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Use of whey peptide fraction in coated cashew nut as functional ingredient and salt replacer

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ABSTRACT

Consumers are increasingly concerned about healthy eating habits. The incorporation and stability of 2% antihypertensive whey peptide extract in a new coating of cashew nuts with reduced salt (less 15 and 30%) was studied. The evaluation of nutritional value, *in vitro* antihypertensive activity and consumer acceptance of final products was assessed. Incorporation of peptide fraction assured the production of a snack with an ACE-inhibitory activity (532.2 μg mL^{-1} IC_{50} value). The amount of lipids present in coated cashew nuts was composed mainly by essential fatty acids, mostly monounsaturated. Glutamic acid, leucine, arginine and aspartic acid were the most abundant essential aminoacids. 70% of the consumers considered both samples (15 and 30%) as "ideal taste". The results suggest that the new coating allowed the development of a new snack with reduced salt content, opening new opportunities as carrier of other ingredients to develop more diversified and efficient functional foods.

1. Introduction

In the last years, nuts have been highlighted as an important constituent of a healthy diet, although their real intake varies remarkably in human populations in different regions of the world. They constitute a good source of certain vital bioactive compounds that could elicit many health benefits in human beings. Several studies show a connection between frequent nut consumption and reduced incidence of several chronic diseases, such as obesity and body weight gain (Ros, 2010), Cashew (*Anacardium occidentale* L.) is a tropical fruit with high content of ascorbic acid. The main product of consumption is the nut, occupying a significant and popular place in the diet of the human population as an appetizer and is one of the most important edible nuts being widely consumed in Europe, USA and Asia (Ogunsina, Owolarafe, & Olatunde, 2008). Cashew nut is reported to be rich in fat (46%), protein (21.2%) and carbohydrates (22.3%); providing 596 kcal of energy per 100 g of intake. In addition, it contains substantial amounts of essential amino acids, vitamins and minerals (Ogunsina & Bamgboye, 2014). Its fatty acid profile has been indicated in the control of cholesterol (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2006) and selenium has shown to play an important role as an antioxidant, participation in thyroid metabolism, and suggesting bioactivity in cancer prevention (Kannamkumarath, Wrobel, Wrobel, Vonderheide, & Caruso, 2002). Usually the processed nuts are consumed as snacks in roasted and salted form, or as ingredients in different food products, including chocolate bars, confectionary and baked goods, candies and ice-cream toppings. Combining nutritional facts of this nuts and facing a demanding consumer society with increasing concern about health problems, cashew nut is a key target to develop a functional food more attractive and more readily usable by the consumer. So, the main objective of this study was the development of a cashew healthy snack with reduced salt content.

Biologically active peptides are food-derived peptides that exert regulatory functions in humans beyond normal and adequate nutrition. They are mainly found in milk (major source of bioactive peptides), egg, meat and fish as also in many plants. Depending on the sequence of amino acids, they can exhibit diverse biological activities, including hearth disorders (Erdmann, Cheung, & Schröder, 2008). In view of current trends of cardiovascular diseases, hypertension represents a major risk factor and their control with alternative therapies to drugs has been widely studied. There are several mechanisms wherein peptides from dairy products are the most successful studied on the effect of inhibiting angiotensin converting enzyme (Marques et al., 2012).

Because of their health-enhancing potential and safety profiles

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biopeptides may be used as ingredient in functional foods. Researches in this field have intensified during the past two decades and have been extensively reviewed. Some products are already in the market or under development by food companies that exploit the potential of food-derived bioactive peptides and which attribute scientifically evidenced health claims to consumption of these functional foods (Phelan, Aherne, FitzGerald, & O'Brien, 2009). For above reasons, whey-derived bioactive peptides are considered as prominent candidates for various health-promoting functional foods targeted at heart, bone and digestive system health as well as improving immune defense, mood and stress control (Korhonen, 2009).

On the other hand, it has become common knowledge that excess intake of sodium ion leads to hypertension and coronary heart disease (Beck, Jekle, & Becker, 2012). In recent years, the industry has highlighted the reduction of salt content of products with minor impact in the taste. The World Health Organization (WHO) recommends a maximum daily consumption of 2 g of sodium or 5 g of salt. Although consumers increasingly seek healthier products, besides the safety problems, the taste is often the most critical purchase factor. It is therefore of crucial importance that salt substitutes have flexibility for use in many products, are not expensive and especially, do not change organoleptic characteristics (Morley, 2012). Furthermore, whey mineral concentrate supplementation, has been found to lower blood pressure. The major electrolyte in whey mineral is potassium, but it also increases the intakes of calcium and magnesium, all of which are regarded as beneficial changes in arterial function (Pal & Radavelli-Bagatini, 2013).

Therefore, considering the nutritional properties, popularity and convenience of cashew nuts, makes this is an ideal carrier to incorporate functional ingredients such as bioactive whey peptide extract (with antihypertensive properties and healthy salt profile) to generate a new functional food to promote health and prevent disease. Thus, the main objective of this study was to develop a new healthy snack by coating cashew nuts with bioactive peptides and reduced salt content, through incorporation of whey peptide fraction with proven antihypertensive activity. In addition, evaluation of their nutritional properties, peptide stability and consumer acceptance were also encompassed.

2. Materials and methods

2.1. Production of whey bioactive peptide fraction

Whey peptide extracts were obtained from a whey mixture of 80% cow, 18% goat and 2% ewe's whey, kindly provided by Saloio (Torres Vedras, Portugal), using a pilot-scale filtration equipment according to the procedure already described by Tavares, Amorim, Comes, Pintado, Pereira, and Malcata (2012) and described in Fig. 1. Whey protein retentate was hydrolyzed with an aqueous extract of *Cynara cardunculus* (Formulab, Maia, Portugal) at an enzyme/substrate ratio of 3% (v/v) incubated for 3 h at pH 5.2 and 55 °C. Hydrolysate extract was nanofiltered using an organic membrane PTI Advanced Filtration (model NF 3838/30-FF), area 6,9 m^2 with 3 kDa cut-off and concentrated by reverse osmosis using a pilot plant module (ORM – Tecnologia e Ciência na Indústria Lda, Belas, Portugal) with 2.5 S Seawater membrane (Advanced Structures Inc., Los Angeles, CA, USA) up to 10% of total solids and freeze dried 0.05 mbar (Martin Christ Alpha 1–4 LD plus, Osterode Germany). The fraction used to coat cashew nuts was molecular weight below 3 kDa (Peptide extract < 3 kDa).

