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Development of a Condiment Containing Ficin for the Tenderization of Beef

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Abstract

Beef is one of the main nutritional sources to people, and its consumption is highly dependent on its sensory characteristics. Changes to the textural and flavor properties of this food can affect the acceptability by consumers. The aim of this work was to develop a condiment containing ficin with the ability to improve the sensory characteristics of beef. Dehydration and triphasic separation methods were used to extract ficin from figs fruit, and its enzyme activity was confirmed through Balls and Hoover method. The protein extract with the fastest enzyme activity as representation of a better activity was selected to develop the condiments. Two formulations containing 0.5 and 1 wt.% of ficin were prepared, and their safe consumption was tested through microbiologic tests based on normative NTE INEN 2532. The activity of condiments was tested on the firmness of beef fillets. Furthermore, the acceptability of texture and flavor of fried beef fillets treated with the condiments was also evaluated through acceptability ranking sensory test. The triphasic separation method favored the obtention of ficin with the best enzyme activity. The beef fillets treated with the condiment at 1 wt.% of ficin exhibited better firmness characteristics and higher acceptability by consumers. These results could be used as base to research condiments containing ficin able to improve the sensory characteristics of meat products with the aim of increasing their consumption.

Keywords

Meat products, Acceptability, Consumers, Enzymatic activity

Introduction

One of the main problems in the food area is associated with the type of meal consumed by the people. The nutrition of the population of developed countries is mainly affected by the high consumption of fatty and protein foods. Instead, the population of developing countries generally suffers problems of malnutrition [1, 2]. During 2022, 390 million adults had insufficient weight, 149 million children exhibited retardancy on their growth and 37 million children suffered obesity [3]. Ecuador is a developing country with high levels of malnutrition. In this country, malnutrition problems in people have emerged since 1993. Between 2014 and 2018, the malnutrition in Ecuador increased from 25% to 27%, and it was mainly shown in children [4]. For this reason, Ecuador is catalogued as the second country with the highest indicators of malnutrition in the world [3, 5]. Some works highlight the importance of essential nutrients such as: proteins, vitamins, carbohydrates, fats, unsaturated fatty acids, monounsaturated fatty acids, and minerals as calcium and iron [6, 7]. The lack of some of them can reduce the physical and

mental performance of people and affect their growth and fertility. The low consumption of meat protein is one of the main lacks associated with malnutrition in the people. In Ecuador, the beef consumption is around of 10 kg per person in the year [8]. This problem has mainly arisen from the lack of quality in meat products, resulting in people with iron deficiency [9, 10].

The food industry is constantly testing different tools and methodologies to improve the quality of meat and increase its consumption. The main factors that affect the quality of beef are: i) the genetic of the animal, mainly associated to the race and muscle structure, ii) the beef cut since the zones where a high muscle amount is created by the weight the animal is generally harder, and iii) post-mortem processing due to the enzyme activity of intrinsic ones affects the texture of meat. Furthermore, a high juiciness in beef cuts always produces a positive perception of this food by consumers, and thus, an increase on its consumption [11-13]. Main methodologies used to improve the quality of meat are electrical, mechanical and chemical processes as well as the use of enzymes [14]. Enzymes are proteins catalysts that facilitate chemical reactions by either building up or breaking down molecules. Enzymes play an important role as additives in the food industry since they are able to alter the way of food processing, and thus, their appearance, texture, nutritional value, flavor and processing time [15]. Enzymes are extensively used to develop food products, highlighting baking, brewing, meat, juice and beverages, dairy, dietary supplements, vegetable processing, fats, and oils [16, 17]. The tenderization of beef is the main purpose of the application of enzymes. These biomolecules can help to obtain bioactive peptides with antioxidant, antihypertensive and antimicrobial properties from beef proteins. Furthermore, its mechanism favors the presence of new tastes and odors, improving the flavor of this food [18, 19]. For these reasons, the use of enzymes to improve the sensory quality of beef is highly preferable in comparison with electrical, mechanical, and chemical methods since they exhibit high efficiency, specificity, and safety [18].

