Archives of Physical Health and Sports Medicine

ISSN: 2639-1805

Volume 1, Issue 2, 2018, PP: 08-14

Evaluation of Protein Supplements Isolated from Vegetables (Soy, Pea and Rice) on Weight Gain and Food Consumption in Wistar Rats

Ingrid Lannay Rodrigues da Silva[1], Alexandre Coelho Serquiz[1]*

[1]Department of Nutrition, University Center of Rio Grande do Norte, Natal, Brazil.
alexandreserquiz@gmail.com

***Corresponding Author:** Alexandre Coelho Serquiz, Department of Nutrition, University Center of Rio Grande do Norte, Natal, Brazil.*

Abstract

Most foods of plant origin have antinutritional factors which, when present in food, can cause adverse physiological effects. The present work aimed to verify the presence of protease inhibitors, as well as weight gain and dietary intakes of protein supplements isolated from vegetables, in wistar rats. The animals were divided into six groups; groups one and two were control groups (positive and negative control); the others received samples of four supplements (2 groups receivedsupplements of soybean, 1receivedsupplements of rice and 1 receivedsupplements ofpea). It was observed that the animals that received the supplementation presented a reduction in the weight gain and reduction of the alimentary consumption. It may be suggested that this reduction was due to satiety caused by the increase of CCK through a mechanism dependent on proteolytic inhibitors present in these supplements. Therefore, it is believedthat the use of these supplements, due to the presence of enzymatic inhibitors, would impair its functionality for hypertrophypurposes.

Keywords: *Serine proteinase inhibitors. Trypsin. Chymotrypsin. Nutritional supplements.*

Introduction

It is common to reinforce stereotyped aesthetic body patterns and, in pursuit of an "aesthetically perfect" body, many people test diets and dietary regimes of any kind in the hope of achieving a new level of well-being or physical performance (BRASIL, 2014).

Proteins are important components of the human diet and play an essential role as structural and functional elements of living systems. These are essential molecules to animals and, therefore, should be present in the diet, in adequate quantities (ASSIS et al., 2018).

However, there is an age-old popular belief among athletes that additional protein increases strength, improves performance, and consequently increases lean mass, which has made supplement use more widely in recent decades (MAHAN; ESCOTT, 2005).

Currently, with the increasing commercialization of new food sources, the increase of vegetable-based proteins is notable, generated from the processing of in natura products such as soy, peas and rice (MOUTE et al., 2006; SACK et al., 2015; ASSIS, 2018).

However, it is common knowledge that most of these plant protein supplements have antinutritional factors and low levels of essential amino acids (FERRI, 2006; PIRES et al., 2006). Antinutritional factors may interfere with the absorption or use of nutrients and, if ingested in high concentrations, they may have harmful effects on health (BENEVIDES et al., 2011), such as causing substantial reductions in protein digestibility, as well as causing a decrease in weight gain and growth (ARAÚJO, 2012; GILANI, COCKELL 2012; SERQUIZ, 2016).

Some studies have shown that inhibitors in adequate concentrations can provide satiety and the probable mechanism that explains this action is the longer presence of proteins in the intestine, which occurs by inhibiting digestive enzymes, thus increasing the cholecystokinin stimulus (CCK), which act on the arcuate nucleus stimulating satiety effects (RIBEIRO, 2013; SERQUIZ, 2016).

Several studies on the purification of these protease inhibitor substances have been positively highlighted in the control of several biological processes, due to their wide spectrum of heterologous actions, acting in the regulation of the hyperactivity of certain enzymes, or even inactivating them and favoring several processes with beneficial effects on health. They are recognized as bioactive molecules, with anticancer, anti-inflammatory, antiobesity functions, among others (FANG et al., 2010; RIBEIRO, 2013; SERQUIZ, 2012).

Thus, the present work aimed to verify the presence of protease inhibitors, as well as the weight gain and dietary intake of supplements based on proteins isolated from vegetables in *wistar* rats.