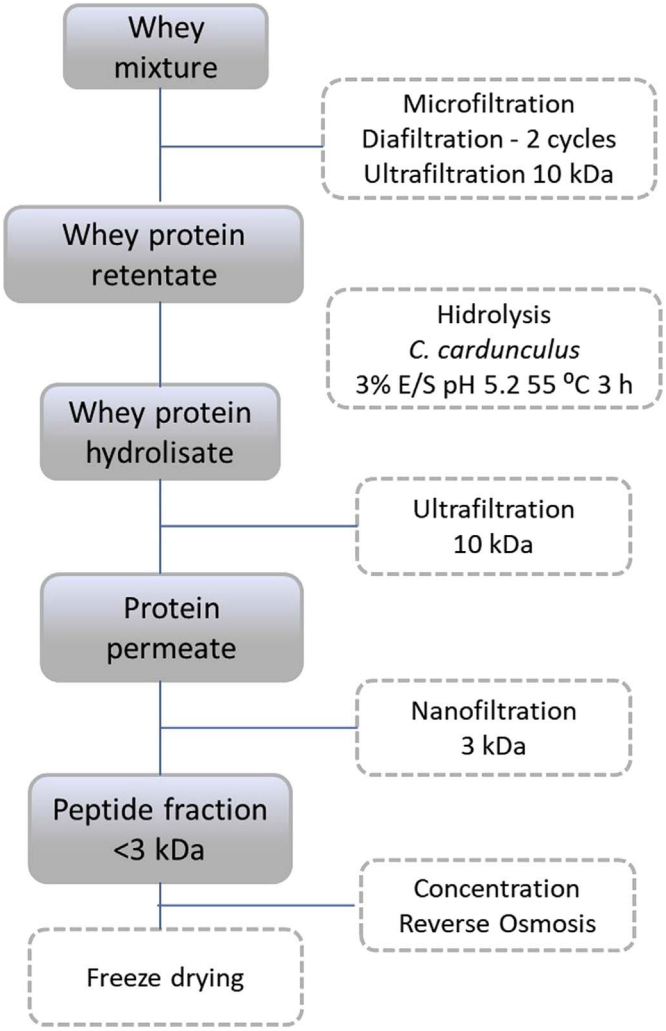

Fig. 1. Flow diagram of pilot-scale whey filtration process combined with enzymatic hydrolysis, used to obtain peptide concentrates.

2.2. Cashew nuts with expanded coating

2.2.1. Samples

Four different samples were made with the procedure describes below: (i) coated cashew nuts without peptide fraction; (ii) coated cashew nuts with 2% of < 3 kDa peptide fraction; (iii) coated cashew nuts with 2% of < 3 kDa peptide fraction and 15% reduction in the salt content and (iv) cashew nuts coated with 2% < 3 kDa peptide fraction and 30% reduction in salt content.

Cashew nut coated with and without peptide fraction was used for compositional analysis and antihypertensive peptide stability IC_{50}. For sensory analysis, salt content of coated recipe was reduced by 15 and 30% and was added 2% of < 3 kDa peptide fraction.

2.2.2. Solutions

All dry ingredients used were food grade and bought in local market (Campinas/Brazil).

2.2.2.1. Gumming solution. Gumming solution was prepared by adding 40% of acacia gum in 60% of water and stirred until complete dissolution of the gum. This solution is able to form a continuous and homogeneous film on the cashew nut surface preventing migration of the fat to the coating.

2.2.2.2. Adhesion syrup. **Sample without peptide extract:** All dry ingredients–33% sugar, 9.5% dextrin and 5.5% refined salt – were mixed and dispersed in water. The solution was heated up to 90 °C with stirring and allowed to stand until cool down (60 °C). A solution of 0.5% Annatto (a natural food coloring from achiote tree seeds) was added to the mixture to provide color. **Sample with peptide fraction**: All dry ingredients–33% sugar, 9.5% dextrin; 5.5% refined salt and 2%

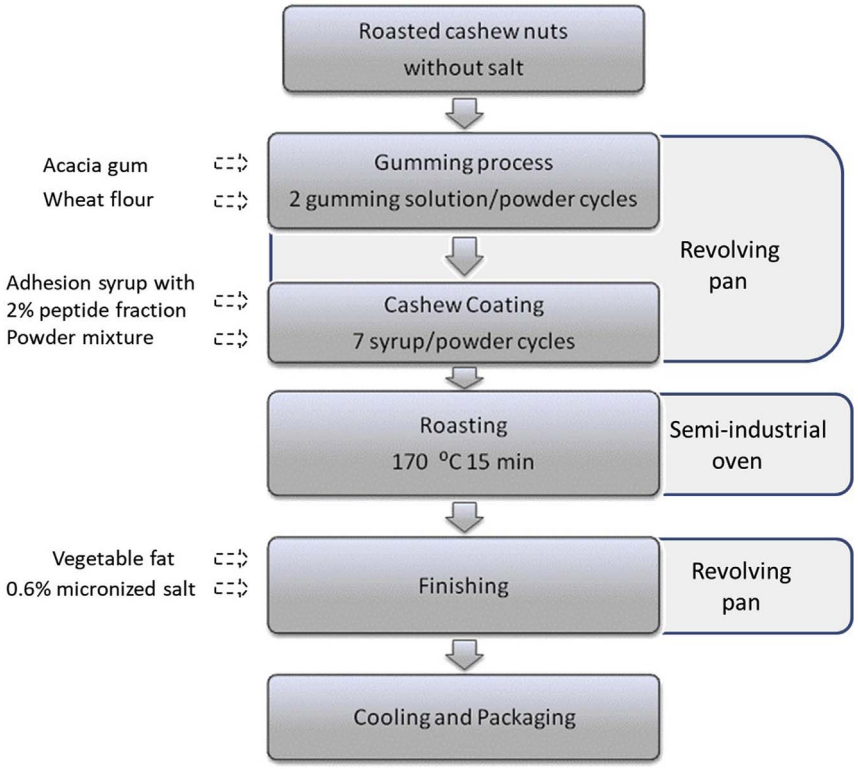

Fig. 2. General flowchart of manufacturing coating of cashew nuts snacks with expanded coating.

