STUDY CONCERNING THE PROTEOLYTIC ACTIVITY OF VEGETABLE ENZYMES ON CURED MEAT QUALITY

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Abstract
The purpose of this paper was to study the proteolytic activity of vegetable proteins reflected by physico-chemical parameters of meat. In this regard have been used the proteins of vegetable origin such as the bromelain and papain. Was used the juice obtained from pineapple respectively papaya. It was studied compressive force of meat in different conditions of exogenous proteins action. They were analyzed the quality parameters of meat: moisture, amino acids, pH. Also, have been analyzed the proteolytic activity and acidity of enzymatic substrates. The addition of exogenous vegetable proteins improves tenderness, protein digestibility and decreases the time of meat processing. Analyses were performed on beef and pork meat. The papaya juice had a proteolytic activity, 24g glycocol/100 g casein, higher than pineapple juice, 20 g glycocol/100 g casein. The samples of meat, cured in papaya juice contain a greater amount of free amino acids than those cured in pineapple juice. As a measure of softness meat, it was found that the compressive force was the lowest in the case of pork meat, matured in papaya juice, 16,45 kPa, compared to beef, where it was 17,45 KPa. The papaya juice had the most powerful proteolytic action on muscle tissue from samples analyzed.

Keywords: bromelain, papain, tenderness meat

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1.INTRODUCTION

The most commonly used method for accelerating the maturation of meat and improve meat tenderness is maturing with plant proteolytic enzymes. For maintaining food safety, meat must be handled as quickly as possible after slaughter, before the state of rigidity to show up. After maturing, the meat acquires a soft consistency becomes more tender and juicy, the taste is pleasant and the color changes from red to red-gray.

Exogenous proteolytic enzymes extracting from plants, bacteria, and fungal sources have been used for centuries to improve tenderness meat by hydrolysis of proteins. United States federal agencies recognize five exogenous enzymes – papain, ficin, bromelain, *Aspergillus oryzae* protease, and *Bacillus subtilis* protease as safe to improve meat tenderness (Chaurasiya, 2015).

Papain, bromelain and ficin are proteolytic enzymes acting plant are extracted from papaya, pineapple and figs. The ginger protease has been extracted of ginger rhizome and using it to maturation led to a tender meat explained by the action of plant proteins on the connective tissue components and myofibrillar proteins (Naveena, 2004; Saranya, Santhi and Kalaikannan, 2015; Abdeldaiem, Hoda, Ali, 2014.).

The treatment with papain and bromelain it affects the structure of the collagen fibers from the muscles and destabilizes the intermolecule bonds (Doneva et al, 2015).

Bromelain first degrades 40% of the collagen in the sarcolemma followed by degradation of myosin in the myofibrillar component and proteolytic effect is enhanced by increasing temperature. (Calkins and Sullivan, 2007)

Bromelain determine at the microstructure level decrease in muscle myosin heavy chains and actin (Ketnawa, 2011).

Papain acts at the Z membrane and determines the degradation of myofibrillar and collagen proteins, yielding protein fragments of several sizes (Calkins and Sullivan, 2007).

The enzymatic conversion of collagen into gelatin is an indicator of the meat tender, more important than the total amount of connective tissue (Rawdkuen and Benjakul, 2012).
The effect of exogenous enzyme action is reflected in the degree of hydrolysis of proteins in high and low connective tissue muscles (Sullivan and Calkins, 2010).
The treatment with proteolytic enzymes is one of the popular methods for meat tenderization.

2. MATERIAL AND METHODS

2.1. Experimental Materials

In order to analyze the efficiency of proteolytic enzymes of plant, was used juice derived from papaya (*Carica papaya*) and pineapple fruits (*Ananas comosus)*. The juice was extracted using a centrifugal machine. Meat was purchased from animals of their own production, 8 hours of slaughter. Rectangular pieces of meat were weighed so as to have 100g and thickness be 1 cm.
Samples were identified as follow:
Cpm - Control sample of pork
Cp1 - Sample of pork matured in 50 ml pineapple juice
Cp2 - Sample of pork matured in 100 ml pineapple juice
Cp3 - Sample of pork matured in 50 ml papaya juice
Cp4 - Sample of pork matured in papaya juice 100ml
Cp5 - Sample of pork matured with salt
Cbm-Control sample of beef
Cb1-Sample of beef matured in 50 ml pineapple juice
Cb2-Sample of beef matured in 100 ml pineapple juice
Cb3-Sample of beef matured in 50 ml papaya juice
Cb4-Sample of beef matured in papaya juice 100ml
Cb5-Sample of beef matured with salt

The samples thus obtained were immersed individually in the variable volume of vegetable juice. To compare the effectiveness of exogenous enzymes, regarding the maturation of meat with other classical processes, a meat sample was treated with sodium chloride. The control sample was not treated with NaCl and was not immersed in vegetable juice.

