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ORIGINAL PAPER

# Bioactive Properties of *Phaseolus lunatus* (Lima Bean) and *Vigna unguiculata* (Cowpea) Hydrolyzates Incorporated into Pasta. Residual Activity after Pasta Cooking

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**Abstract** The aims of the study were to study the inclusion of *P. lunatus* (PLH) and *V. unguiculata* (VUH) protein hydrolyzates with bioactive properties into a pasta-extruded product and determine residual activity after extrusion or pasta cooking. Both protein hydrolyzates showed angiotensin-converting enzyme inhibition (ACEI) and antioxidant activity (TEAC). PLH showed higher ACEI but lower TEAC than VUH (97.19 ± 0.23 vs. 91.95 ± 0.29 % and 244.7 ± 3.4 vs. 293.7 ± 3.3 μmol Trolox/g, respectively). They were included at 5 or 10 % into wheat pasta. Control pasta had the lowest ACEI activity or TEAC (22.01 ± 0.76 % or 14.14 ± 1.28 μmol Trolox/g, respectively). Higher activity remained in pasta with PLH than VUH after extrusion, and higher the level of addition, higher the ACEI was. Pasta had practically the same ACEI activity after cooking, thus active compounds were not lost by temperature or lixiviation. Regarding TEAC, higher activity remained in pasta with 10 % VUH (31.84 ± 0.17 μmol Trolox/g). Other samples with hydrolyzates had the same activity. After cooking, pasta with hydrolyzates had higher TEAC values than control, but these were not modified by the level of incorporation. Moreover, the profile changed because pasta with PLH had the highest TEAC values (21.39 ± 0.01 and 20.34 ± 0.15 for 5 or 10 % hydrolyzates, respectively). Cooking decreased this activity (~ 20 %), for all samples. Although a certain loss of antioxidant activity was observed, pasta could be a good vehicle for bioactive compounds becoming a functional food.

**Keywords** Lima bean · Cowpea · Functional foods · Antihypertensive · Antioxidant activity

**Abbreviations**

| | |
|---|---|
| PLH | *Phaseolus lunatus* hydrolyzate |
| VUH | *Vigna unguiculata* hydrolyzate |
| ACEI | Angiotensin-converting enzyme inhibition activity |
| TEAC | Trolox equivalent antioxidant capacity |
| PC | Protein concentrate |
| PC-PL | *Phaseolus lunatus* protein concentrate |
| PC-VU | *Vigna unguiculata* protein concéntrate |
| NFE | Nitrogen-free extract |
| d.b | Dry base |

## Introduction

Legumes are widely consumed in south-eastern México as major dietary carbohydrate and protein sources in human and animal diets. Both, lima bean (*P. lunatus*) and cowpea bean (*V. unguiculata*) are broadly grown and harvested in Latin America, the southern United States, Canada, and many other regions worldwide. These legumes have high protein content (20–35 %) and are especially rich in essential amino acids such as lysine and threonine. Also, they provide complex carbohydrates (starch and dietary fiber), vitamins (B complex) and minerals (zinc, iron and calcium) [1, 2]. Besides quality protein, lima bean and cowpea proteins have good functional properties, allowing broad technological applications in food processing.

Partial hydrolysis of protein structure using proteases working in moderate pH and temperature conditions

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(pH 5–9; 40–60 °C) may contribute to the development of new peptide fractions with nutritional or functional characteristics [3]. Bioactive peptides from food protein hydrolyzates have numerous potential activities such as antihypertensive and antioxidant. In this regard, antihypertensive effect mediated by inhibition of angiotensin-converting enzyme I (ACE-I) is one of the most studied mechanisms for food peptides [4]. On the other hand, antioxidant peptides from food protein hydrolyzates may prevent the generation of lipid radicals (peroxides and hydroperoxides) [4], which have a negative impact on flavor, texture, nutritive value, as well as shelf life of food products [5]. Therefore, because of their potential bioactivity, protein hydrolyzates may be important food ingredients. The use of protein hydrolyzates in extruded products represents a viable alternative, since most of the protein used in human diets comes from cereals and legumes [6]. The mixture of these products for the preparation of functional foods also leads to the complementation of essential amino acids, obtaining products with better protein quality [7]. Segura-Campos et al. [8] evaluated *in vitro* bioactivity, nutritional and sensory properties of semolina pasta added with hard-to-cook bean hydrolyzate obtained with a sequential enzymatic system (Alcalase®-Flavourzyme®). In that work, the authors reported low bioactivity in dry or cooked pasta. Thus, other type of legume proteins and different hydrolysis systems could be proved to obtain a product with high residual bioactivity. The aims of this work were to study the inclusion of protein hydrolyzates of *P. lunatus* and *V. unguiculata* with bioactive properties into a pasta-extruded product and evaluate residual activity after extrusion or pasta cooking, in order to obtain a functional food.