of < 3 kDa peptide extract – were mixed and dispersed in water, and following the procedure described above. S**amples with low content of salt**: all ingredients and procedure previously described for sample with 2% of < 3 kDa peptide fraction, except for refined salt, which was added 0.83 and 1.65% (reduction of 15 and 30% of salt content).

2.2.2.3. Powder mixture. Mixture consisting of 75% of wheat flour, 25% of pregelatinized starch and 2.6% of refined salt.

2.2.3. Cashew nuts coating: process description

Four batches were performed (one for each sample - 4 samples). Each batch was formed by the following steps (Fig. 2): **Gumming**: 500 g of cashew nuts (local market, Campinas, Brazil) were placed in a pilot revolving pan - capacity 5 L (Incal, JAA 110 Model) 12 rpm and were added 2 cycles of gumming solution/wheat flour: each cycle took 6 min and consisted of adding 20 mL of gumming solution and 30 g of wheat flour. **Cashew coating**: to gumming/flour cashew nuts were applied 7 cycles of adhesion syrup (30 mL/each cycle) and dry powder mixture (40 g/each cycle), promoting a rapid buildup of cashew's shell. Each syrup/powder cycle was applied during 4 min. **Roasting**: the coated cashews were placed in a semi-industrial oven (Vipinho 045002126, Curitiba, Brasil) at 170 °C for 15 min. **Finishing**: in order to add visual and taste appeal, the coated cashews were finally coated with 1% of vegetable fat and 0.6% of micronized salt per for 100 g of coated cashew, in a revolving pan previously used. Fig. 3 represents the process and final appearance of cashew nuts.

2.3. Physicochemical analysis

Total solids, crude protein (N x 5.3), ash and carbohydrates content of coated cashew nuts and < 3 kDa peptide fraction were determined by the AOAC procedures (Horwitz, 2010). Fatty acid composition was made according Firestone (2009). Total fiber was determined according to enzymatic-gravimetric technique (Prosky, Asp, Schweizer, DeVries, & Furda, 1988). The carbohydrate content was estimated: 100 - (moisture + protein + lipid + dietary fiber + ash). The amino acids levels were determined by reverse phase-high performance liquid chromatography (RP-HPLC) following White, Hart, and Fry (1986) methodology. Quantification was performed by internal calibration, using detection at 254 nm. All analysis were made in triplicate.

2.4. ACE inhibitory activity in vitro assay

Angiotensin converting enzyme - ACE-inhibitory effect of coated cashew nuts and whey peptide fraction < 3 kDa was measured using the fluorimetric assay (Quirós et al., 2007). The protein content of the peptide fractions was determined by bicinchoninic acid assay (Pierce, Rockford IL, USA), using bovine serum albumin as a standard and absorbance was measured at 562 nm in a microplate reader (FLUOstar Optima, BMG, Germany). Data non-linear fitting was performed to calculate the IC_{50} values and all analysis were made in triplicate.

2.5. Peptide analysis by RP-HPLC-MS/MS

Peptide sequence of < 3 kDa peptide faction was analyzed by RP-HPLC-MS/MS using an 1100 HPLC system (Agilent Technologies, Waldbron, Germany) with a Hipore® RP318 C18 column (250 mm of length, 4.6 mm of inner diameter and 5 µm of particle size) from Bio-Rad (Richmond CA, USA) and mobile phases composed for: solvent A - mixture of water and trifluoroacetic acid (TFA) (1000:1, v/v), and solvent B - acetonitrile and TFA (1000:0.8, v/v). The peptides were eluted with a linear gradient of solvent B in A going from 0% to 20% over 60 min, at a flow rate of 0.5 mL/min. HPLC system was connected online to an Esquire 3000 quadrupole ion trap instrument (Bruker Daltonik, Bremen, Germany), equipped with an electrospray ionization source with methodology described by Hernández-Ledesma, Dávalos, Bartolomé, and Amigo (2005). Spectra were recorded over the mass/charge (m/z) range 100–1500. BioTools (version 2.1; Bruker Daltoniks) was used to process the MS(n) spectra and to perform peptide sequencing.

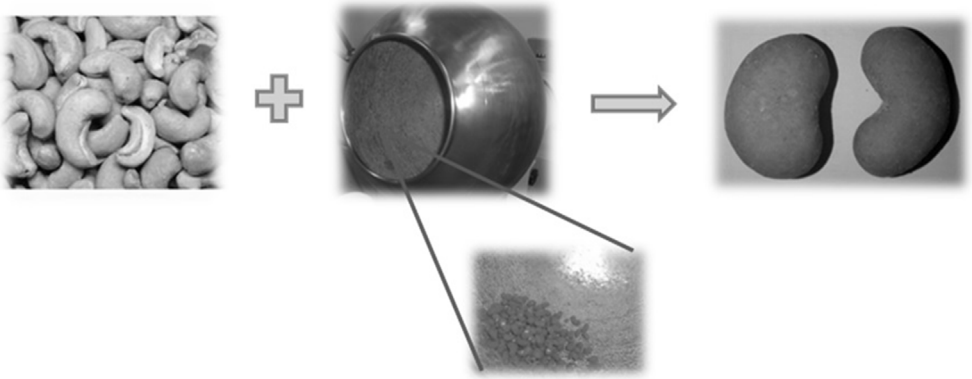

Fig. 3. Images of cashew coating process making evidence of the more representative transformation steps and of final appearance of coated cashew nuts.

