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Baits for *Anastrepha fraterculus* with Hydrolyzed Protein from Agroindustrial By-products Pretreated with Gamma Radiation

Cebos para *Anastrepha fraterculus* con Proteína Hidrolizada de Subproductos Agroindustriales Pretratados con Radiación Gamma

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Abstract

The fruit fly (*Anastrepha fraterculus*) affects several Ecuadorian crops with export potential. Currently, a costly imported bait is used to monitor and control this pest. The aim of this research is to formulate baits for the fruit fly that could replace the commercial bait. Soy cake, palm kernel cake, bovine blood and whey were used as raw material. Each material was irradiated with a dose of 20 kGy with a Cobalt-60 source as a pretreatment. Then, the protein was extracted and hydrolyzed with a 0.025 AU mL^{-1} bromelain solution at pH 7,0 and 50°C, for 30 min. The baits were formulated with hydrolyzed protein, molasses, water, and borax, and they were placed in McPhail traps. The field evaluation was carried out in cherimoya (*Annona cherimola*) and guava (*Psidium guajava*) orchards. The hydrolysis degrees that were reached in the enzymatic process had values between 19.16 and 26.64%. According to an SDS-PAGE electrophoresis, the hydrolysates had peptides with molecular weights between 5 and 20 kDa. The bait made with palm kernel cake hydrolyzed protein and the commercial bait were statistically equal in the number of captured flies, whereas the bait made with whey protein had a higher FTD index (flies caught per trap, per day). The formulated baits could be an inexpensive alternative to the commercial bait for monitoring fruit flies in Ecuador.

Keywords: Attractant baits, protein hydrolysates, ionizing radiation, fruit fly.

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Resumen

La mosca de la fruta (*Anastrepha fraterculus*) afecta a varios cultivos ecuatorianos con potencial de exportación. En la actualidad, para el monitoreo de esta plaga, se emplea un cebo importado que tiene un alto costo. La presente investigación tiene como objetivo formular cebos atrayentes de mosca de la fruta que puedan reemplazar al cebo comercial. Como materia prima se empleó torta de soya, torta de palmiste, sangre bovina y suero; cada material fue irradiado con una dosis de 20 kGy, en una fuente de Cobalto-60 como pretratamiento. Luego, la proteína se extrajo y se hidrolizó con una solución de bromelina de 0,025 UA mL^{-1}, a pH 7,0 y 50°C durante 30 min. Los cebos fueron formulados con proteína hidrolizada, melaza, agua y bórax y se colocaron en trampas McPhail. La evaluación en campo se llevó a cabo en cultivos de chirimoya (*Annona cherimola*) y guayaba (*Psidium guajava*). En el proceso enzimático se alcanzaron grados de hidrólisis entre 19,16 y 26,64%. Por electroforesis SDS-PAGE se determinó que los hidrolizados proteicos contenían péptidos con pesos moleculares entre 5 y 20 kDa. Se encontró que el cebo de proteína hidrolizada de palmiste y el cebo comercial fueron estadísticamente iguales en la cantidad de moscas atrapadas, mientras que el de suero presentó un mayor índice MTD (moscas capturadas por trampa, por día). Los cebos formulados podrían ser una alternativa más económica que el cebo importado para el monitoreo de la mosca de la fruta en el Ecuador.

Palabras clave: Cebos atrayentes, hidrolizados proteicos, mosca de la fruta, radiaciones ionizantes.

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1 Introduction

The increase in exports of non-traditional Ecuadorian products such as pitahaya, mango and passion fruit between 2012 and 2017 outpaced the growth of several traditional products such as bananas, cocoa and industrialized coffee (Verdugo and Andrade, 2018). However, markets for other fruits with high export potential have not been opened due to persistent inadequate fruit fly management (Vilatuña et al., 2010). There are international markets that do not admit the entry of fruit without adequate treatment from countries affected by this pest, such as the United States, Japan and the European Union (Vilatuña et al., 2016; IAEA, 2019). Phytosanitary requirements imposed by importing countries seek to prevent the entry of this pest into areas considered free (García-Rosero et al., 2015).

Fruit flies belong to the *Tephritidae* family of the *Diptera* order. In Ecuador predominates the genus Anastrepha, native to Central and South America, and *Ceratitis*, a genus introduced from the Mediterranean around 1976 (Vilatuña et al., 2010). They are considered one of the main pests of economic interest worldwide, due to the damage caused on fruits and crops in tropical and subtropical regions (White and Elson-Harris, 1992; Hafsi et al., 2016). Fruit flies can adapt to various environmental conditions and infect fruit at different ripening stages. The females are attracted by certain aromas that fruits emit when they begin to ripen, and when this occurs they deposit eggs inside the fruit. Upon hatching, the larvae feed on the pulp and form galleries that facilitate the entry of pathogenic agents such as fungi and bacteria, causing fruit rot and consequent rejection for consumption, export and agro-industry (INIAP, 2004).

In addition, the presence of fruit flies increases production costs because of research expenses and the application of monitoring and control measures (Salcedo-Baca et al., 2009). For example, the export of Ecuadorian mangoes to markets such as the United States has been achieved (Fundación Mango Ecuador, 2019), but by using pesticides during cultivation and post-harvest hydrothermal treatment of the fruits (AGROCALIDAD, 2016). Due to the growing importance of this and other crops in the country (MAG-CGINA, 2022), there is interest in controlling this pest through more eco-friendly options.