Enzymes can be extracted from different sources such as bacteria, fungi, yeasts, archaea, animal organs, or plant extracts [20]. Plants such as: soy, wheat, corn, rice, barley, potatoes, algae, and fruits as well as their different parts (leaves, fruits, peel, stem) have constituted the main sources to obtain enzymes [21]. The peel of fig (*Ficus carica L.*) is one natural source enriched with latex, whose main compound to be extracted is ficin. In Ecuador, the production of figs is being highly promoted due to its potential as exportation fruit. A green fig can produce between 10 and 15 g of latex where 100 to 150 mg of proteases as ficin can be contained. Ficin represents approx. 70 to 90% of the total protein content of latex [22]. This enzyme is a cysteine-protease widely used in the textile, pharmaceutical and food industries. Ficin is activated with the presence of reductor agents, and its main function is to catalyst the hydrolysis of peptide bonds in proteins. In this way, meat products can be subjected to tenderization process using ficin [23].

Nowadays, the consumers also require meat products with high sensory quality. The organoleptic characteristics of beef such as color, juiciness, texture, and flavor are the main parameters to be judged by consumers [24]. The texture of beef is a key factor for the industry. A product with a good texture shows tenderness and juiciness, favoring in turn excellent sensory perceptions of flavor and promoting its acceptability by consumer. Therefore, the use of enzymes as ficin has been a novel strategy to improve the texture characteristics of beef [18, 25, 26].

The aim of this research was to develop a condiment containing ficin to improve the sensory characteristics of the texture and flavor of beef fillets from the flank section of animal. It is important to highlight that the selection of this meat cut with high firmness was done to evidence in the best way the tenderization effect of condiment in the beef.

Materials and Methods

Ingredients and reagents

Onions, oregano, cumin, coriander, and garlic in powder were obtained from the local supermarket. Ammonium sulphate, tert-butyl alcohol, and distilled water obtained from Labomersa (Quito, Ecuador) were used as reagents. Beef fillets (flank section) were purchased from Supermaxi (Riobamba, Ecuador).

Development of condiment containing ficin with softener properties to beef

Extraction of ficin from figs

Fig fruits with a green color and homogenous size were selected to obtain the ficin enzyme. The fruits were washed two times with distilled water to eliminate the impurities. Several cuts were done in the figs from the peduncle to ostiole owing to promote the drip of latex. The cuts were done with a surgical knife every five minutes. Latex was collected into a glass container and subjected to the following methods to extract ficin:

- **Dehydration:** 12 ml/g of latex were mixed with 18 ml of ethanol 96% and stored at -4 °C for 7 days. The mixture was centrifuged at 4500 rpm for 20 min, and the supernatant was removed. The precipitate was put into porcelain capsule and dried at 35 °C for 6 h. The dry precipitate was subjected to a milling process with a mortar to obtain a powder.
- **Triphasic separation:** 10 ml/g of latex were mixed with distilled water with a volume ratio of 1:0.5. The mixture was centrifuged at 5000 rpm and 4 °C for 15 min to eliminate the rubber and impurities. The insoluble phase was removed. Ammonium sulphate was added to supernatant to reach a concentration of 40% (m/v). The solution was stirred, and later, kept at rest for 45 min. Later, tert-butyl alcohol was added to the solution at a ratio of 1:0.75, and the mixture was stirred. The mixture was centrifuged at 3000 rpm and 4 °C for 10 min to facilitate the separation of three phases. The upper, intermediate and over phases were carefully eliminated to recuperate the precipitate (latex). The precipitate was put into porcelain capsule and dried at 20 °C to obtain a powder.

To verify the successful extraction of ficin, Balls and Hoover method was carried out [27]. This test evidences the enzymatic activity through the degradation of a patron protein as casein. 10 mg of ficin solution (0.05 g of ficin in 10 g of acetic acid at 0.01% v/v) was added into 10 ml of a solution of milk at 2.5% (w/v). The sample was boiled at 50 °C and stirred. The result was expressed as the time when the first coagula was formed.

Preparation of condiment containing ficin for beef tenderization

Two formulations were proposed to prepare the condiment containing ficin. All formulations were composed of onion (20 wt.%), oregano (10 wt.%), cumin (5 wt.%), coriander (10 wt.%), garlic (30 wt.%) and salt (25 wt.%). The ficin was added at 1 and 0.5 wt.%, and the formulations were named as condiment_1 and condiment_0.5 [28]. A control sample without ficin was also prepared and named condiment_0. Microbiologic tests based on normative NTE INEN 2532 were done owing to ensure the safety of the condiments. This normative informs different methodologies and normative applied to evaluate the presence of mesophilic aerobics, molds, and yeasts, coliforms, *Escherichia coli* and *Salmonella*. The methods are mainly based on the agar depth in the plate method. Furthermore, normative NTE INEN 2532 establishes the following microbiological maximum limits: 10^5 cfu/g for mesophilic aerobics, 10^3 cfu/g for molds and yeasts, 10^2 cfu/g for coliforms, < 10 cfu/g for *E. coli* and absence for *Salmonella*.