2.6. Sensory analysis

To evaluate the acceptability of the new product, consumers panelists have been recruited among staff and interns ITAL (Campinas, Brazil) including 60 consumers of nuts/peanuts, aged between 18 and 60 years. Participants were informed about its general aim and procedures for handling personal data, and gave written informed consent prior to participation. They were informed that coated products possed functional ingredients with biological properties, namely potential antihypertensive activity. All samples were produced and prepared according to good hygiene and manufacturing practices. The samples were evaluated for the overall acceptability and in particular to appearance, aroma, flavor and crispness and liking ratings (OL) were provided on a 9-point hedonic scale. The intensity of salt through a 5 points scale (5 = much too salty, 3 = just about right, 1 = not at all salty enough) and the purchase intent through a 5-point scale (5 = certainly buy, 3 = maybe buy, maybe not, 1 = certainly not buy). Panelists were also asked to describe what product they liked best and least (Meilgaard et al., 2006).

The test was conducted in individual booths with fluorescent lighting and equipped with computerized Compusense Five software version 4.8 (Guelph, Ontario, Canadá) for collecting and analyzing data.

In addition to issues related to product evaluation, panelists answered questions about their consumption patterns and characteristics related to age and social class definition.

2.7. Statistical analysis

Results were expressed as mean ± standard deviation and compared by analysis of variance (ANOVA) followed by the Tukey's test. Differences were considered statistically significant at $p \leq .05$. Statistical analysis was performed using the SPSS software program (SPSS 16.0, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Production of whey protein and whey peptide concentrates

Membrane technologies have been proving to be an asset and easily applicable to a wide range of processing namely to purify peptide fractions from a complex mixture (Amorim et al., 2016). Protein fractions and peptide extracts were obtained through sequential steps combining pilot filtration process and hydrolysis applied to whey mixture (bovine, ovine and caprine whey), Fig. 1. Peptide fraction < 3 kDa was selected, since besides having a high content of peptides - ca. 68% of protein content, also contains a mixture of minerals – sodium, potassium, phosphorous and calcium (Table 1), which are favorable in the treatment of coronary diseases and is also commonly used for maintaining healthy body weight in humans (Zhou, Keenan, Losso, Raggio, Shen, McCutcheon, et al., 2011) and can be used as substitute of salt by reducing its content in final product, being already referred several studies in which they use milk minerals as salt replacers (Cepanec, Vugrinec, Cvetković, & Ranilović, 2017; Engeloug, Yi, Egelandsdal, Haug, & Nordvi, 2017; Smith, Metzger, & Drake, 2016).

Table 1
Protein, sugar and mineral content (% w/w, dry basis) of < 3 kDa peptide fraction obtained in semi-industrial system.

	< 3 kDa peptide fraction (% of dry basis)
Protein	68 ± 0.3
Sugars	20 ± 0.10
Ash	8 ± 0.09
Minerals	
K	2.12 ± 0.05
Ca	0.38 ± 0.01
P	1.70 ± 0.03
Na	3.80 ± 0.13

Results were expressed as mean ± standard deviation.

3.2. Physicochemical properties of coated cashew nut

It is important to assure an equilibrated nutritional composition a food when it is being a carrier for a functional ingredient and specially if processed. The nutritional composition of peptide coated cashew nuts is presented in Table 2. All analysis was performed to final product (roasted coated cashew nut).

Regarding fat content (26.0 g 100 g $^{-1}$ sample) is lower when compared with roasted and salted cashew nuts with values for total lipids ca. 46.3 g 100 g $^{-1}$ sample (TACO, 2011), due the cashew base used for coating was a non-processed cashew and, during coating, only low amounts of vegetable oil were added. This fact is a favorable factor, since the lipids present in coated cashew nuts are represented mainly by natural essentially fatty acids present in the nut and not extra saturated lipids introduced by traditional processing of these kind of snacks. As essentially fatty acids present in non-processed cashew are mostly monounsaturated fatty acids, which are in accordance with the literature (Rico, Bulló, & Salas-Salvadó, 2016) and this kind of nuts are a good dietary source of unsaturated fatty acids, tocopherols, squalene and phytosterols, being a source of bioactive constituents that elicit cardio-protective effects (Ryan et al., 2006). The amount of carbohydrates is increased (50.3 g 100 g^{-1} sample) towards TACO reference values 29.1 g 100 g^{-1} due to the cashew coating, which was performed based on wheat flour, sugar and dextrin. In future studies, the concentration of these additives can be manipulated, in order to reduce

Table 2
Nutritional composition and fatty acid profile of coated cashew nut with 2% of peptide fraction.

Analysis	g100g^{-1} sample
Moisture	2.7 ± 0.0
Total fat	26.0 ± 0.8
Crude protein (N x 5.3)	14.2 ± 0.2
Ash	3.4 ± 0.1
Total dietary fiber	3.5 ± 0.1
Total carbohydrate[a]	50.3
Energy (kcal)	492
Fatty Acids	**g100g^{-1} sample**[b]
Saturated	7.8
Monounsaturated	26.7
Total Poliunsaturated	8.2
Ômega 3	0.1
C16:0 palmitic	3.98
C18:0 stearic	3.45
C20:0 arachidic	0.23
22:0 behenic	0.06
C 24:0 lignoceric	0.06
16:1 ω 7 palmitoleic	0.13
18:1 ω 9 oleic	26.50
20:1 ω11 *cis*-11-eicosenoic	0.08
18:2 ω6 linoleic	8.07
18:3n-3 ω 3 α linolenic	0.08
18:1t	0.11
18:2t	ND
ω 6/ω 3*	100.87

[a] Calculated as: 100 - (g100g-1 moisture + g100g-1 ash + g100g-1 crude protein + g100g-1 total fat + g100g-1 total dietary fiber).
[b] Area x % Total fat/100 × Conversion Factor (F = 0.956). Detection limit of the method = 0.01 g/100 g. ω = omega, α = alfa. N.D. = Not detected. *rates linoleic acid (ω 6) and linolenic acid (ω 3). The caloric value of the sample was calculated as the sum of the percentages of protein and carbohydrates multiplied by factor 4 (kcal/g) plus the total lipid content multiplied by the factor 9 (kcal/g).

their content and make coated cashew nuts more similar to commercial snacks, or alternatively replace them by non gluten carbohydrates and preferncially higher fiber content to improve functional properties. Values were compared with reference values for roasted and salted cashews described by Brazilian Food Composition Table – Taco (TACO, 2011), this type of cashew was selected for comparison, since that was the most similar with the product developed in this work.