In 2014, the Agency for Phytosanitary and Zoosanitary Regulation and Control (AGROCALIDAD) started the "National Fruit Fly Management Project", with the purpose of declaring free or low prevalence areas for fruit production, through management strategies in the host crops of the fly (Vilatuña et al., 2016). Integrated management requires knowledge of the population density of the pest and its variations over time (Vilatuña et al., 2010). Baits formulated from organic compounds such as putrescine, ammonium acetate or liquid hydrolyzed protein have been effectively used as attractants in programs for the detection and monitoring of several fruit fly species (Heath et al., 1997; López-Guillén et al., 2010). Hydrolyzed protein is used, due to the content of nitrogenous compounds associated with the attraction of flies to fruit (Bateman et al., 1981; Mazor, 2009). The presence of essential amino acids for insects, such as methionine (Dadd, 1985), can also contribute to the attracting power of the baits (Díaz-Fleischer and Castrejón-Gómez, 2012).

The bait chosen by the National Fruit Fly Management Project is a commercial product that has hydrolyzed vegetable protein as its main component (Vilatuña et al., 2016); it is manufactured in Argentina and costs USD 14.60 per liter of concentrate (Edifarm, 2016). Therefore, there is an opportunity to formulate a bait that can be produced in the country at a lower cost, possessing similar or greater effectiveness and generating environmental and social benefits. From this perspective, agroindustrial by-products that contain protein and have a low commercial value can be used as raw material (Zahari and Alímon, 2005). In Ecuador, palm kernel and soybean cakes are available from vegetable oil extraction industries, as well as whey and bovine blood from cheese production and cattle slaughtering, respectively. These are produced in large quantities and are commonly discarded or destined for animal feed (Figueroa et al., 2012).

The inclusion of a pretreatment of the raw materials (agroindustrial by-products) can increase the yield of the extracted protein. Herrero et al. (2009) indicate that ionizing radiation could modify the structure of proteins and cause, depending on their intensity, even deamination, decarboxylation, re-

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duction of disulfide bonds and other changes that would facilitate their separation in extraction processes. These proteins, after enzymatic hydrolysis with proteases such as bromelain (Guadix et al., 2000), could be used for creating attractant baits (Barrera, 2006), due to their high content of ammonia compounds and their high solubility (Benítez et al., 2008). In addition, irradiation at intermediate and high doses allows reducing the microbial load (Kuan et al., 2013).

The aim of the research was to formulate fruit fly (*Anastrepha fraterculus*) attractant baits, based on hydrolyzed protein obtained from the agro-industrial by-products mentioned above, and to evaluate their performance in monitoring the pest in the field. In addition, the pretreatment effect of raw materials with gamma irradiation on protein extraction yield was determined.

2 Materials and Methods

2.1 Assessment of baits in the field

2.1.1 Area of study

The field trial was conducted for 5 weeks, from August 18 to September 22, 2017, in Puéllaro, a rural cantonal head of Quito, Pichincha province. Four areas near the parish were identified, called Estadio, La Esperanza, La Merced and Sigsihuayco, in which there are custard apple (*Annona cherimola*) and guava (*Psidium guajava*) crops, which are host species of the fruit fly (Supplementary Material 1).

2.1.2 Traps

Two McPhail type traps were used for each type of bait, which were placed in custard apple or guava trees with a minimum separation of 30 m, on detection points determined based on the monitoring routes managed in the "National Fruit Fly Program". Each trap had 250 mL of attractant solution.

The attractant capacity of each bait was quantified every 7 days using the FTD index, which corresponds to the average number of flies captured per trap daily (Imbachi et al., 2012) (Supplementary Material 2). Then, traps were washed and 250 mL of fresh attractant solution (Asaquibay et al., 2010) were placed to keep them operative during the experimental period (Vilatuña et al., 2010).

2.1.3 Experimental design for evaluating baits in the field

The bait effectiveness evaluation described corresponded to a $5 \times 5 \times 4$ trifactorial randomized block design, in which the factors were bait type (5 levels), week (5 levels) and sector (4 levels), while the blocks corresponded to subdivisions made in each sector. The response variable was the FTD index. The data were analyzed in the Statgraphics Centurion XVIII program, through a variance analysis (ANOVA) with 95% confidence and a multiple range test with Fisher's method or least significant difference (LSD).

2.2 Raw materials and reagents

Raw materials used for preparing fruit fly attractant baits were: soybean cake, palm kernel cake, whey and bovine blood. The commercial bait currently used in Ecuador for fruit fly monitoring was also used (Supplementary Material 3).

