35	34.74±0.0	0.32

Data are mean ± standard error of the mean. Between parentheses are the number of subjects

Table 7

Biochemical parameters in serum at basal and 24 hours after intake of 30 g of sachu inchi flour or 30 g of soy flour.

	Sachu inchi (n=15)		Soy (n=15)	
	BASAL	24 h	BASAL	24 h
Urea (mg/dl)	26.97 ± 1.77	26.46 ± 1.57	26.82 ± 2.24	26 ± 1.77
Creatinine (mg/dl)	0.71 ± 0.03	0.73 ± 0.04	0.69 ± 0.03	0.74 ± 0.03
Uric acid (mg/dl)	5.05 ± 0.28	4.6 ± 0.25	4.3 ± 0.26	5.33 ± 0.29
Cholesterol (mg/dl)	169.87 ± 14.37	173.6 ± 8.36	168.87 ± 7.81	189.07 ± 9.80
HDL (mg/dl)	43.2 ± 2.49	43.87 ± 2.72	43.67 ± 2.50	42 ± 2.22
LDL (mg/dl)	118.67 ± 8.99	115.13 ± 6.96	108.4 ± 6.34	126.73 ± 9.14
VLDL (mg/dl)	27.13 ± 5.52	20.47 ± 2.06	15.93 ± 1.57	14.53 ± 0.89
Triglycerides (mg/dl)	105.93 ± 10.1	101.8 ± 10.24	79.67 ± 7.81	72 ± 4.38
CHOL/HDL	4.42 ± 0.35	4.07 ± 0.20	4.01 ± 0.22	4.64 ± 0.32
LDL/HDL	2.9 ± 0.30	2.69 ± 0.17	2.57 ± 0.19	3.13 ± 0.27
CRP (mg/l)	<6	<6	<6	<6
IL 6 (uIU/mL)	35.62 ± 24.94	28.63 ± 12.16	96.34 ± 44.75	15.63 ± 2.40

Data are mean ± standard error of the mean.

p<0.01 with respect to basal values. CRP= C-reactive protein. Between parentheses are the number of subjects

Table 8

Nitrogen Balance in volunteers consuming sachu inchi meal or soy meal.

	Sachu Inchi (15)	Soy (15)	P value
Nitrogen intake (g/day)	4.66±0.02	4.59±0.03	0.01
Nitrogen intake (mg/kg/day)	76±4	71±4	>0.05
Protein intake (g/kg/day)	0.475±0.022	0.440±0.027	>0.05
Ureic nitrogen excretion (g/day)	9.52±0.72	8.77±0.71	>0.05
Non ureic nitrogen excretion (g/day)	0.56±0.04	0.60±0.04	>0.05
Fecal nitrogen excretion (g/day)	0.46±0.002	0.45±0.003	>0.05
Skin nitrogen excretion (g/day)	0.33±0.021	0.32±0.01	>0.05
Total Nitrogen excreción (g/day)	10.88±0.75	10.15±0.75	>0.05
Nitrogen Balance (g/day)	-6.21±0.75	-5.56±0.73	>0.05

Data are mean ± standard error of the mean. Between parentheses are the number of subjects