Ash analysis provides advance information on the nutritional value regarding to mineral content. In the coated cashew is slightly higher $p \leq .05$ (3.4 g 100 g^{-1}) than the reference cashew (2.6 g 100 g^{-1}), due to the presence of minerals in the peptide fraction. There are studies that use of whey powder for the replacement of refined salt (Petersen et al., 2014). Thereby, in this study this factor was taken into account and salt content was reduced and use the peptide fraction as a source of natural minerals beyond the antihypertensive activity. Concerning amino acid content, as shown in Table 3, cashew nuts contain 18 of the 22 amino acids used by humans, including all of the essential ones (NutriBase, 2001). As a result, cashew nuts are considered a high-quality or complete source of protein usually difficult to find in a vegetable protein source. Cashew are particularly high in glutamic acid, leucine, arginine and aspartic acid, but relatively low in cystine and methionine. Concerning essential amino acids, coated cashew nuts had values according reference values indicated by FAO/WHO/UNU (2007), being at higher concentrations for almost all essential amino acids.

3.3. In vitro biological activities

Protein hydrolysates produced with enzyme preparations can be used to manipulate peptide molecular weight distributions and free amino acid content, therefore increasing yields of specific hydrolysates containing potentially bioactive, low-molecular-weight peptides (Gilmartin & Jervis, 2002). These small peptides make a considerable contribution to a biological potential of protein hydrolysates, and are good candidates to be *in vivo* physiologically bioactive agents. Considering the above, the peptide fraction with Mr < 3 kDa was analyzed to determine their ACE inhibitory activity.

Table 3
Amino acid composition of coated cashew nut and FAO reference values.

Amino acids	Peptide coated cashew nut g 100 g^{-1} protein	Amino acid requirement* g 100 g^{-1} protein
Threonine**	3.22	2.30
Histidine**	2.20	1.50
Valine**	4.82	3.90
Methionine + cystine**	1.09	2.20
Cystine**	0.71	0.6
Isoleucine**	3.58	3.00
Leucine**	7.02	5.90
Tryptofan**	5.46	0.60
Phenylalanine + Tyrosine**	4.60	3.80
Lysine**	3.17	4.50
Aspartic Acid	7.43	N/E
Glutamic Acid	24.91	N/E
Serine	5.31	N/E
Glycine	4.24	N/E
Tyrosine	3.01	N/E
Arginine	9.84	N/E
Proline	7.33	N/E
Alanine	3.36	N/E

Data are the average of three determinations (g amino acid100 g^{-1} cashew nuts).
*Reference values, adult indispensable amino acid requirements (FAO/WHO/UNU, 2007).
**Essential amino acids.
N/E Not essential.

ACE-inhibitory activity of whey peptide extract was measured and calculated the IC_{50}, as it represents the protein extract concentration required for 50% *in vitro* enzyme inhibition. The peptide fraction with Mr < 3 kDa produced with this sequential system exhibited high ACE-inhibitory activity (IC_{50} 12.8 μg mL^{-1} of protein content), regarding peptide fraction with Mr > 3 kDa (Table 4), suggesting peptides released from the proteins are the agents behind inhibition.

Peptides with antihypertensive activity consist of only two to nine amino acids and that most are di or tripeptides, making them resistant

Table 4
IC_{50} values for < 3 kDa peptide fraction and coated cashew nuts with and without peptide fraction. Predominant peptide sequences identified by RP-HPLC-MS/MS in < 3 kDa peptide fraction.[a]

Samples	IC50 μg mL^{-1} protein
Peptide fraction > 3 kDa	92.0 ± 0.8
Peptide fraction < 3 kDa	12.8 ± 0.2
Cashew nuts with peptide coating	532.2 ± 1.4
Cashew nuts coated without peptide fraction	No inhibition

Sequence[a]	Mass (Da)	Protein source	Source
KGYGGVSLPEW	1191	α – Lactoalbumin	Cow
DKVGINYW	993.1	α – Lactoalbumin	Cow
MAIPPKKNDQD	1140	k-Casein	Cow
VQVTSTAV	803.4	k-Casein	Cow
KTEIPTIN	914.51	k-Casein	Cow
TSTAVVQV	804	k-Casein	Cow
RELEEL	787.41	β-Casein	Cow
LEPLQGAV	826.38	Lactoferrin	Cow
REQEEL	803.32	β-Casein	Goat
PHLSFMAI	915.0	k-Casein	Goat

[a] Predominant peptide sequences with identity confirmed ($p < .05$).

to endopeptidase action in the digestive tract (Kitts and Weiler (2003). The ACE-inhibitory activity in the hydrolysate studied here was higher than reported by Contreras, Carrón, Montero, Ramos, and Recio (2009) for the supernatants from the casein hydrolysates prepared with pepsin (22.19 μg mL^{-1} of protein content) and bovine lactic casein hydrolysate (74 μg mL^{-1} of protein content) using a combination of three enzymes, namely, subtilisin, bacillolysin, and trypsin (Yamada et al., 2013).