Beef fillets with a thickness of approx. 1.5 cm and a length of 15 cm were subjected to tenderization process by effect of condiments containing ficin. The fillets were randomly divided into two groups: beef treated with condiment_0.5 (BC_0.5) and beef treated with condiment_1 (BC_1). The fillets were treated with both condiments and maintained in contact for 5 min. Fillets treated with condiment without ficin were used as control and named as BC_0.

Firmness and sensory evaluation

Firmness analysis

The treated and untreated raw fillets with condiments containing ficin were subjected to a firmness analysis by using a Humboldt Universal Penetrometer H-1240.4F (Chicago, USA) fitted with a 3 mm diameter plunger. The plunger of penetrometer was put over the beef surface (specifically in the central part of fillet), and a force was applied for 10 s. The firmness was determined by the length of penetration into each fillet (mm). The experiment was carried out by triplicate.

Sensory analysis

The effect of condiment containing ficin in the sensory properties of beef was evaluated through an acceptability ranking sensory test. Thirty untrained beef consumers participated in this study (18 women and 12 men, from 14 to 60 years old). The participants were randomly, and voluntary recruited from Sciences Faculty at Escuela Superior Politécnica de Chimborazo. The participants evaluated the effect of condiments containing ficin in the texture and flavor of fried beef pieces. The test was carried out in one session (from 09:00 am to 15:00 pm).

Treated and untreated beef fillets with condiments containing ficin were subjected to frying process in sunflower oil. Each side of the fillet was fried for 3 min. The frying temperature oscillated between 165 and 180 °C, and the pH ranged between 5.5 and 6 during this procedure [29]. Subsequently, the samples were cut into pieces of 2 × 2 cm and put into transparent plastic glasses covered with alumina. The weight of each sample was approximately 2 g, and the glasses were codified with a label containing three-digit random numbers. A glass with a solution of citric acid at 5% (w/v) and a soda cracker were provided to participants for rinsing their mouths after each evaluated sample. The samples were delivered in an established order to participants. They tasted and evaluated the acceptability of texture and flavor of the samples by using an ordering sensory test. The rank of scale for each parameter started from 1 = Higher acceptability, 2 = Medium acceptability, and 3 = Lower acceptability.

Statistical analysis

One-way analysis of variance (ANOVA) with $\alpha = 0.05$ was performed on the firmness and acceptability data obtained to determinate the significant differences ($p \leq 0.05$) between the samples. Once a significant effect of the sample was found, Tukey test was used to compare them. InfoStat program (student version) was used for statistical analysis.

Results and Discussion

Extraction of ficin and activity enzyme

Figure 1 shows the extracts of ficin obtained by using dehydration and triphasic separation. As it is shown in figure 1A, dehydrated extract exhibited high stickiness. Instead, the extract obtained by triphasic separation was in powder format (Figure 1B). This fact could be associated with the extraction method used to obtain the enzyme. The high stickiness of the dehydrated sample would be associated with the presence of remanent rubber and impurities that were not eliminated during the extraction method. Compounds such as: phenolic compounds from latex, amino acid products by the partial hydrolysis of proteins, salts, and sugar residues are the main remnants of extraction process of ficin [23]. Instead, a better manipulable extract with ficin enzyme was reached with the triphasic separation method which favored the obtaining of an extract in powder format.

Ficin is a polypeptide able to produce the hydrolysis of casein and favors the formation of clots. Regarding to a previous study, the needed time to the formation of casein clot

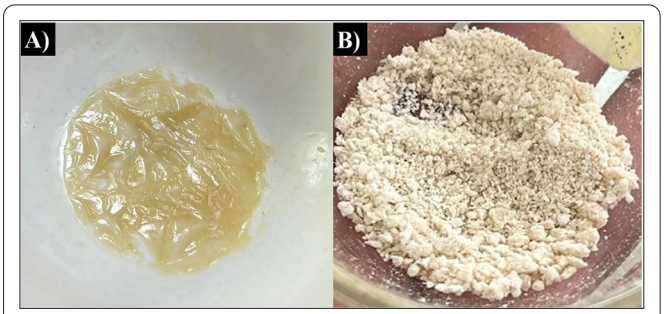


Figure 1: Ficin extracted by **(A)** dehydration, and **(B)** triphasic separation.