2.2. Experimental Design

Chemical analyzes were performed at baseline, to 24 hours and 48 hours, being kept at 0-4^{o}C. To evaluate the dynamics of the protein-system of the meat, there was used the biochemical analyses of amino acids. Determination of amino acids was performed by the method Sorensen. The water content was analysed by drying at oven, for 1 hour at 150^{o}C.
For determine the activity of proteolytic enzymes, enzymatic extract made from papaya and pineapple has acted on a casein solution of known concentration at a temperature of 37 to 40°C. The free amino acids were dosed after Sorensen method. Determination of total acidity was done by titration with NaOH in the presence of phenolphthalein on papaya and pineapple juices.
In order to measure the tenderness of a small sample of raw or processed meat is used device that uses the compression to crushing. the device consists of a stainless steel cylinder with a height 85 mm and diameter 60 mm. Inside the cylinder a plunger slides over the bottom with a rubber gasket which executes the sample pressing which has a thickness of 1.27 cm. The plate on the bottom of the cylinder has a hole of 28 mm. The piston is pressed with weights calibrated before the first pieces of meat are pushed through the hole. Reads the total weight that was acted. The test piece of meat is placed in such a way that the muscle fibers to be perpendicular to the geometry axis of the hole bottom.

3. RESULTS AND DISCUTION

Papaya and pineapple juices were analyzed after production. The hydrolysis degree of proteins was evaluated by the amount of free amino acids in samples.
The results of determining proteolytic capacity are shown in figure 1.

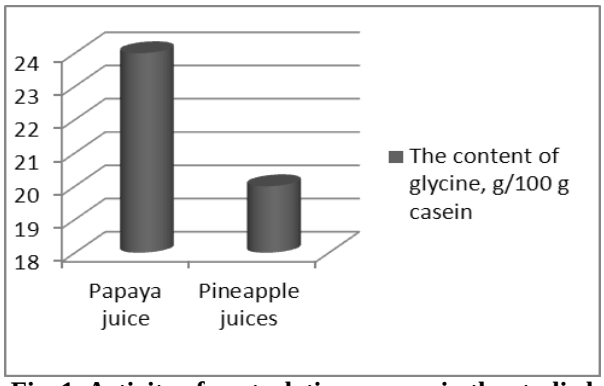

Fig. 1. Activity of proteolytic enzymes in the studied samples

Proteolytic activity of enzymes in papaya juice is higher than in pineapple juice.
As a result of analysis for the determination of total acidity papaya juice and pineapple juice, it has been found that papaya juice had an acidity 0,0281 g acid citric/100ml and 0,037 g acid citric/100ml pineapple juice. (figure 2)

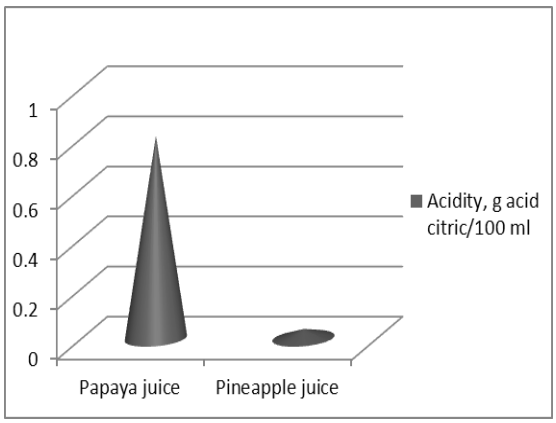

Fig. 2 The total acidity of the studied samples

The values obtained from determining the moisture content and pH of pork are shown in figure 3.
The samples of pork and beef had pH between 5.5 - 6. These values are favorable action of plant enzymes. According to Calkins and Sullivan (2007) optimal pH is 4-6 for bromelain, and 5-6 for papain, so that the two enzymes act effectively to change the structure of proteins.

Matured pork meat with salt had the lowest water content 57,67 % and with papaya juice with the highest content 75,58%. The same trend it was for beef.