In vitro biological potential observed in the enzymatically hydrolyzed whey proteins was higher than the 140 μg mL^{-1} of protein content presented by Li, Qu, Wan, and You (2007) for a rice protein hydrolysate. Also, enzymatic hydrolysates from different plant protein sources with IC_{50} values ranging from 200 to 246, 700 μg mL^{-1} of protein content have been shown *in vitro* ACE inhibitory activity as well as antihypertensive activity *in vivo* (Pihlanto & Ma¨kinen, 2013).

Tavares, Sevilla, Montero, Carron, and Malcata (2012), have proved *in vivo* the activity of peptide extract from whey proteins, which was similar to peptide fraction used in this work. In that study, after a single oral administration in spontaneously hypertensive rats (SHR), the extract exhibits an antihypertensive effect. This result leads us to elucidate the effectiveness of peptide extract produced and used in cashew coating, suggesting this use as a physiologically functional ingredient with potential benefits in the prevention and/or treatment of hypertension.

When incorporated in cashew coating, the hydrolysate bioactivity (12.8 μg mL^{-1} of protein content) declined notably (532.2 μg mL^{-1} of protein content) as expected, although the final product must be considered with high incorporation efficiency, once only 2% of peptide fraction was added and there was a high dilution in the matrix; however, the manipulation of formulation in future by increasing the concentration of peptides in the product may assure modulated level of antihypertensive activity. However, is important to highlight that the antihypertensive activity was not lost even when using high temperatures during adhesion syrup preparation and roasting of the product, proving the high stability of the ingredient, this is since low molecular weight peptides are more likely to be stable to the heating process, whereas, the proteins with secondary, tertiary and quaternary structure (Elavarasan, Shamasundar, Badii, & Howell, 2016). However, considering functional food with antihypertensive activity there are several studies reporting IC50 *in vitro* values higher than those obtained in this study, as in the case of (Miguel, Manso, López-Fandiño, Alonso, and Salaices (2007)) which reported IC_{50} values of 4785 μM for κ-casein macropeptide, being about 3 g/L of protein. Thus, this suggests that even in roasted coated cashew nut was obtained higher values for IC_{50} than for the isolated extract, they are within the values reported for other functional products claiming antihypertensive activity, such Ameal S® contains casein hydrolysate, Valio® - fermented milk product and other similar products with contains ACE inhibitory peptides as active component (Mine, Li-Chan, & Jiang, 2011). Nevertheless, in future, further *in vivo* studies with cashew nuts must be performed, with all, this study represents the beginning of a different approach in healthy snacks formulation with peptides from whey proteins.

3.4. Peptide identification

Table 4 also shows amino acid sequence of 10 peptides corresponding to the most representative peptides present in < 3 kDa fraction analyzed by HPLC-MS/MS in an attempt to identify the most likely responsible for the aforementioned bioactivity.

Wu, Aluko, and Nakai (2006) proposed structural characteristics for ACE-inhibitory peptides considering sequences with hydrophobic amino acid residues at the amino terminus and aromatic amino acids at the carboxyl terminus. In this study, all identified peptides contain between 6 and 11 residues and they are particularly rich in isoleucine, valine and tryptophan residues, amino acids related to the ACE-inhibitory activity, as well as, proline - Pro, which has been recognized as an enhancer of binding to the active site of ACE (Pan, Cao, Guo, & Zhao, 2012).

Peptides with the sequence KGYGGVSLPEW and DKVGINYW identified in our extract, entail an antihypertensive effect *in vivo*, upon oral administration to spontaneously hypertensive rats SHR by gavage demonstrated by Tavares et al. (2012). This support the potential antihypertensive effect in our whey peptide fraction used in cashew coating.

Table 5
Acceptability profile obtained for cashew nuts samples - coated cashew nuts without peptide extract; coated cashews with 2% of < 3 kDa peptide fraction and 15% reduction in the salt content and coated cashew nut with 2% of < 3 kDa peptide fraction, concerning to overall appearance, aroma, flavor and crispness, and regarding the optimal intensity of salt and the purchase intention.

Evaluation		Samples			
		Standard	15% reduction salt	30% reduction salt	M.S.D.
Acceptability	Appearance	7.4 ^{a} ± 1.2	7.4 ^{a} ± 1.1	7.3 ^{a} ± 0.8	0.32
	Aroma	7.3 ^{a} ± 1.2	7.3 ^{a} ± 1.2	7.3 ^{a} ± 1.0	0.39
	Flavor	7.2 ^{a} ± 1.2	7.3 ^{a} ± 1.1	7.2 ^{a} ± 1.2	0.49
	Crispness	7.5 ^{a} ± 1.3	7.2 ^{a} ± 1.3	7.4 ^{a} ± 1.1	0.46
	Overall	7.2 ^{a} ± 1.1	7.3 ^{a} ± 1.0	7.4 ^{a} ± 0.8	0.38
Intensity of salt		2.7 ^{b} ± 0.5	3.2 ^{a} ± 0.6	2.8 ^{b} ± 0.7	0.20
Purchase intent		4.0 ^{a} ± 1.1	3.9 ^{a} ± 1.1	4.2 ^{a} ± 0.9	0.42

Results are expressed as mean ± standard deviation. MSD: minimal significant difference at the level of error of 5% by Tukey test. In each row, values followed by the same letter do not differ statistically among themselves the error level of 5%. Liking ratings were provided on a 9-point hedonic scale.

3.5. Sensorial evaluation – acceptability test

Nine questionnaires were either incomplete or contained invalid answers. Their data was thus excluded from further analysis, yielding 51 valid questionnaires. The results obtained from the acceptability tests namely appearance, aroma, flavor and crispness, the intensity of salt and the intention to purchase the products are presented in Table 5. Both samples–15 and 30% of salt content reduction presented means that positioned in the range between like moderately and like very much for all the attributes evaluated and did not differ significantly from each other ($p \geq .05$).

The attribute intensity of salt was the only exception, with 15% salt reduction sample differing significantly ($p \leq .05$) from the remaining samples. Therefore, as can be shown in Fig. 4, 70 and 60% of the consumers considered both samples as “ideal taste”, namely, 2% peptide fraction with 15% salt reduction and 2% peptide fraction with 30% salt reduction samples, respectively. In addition, the sample with the highest frequency of positive purchase intent (certainly buy or probably buy) was 30% salt reduction (84%), followed by the sample with 15% salt reduction (73%) and without peptide fraction (59%).