## Materials and Methods

### Raw Material and Reagents

Lima bean (*P. lunatus*) and cowpea (*V. unguiculata*) were obtained in local markets of Yucatán State, México. Analytical grade reagents were purchased from J.T. Baker (Phillipsburg, NJ, USA), Sigma (Sigma Chemical Co., St. Louis, MO, USA), Merck (Darmstadt, Germany) and Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The enzymes pepsin (EC 3.4.23.1) and pancreatin (232–468-9) were purchased from Sigma.

### Flours, Concentrates and Hydrolyzates

#### *P. lunatus (Lima Bean) and V. unguiculata (Cowpea) Flours*

Lima beans were dried in an oven at 60 °C during 4 h and then were ground successively on a roller mill (Cemotec 1990 and Ciclotec Tecator). The flour obtained was sieved through a 200-mesh to obtain a particle size of 500 μm [9]. Cowpea beans were subjected to drying at 60 °C using a convection oven for 4 h, then were broken in a manual roller mill and underwent separation of particles with compressed air, in order to eliminate grain shell. Then the beans were ground in an impact mill Ciclotec (Tecator). The flour was sieved by 200-mesh to obtain a particle size of 500 μm [2]. Wheat flour from commercial durum wheat was provided by Buhler S.A.

#### *Preparation of Protein Concentrates (PC)*

Protein concentrates (PC) were prepared according to Betancur-Ancona et al. [10]. Briefly, 2 kg of each bean flour was suspended in distilled water at a 1:6 (*w*/*v*) ratio. The pH was adjusted to 11 with 1 mol/L NaOH. After mechanical stirring for 1 h (Caframo RZ-1) at 400 rpm, the suspensions were passed through 180 and 150 μm screen. Residues were washed five times with 2.8 L water, and the filtrate incorporated to the filtrate. The pH of filtrate was adjusted to the protein isoelectric point (4.5) with 1 mol/L HCl. The suspension was centrifuged at 1317×g for 15 min and the precipitate was freeze-dried (Labconco, Kansas City, MO, USA). Two concentrates were produced: *Phaseolus lunatus* (PL-PC) and *Vigna unguiculata* (VU-PC).

#### *Enzimatic Hydrolysis of PC*

The hydrolysis was carried out in a 2 L reactor with mechanical agitation at 300 rpm (Caframo RZ-1) and temperature control (Bronswick model R76) at 37 °C. Hydrolysis of PL-PC was run during 90 min using a 4 g/100 mL protein suspension and pepsin (P7000, Sigma) at E/S ratio of 1/10 (*w*/*v*). The pH of the system was adjusted to 2 with 0.1 mol/L HCl [11]. In the case of VU-PC, protein hydrolysis was performed involving digestion with pepsin for 45 min followed by digestion with pancreatin for 45 min [9]. For pepsin hydrolysis, a suspension of 4 g/100 mL protein and pepsin (P7000, Sigma) at E/S ratio of 1/10 (*w*/*v*) were used. The pH of the system was adjusted to 2 with 0.1 mol/L HCl. After 45 min, the pH was adjusted to 7.5 with 0.1 N NaOH for pancreatin (P32292, Sigma) hydrolysis using an E/S ratio of 1/10 (*w*/*v*). The hydrolysis reaction was stopped by heating at 80 °C for 20 min in a water bath Lindberg blue M1110. Protein hydrolyzates were freeze- dried (Labconco, Kansas City, MO, USA). Two hydrolyzates were obtained: PLH (*P. lunatus* hydrolyzate) and VUH (*V. unguiculata* hydrolyzate). Degree of hydrolysis (DH) was calculated by determining free amino groups with o-phthaldialdehyde following Nielsen et al. [12]. $DH = h/htot \times 100$; where *htot* is the total number of peptide bonds *per* protein equivalent (mEq/g protein), and *h* is the number of hydrolyzed bonds. The *htot* factor is dependent on amino acid composition of raw material.