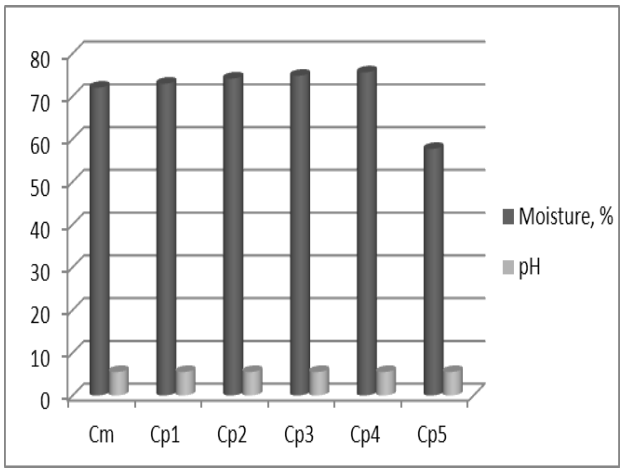

Fig. 3 The evolution of water content and pH in pork meat

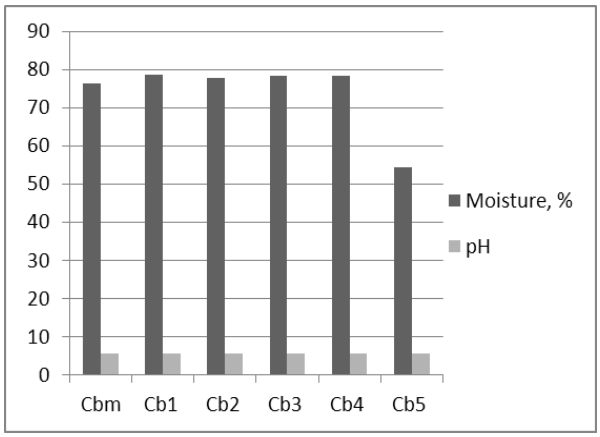

Fig. 4 The evolution of water content and pH in beef

The degree of hydrolysis of proteins was evaluated according to the quantity of free amino acids in samples. The values obtained from dosing amino acids in meat, through the method Sorensen are shown in Table1.

Table 1. The amino acid content of the meat samples cured in different conditions

Sample	Amino nitrogen, g/100 (after 24 h)	Amino nitrogen, g/100 (after 48 h)
Cpm	0,2641	0,2777
Cp1	0,2800	0,2941
Cp2	0,2884	0,3619
Cp5	0,2107	0,2549
Cb3	0,1386	0,3137
Cb4	0,2244	0,3762

Samples matured in pineapple juice contain a higher amount of amino acids than those matured with NaCl. After 24 hours, in the case of samples of pork and beef ripened at different concentrations of pineapple juice, the amine nitrogen values obtained were lower than in the case of samples of pork ripened under the same conditions during 48 hours.
The juice volume was influenced directly proportional the amount of amino acids so better results were obtained in the case of maturation with 100 ml juice.
The compressive force values results after determining tenderness of pork samples are presented in Table 2.

Table 2. The compressive force values for pork meat seamples

Sample	Compressive force, kPa
Cpm	32.92
Cp1	31.16
Cp2	17.50
Cp3	24.51
Cp4	16.45
Cp5	38.16

After determining the tenderness of the pork meat, it was found that in the case of samples of pork ripened in a smaller amount of juice, respectively 50 ml, compressive force is higher and therefore less tender than samples of pork ripened in a large amount of juice, respectively 100 ml. In the case of the sample of pork matured in 20 g salt, compressive force has a maximum value, 38,16 kPa, which means that respective sample is less tender than other samples.

Table 3. The compressive force values for beef meat seamples

Sample	Compressive force, KPa
Cbm	40.15
Cb1	22.76
Cb2	17.50
Cb3	24.16
Cb4	22.76
Cb5	45.52

Also, in the case of a control sample, the compressive force is large compared to the samples ripened in various volumes of juices which means that the proteolytic enzymes in the juice of pineapple and papaya acts on the proteins of the meat samples, reducing the polypeptide chain, obtaining simultaneously tenderizing meat.
After determining the tenderness of the beef meat, it was found that in the case of samples of pork ripened in a smaller volume of juice, respectively 50 ml, compressive force is higher, 22,76 kPa and therefore less tender than samples of pork ripened in a large volume of juice, respectively 100 ml.
In the case of the sample of beef matured in 20 g salt, compressive force has a maximum value, 45,52 kPa
Following the determination of tenderness meat, be it of pork or beef samples, meat samples ripened in pineapple juice or papaya juice were more tender than the other samples, which means that the enzymes proteotitice (papain, bromelain) present in papaya juice and pineapple juice, plays a positive role in regard to the texture of meat.

4. CONCLUSIONS

In the food industry, proteolytic enzymes have broad applications because they represent the key role in diversity of transformations of the protein substances.
The maturation of meat can be achieved with proteolytic enzyme preparations, leading to economic benefits and improve meat quality. The cooking time of meat treated with enzyme preparations is reduced by 1/3. Due to this fact increases the nutritional value of the meat and thus the bioavailability of nutritional compounds formed following proteolysis (amino acids).
Superior efficiency had the papaya juice because compression force applied to these samples was less than for maturation in pineapple juice.

6. REFERENCES

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