The following reagents were used for protein extraction, enzymatic hydrolysis and bait formulation: NaOH (JTBaker; 98.5% purity), HCl (Riedel de Haen; 37%), bromelain (Sigma-Aldrich; B4882), flavourzyme (Granotec), casein (Merck, 102244, analytical grade), sodium dibasic phosphate (Sigma; S9763), trichloro acetic acid (Analar; 99% purity), bovine whey albumin (Sigma; A4503, electrophoresis grade), sodium carbonate (Merck; 99.5% purity), cupric sulfate (Sigma; 99% purity), sodium potassium tartrate (Merck, 99% purity), phenol reagent according to Folin-Ciocalteu (Sigma; F9252), ammonium persulfate (Promega; 99% purity), borax (technical grade) and 2-mercaptoethanol (Merck; CAS 60-24-2). The following reagents used for molecular determination had an electrophoresis grade: acrylamide (Bio-Rad), tetrabromophenol brilliant blue (Sigma), bisacrylamide (Bio-Rad), Coomassie brilliant blue (Merck), sodium dodecyl sulfate (Sigma) and Tris HCl (Sigma-Adrich). Biuret's reagent was prepared with cupric sulfate pentahydrate (Fluka, Biochemika; 99% purity), EDTA (J.T. Baker, analytical grade), potassium iodide (Panreac; 99% purity) and 6 N NaOH solution (J.T. Baker; 98.5% purity).

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2.3 Obtaining of protein extracts

2.3.1 Soybean cake

Protein was extracted from ground soybean cake. For this, the cake was placed in water, in a 1:5 ratio of solid to liquid, and alkalinized with NaOH 6 N; then, an isoelectric precipitation of the solubilized protein was performed, as indicated in the method described by Vioque et al. (2001), which is detailed in Supplementary Material 4.

2.3.2 Palm kernel cake

The palm kernel flour was mixed with a 0.03 M NaOH solution, in a 1:30 ratio of solid to liquid. This mixture was stirred for 45 min at 35°C (Zarei et al., 2012) and filtered to obtain a supernatant free of solids and fat residues. Finally, the supernatant was dried for 16 h at 50°C and stored under refrigeration (Arifin et al., 2009).

2.3.3 Whey

Samples of 100 mL of whey were homogenized and pH was adjusted to 5.2 with a 6 N HCl solution. In order to separate the fat from the whey, the samples were centrifuged at 210*g* for 15 min; the aqueous phase was poured into beakers, and these were heated at 93°C for 30 min (Supplementary Material 5). The samples were cooled for 20 min and filtered for 3 h on filter paper (Vázquez et al., 2010).

2.3.4 Bovine blood

Samples of 100 mL of irradiated blood were dried at 110°C, for 6 h in a Memert DIN 40 050-IP 20 oven, until a concentrate with 5 to 10% humidity was obtained (Figueroa et al., 2012). The concentration of soluble protein in all samples was determined using the Biuret method described by Fernández and Galván (2010).

2.4 Pretreatment evaluation of raw materials with gamma radiation

2.4.1 Pretreatment with gamma radiation

The effect of gamma radiation on protein extraction yield was evaluated for each raw material. The doses evaluated for soybean and palm kernel cakes were 15, 20 and 25 kGy, while for whey and bovine blood were 10, 15 and 20 kGy. In each case, the protein percentage obtained was determined (Supplementary Material 6).

In the protein extraction assays, the experimental units for the soybean and palm kernel cakes were polyethylene bags with 1 kg of sample; for the whey and bovine blood they were 3 L bags. The samples were placed 30 cm from the Cobalt-60 source in the irradiation chamber of the Radiation Technology Laboratory of the National Polytechnic School. The samples were flipped halfway through the exposure time to guarantee dose uniformity (Maity et al., 2009). The experiment had three replicates.

2.4.2 Experimental design for pretreatment assessment

A completely randomized design was used for each raw material. In each of these designs, the design variable was the irradiation dose; its levels were 15, 20 and 25 kGy for raw plant materials, and 10, 15 and 20 kGy for raw animal materials. The response variable was the amount of protein obtained. There were three replicates. Statistical analysis was performed using Statgraphics Centurion XVIII program; an ANOVA was performed with 95% confidence and a multiple range test with Fisher's method.

2.5 Enzymatic hydrolysis of protein extracts

The substrate and enzyme concentrations for the hydrolysis processes were selected experimentally, as described below.

2.5.1 Selection of substrate concentration

Protein isolates were solubilized in 0.1 M pH 7.0 sodium phosphate buffer, at concentrations of 20, 40, 60, 90, 120, 120, 150 and 200 mg mL^{-1}; the solutions were shaken for 10 min at 900 rpm and subsequently centrifuged at 1,698 $\times g$ for 15 min. Then, 600 μL of the supernatant was mixed with 2,400 μL of phosphate buffer (Cheftel et al., 1989) and the amount of soluble protein was determined using the Biuret method (Fernández and Galván, 2010). The concentration that allowed obtaining the highest amount of soluble protein for each substrate was selected (Supplementary Material 7).

