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EFFICIENCY OF PLANT PROTEASES BROMELAIN AND PAPAIN ON TURKEY MEAT TENDERNESS

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Abstract: The main subject of study is the effect the plant proteases bromelain and papain exert on turkey meat tenderness. Experiments are conducted with samples of raw meat in 3 different concentration levels of the enzyme solutions (50U/ml 100U/ml and 200 U/ml) and in 3 different time periods (duration) of treatment (24 h, 48 h, 72h). An increase in enzyme concentration and treatment duration results in a higher degree of protein hydrolysis in the turkey meat. The optimal conditions for hydrolysis with minimal loss of protein and highest retention of organoleptic qualities of the meat samples are established.

Key words: tenderizing, turkey meat, bromelain, papin

Introduction

Tenderness belongs to the most important meat quality traits. There are several factors that determine meat tenderness: sarcomere length, myofibril integrity and connective tissue integrity. The latter one determines the quality of background toughness. (*Chen et al., 2006*) There are two different components to meat toughness: *actomyosin toughness* and *background toughness*. Actomyosin toughness is attributed to myofibrillar proteins, whereas background toughness is due to connective tissue presence.

In the recent years interest is growing in the development of better methods to produce meat with improved tenderness whilst preserving its nutritional qualities. (*Koohmaraie, 1996*) Various physical and chemical methods have been developed to improve meat tenderness. (*Qihe et al., 2006*) These methods attempt to reduce the amount of connective tissue in the meat without that resulting in the breakdown of myofibrillar proteins.

Such meat tendernization methods include muscle stretching and electrical stimulation. The basic methods used in meat tendernization are marination and ion/organic acid injections. The aforementioned physical and chemical methods improve meat tenderness with little effectiveness.

Another interesting method in meat tenderization is the application of exogenous enzymes of vegetable, bacterial or fungal origin. It is used to examine

the alteration of the structure of the connective tissue and the integrity of the myofibrils.

The same enzymes can be used not only with beef, pork and poultry, but also with many marine products. Most of these enzymes are vegetable proteases. (*Ashie et al., 2002; Minh et al., 2012.)* In recent years, from all the exogenous proteolytic enzymes used in meat tenderization, the cysteine proteases have attracted considerable interest, in particular, vegetable cysteine proteases. Some of them have long been used in cooking. (*Sullivan and Calkin, 2010*).

The main objective of this project is to study the effect the two trade vegetable enzyme proteases (papain and bromelain) have on raw turkey meat and their capacity to hydrolyze protein complexes, present in the meat and its connective tissue.

Materials and Methods

Materials: Meat – turkey drumsticks (*Meleagris gallopavo*); Enzyme solutions – papain (Merck), bromelain (Merck).

Methods

Enzymatic processing of turkey meat samples – The meat samples are treated with bromelain or papain with alternating enzyme concentration and duration of the process.

Enzyme solutions - Both enzyme solutions are with the following caseinolytic activity – I (50U/ml), II (100U/ml), III (200U/ml). The enzymes are dissolved in a solvent containing 0,9% NaCl, sodium hydrogen carbonate and citric acid. The active acidity of the enzyme solutions is pH 6,30.

Measuring the Water Retention Capacities (MRC) - Meat samples of 3-5 g are wiped with filter paper to remove surface water and to weigh accurately in milligrams. This value is noted as raw met weight (starting weight). The samples are then treated with bromelain and papain solutions at 4°C for 24, 48 and 72 hrs. Then, the surface water is removed with filter paper. Alongside the samples, controls are assigned every full hour of treatment, in which the meat is placed inside enzyme-free marinate. The processed meat is weighted and is assigned value after enzyme treatment (final weight). A water retention percentage is determined.

Enzymatic activity - The caseinolytic activity of the proteases papain and bromelain is measured by the substrate casein in a 50mM Tris/ HCl boofer at pH 8.0 with 1mM $CaCl_2$, in accordance with *Chen's, Zhang et al. 2003's* method. One unit of enzyme activity is defined as the amount of enzyme needed to release 1 μg tyrosine from caseine for 1 minute.

Quantity assessment of free amino acids – ninhydrin test

The concentration of free amino acids in the dissolved fractions after enzyme hydrolysis is assessed by a ninhydrin test. *(Murariu et al., 2003*)

Collagen and elastin hydrolysis experiment in the already utilized enzyme solutions - 2.0 g connective tissue samples from the turkey drumsticks are inundated with 40 ml of the respective enzyme solution and incubated at room temperature for 24-96 hours. The experimental samples are then removed from the solution, leached with distilled water and lyophilized. Lastly, they undergo electrophoretic analysis.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

SDS–PAGE was preformed with the method outlined by *Laemmli (1970).*

Polyacrylamide gel - 6% stacking and 10 % separating gel:

Electrolyte buffer: Tris – glycine, pH 8,5 with 0,1 % SDS;

Statistical analysis - All data are presented as means ±SD (standard deviation) for at least three replications for each prepared sample. Statistical analysis was performed using two-sample t-test. The results are considered to be significant when $P<0,05$. All statistical analyses were performed using Excel 2013.