Materials and Methods

The cross-sectional experimental study used as a source samples of 4 protein supplements isolated from national and imported vegetables, purchased in the local market of the city of Natal - RN, Brazil, being two supplements based on isolated protein of soy, one supplement based on isolated protein of rice and one of pea, totaling 4 samples.

The Obtainment of Gross Extract

Supplement samples were extracted separately in 0.05 M Tris-HCl buffer in the ratio 1:10 (w / v). Each sample was then maintained for about 12 hours at 8 °C and centrifuged at 12,000 x g for 30 minutes at 4 °C to obtain precipitated proteins. The precipitate was discarded and the supernatant was obtained as gross extract.

Evaluation of the Inhibitory Activity of Digestive Enzymes

Determination of Antitryptic Activity of the Extract and Protein Fractions

The antitryptic activities of the supplements studied were determined using the following methodology: 20 µL aliquot of the bovine trypsin solution (0.3 mg / ml HCl 2.5 mM) was preincubated with 560 µl of 0.05 M Tris-HCl buffer, 7.5 pH, 120 µl of 2.5 mM HCl and 100 µl of supplement for 15 minutes at 37ºC. After this period, the reaction was started by adding 500 µL of specific substrate solution (BApNA 1.25 mM). The reaction was processed for another 10 minutes under the same incubation conditions and then interrupted by adding 120 µl of 30% acetic acid solution. All the trials were done in triplicates and blank tests were performed. The absorbance was measured at 405 nm. Results were expressed as percentage of inhibition.

Determination of Anti-Chymotryptic Activity of the Extract and Protein Fractions

The anti-chymotryptic activity was determined by incubating 100 mL of the protein isolate from the three food products analyzed, the enzyme chymotrypsin at 0.02 mg mL-1 and 1% azocasein as the substrate. Substrate hydrolysis was measured by reading absorbances at wavelength at 440 nm measured by spectrophotometer. Blank tests were performed and the trials were done in triplicate.

In Vivo Trial with Extracts of Protein Concentrate Extracts of Vegetables (Soy, Rice and Pea)

After approval by the ethics committee of use on animals (CEUA), the animals were evaluated by a veterinarian regarding their health. The research complied with current national laws, and procedures involving animals and their care were in accordance with institutional guidelines, in accordance with national and international law and Guidelines for the Use of Animals in Biomedical Research. The animals were kept in the breeding room of the Universidade Federal do Rio Grande do Norte (UFRN - Federal University of Rio Grande do Norte), in Natal-RN, Brazil, under controlled temperature (20-24ºC) and with a light-dark cycle of 12 hours. They were separated in a box of propylene, lined with wood sawdust, which in turn was changed daily. Feed and water were supplied *ad libitum* throughout the experiment.

Animals

After 6 days of adaptation, *wistar* rats were randomly distributed in 6 groups of male *wistar* rats(n=6), for weight and consumption assessment. The diet used by the negative control group was Labina purine commercial. The positive control group received supplementation with purified soybean inhibitors. Groups 3 to 6 were supplemented with isolated vegetable-based protein; for groups 3 and 4, soy-based supplements (distinct brands) were used; for group five, rice-based supplements were used; and finally for group six, pea-based supplements were used. All diets were offered orally and administered by gavage at the dosage of 25 mg / kg, adapted from Serquiz, 2012.

Food Consumption

After establishing the usual dietary consumption pattern of each group of animals (average in g of diet consumption) during the 6 days of adaptation, the animals were submitted to tests with the diets and they were evaluated for food consumption (g) by means of the calibrated balance. Thus, the difference between the diet provided (before consumption) and the diet consumed was obtained on a weekly basis: Dietary intake per week (g) = Diet Provided - Diet consumed, and the result was expressed as the average of the food consumption, per week, per group (g), for 14 weeks, considering the standard individual food consumption of the animals. Diets, water intake and weight gain were monitored daily.

Evolution of Body Weight

The rats were weighed individually on a calibrated balance once a week for a period of 14 weeks, in each group. In this way, the mean weight (g) was measured.