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2.5.2 Selection of enzyme concentration and hydrolysis time

First, bromelain and flavourzyme enzymes were verified to retain their proteolytic activity according to the method described by Castillo et al. (2012) (Supplementary Material 8). Solutions of 0.020 AU mL^{-1} bromelain, 24.0 LAPU mL^{-1} flavourzyme and 10 mg mL^{-1} casein were prepared to guarantee that the enzyme concentration was lower than the substrate concentration and to generate saturation conditions (Nelson and Cox, 2013). Also, 100 μL of each enzyme solution were added to 1,100 μL of casein, and incubated for 20 min at 37°C. Then, 1,800 μL of 5% TCA were added to each mixture to stop the reaction and precipitate the soluble protein. Subsequently, the samples were centrifuged at 2,821 $\times g$ for 20 min; 1,000 μL aliquots were taken from the supernatant and their absorbance was measured at 280 nm in a Hitachi U-19000 UV-VIS spectrophotometer (Castillo et al., 2012). Blanks were prepared in the same way, but TCA was added immediately after mixing each enzyme solution with the substrate. The assay was performed in duplicate.

Hydrolysis assays were then performed with different concentrations of each enzyme. For each substrate, selected concentrations were used as indicated in section 2.4.1. The following concentrations were evaluated for bromelain: 0.002; 0.006; 0.015; 0.020 and 0.025 AU mL^{-1} and for flavourzyme: 2.0; 4.7; 6.0; 12.0; 24.0 and 40.0 LAPU mL^{-1}. The hydrolysis conditions for bromelain were pH 7.0; 40°C for 1 h and for flavourzime pH 7.0; 50°C for 5 h (Benítez et al., 2008). Then, the enzyme concentration that allowed obtaining the highest amount of soluble protein in the shortest time was chosen for each raw material (Supplementary Material 9).

In order to quantify the soluble protein, 200 μL aliquots of the reaction mixture were taken at different times. For reactions with bromelain, aliquots were extracted at 0, 1, 3, 5, 10, 20, 30 and 60 min; while for reactions with flavourzyme at 0, 15, 30, 60, 120, 180, 240 and 300 min. In each case, 2,000 μL of TCA (10%) were added to stop the enzymatic reaction. Then, the samples were centrifuged at 2,821 $\times g$ for 15 min, the supernatant was taken from each sample and the soluble protein content was measured by the Biuret method (Fernández and Galván, 2010).

Finally, product formation rate curves were made considering time for all concentrations of each enzyme, and the one that allowed obtaining the highest concentration of protein hydrolysates in the shortest reaction time was selected (Supplementary Material 10, Supplementary Material 11).

2.5.3 Degree of enzymatic hydrolysis

The degree of hydrolysis was determined with equation 1. The amount of soluble protein was determined during hydrolysis of each protein isolate for 1 h at the conditions previously indicated. Aliquots of 1 mL were taken at different times (0, 3, 5, 10, 20, 30 and 60 min), each aliquot was mixed with 1 mL of 10% TCA and centrifuged at 2,821 $\times g$ for 10 min (Molina-Ortiz and Wagner, 2002). Total protein content was determined by hydrolysis of 0.05 g of each isolate with 2 mL of 6 N HCl at 110°C for 48 h. The hydrolysates were then centrifuged at 2,821 $\times g$ for 10 min (Wilchek and Miron, 2003). Soluble and total protein contents were quantified in the supernatant by the Biuret method (Supplementary Material 12).

$$HD(\%) = \frac{\text{Soluble protein in TCA}(10\%)}{\text{Total protein}} \times 100 \quad (1)$$

2.5.4 Molecular size determination of the hydrolysates obtained by SDS-PAGE electrophoresis

The determination of molecular weights for each raw material was performed by SDS-PAGE electrophoresis (Supplementary Material 13), following the procedure described by Laemmli (1970) and modified by Castillo et al. (2012).

2.6 Formulation of fruit fly baits with hydrolyzed protein

To formulate the attractant baits, the concentration of soluble protein in the hydrolysates obtained was determined and the commercial bait was characterized (Supplementary Material 14). Then, the amount of each type of protein hydrolysate to be placed in the traps was established, so that all the resulting solutions have the same amount of protein (Supplementary Material 15, Supplementary Material 16). The reference formulation contains 10% of commercial bait, 3% borax and 87% water (OIEA, 2005).

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3 Results

3.1 Evaluation of a pretreatment of raw materials with gamma irradiation

Figure 1 shows the amount of protein extracted from raw materials irradiated with doses from 0 (control) to 25 kGy. Irradiation had a significant effect ($p < 0,05$) on the extraction process in soybean cake, palm kernel cake and whey. The highest yield was obtained from the soybean cake irradiated with the 20 kGy dose, being 10 % higher than the one obtained with the control sample. Significant increases were observed in the palm kernel cake in the 20 and 25 kGy treatments; 12 % more protein was extracted at 20 kGy compared to the control sample, as shown in (a). At the same dose, the highest extraction yield was obtained for whey, with a 0.82 % increase with respect to the control sample. The blood samples did not show significant differences compared to the control, as shown in (b), because the extraction process included drying of the blood samples as a way of concentrating the protein. This method is not based on protein denaturation and precipitation (Figueroa et al., 2012), therefore, it was not benefited by the treatment with ionizing radiation, which, on the other hand, favors precipitation (Gaber, 2005).

Consequently, the irradiation dose selected for all the raw materials was 20 kGy, because it gave better yields from the soybean cake and whey. In the case of palm kernel cake, treatment with 20 kGy would generate results statistically equal to those obtained with the 25 kGy dose, but would represent less irradiation time and cost. As for bovine blood, no increase in the percentage of recovered protein would be achieved, but other benefits would also be obtained, such as microbial decontamination.