**Proximate Composition**

Standard AOAC [13] procedures were used to determine nitrogen (method 954.01), fat (method 920.39), ash (method 925.03), crude fibre (method 962.09), and moisture (method 925.09) contents of milled beans. Nitrogen content was quantified with a Kjeltec Digestion System (Tecator, Höganäs, Skåne län, Sweden). Protein content was calculated as nitrogen × 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference from the sum of the protein, fat, ash, and crude fibre content.

***In Vitro* Biological Activities**

*Angiotensin-Converting Enzyme Inhibition Activity (ACEI)*

Angiotensin-converting enzyme inhibition activity (ACEI) was analyzed with the method of Hayakari et al. [14], which is based on the fact that ACE hydrolyses hippuryl-L-histidyl-L-leucine (HHL) to yield hippuric acid and histidyl-leucine. Hippuric acid reacts with 2,4,6-trichloro-S-triazine (TT) in a 0.5 mL incubation mixture containing 40 μmol potassium phosphate buffer (pH 8.3), 300 μmol sodium chloride, 40 μmol HHL in potassium phosphate buffer (pH 8.3), and 100 mU/mL ACE. This mixture was incubated at 37 °C for 45 min and the reaction stopped by addition of TT (3 mL/100 mL) in dioxane and 3 mL 0.2 mol/L potassium phosphate buffer (pH 8.3). After centrifuging the reaction mixture at 10,000×g for 10 min, enzymatic activity was determined in the supernatant by measuring absorbance at 382 nm. All runs were done in triplicate over two samples ($n$ = 6). Results were expressed in percentage of angiotensin-converting enzyme inhibition. Hydrolyzates were evaluated at 1 g d.b/100 mL.

*Trolox Equivalent Antioxidant Capacity (TEAC)*

To determine the antioxidant capacity, ABTS• + radical cation decolorization assay according to Cian et al. [15] was used. To estimate the TEAC, a concentration-response curve for the absorbance at 734 nm for ABTS• + as a function of concentration of standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution (0–2.5 mmol/ L) in 0.01 mmol/ L (PBS, pH 7.4) was performed. The absorbance reading was taken at 6 min after initial mixing. Hydrolyzates were evaluated at 1 g d.b./100 mL. All runs were done in triplicate over two samples ($n$ = 6).

**Spaghetti Added with Bioactive Hydrolyzates**

Wheat flour and legume hydrolyzates at 95:5 and 90:10 rates were first mixed for 1 min at 60 rpm in a Brabender p-600 mixer. Based on the flours properties and the operator experience, hydration levels of 33 and 32 % (d.b) were utilized for the production of pasta with 5 or 10 % replacement with hydrolyzates, respectively. Wheat pasta with 35 % hydration level was elaborated as control. Samples were conditioned by adding water to reach the moisture level corresponding to each experimental sample, 2 h before each run. Mixtures were extruded like spaghetti in a Brabender 10 DN extruder with a non-compression screw and a teflon die nozzle of 28 mm with three 1.5 mm diameter holes. Extrusion temperature of 40 °C and 100 rpm screw speed were used. The feeding rate of the extruder was at full capacity. The products were dried at low temperature (40 °C) and at 70 % relative humidity during 24 h. The experiment was carried out in duplicate.

*Spaghetti Cooking Time*

Ten grams sample of spaghetti were placed into a 500 mL beaker with 200 mL of boiling distilled water. Every 30 s, during cooking, the core strand of the spaghetti was observed as it was squeezed between two transparent glass slides. The cooking time (T0) was determined as the time when the white core disappeared [16]. The assay was performed by triplicate.

*Cooking Losses and Water Absorption*

Ten grams of spaghetti (d.b) samples of 10 cm long were placed into a 500 mL beaker with 200 mL of boiling distilled water. After the required cooking time, the cooked pasta was drained for 3 min and an aliquot was weighed and dried at 105 °C until constant weight, in order to determine water absorption (WA). It was reported as g of water/g spaghetti (d.b). The cooking water was then collected and placed into an air oven at 105 °C and evaporated to dryness. The residue was weighed and reported as the percentage of the starting material. All analyses on the cooked pasta were made at optimal cooking time. For each time, five determinations were performed and the analytical method was made twice ($n$ = 10).