Statistical Analysis

Data were tabulated and analyzed using descriptive statistics, using central tendency and dispersion measures (mean and standard deviation) using the Microsoft Excel® program, version 2015.

Results and Discussion

Foods of plant origin are important source of protein and energy, but they have significant toxicity of compounds known as antinutritional substances, which reduce the nutritive value of these foods when consumed (SILVA; SILVA 2000).

Vegetables may interfere with the digestibility, absorption or utilization of nutrients and, if ingested in high concentrations, they may have harmful effects on health (SANTOS, 2006), because they have an inhibitory action of digestive enzymes, such as trypsin and chymotrypsin, reducing the biological availability of essential amino acids and minerals, as well as causing irritations and lesions of the gastrointestinal mucosa, thus interfering with the selectivity and efficiency of biological processes (SGARBIERI, 1987; SERQUIZ, 2017).

Plant inhibitor levels may vary, depending on the maturation stage, their location in the tissues, the time of harvest and storage, as well as the plant variety, and different classes of inhibitors may coexist in a single tissue and organ (RYAN, 1990; RICHARDSON, 1997; BRZIN; KIDRIC, 1995).

Table 1 shows the protease inhibitory activity present in the commercially available protein supplements based on plants used in the research. In the analysis, it was found that there was a greater amount of inhibition in the soybean supplements, being around 90% of inhibitory activity for trypsin and 60% for chymotrypsin, overcoming rice- and pea-based supplements, which maintained percentages of inhibition for trypsin around 50% and chymotrypsin with 45% and 38%, respectively.

Table 1. *Inhibitory activity of tryps in from vegetable protein supplements, (SV1) Soy Protein Concentrate Supplement, (SV2) Soy Protein Concentrate Supplement, (SV3) Rice Protein Concentrate Supplement, (SV4Pea Protein Concentrate Supplement. *statistical difference with p<0.05.*

	Inhibitory Activity for Chymotrypsin	Inhibitory Activity for Trypsin
Soy Inhibitor	90 %	100 %
SV1 (soy) 1	60%	90%
SV2 (soy) 1	58 %	86 %
SV3 (rice)	45%	53%
SV4 (pea)	38%	48%

The data obtained in this research corroborate those presented by Assis et al. (2018), as they evaluated inhibitory activity for proteolytic enzymes in 15 plant-based supplements sold in international and national trade. The authors observed that all supplements analyzed showed inhibitory activity for the enzymes trypsin, chymotrypsin and pepsin. However, soybean supplements showed higher inhibitory activity, which was expected, since the natural occurrence of legume grains occurred (CONCEIÇÃO, 2010; MONTEIRO et al., 2004).

The higher the amount of inhibitors present in legumes, the lower their nutritional quality (PETROVIC et al., 2014). When consumed in excess, these foods / supplements cause complexation of the enzymes trypsin and chymotrypsin secreted by the pancreas, preventing the proteolytic action (MONTEIRO et al., 2004; MIURA et al. 2005; SERQUIZ et al., 2012). Inhibitors also act in a damaging manner in relation to protein utilization (ZANG et al., 2006). The proteins are reduced in the digestive processes, thus interfering in the bioavailability and absorption of amino acids (CONCEIÇÃO et al., 2010).

This inhibition is capable of causing excessive fecal loss of proteins secreted by the pancreas, generating negative nitrogen balance, a fact that generates injury to individuals (RACKIS; GUMBAMNN, 1982; BENDER, 1987; XAVIER-FILHO; CAMPOS, 1989; VAZ PATTO et al., 2015).

Despite these data in the literature, other studies show that, when consumed in adequate doses, they generate beneficial effects on health, in the control of various biological processes, by their broad spectrum of heterologous actions, acting on the regulation of hyperactivity of certain enzymes or even inactivating them. They are recognized as bioactive molecules, with anticancer, anti-inflammatory, antiobesity functions, among others (FANG et al., 2010; RIBEIRO, 2013; SERQUIZ, 2012).