As to the positive aspects described by consumers when questioned what they liked in each of the samples, there were 34–36 positive mentions for crispness and from 17 to 25 that refer to the flavor. About the reasons for dissatisfaction, the standard samples, 15% and 30% salt reduction showed 11 and 14 that refer to the flavor, respectively.

4. Conclusion

The results confirm that proposed process for the development of a new functional coated cashew nut could assure the efficient incorporation and stability of antihypertensive peptides (< 3 kDa) obtained from the fractionation of whey proteins hydrolysate. However, although the final *in vitro* antihypertensive activity (532.2 μg mL^{-1} of protein content) of coated cashew nuts was close to other products in the market with equivalent activity, the increase of peptide fraction in the formulation may assure improved level of antihypertensive activity

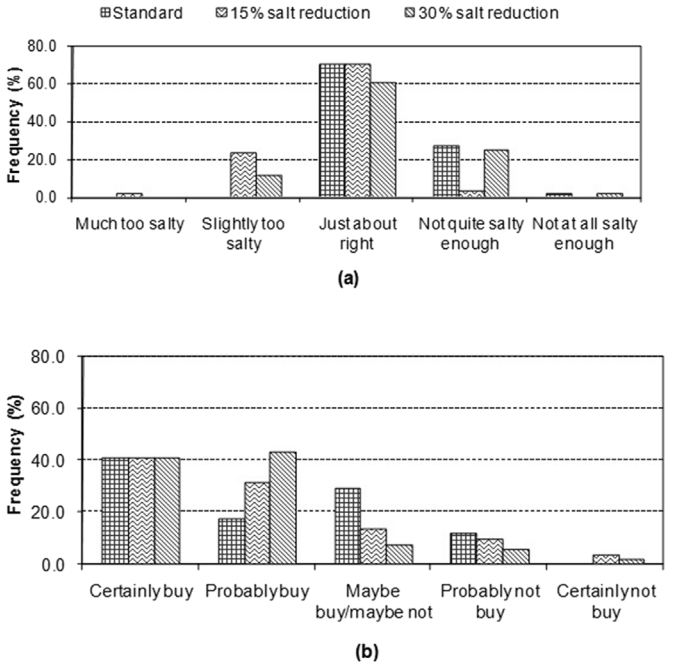

Fig. 4. Frequency distribution of liking ratings given by the consumers to samples of cashew nuts with peptides as the optimal intensity of salt (a) and purchase intention (b).

according the expected final claim. Moreover, this extract was able at low concentrations (2%) to replace successfully 30% of the conventional salt, thereby directly reducing the salt content in the snack, assuring a positive health impact. The sensory analysis of the plain coated cashew nuts, developed for the first time had a very good acceptance by consumers, and the addition of peptide fraction combined with the reduction of up to 30% of salt, did not affect significantly the appearance and taste as well as the very good acceptance or purchase intention by consumers. All this suggests that the application of peptides extracts with biological activity in the development of new crunchy cashew nut coating for preparation a snack with reduced salt content was possible, opening up a new trend in the development of new value-added food products.

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References

Amorim, M., Pereira, J. O., Gomes, D., Pereira, C. D., Pinheiro, H., & Pintado, M. (2016). Nutritional ingredients from spent brewer's yeast obtained by hydrolysis and selective membrane filtration integrated in a pilot process. *Journal of Food Engineering, 185*, 42–47.

Beck, M., Jekle, M., & Becker, T. (2012). Sodium chloride – sensory, preserving and technological impact on yeast-leavened products. *International Journal of Food Science and Technology, 47*(9), 1798–1807.

Cepanec, K., Vugrinec, S., Cvetković, T., & Ranilović, J. (2017). Potassium chloride-based salt substitutes: A critical review with a focus on the patent literature. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 881–894.

Contreras, M. d. M., Carrón, R., Montero, M. J., Ramos, M., & Recio, I. (2009). Novel casein-derived peptides with antihypertensive activity. *International Dairy Journal, 19*(10), 566–573.

Elavarasan, K., Shamasundar, B., Badii, F., & Howell, N. (2016). Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven-and freeze-dried protein hydrolysate from fresh water fish (*Cirrhinus mrigala*). *Food Chemistry, 206*, 210–216.

Engeloug, A. M., Yi, G., Egelandsdal, B., Haug, A., & Nordvi, B. (2017). Commercial mineral enhanced dairy by-products as sodium replacers, antioxidants and calcium fortifiers in sausages. *Journal of Food Science, 82*(6), 1302–1309.

Erdmann, K., Cheung, B. W., & Schröder, H. (2008). The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. *The Journal of Nutritional Biochemistry, 19*(10), 643–654.

FAO/WHO/UNU, J. (2007). In W. T. R. Series (Ed.). *Protein and amino acid requirements in human nutrition: Report of a joint FAO/WHO/UNU expert consultation*. World Health Organization.

Firestone, D. (2009). *Official methods and recommended practices of the American Oil Chemists Society*. AOCS.

Gilmartin, L., & Jervis, L. (2002). Production of cod (*Gadus morhua*) muscle hydrolysates. influence of combinations of commercial enzyme preparations on hydrolysate peptide size range. *Journal of Agricultural and Food Chemistry, 50*(19), 5417–5423.

Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., & Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry, 53*(3), 588–593.

Horwitz, William (2010). *Official methods of analysis. Vol. 222*. Washington, DC: Association of Official Analytical Chemists.

Kannamkumarath, S. S., Wrobel, K., Wrobel, K., Vonderheide, A., & Caruso, J. A. (2002). HPLC–ICP–MS determination of selenium distribution and speciation in different types of nut. *Analytical and Bioanalytical Chemistry, 373*(6), 454–460.