*Spaghetti Bioactive Properties*

Dry pasta was milled and 100 mg were extracted with 10 mL buffer and centrifuged at 13,698×g for 10 min. In the case of cooked pasta, the samples were cooked as was mentioned before at cooking time. After cooking, the water was drained and 4 g cooked pasta were homogenized with 10 mL of the corresponding buffer using a homogenizer ProScientific, and centrifuged at 13,698×g for 10 min. The supernatants were taken from each sample and processed regarding bioactive properties.

### Statistical Analysis

All results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and by Duncan's multiple range test using the software Statgraphics Centurion XV 15.2.06.

## Results and Discussion

### Proximate Composition of Flours, Concentrates and Hydrolyzates

Results of proximal composition of flours, concentrates and hydrolyzates are shown in Table 1. The composition of flours was similar to that reported in other studies [9, 17]. Protein content of PL-C and VU-PC was high and similar to those reported by Guzmán-Méndez et al. [9]. As expected, the protein content of PLH and VUH was similar to that found for PL-C and VU-PC (Table 1).

Regarding hydrolyzates, PLH showed a DH of 17.38 %, after 90 min pepsin hydrolysis. It could be classified as extensive hydrolyzate (DH >10 %) [18], which is very interesting in regards to bioactive peptides, since low peptide chain long is related with higher ACEI and antioxidant properties [4]. VUH presented a DH 30.75 %, which was higher than the value of PLH, since VU-PC was hydrolyzed by a sequential hydrolysis with pepsin-pancreatin. Also, it can be classified as extensive hydrolyzate. Pepsin is the major gastric enzyme that degrades proteins in the stomach during digestion. Likewise, it presents endopeptidase activity, which hydrolyzes at the C-terminal aromatic amino acids (phenylalanine, tyrosine and tryptophan). Its action breaks the polypeptide chains in shorter sections, in other words, for its action free amino acids are produced, but most of products are oligopeptides. Pancreatin includes proteases such as trypsin, chymotrypsin and elastase, which are serine proteases having endo-exo-peptidase activity.

### Pasta Quality Evaluation

#### *Cooking Times*

Table 2 shows cooking time (T0) of different samples. They were between 7.13 and 9.18 min. The results show a decrease of T0 as wheat semolina is replaced by increased protein hydrolyzates level. Gallegos-Infante et al. [19] observed a diminution of T0, from 10 min (control) to 8.45 min in spaghetti with 30 % of bean flour. Similar results were observed by Gallegos-Infante et al. [20], who developed a paste adding *Phaseolus vulgaris* flour. They suggested T0 is related to the network formed by the gluten during processing of the dough, thus the addition of other compounds significantly affect this network.

#### *Water Absorption and Cooking Solid Losses*

Table 2 shows water absorption (WA) corresponding to pasta at T0. WA values were between 2.30 and 2.76 g water / g of pasta. WA of pasta added with VUH was lower than that found for control ($p < 0.05$). In this sense, Giménez et al. [21] also observed a decrease of pasta water absorption at 10 % level of wheat substitution by *Vicia faba* flour. However, pasta added with PLH absorbed more water. This could be due to PLH had lower DH than VUH. Therefore, most of the secondary structure of protein could remain after hydrolysis process, which will result in an increase of protein-water interaction of product.

Solid losses of the different samples are shown in Table 2**.** It is observed that pasta added with PLH showed higher cooking losses and 10 % replacement level increased the amount of solid losses. The results were similar to those reported by Petitot et al. [22], who obtained 5.6 % of solids losses for the control, 7 % for pasta added with chickpea flour, and 6.8 % for pasta fortified with bean flour. Also, Zhao et al. [23] found that the addition of legumes as green peas, chickpeas and lentils increase solids losses by cooking. The solid losses during cooking are undesirable because they are produced by the solubility of compounds

**Table 1** Proximate composition of *P. lunatus* (PL) and *V. unguiculata* (VU) flours, protein concentrate and hydrolyzates