In general, radiation treatments increased the protein extraction yield. Consistently, Castillo et al. (2019) reported the increase in the percentage of extracted protein in chicken feathers exposed to 25 kGy. Likewise, Kuan et al. (2013) pointed out the modifications in the secondary structure of proteins when exposed to doses higher than 10 kGy, favoring their extraction.

3.2 Enzymatic hydrolysis of protein extracts

3.2.1 Selected substrate concentration

The substrate concentration of 15 % weight by volume (w/v) was selected for the soybean and palm kernel cake, because it produced the highest amount of soluble protein. In the case of whey and bovine blood, 12 and 15 % (w/v) were chosen, since the solubility of the protein decreased at higher concentrations, maybe due to the excess of solute (Cheftel et al., 1989).

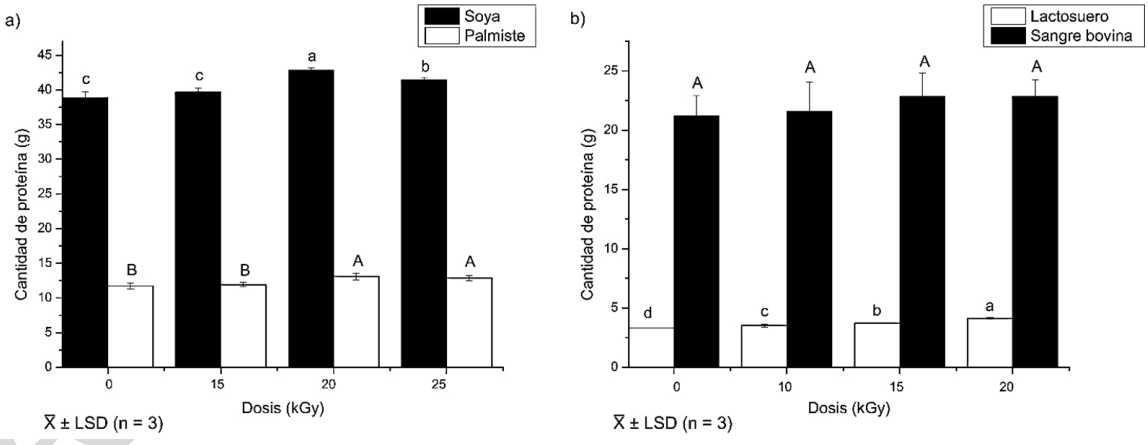

Figure 1. Effect of dose on the amount of protein extracted from irradiated raw materials a) soybean and palm kernel cakes, b) whey and bovine blood.

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3.2.2 Selected enzyme concentration and hydrolysis time

Although both enzymes showed proteolytic activity to be used in the hydrolysis of protein isolates, the reactions catalyzed with flavourzyme required longer reaction time and higher enzyme concentration than the reactions with bromelain to achieve similar concentrations of soluble protein in the hydrolysates. Therefore, bromelain was selected for the subsequent trials. The combined use of bromelain and flavourzyme was discarded because no synergistic effects were observed.

Figure 2 shows the plots of soluble protein concentration considering time, which were obtained by hydrolyzing protein concentrates of the raw materials with bromelain at concentrations between 0.002 and 0.025 AU mL^{-1}. It is shown that the highest soluble protein concentration values are achieved for all substrates with a concentration of 0.025 AU mL^{-1} of bromelain. This is an advantage presented by the hydrolyzates compared to the original protein, because the number of polar groups increases when it is broken into peptides of lower molecular weight, due to the increased exposure of carboxylic groups and free amines, which improves its solubility (Benítez et al., 2008). The chosen reaction time was 30 min, since it allowed reaching protein concentrations of 11.94, 3.23, 7.24 and 8.53 mg mL^{-1} for soybean, palm kernel, whey and bovine blood, respectively, which were close to the maximum values observed at 60 min.