Kitts, D., & Weiler, K. (2003). Bioactive proteins and peptides form food sources. Applications of bioprocesses used in isolation and recovery. *Current pharmaceutical design, 9*, 1309–1325.

Korhonen, H. (2009). Milk-derived bioactive peptides: From science to applications. *Journal of Functional Foods, 1*(2), 177–187.

Li, G.-H., Qu, M.-R., Wan, J.-Z., & You, J.-M. (2007). Antihypertensive effect of rice protein hydrolysate with in vitro angiotensin I-converting enzyme inhibitory activity in spontaneously hypertensive rats. *Asia Pacific Journal of Clinical Nutrition, 16*.

Marques, C., Amorim, M. M., Pereira, J. O., Pintado, M. E., Moura, D., Calhau, C., et al. (2012). Bioactive peptides: Are there more antihypertensive mechanisms beyond ACE inhibition? *Current Pharmaceutical Design, 18*(30), 4706–4713.

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (2006). *Sensory evaluation techniques*. CRC press.

Miguel, M., Manso, M. A., López-Fandiño, R., Alonso, M. J., & Salaices, M. (2007). Vascular effects and antihypertensive properties of κ-casein macropeptide. *International Dairy Journal, 17*(12), 1473–1477.

Mine, Y., Li-Chan, E., & Jiang, B. (2011). *Bioactive proteins and peptides as functional foods and nutraceuticals, Vol. 29*. John Wiley & Sons.

Morley, W. (2012). *Novel strategies for reducing sodium, Vol. 66*. Chicago, IL: ETATS-UNIS: Institute of Food Technologists.

NutriBase (2001). *The NutriBase complete book of food counts*. Penguin.

Ogunsina, B. S., & Bamgboye, A. I. (2014). Pre-shelling parameters and conditions that influence the whole kernel out-turn of steam-boiled cashew nuts. *Journal of the Saudi Society of Agricultural Sciences, 13*(1), 29–34.

Ogunsina, B. S., Owolarafe, O. K., & Olatunde, G. A. (2008). Oil point pressure of cashew (*Anacardium occidentale*) kernels. *International Agrophysics, 22*(1), 53–59.

Pal, S., & Radavelli-Bagatini, S. (2013). The effects of whey protein on cardiometabolic risk factors. *Obesity Reviews, 14*(4), 324–343.

Pan, D., Cao, J., Guo, H., & Zhao, B. (2012). Studies on purification and the molecular mechanism of a novel ACE inhibitory peptide from whey protein hydrolysate. *Food Chemistry, 130*(1), 121–126.

Petersen, B. L., Bastian, E. D., Ward, L. S., Paulsen, S. J., Wrobel, S. A., & Marshall, J. A. (2014). *Acidified whey protein compositions and methods for making them* (Google Patents).

Phelan, M., Aherne, A., FitzGerald, R. J., & O'Brien, N. M. (2009). Casein-derived bioactive peptides: Biological effects, industrial uses, safety aspects and regulatory status. *International Dairy Journal, 19*(11), 643–654.

Pihlanto, A., & Ma¨kinen, S. (2013). Antihypertensive properties of plant protein derived peptides. *Bioactive food peptides in health and disease*. InTech.

Prosky, L., Asp, N.-G., Schweizer, T., DeVries, J., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *Journal AOAC International, 71*(5), 1017–1023.

Quirós, A., Ramos, M., Muguerza, B., Delgado, M. A., Miguel, M., Aleixandre, A., et al. (2007). Identification of novel antihypertensive peptides in milk fermented with Enterococcus faecalis. *International Dairy Journal, 17*(1), 33–41.

Rico, R., Bulló, M., & Salas-Salvadó, J. (2016). Nutritional composition of raw fresh cashew (*Anacardium occidentale* L.) kernels from different origin. *Food Sciences and Nutrition, 4*(2), 329–338.

Ros, E. (2010). Health benefits of nut consumption. *Nutrients, 2*(7), 652–682.

Ryan, E., Galvin, K., O'Connor, T., Maguire, A., & O'Brien, N. (2006). Fatty acid profile, tocopherol, squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts. *International Journal of Food Sciences & Nutrition, 57*(3–4), 219–228.

Smith, S. T., Metzger, L., & Drake, M. A. (2016). Evaluation of whey, milk, and delactosed permeates as salt substitutes. *Journal of Dairy Science, 99*(11), 8687–8698.

TACO (2011). *Tabela brasileira de composição de alimentos* (4.ed). Campinas: NEPAUNICAMP 161p.

Tavares, T. G., Amorim, M., Comes, D., Pintado, M. E., Pereira, C. D., & Malcata, F. X.

(2012b). Manufacture of bioactive peptide-rich concentrates from Whey: Characterization of pilot process. *Journal of Food Engineering, 110*(4), 547–552.
Tavares, T., Sevilla, M. A., Montero, M. J., Carron, R., & Malcata, F. X. (2012a). Acute effect of whey peptides upon blood pressure of hypertensive rats, and relationship with their angiotensin-converting enzyme inhibitory activity. *Molecular Nutrition & Food Research, 56*(2), 316–324.
White, J. A., Hart, R. J., & Fry, J. C. (1986). An evaluation of the waters picotag system for the amino acid analysis of food Materials. *Journal of Automatic Chemistry, 8*, 170–177.
Wu, J., Aluko, R. E., & Nakai, S. (2006). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure-activity relationship study of di-and tripeptides. *Journal of Agricultural and Food Chemistry, 54*(3), 732–738.
Yamada, A., Sakurai, T., Ochi, D., Mitsuyama, E., Yamauchi, K., & Abe, F. (2013). Novel angiotensin I-converting enzyme inhibitory peptide derived from bovine casein. *Food Chemistry, 141*(4), 3781–3789.
Zhou, J., Keenan, M. J., Losso, J. N., Raggio, A. M., Shen, L., McCutcheon, K. L., et al. (2011). Dietary whey protein decreases food intake and body fat in rats. *Obesity, 19*(8), 1568–1573.