In addition, the present study also evaluated the action of plant protein supplements on weight gain and food intake. The analysis showed that the animals that received the vegetal supplements obtained a reduction in the weight gain when compared to those of the negative control group. The group that received the soybean inhibitor (positive control) was the one that obtained the greatest reduction in the weight gain (30.67 ± 3.005), followed by the supplemented groups, SV1 (36.67 ± 4.041), SV3 (38.0 ±2.0), SV2 (39.33 ± 1.528) and SV4 (40.33 ± 1.528). The results are shown in figure 1A.

Food intake was lower in the groups that ingested the supplements. In Figure 1B, it is possible to observe that the negative control group was the one that had the highest average consumption, reaching 34.67 ± 2.082. On the other hand, the intake of the soybean inhibitor (positive control) caused the animals to obtain the lowest mean (17 ± 2.0). Finally, it can be observed that the animals supplemented with vegetable-based protein obtained approximate average food consumption, SV1 (24.33 ± 1.528), SV2 (27 ± 1.0), SV3 (26.33 ± 1.528) and SV4 (28 ± 2.082).

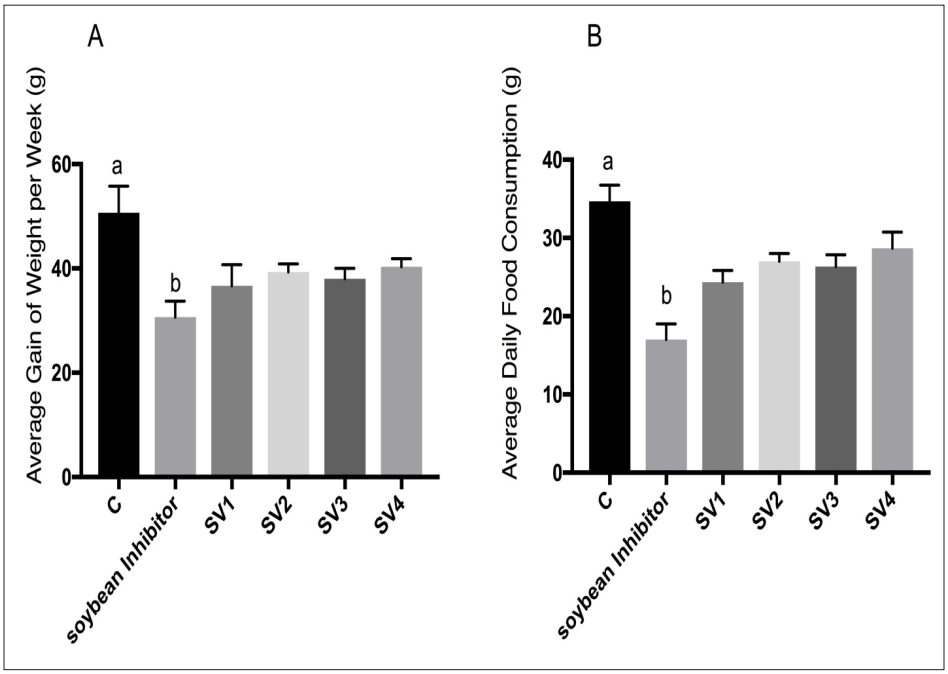

Fig 1. *Effects on weight gain and feed intake of rats supplemented with plant-based protein supplements, (C) Negative control, Soy inhibitor (positive control), (SV1) Soy protein concentrate supplement, (SV2) Soy protein concentrate supplement, (SV3) Rice protein concentrate supplement, (SV4) Pea protein concentrate supplement. *Different letters mean statistical difference with p <0.05.*

A recent study evaluated the inhibitory activities for trypsin, chymotrypsin and pepsin in supplements based on isolated plant proteins. The authors concluded that the use of these supplements has a negative influence due to the presence of enzymatic inhibitors, diminishing their functionality for hypertrophy purposes (ASSIS et al., 2018). However, further studies are necessary to prove this assumption of the authors.