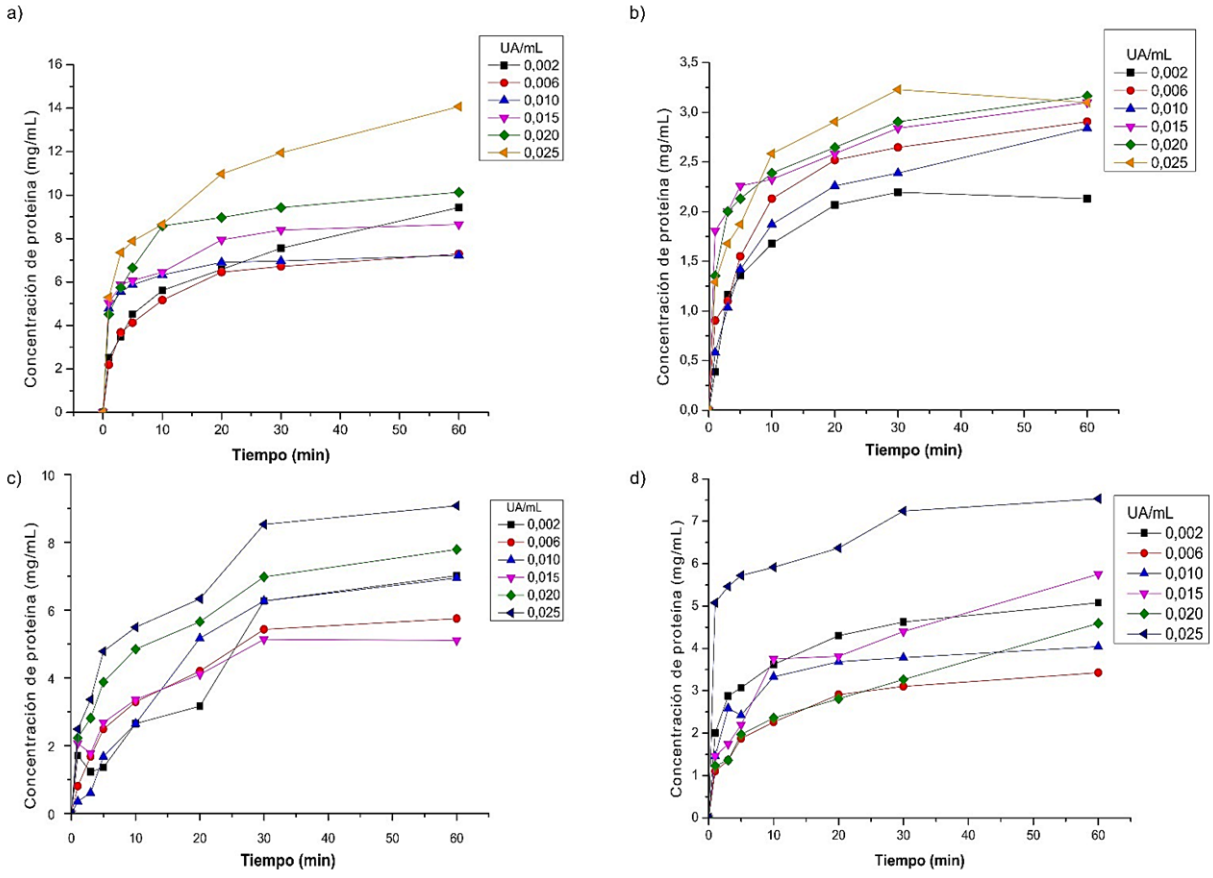

Figure 2. Soluble protein concentration according to hydrolysis time with bromelain at different concentrations, for a) soybean, b) palm kernel, c) whey, d) blood.

3.2.3 Hydrolysis degree

Figure 3 shows the hydrolysis degrees of the protein concentrates corresponding to each raw material, during 60 min of reaction, with 0.025 AU mL^{-1} of bromelain, under the pH and temperature conditions established.

It can be observed that after 30 min of reaction, hydrolysis percentages of 33.87 for soybean, 38.89 for palm kernel, 18.42 for whey and 20.24 for blood were obtained. These hydrolysates are extensive and can be used for the formulation of liquid protein substances, due to their high solubility and the fact that these types of hydrolysates are easily absorbed by living organisms (Vioque et al., 2001). the values of the hydrolysis degree did not present increases at 60 min that would justify a longer reaction time; for this reason, the hydrolysates corresponding to 30 min of reaction were used in the following stages.

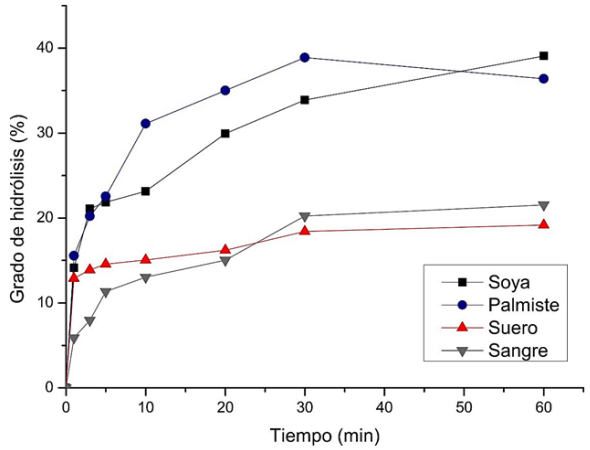

Figure 3. Hydrolysis degree for each raw material during the reaction with bromelain.

3.2.4 SDS-PAGE electrophoresis

Figure 4 shows the results of the molecular characterization by SDS-PAGE electrophoresis. Peptides with molecular weights between 10 and 70 kDa were identified in the commercial bait. The soybean hydrolysates exhibited bands corresponding to molecular weights of 10 to 20 kDa and in palm kernel, mostly lower than 15 kDa (Figure 4.a). Peptides from blood hydrolysate had molecular weights from 10 to 70 kDa and whey from 10 to 15 kDa (Figure 4.b). The commercial bait and the hydrolysates of the raw materials presented peptides with molecular sizes between 10 and 15 kDa, highly digestible fractions that would enable to formulate a bait that could replace the one currently imported.

3.3 Field evaluation of fruit fly baits

The attractant solutions placed in the traps for field evaluation were formulated to present a soluble protein concentration of 12.55 mg mL^{-1} equal to that of the commercial bait, i.e., different amounts of the protein extracts were placed to reach the same amount of protein in each bait.