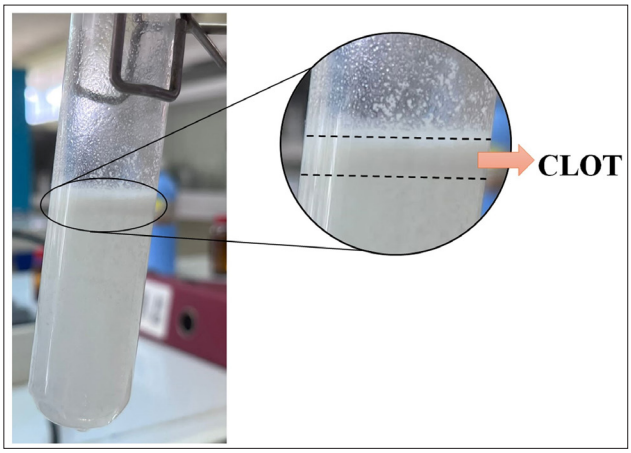


Figure 2: Test of enzyme activity of ficin.

is between 20 and 150 s [30]. The enzyme activity of extracted ficin was assessed through the Balls and Hoover method. As figure 2 shows, the formation of a casein clot confirmed the successful obtention of enzyme through dehydration and triphasic separation. However, the enzymatic activity of dehydrated extract (45 s) was slower than the extract obtained by triphasic separation (38 s). This fact would be associated with the amount of ficin contained into extract since the high complexity of the triphasic separation method could favor a higher amount of this enzyme, and thus, a faster enzymatic activity in the milk. According to these results, extract obtained by triphasic separation method was selected to the preparation of condiment.

Obtaining of condiment containing ficin

Three condiments containing ficin were prepared, and their innocuity was assessed by microbiologic tests. Table 1 shows the results obtained from bacterial count.

As can be evidenced, all formulations maintained a high innocuity. The bacterial count values for each microorganism established in the normative NTE INEN 2532 were not exceeded. This result would be associated with the presence of compounds with antimicrobial property. It is widely known that onion, oregano, cumin, coriander, and garlic are potential sources of antibacterial and antifungal compounds. Previous studies have evidenced the presence of a great variety of antimicrobial compounds in these substances, such as: phenolics, organosulfur, flavonoids, carvacrol, thymol, carvacrol, flavonoids, linalool, among others, and their effective antimicrobial property against bacteria, molds, and yeast [31-35].

Firmness analysis

Fillets selected of flank beef were used to evaluate the effective action of condiments containing ficin. Results of firmness are shown in table 2.

The results showed significant differences between the samples. Furthermore, the increase of concentration of ficin in the condiment favored a higher penetration in the fillets. The main proteins of beef are myosin, actine, and collagen. Myosin and actine are contractile proteins that are part of muscle fibers. Instead, collagen is a structural protein of connective tissue. The enzymes are able to decrease the presence of connective tissue in the proteins, favoring the tenderization of beef products. One of the main enzymes used to the tenderization process of beef products are proteases. This type of enzyme comes from plants such as: papaya (papain), pineapple (bromelain), and figs (ficin) [36]. Ficin is an enzyme with the ability to hydrolyze specific peptide bonds present in muscle proteins and collagen molecules and transform it to aromatic compounds. This fact favors the reduction of firmness of beef giving rise to better tenderness of food since it exhibits a new physical behavior as gelatine [18, 37, 38].

Table 2 also shows the relation response:dose since a higher concentration of ficin favored a higher penetration in the fillets due to the hydrolysis of peptide bonds in the food. Similar results were obtained in the research carried out by Zhu et al. [39]. Authors evidenced a lower tenderization process in beef briskets when a lower concentration of actinidin enzyme extracted from kiwi was applied. This result demonstrated that high resistance of beef briskets was associated with a higher presence of peptide bonds [39]. On the other hand, the effectiveness of enzymes depends on medium conditions and the characteristics of peptide. Research carried out by Azmi et al. [18] indicated that plant proteases are highly effective to the beef tenderization, but their effectiveness depends on enzyme concentration, pH, time, and temperature. In this context, a

Table 1: Results of bacterial count of different condiments.