| Components (g/ 100 g d.b) | Flours | | Protein concentrate | | Hydrolyzates | |
|---|---|---|---|---|---|---|
| | PL | VU | PL | VU | PL | VU |
| Moisture | 11.02 ± 0.09 | 8.46 ± 0.17 | 3.38 ± 0.04 | 5.07 ± 0.07 | 4.28 ± 0.14 | 11.04 ± 0.08 |
| Ash | 4.01 ± 0.15 | 4.16 ± 0.04 | 2.99 ± 0.01 | 5.72 ± 0.18 | 3.20 ± 0.02 | 3.06 ± 0.05 |
| Protein | 23.82 ± 0.23 | 22.92 ± 0.62 | 74.06 ± 0.30 | 68.29 ± 0.73 | 64.85 ± 0.77 | 63.14 ± 0.54 |
| Crude fibre | 5.83 ± 0.11 | 1.77 ± 0.10 | 0.11 ± 0.00 | 0.08 ± 0.00 | 0.48 ± 0.00 | 1.34 ± 0.02 |
| Fat | 1.19 ± 0.00 | 1.16 ± 0.07 | 3.84 ± 0.21 | 2.97 ± 0.18 | 3.31 ± 0.17 | 0.34 ± 0.00 |
| NFE | 65.15 ± 0.13 | 69.99 ± 0.50 | 19.0 ± 0.52 | 22.94 ± 0.21 | 28.16 ± 0.53 | 32.12 ± 0.24 |

Results are expressed as mean ± SD, in dry base (d.b); *NFE*: nitrogen-free extract

**Table 2** Pasta quality indicators

| Pasta | Cooking time (min) | Water absorption (g water/g spaghetti d.b) | Solid losses (g solids/100 g d.b) |
|---|---|---|---|
| *Control* | 9.18 ± 0.07 $^{c}$ | 2.35 ± 0.04$^{b}$ | 7.24 ± 0.18$^{a}$ |
| *P. lunatus* 5 % | 8.27 ± 0.08 $^{b}$ | 2.76 ± 0.03$^{c}$ | 9.65 ± 0.11$^{c}$ |
| *P. lunatus* 10 % | 7.37 ± 0.10 $^{a}$ | 2.67 ± 0.04$^{c}$ | 10.76 ± 0.17$^{d}$ |
| *V. unguiculata* 5 % | 8.18 ± 0.07 $^{b}$ | 2.40 ± 0.04$^{a}$ | 7.63 ± 0.05$^{a}$ |
| *V. unguiculata* 10 % | 7.13 ± 0.09$^{a}$ | 2.30 ± 0.05$^{a}$ | 8.83 ± 0.31$^{b}$ |

Different letters in a column mean significant differences between samples ($P < 0.05$)

such as starches, protein and minerals, which pass into the cooking water causing pasta lose its shape when left longer submerged in hot water. They are also an indicator of yield during cooking since lower amounts of solid into cooking water indicate high quality pasta [24]. Solid losses during cooking are attributed to the dilution effect exerted by the ingredient replacing gluten semolina, which weakens the overall structure of the dough [25]. This effect was more evident by increasing the substitution level of semolina by hydrolyzates.

## Bioactive Properties

### *Angiotensin-Converting Enzyme Inhibition Activity (ACEI)*

It was observed that hydrolyzates presented high ACEI (higher than 90 %) and PLH showed higher ACEI than VUH (97.19 ± 0.23 vs. 91.95 ± 0.29 %, respectively). Table 3 shows ACEI (%) for pasta added with 5 or 10 % hydrolyzates, before and after cooking. Control sample (semolina) had the lowest ACE inhibition activity. Pasta with PLH has higher activity than VUH after extrusion, and higher the level of addition, higher ACEI was. After cooking, the results follow the same tendency: pasta with hydrolyzates had higher values than control, and at higher level, higher the activity was. Cooking increased this activity, except for pasta with VUH at 10 % and with PLH at 5 %. This could be related with higher extraction of active compounds in cooked samples. It is very important to point out that samples had practically the same activity after cooking, thus active compounds were not loss by temperature or lixiviation. Pasta with PLH at 10 % had the highest ACEI before and after cooking. There are few reports about bioactive properties of peptides after cooking treatments. Segura-Campos et al. [26] studied the addition of *chia* (*Salvia hispanica* L.) protein hydrolyzates at two levels to white bread and carrot cream and observed that products with *chia* improved ACEI compared to the controls. Cian et al. [15] evaluated ACEI from extruded products with added hydrolyzates and observed they had higher activity than maize control; however, the extrusion process modified ACEI formerly present in different hydrolyzates. Extruded maize added with red seaweed showed higher dialyzability of angiotensin-converting enzyme inhibitor compounds than extruded maize, demonstrating this high-temperature–short-time (HTST) process did not impair this activity [27].