The ANOVA indicated that the type of bait used did have a significant effect ($p < 0,05$) on the number of flies trapped. Figure 5 presents the means of the FTD index for the bait type. It is observed that the whey bait showed the highest FTD index (LSD, 95%). The effectiveness of the palm kernel and blood baits was statistically equal to that of the commercial bait. Therefore, these could be alternatives for fruit fly monitoring and control in Ecuador. The soybean bait showed a lower FTD index than the commercial bait.

The difference in effectiveness of the baits was possibly due to the molecular size and nature of the peptides obtained by the proteolytic action of bromelain. Bromelain is known to catalyze the hydrolysis of peptide bonds formed with non-polar residues (Benítez et al., 2008). The different result obtained with palm kernel and soybean bait could be due to the variation between the amino acid profiles of the corresponding protein extracts, since palm kernel protein has 7 out of the 8 hydrophobic amino acids (Alimon, 2004; Nelson and Cox, 2013), while soybean protein only has 5 (Calderón de la Barca et al., 2000). The efficiency of whey bait in capturing fruit flies was possibly due to the presence of nitrogen-rich peptides with molecular weights between 10 and 15 kDa. According to Canal et al. (2010), fruit flies easily perceive and assimilate short-chain peptides with low molecular weight. In addition, whey proteins have abundant presence of lysine, providing a higher amount of nitrogen than other protein sources (Jovanović et al., 2005). Nitrogen is a necessary factor for dipteran growth, which allows to reach sexual maturity (Montoya et al., 2010).

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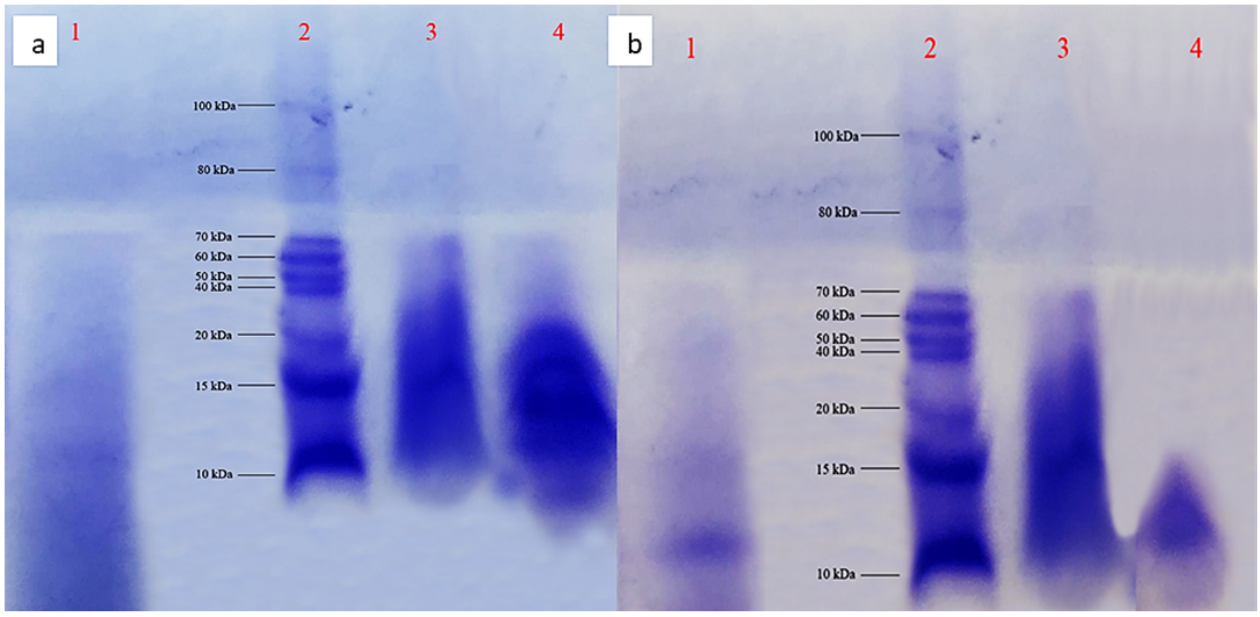

Figure 4. Electrophoresis gel a) 1. palm kernel hydrolysate, 2. standard, 3. commercial bait and 4. soy hydrolysate b) 1. blood hydrolysate, 2. standard, 3. commercial bait and 4. whey.

Figure 6 shows the FTD index considering time for each bait. In all cases, FTD values higher than 1 were obtained; therefore, Puéllaro can be considered a fruit fly infested area. Palm kernel and whey baits presented the highest values during the time evaluated. The FTD peaks could be due to the first rains of the season prior to the installation of the traps, which could favor the development of pest pupae and consequently increase the incidence of adults. Consistently, Tucuch-Cauich et al. (2008) pointed out a direct relationship between the presence of rainfall and the increase in the incidence of *Anastrepha* spp. It is believed that the affinity of fruit flies for the protein hydrolysates used in this research was influenced by factors such as climate, phenological state of the flies, crop type, trap location, trapping density and population level (Canal et al., 2010).