Formulation	Bacteria	Bacteria count (cfu/g)	Reference value (cfu/g)
Condiment_0	*E. coli*	< 10	< 10
	Meshopilic aerobic	< 10	1×10^6
	Total coliforms	< 10	1×10^3
	Yeasts and molds	< 10	1×10^4
	Salmonella	ND	ND
Condiment_0.5	*E. coli*	< 10	< 10
	Meshopilic aerobic	< 10	1×10^6
	Total coliforms	< 10	1×10^3
	Yeasts and molds	< 10	1×10^4
	Salmonella	ND	ND
Condiment_1	*E. coli*	< 10	< 10
	Meshopilic aerobic	< 10	1×10^6
	Total coliforms	< 10	1×10^3
	Yeasts and molds	< 10	1×10^4
	Salmonella	ND	ND

Note: ND = Non detectable. Reference values are according to normative NTE INEN 2532.

Table 2: Analysis of firmness of beef fillets treated with different condiments.

Beef sample treated with condiment	Length of penetration
BC_0	116.67 ± 5.51^a
BC_0.5	148.00 ± 5.00^b
BC_1	195.33 ± 3.06^c

Note: Lower case letters a-c indicates significant differences among samples ($p < 0.05$) according to ANOVA analysis and Tukey's test. The number of raw fillets treated and untreated with condiment (n) was three.

previous study evaluated the effect of temperature and pH on the enzyme activity of ficin. Results showed that the enzyme action was not affected when a temperature of 70 °C and a pH of 8 were used [23]. This fact is important to highlight since in our research the conditions employed during the extraction of enzyme by triphasic separation did not affect the ficin activity. Furthermore, the enzyme tenderization effect of condiment with ficin was not affected despite the frying process conditions (temperature = 165 - 180 °C and pH = 5.5 - 6). This fact could be corroborated by the sensory test since panelists were able to differentiate the level of firmness of the samples once time that they were treated with the condiments and later subjected to the frying process. However, a chemical analysis is proposed for future studies to confirm this hypothesis.

Sensory analysis

Table 3 shows the values of acceptability of texture and flavor of fried beef fillets treated with the different condiments. The samples were evaluated and rated as 1 = Higher acceptability, and 3 = Lower acceptability.

The texture and flavor of beef fillets treated with the condiment containing the highest concentration of ficin (BC_1) were the most accepted by participants. As it was shown in table 2, the high concentration of ficin promoted a lower firmness of beef sample BC_1, and thus, a fried product with a texture and flavor highly acceptable. This result would be associated to the action of cysteine contained in the ficin that is able to act on the beef proteins as the elastin. Thus, this amino acid promotes the hydrolysis and solubility of peptide bonds of beef proteins [18, 19, 25]. Previous research evidenced changes in the texture of beef products by the effect of ficin concentration [40].

The concentration of ficin also affects the water retention percentage in beef products, and thus, its texture can be modified. The presence of water in this food favors its tenderization process when it is fried. This fact is evidenced due to the beef can maintain its texture characteristics such as: juiciness and tenderness [25]. For instance, Li et al. [40] determined that beef treated with ficin at 0.1 g/L and incubated at 55 °C for 60 min maintained its texture characteristics. Furthermore, the modification of texture characteristics of beef can promote changes in the flavor perception by consumers [25]. An excess of tenderization on beef products can be promoted by ficin action. This fact in turn can generate a low quality in the product since its texture and flavor are not accepted by consumers [19].

Conclusion

A condiment containing ficin was prepared and its tenderizer property was tested in beef. The triphasic separation was the most effective method to obtain ficin extract highly pure from fig fruits. The enzymatic effectiveness of ficin extract in the milk with a coagulation time of 38 s extract was validated through the Balls and Hoover method. Two condiments with different ficin extract concentrations were prepared, and their enzymatic activity was tested on beef fillets. The condiment containing the highest concentration of ficin promoted the obtention of beef fillets with the lowest firmness. Furthermore, the enzymatic activity of this condiment was verified through sensory analysis. Consumers ranked with the highest qualifications to the texture and flavor of fried beef fillets that were previously treated with the condiment that contained 1 wt.% of ficin. These results would favor research about the development of condiments containing ficin to be applied on meat products with the aim of improving their sensory characteristics and increasing their consumption.

Table 3: Acceptability of fillets treated with different condiments.

Beef sample treated with condiment	Texture	Flavor
BC_0	2.77 ± 0.43^{c}	2.83 ± 0.38^{c}
BC_0.5	2.17 ± 0.53^{b}	1.90 ± 0.66^{b}
BC_1	1.07 ± 0.25^{a}	1.27 ± 0.45^{a}

Note: Lower case letters a-c indicates significant differences among samples ($p < 0.05$) according to ANOVA analysis and Tukey's test. The number of people to evaluate the texture and flavor of fried beef (n) was thirty.

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Conflict of Interest

None.

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