### *Trolox Equivalent Antioxidant Capacity (TEAC)*

Hydrolyzates presented high TEAC values, which corresponded to high ABTS inhibition (74.8 and 83 % for PLH and VUH, respectively). It was observed that VUH showed higher antioxidant capacity than PLH (293.7 ± 3.3 vs. 244.7 ± 3.4 μmol Trolox/g, respectively). As it is known, the antioxidant properties of peptides are highly influenced by the composition, sequence and molecular mass [28]. Most of the reported peptides exhibiting antioxidant activity were those with low molecular weights [1, 5]. In this sense, Dávalos et al. [29] suggested that hydrolyzates with high DH obtained with sequential proteases systems, such as pepsin-pancreatin, had higher proportion of low molecular weight peptides, which would access more easily to the oxidant

**Table 3** Angiotensin-converting enzyme inhibition activity (ACEI) and Trolox equivalent antioxidant capacity (TEAC) for pasta added with 5 or 10 % hydrolyzates

| | | ACEI (%) | | TEAC (μmol Trolox/ g d.b) | |
|---|---|---|---|---|---|
| Pasta | Levels | Before cooking | After cooking | Before cooking | After cooking |
| Control | - | 22.01 ± 0.76$^{a}$ | 43.68 ± 0.23$^{b}$ | 14.14 ± 1.28$^{b}$ | 5.95 ± 0.05$^{a}$ |
| *V. unguiculata* | 5 | 43.68 ± 0.99$^{b}$ | 76.80 ± 0.18$^{d}$ | 27.44 ± 0.54$^{e}$ | 16.63 ± 0.25$^{c}$ |
| hydrolyzate | 10 | 79.93 ± 0.06$^{f}$ | 78.08 ± 0.35$^{e}$ | 31.84 ± 0.17$^{f}$ | 17.89 ± 0.07$^{c}$ |
| *P. lunatus* | 5 | 77.42 ± 0.41$^{de}$ | 69.24 ± 0.35$^{c}$ | 26.30 ± 1.19$^{e}$ | 21.39 ± 0.01$^{d}$ |
| hydrolyzate | 10 | 90.30 ± 0.06$^{g}$ | 91.95 ± 0.58$^{h}$ | 26.09 ± 0.99$^{e}$ | 20.34 ± 0.15$^{d}$ |

Different letters in columns and rows mean significant differences among samples ($P < 0.05$)

system and lead to high values of TEAC. Taking into account that VUH presented higher DH than PLH, the higher antioxidant capacity of VUH could be due to the presence of low molecular weight peptides in this hydrolyzate.

Table 3 shows TEAC for pasta added with 5 or 10 % hydrolyzates, before and after cooking. Before cooking, control sample (semolina) had the lowest TEAC value. When hydrolyzates were incorporated into pasta, higher activity remained in pasta with 10 % VUH. Other samples with hydrolyzates had the same activity. After cooking, the results follow the same tendency: pasta with hydrolyzates had higher values than control, but the level of incorporation did not modify the activity. Moreover, the profile changed because pasta with PLH had the highest values of TEAC. Cooking decreased this activity, for all samples. This could be related with loss by temperature or lixiviation of active antioxidant compounds. Segura-Campos et al. [26] observed that chia protein hydrolyzates addition had no effect on antioxidant activity in white bread and only a slight effect in carrot cream, indicating the level 2.5 or 5 mg / g product or the process (bread making or cooking) were responsible of low residual antioxidant activity. Also, Cian et al. [14] evaluated TEAC from extruded products with added hydrolyzates and observed they had higher activity than maize control; however, the extrusion process modified TEAC formerly present in different hydrolyzates, changing the activity of hydrolyzate after cooking extrusion.

## Conclusions

There are many works about bioactive properties of protein hydrolyzates, but few related with such activity after food incorporation or after cooking.

This work has demonstrated that hydrolyzates of *P. lunatus* or *V. unguiculata* incorporated at 5 or 10 % into pasta retain most of the activity after pasta processing, moreover after pasta cooking, demonstrating that nor lixiviation or inactivation trigger the loss of bioactivity, although a certain loss of antioxidant activity was observed. Pasta could be a good vehicle for bioactive compounds becoming a functional food. Since hydrolyzates have bioactive properties that remained after extrusion and water cooking, it could be very interesting study their incorporation in other food products such as baked goods or corn Mexican tortillas.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare no conflicts of interest.

**Human and Animals Studies** This article does not contain any studies with human or animal subjects.

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