Results and Discussion

Turkey drumstick meat undergoes enzyme catalysis. The main objective is to study the effect the enzymes bromelain and papain exert on raw meat samples. In the conducted experiments 3 different enzyme solutions were used (50 U/ml, 100 U/ml and 200 U/ml). The experiments varied in their duration – 24h, 48h, 72h. Water retention capacity changes in the meat samples are recorded. The diagrams below indicate the varied rates in water retention capacity.

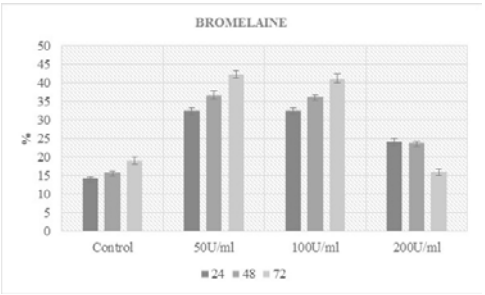

Figure 1. Water retention capacity after bromelain processing (±SD)

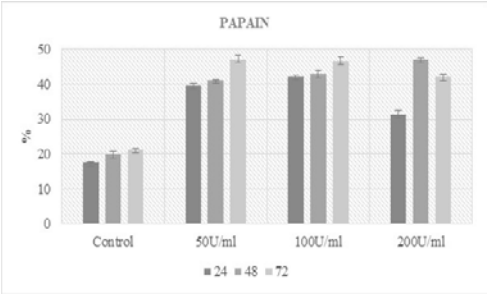

Figure 2. Water retention capacity after papain processing (±SD)

The obtained results establish that both parameters (enzyme concentration and processing duration) affect water retention capacity. Both bromelain and papain variants indicate higher water retention rate.

Bromelain and papain are vegetable cysteine endopeptidases. The improved meat tenderization with vegetable cysteine proteases is due to the higher breakdown of myofibril proteins and the disruption of the muscular fibril structure in the experimental samples compared to the control ones *(Jorgova et al.,1989).*

Due to partial enzyme hydrolysis of the meat proteins a higher affinity for water molecules is established. The augmented hydrophilicity is determined by the many hydrophilic groups (hydroxilic – OH, carboxilic –COO- amino – NH-3, thiolic – SH, amide – $CONH_2$), which are released at the surface of the protein molecules. Consequentially, in the processing with solutions of 50 U/ml and 100 U/ml caseinolytic activity, the water retention rate is higher. In hydrolysis with 200 U/ml, the more time is elapsed during treatment, the lower the water retention rate is, observed mostly in the use of bromelain. This is caused by the higher rate of hydrolysis of the meat proteins, respectively by the gelatinization of the samples and the release of terminal peptides and amino acids.

In order to assess the rate of full hydrolysis the enzymes induce in the solutions a test was conducted to determine the quantity of free amino acids in the reactive liquid. The analysis was done only for the samples with the longest duration in the enzyme solution (72 hrs). The obtained results are indicated below in table 1.

Statistically significant difference is found in the amino acid concentration between the control and experimental variants.

The highest concentration is noted in the reactive liquid containing 200U/ml caseinolytic activity of the respective enzyme. These results correlate with the water retention rate values. The higher the concentration of the enzyme is, the higher the chance of complete hydrolysis to occur in the meat proteins, deteriorating the appearance and taste of the meat.

Table 1. Free amino acid content in the reactive liquid after enzyme hydrolysis (±SD)

Variants	Concentration mg/ml		Significance[1]	Concentration mg/ml		Significance[1]
	Bromelain	Control		Papain	Control	
I	1,262±0,017	0,775± 0,082	**	1,218±0,172	0,728±0,116	*
II	1,210±0,028	0,867±0,004	***	1,335±0,097	0,856±0,088	*
III	1,490±0,021	0,772±0,036	***	1,577±0,576	0,856±0,045	*

[1] Significantly different from the control group at: *p < 0,05; ***p < 0,01; ***p < 0,001.

In the following visual materials (fig. 3 and 4) it is clear that the meat samples processed with 50 and 100 U/ml bromelain and papain for 24 hrs retain their colour and fresh look. On the outside the structure of the experimental variants is similar to the control ones and the muscle fibers are intact. The 48hr samples and, exceedingly most of all, the 72hr samples show muscle fiber deformation and breakdown, the colour fades and the surface turns mucous. The dissociation of the muscle fibers at such high rate is to be avoided since the outward appearance of the product is of major importance to the consumers.