Data present in the literature confirm that the consumption of foods with high inhibitory activity for proteolytic enzymes, when ingested in excess, has the capacity to reduce the protein digestion of food, providing a decrease in the weight gain and growth of the animals (MONTEIRO et al., 2004; MIURA et al. 2005; SERQUIZ et al., 2012; PEACE et al., 1991; BRUNE et al., 2010; RIBEIRO et al., 2015; CARVALHO et al., 2016).

Grant et al., (1988) and Hasdai et al., (1989) observed that the ingestion of soybean meal and raw beans by animals resulted in depression of the body weight gain or weight loss of animals. Serquiz et al. (2016) showed that an isolated peanut protein with anti-trypsin activity (AHTI) was able to significantly reduce fasting glucose, food intake and weight gain in a healthy experimental model.

The reduction in weight gain can be explained by the action of the inhibitors present in these foods, since they can act by suppressing the regulation of pancreatic secretion, by increasing the plasmatic level of the hormone cholecystokinin (CCK) by the intestinal mucosa (LIDDLE et al., 1984; LIENER, 1994; NAKAJIMA et al., 2011). This hormone is released in response to the presence of fat and protein in the gastrointestinal tract (KONTUREK et al., 2004). Presenting great relevance in digestive processes and satiation, interfering in food consumption (NAKAJIMA et al., 2011; KOMARRNYTSKY; COOK; RASKIN, 2011; CHEN et al., 2012; SERQUIZ, 2012).

Other studies corroborate the reduction of food intake by trypsin inhibitors, attributing this effect to the induction of CCK (CHEN et al., 2012; LIMA et al., 2016; RIBEIRO et al., 2015; KOMARNYTSKY et al., 2007; SERQUIZ et al., 2016). In 1982, Pi-Sunyer et al. described for the first time the effect of CCK on human satiety using infusion of exogenous CCK. Ballinger et al., (1995) reported a 20% reduction in caloric intake following a physiological infusion of CCK.

In 1992, Weller et al. carried out studies with neonatal rats which were given soybean trypsin inhibitor (1 mg in 230 mL of saline solution), and an increase of 87% in plasma concentrations of CCK was observed. Komarnytsky et al., (2011) also reported that oral administration of a protease inhibitor concentrate was effective in reducing food intake and weight gain in healthy rats by increasing circulating levels of CCK through a trypsin-dependent mechanism.

It is believed that the CCK effect on satiety can be explained by its synergistic effect with other hormones, increasing leptin and inhibiting the secretion of ghrelin, which are also responsible for the control of appetite (GOMES et al., 2004; PETERS et al. 2004).

Final Considerations

From the data in the present study, and based on findings from the literature, it can be suggested that the reduction in weight gain observed in the studied animals was probably due to satiety, which consequently caused them to reduce their food consumption and, in consecutive, caloric consumption. As previously mentioned, it is suggested that this reduction in appetite is due to the increase of CCK levels, through the trypsin- and chymotrypsin-dependent mechanism present in these foods.

Therefore, it is assumed that these supplements of vegetable origin are not the best alternative for the gain of muscle mass, as expected, due to a decrease in digestibility and even due to increased satiety, factors that are not interesting for protein synthesis. Therefore, it is evident the need for further *in vivo* studies that can evaluate and investigate these physiological and biochemical mechanisms, being able to prove the real reason for weight loss.

References

[1] ARAÚJO, J. M. A. **Química de Alimentos:** Teoria e Prática. Viçosa. UFV. 2011.

[2] ASSIS, L.O.; et al. Determinação Da Atividade Inibitória De Enzimas Digestivas Em Suplementos Vendidos No Comercio Internacional Á Base De Proteína Vegetal. **RevistaBrasileira de NutriçãoEsportiva.** 2018.

[3] BALLINGER A; MCLOUGHLIN L; MEDBAK S, CLARK M. Cholecystokinin is a satiety hormone in humans at physiological post-prandial plasma concentrations. **ClinSci (Lond).** 1995.