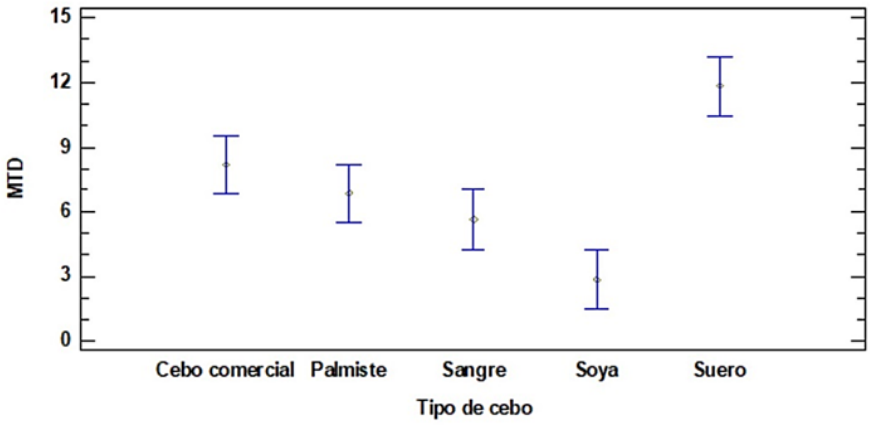

Figure 5. FTD index as a function of the type of bait during field evaluation (mean ± LSD, 95 %).

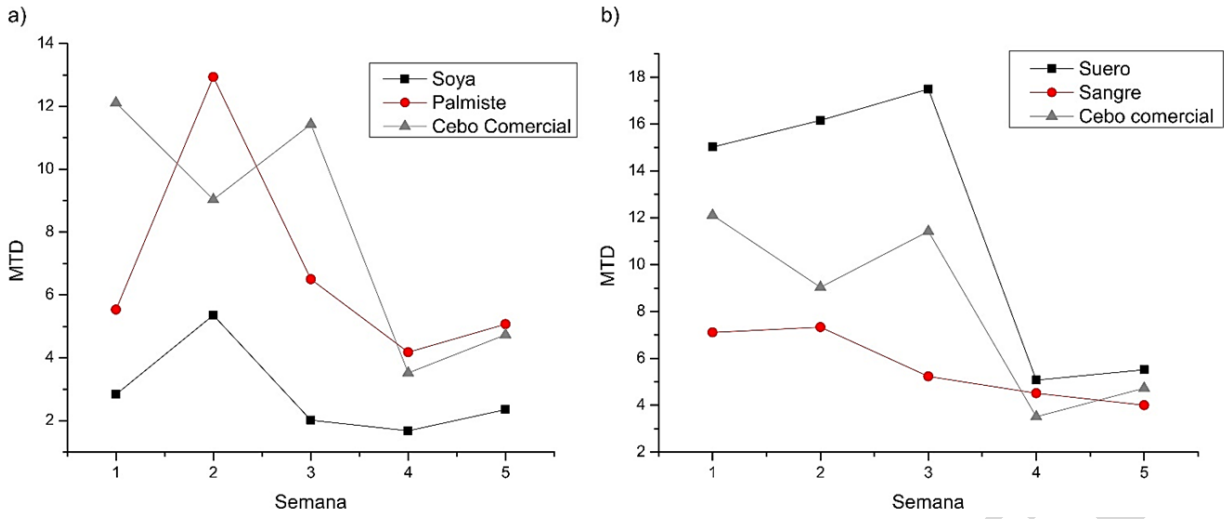

Figure 6. FTD variation of fruit fly from week 1 to week 5.

4 Conclusions

The application of a gamma radiation dose of 20 kGy as pretreatment allowed obtaining a higher yield in protein recovery for soybean with values of 67.24% for palm kernel 60.53% and 35.7% for whey. Radiation treatments did not show significant differences on the protein concentration yield for bovine blood.

The conditions selected for enzymatic hydrolysis were: pH 7.0; 50°C; substrate concentration (w/v) 15% (soybean cake, bovine blood and palm kernel cake) and 12% (whey); 0.025 AU mL^{-1} of bromelain and time of 30 min. The hydrolysis percentages were 33.87 for soybean, 38.89 for palm kernel, 24.78 for whey and 19.94 for blood, after 30 min of reaction. Protein hydrolysates with molecular weights similar to those found in commercial bait (10 to 50 kDa) were obtained.

Hydrolyzed whey protein in the first place, or palm kernel and bovine blood protein in the second could be an alternative to the protein used in the commercial bait, based on the results of the FTD index. It is necessary to investigate the performance of the proposed baits in larger experiments, in different crops and locations in Ecuador, where the presence of fruit flies has been detected. This is because one type of attractant may be effective for a given fruit fly species but not for another, even if they belong to the same genus (Aluja et al., 2001).

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Declaration of interest

No conflicts of interest are declared for any of the authors.

Authors contribution

MS was the director of the research project; he supervised the pretreatment of the raw materials with ionizing radiation, the experimental designs and statistical analyses, as well as the discussion of the results. PC supervised the processes of obtaining hydrolyzed protein. GJ supervised the field evaluation of the baits; CC and MC performed the laboratory tests and the traps.

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Supplementary material

Supplementary material to this article can be found online at https://bit.ly/3DKQg5x.

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