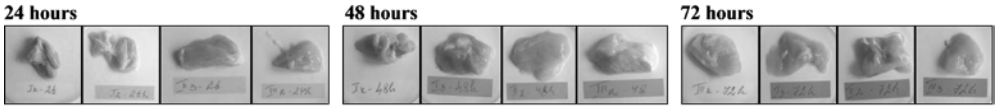

Figure 3. Control and experimental variants processed with bromelain

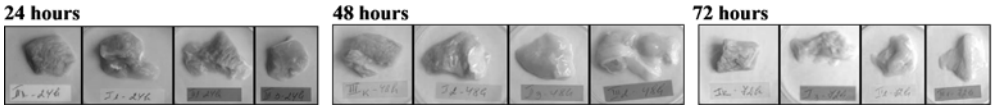

Figure 4. Control and experimental variants processed with papain

Turkey ligaments are mostly composed of collagen type I joined in firm fibers, which give the meat its toughness. With tenderization we aim for partial breakdown of this type of tissue and preservation of the muscle fibers. Treatment with proteolytic enzymes causes disorganization and disintegration of the collagen's structural elements by loosening its intermolecule bonds.

Electrophoresis in polyacrylamide gel SDS-PAGE is conducted with lyophilized samples of turkey ligaments after proteolytic enzyme treatment for 24 and 96 h. Figure 5 shows a photo of the conducted electrophoretic analysis on lyophilized samples of turkey ligaments post enzyme treatment with bromelain and papain.

The basic building unit of collagen – the tropocollagen molecule is composed of a triple coil with 3 polypeptide chains (two $\alpha 1$ and one $\alpha 2$). The control sample shows the typical visual representation of collagen. Two high-molecule mass fractions are observed. They most likely resemble the tropocollagen molecule in collagen type 1 (dimers and trimers).

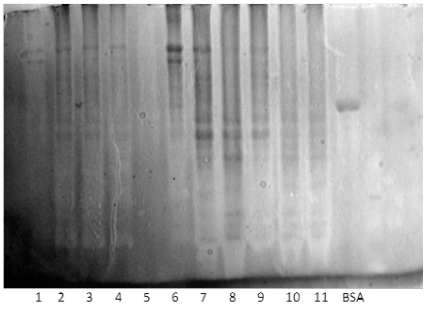

Figure 5. SDS-PAGE lyophilized turkey ligament samples post proteolytic enzyme treatment (bromelain or papain) for 24 or 96 h: 11 processing variants (1- *Control 24 h;* ***2*** *– variant I bromelain 24 h;* ***3*** *- variant II bromelain 24 h;* ***4*** *- variant III bromelain 24 h;* ***5*** *- Control 96 h;* ***6*** *- variant I bromelain 96 h;* ***7*** *- variant II bromelain 96 h;* ***8*** *- variant III bromelain 96 h;* ***9-*** *variant I papain 96 h;* ***10*** *- variant II papain 96 h;* ***11-*** *variant III papain 96 h* ***), BSA – standard – beef serum albumin.***

The rate of hydrolysis is contingent on the following factors – enzyme type, volume of enzyme concentration in the solution and treatment duration. Fractions with lower molecule mass are observed in the experimental variants. According to *Minh et al. (2012),* higher concentrations of the papain and bromelain preparations were able to hydrolyse the meat connective tissue proteins in a non-specific manner and generated a SDS–PAGE time course protein fragment profile. The samples treated with bromelain are noted to possess a smaller amount of fractions and a

lower breakdown rate, whereas the papain samples, particularly the high-concentrated ones, undergo complete breakdown of the connective tissue (multiple fractions with high Rf-value). Such intense hydrolysis leads to protein loss and deterioration of the organoleptic qualities of the meat.

Conclusion

Treatment with proteolytic enzymes results in disorganization and disintegration of the collagen structural elements, which in turn loosens and disrupts the intermolecule bonds. The higher the enzyme concentration and the higher the duration of processing are, the higher the rate of hydrolysis is.

The experimental variants processed with 50 and 100 U/ml bromelain and papain for the duration of 24hrs augment their water retention capacity by 20-25% with preserved colour and fresh look.

Enzyme concentration higher than 200 U/ml results in unwanted intense hydrolysis of the meat proteins and deteriorates the overall appearance and the gustatory qualities of the meat.

The optimal variant for hydrolysis with minimal protein loss and optimal preservation of the orgonoleptic qualities of the product is recorded in the processing of the raw turkey samples in bromelain and papain solutions with 50U/ml caseinolytic activity up to 24 hours.

Uticaj biljnih proteaza bromelaina i papaina na mekoću ćurećeg mesa

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Rezime

Glavni predmet proučavanja je efekat biljnih proteaza bromelaina i papaina na mekoću ćurećeg mesa. Eksperimenti su izvedeni sa uzorcima sirovog mesa u 3 različita nivoa koncentracije rastvora enzima (50U/ml 100U/ml i 200 U/ml) i u 3 različita vremenska perioda (trajanja) tretmana (24 h, 48 h, 72h).

Povećanje koncentracije enzima i trajanja tretmana dovodi do većeg stepena hidrolize proteina u ćurećem mesu. Uspostavljeni su optimalni uslovi za hidrolizu sa minimalnim gubitkom proteina i najvišim zadržavanjem organoleptičkih osobina uzoraka mesa.

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