[4] BENEVIDES, C. M. J. et al. Fatores antinutricionais em alimentos: revisão. **Segurança Alimentar e Nutricional**, Campinas, v. 18, n. 2, p. 67-79, 2011.

[5] CARVALHO, F.M.C ; BEZERRA, A.D.L.; SANTOS, E.A.; MACHADO, R.J.A.; DANTAS, M.B.V.C.; ARAUJO, A.H. Phenolic compound sandantitryptic activity from pulpls and seeds of three varieties of grapes. **Revista Brasileira de Inovação Tecnologica em Saúde,** Natal, v.4, p.39-50, 2014.

[6] CHEN W, HIRA T, NAKAJIMA S, TOMOZAWA H, TSUBATA M, YAMAGUCHI K, et al. Suppressive Effect on Food Intake of a Potato Extract Involving Cholecystokinin Release in Rats. **BiosciBiotechnolBiochem.** 2012.

[7] CONCEIÇÃO, L. L.; DUARTE, M. S. L.; PEREIRA, C. A. S.; SOUZA, E. C. G. Determinação da atividade in vitro de Inibidores de Tripsina em feijão (PhaseolusVulgaris L.)-preto, albumina e globulina. Alimentos e Nutrição, A FANG, E. F.; WONG, J. H; BAH, C. S. F.; LIN, P.; TSAO, S. W; Ng, T. B.**; Biochem. Biophys. Res. Commun**. Vol. 396. Num. 806. 2010.

[8] FERRI, P. Extração de proteínas de folhas de mandioca (ManihotesculentaCrants) para obtenção de concentrado protéico. 2006. 112f. Dissertação (Mestrado em Engenharia Agrícola) – Universidade Estadual do Oeste do Paraná (Unioeste), Cascavel, PR GIRI, A. P.; KACHOLE, M.S. Amylase inhibitors of pigeon pea (Cajanuscajan) **seeds. Phytochemistry**. Vol. 47. p. 197-202. 2004.

[9] GOMEZ, G.; Englander, E.W.; Greeley, G.H., Jr. **Nutrient inhibition of ghrelin secretion in the fasted rat. Regul**. Pept. 2004.

[10] GRANT, G., WATT, W.B., STEWART, J.C., BARDOCZ, S., PUSZTAI, A. Intestinal and pancreatic responses to dietary soyabean (Glycine max) proteins. **Biochemical Society Transactions**, Colchester, v.16, n.4, p.610-611, 1988.

[11] HASDAI, A., NITSAN, Z., VOLCANI, R. Growth, digestibility, and enzyme activities in the pancreas and intestines of guinea-pigs fed on raw and heated soya-bean flour. **British Journal of Nutrition**, London, v.62, n.3, p.529-537, 1989.

[12] KOMARNYTSKY S, COOK A, RASKIN I. Potato protease inhibitors inhibit food intake and increase circulating cholecystokinin levels by a trypsin-dependent mechanism. **Int J Obes**. 2011.

[13] LIDDLE RA, GOLDFINE ID, WILLIAMS JA. Bioassay of plasma cholecystokinin in rats: effects of food, trypsin inhibitor, and alcohol. **Gastroenterology**. 1984.

[14] LIENER, I.E. Implications of antinutritional components in soybean foods. CRC Critical Reviews in Food Science and Nutrition, **Boca Raton**, v.34, n.1, p.31-67, 1994.

[15] LIMA, V.C.O.; et al. Gastroprotective and antielastase effect of protein inhibitors from Erythrinavelutina seeds in na experimental ulcer model. **Biochem. Cell Biol.** 2016.

[16] MAHAN LK, ESCOTT-STUMP S. **Alimentos, Nutrição&Dietoterapia**. São Paulo: Roca, 2005.

[17] MONTEIRO MRP, COSTA NMB, OLIVEIRA MGA, PIRES CV, MOREIRA MA. Qualidade protéica de linhagens de soja com ausência do inibidor de tripsina kunitz e das isoenzimaslipoxigenases**. RevNutr**. 2004.

[18] NAKAJIMA S, Hira T, Tsubata M, et al. Potato extract (Potein) suppresses food intake in rats through inhibition of luminal trypsin activity and direct stimulation of cholecystokinin secretion from enteroendocrine cells. **J Agric Food Chem** 2011.

[19] PETERS, J.H.; KARPIEL, A.B.; RITTER, R.C.; SIMASKO, S.M. Cooperative activation of cultured vagal afferent neurons by leptin and cholecystokinin. **Endocrinology** 2004.

[20] PETROVIC, G.; NIKOLIC, Z.; DORDEVIC, V. Endonuclease heteroduplex mismatch cleavage for detecting matation genetic variation of trypsin inihibitors in soybean. **Instituteof Field andVegetablesCrops**. Vol. 49. Num. 2. p. 102-108. 2014.

[21] PIRES CV, OLIVEIRA MGA, ROSA JC, COSTA NMB. Qualidade nutricional e escore químico de aminoácidos de diferentes fontes protéicas. Ciência e Tecnologia de alimentos (Campinas) 2006.

[22] PI-SUNYER X, KISSILEFF HR, THORNTON J, SMITH GP. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. **PhysiolBehav**. 1982.

[23] RIBEIRO J, SERQUIZ A, SILVA P, et al. Trypsin inhibitor from Tamarindusindica L. seeds reduces weight gain and food consumption and increases plasmatic cholecystokinin levels. **Clinics** 2015.

[24] RICHARDSON, M. Thue proteinase inhbitors of plants and micro-organisms. **Phytochemistry,**Inglaterra, v. 16, 1997.

[25] RYAN, C. A. Protease inhibitors in plants: genes for Improving defeses against insects and pathogens. **Anuu. Re. Phytopathol.** V. 28, p. 425-449, 1990.

[26] SACK, M.; e colaboradores. The increasing value of plant-made proteins. **CurrentOpinion In Biotechnology**. Vol. 32. p. 163-170. 2015.

[27] SANTOS EA, OLIVEIRA AS, RABELO LMA, et al. Affinity chromatography as a key tool to purify protein protease inhibitors from plants. **Affinity Chromatography. In Tech**. 2012. p. 212–44.

[28] SANTOS, E.A.; OLIVEIRA, A.S.; RABÊLO, L.M.A.; UCHÔA, A.F.; MORAIS, A.H.A. Affinity Chromatography as a Key Tool to Purify Protein Protease Inhibitors from Plants (2012). **AffinityChromatography** SERQUIZ AC. Efeito sacietogênico de um novo inibidor de tripsina da paçoca do amendoim com aumento plasmático de colecistocinina (CCK). Universidade Federal do Rio Grande do Norte; 2012.

[29] SERQUIZ, A. C.; e colaboradores. Supplementation with a new trypsin inhibitor from peanut is associated with reduced fasting glucose, weight control, and increased plasma CCK secretion in an animal model. **Journal of Enzyme Inhibition and Medicinal Chemistry**. Vol. 31. Num. 6. p.1261-1269. 2016.

[30] SGARBIERI VC. **Alimentação e Nutrição**. São Paulo: Almed; 1987.

[31] SILVA, M. R.; SILVA, M. A. A. P. Fatores antinutricionais: inibidores de proteases e lectinas. **Revista de Nutrição,** Campinas, v. 13, n. 2, jan/abr., 2000.

[32] WELLER A, et al. Trypsininhibitorand maternal reunionincrease plasma cholecystokinin in neonatal rats. **Peptides.** 1992.

[33] ZANG J, LI D, PIAO X, TANG S. Effects of soybean agglutinin on body composition and organ weights in rats. **ArchAnim Nutr**. 2006.

Citation: **Ingrid Lannay Rodrigues da Silva, Alexandre Coelho Serquiz.** *Evaluation of Protein Supplements Isolated from Vegetables (Soy, Pea and Rice) on Weight Gain and Food Consumption in Wistar Rats. Archives of Physical Health and Sports Medicine. 2018; 1(2): 08-14.*