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Application of kiwifruit protease enzyme for tenderization of spent hen chicken

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Abstract
Kiwifruit enzyme is a plant protease enzyme and protease enzyme has multifunctional role in food industry such as in dairy as milk coagulant and in meat industry for tenderization. Meat tenderness is one of the most important eating quality parameter. The use of spent hen chicken has been limited in scope because of its unacceptable toughness. Henceforth the present study was designed to assess the effect of kiwifruit protease enzyme on tenderization of spent hen chicken. For this, first, slices of 2x2 cm^2 thickness were made. Kiwifruit enzyme was extracted, partially purified, characterized and then optimized for tenderization. The optimized concentration of enzyme was injected in the perpendicular of muscle fibers of spent hen chicken. Tenderness was measured with texture analyzer, physicochemical and sensory qualities were also assessed. The injection of 10 per cent kiwifruit enzyme improved tenderness and the panelist rating for juiciness, tenderness, texture and overall acceptability.

Keywords: Kiwifruit enzyme, tenderization, partial purification, characterization, spent hen chicken

Introduction

Meat tenderness is one of the most important eating quality parameters (Gonzalez *et al.*, 2001; Nowak, 2011) [7, 17]. The use of spent layer has been limited in scope and it has been a problem to the food industry because of its unacceptable toughness. The toughness associated with spent layer's meat is primarly due to the increased cross linking in the connective tissue of older animals (Bailey and Light, 1989) [2]. Meat tenderness is considered as the most important palatability attribute of meat (Chuaynukool *et al*., 2007) [4] which determines whether consumers are repeat buyers (Koohmaraie *et al.*, 1990) [12]. Therefore, it should be ensured that meat reaches an optimum level of tenderness before consumption. There are a number of methods to improve meat tenderness and the overall quality of muscle foods. Of these methods, ageing of the meat (e.g. animal carcasses stored at chill temperatures for 10-14 days) has long been recognized and employed by the meat industry (Lawrie, 1998) [13]. However, the large storage space needed, and the energy consumption and labour costs make the meat products less competitive in the market place (Farouk *et al.*, 1992) [6]. Therefore, it is crucial to develop safe tenderization methods which can improve meat tenderness and consistency more efficiently and economically for the meat industry. This in turn will also benefit the meat end-users in the market place by having more tender meat. Thus alternative methods for meat tenderization are still needed. Treatment by exogenous proteases is one of the most progressive methods used for meat tenderization. Plant proteases are superior to bacterially derived enzymes mainly because of safety problems, such as pathogenicity, or other disadvantageous effects with the latter (Chen *et al.*, 2006) [3]. These enzymes can digest muscle protein when they are mixed with meat. They also can hydrolyse the proteins of collagen and elastin, which lessens the toughness of meat. However, the proper quantity of enzymes needs to be considered because an excessive amount would result in meat decomposition. There is greater extent of research over the use of plant proteases such as ficin, bromelain and papin, but little reporting on the use of kiwifruit enzyme. Therefore, the present study focused on the application of kiwifruit protease enzyme for the tenderization of spent hen chicken.

''Kiwifruit" is potentially an alternative source for proteolytic enzymes. The fruit is very popular in human diet due to its pleasant taste and high content of vitamin C, minerals (potassium, phosphorus, iron) and low calorific value. Kiwifruits are good sources of folate, potassium and contain large amounts of vitamin E in the seeds. However, there is little information describing whether or not the proteases contained in kiwifruits have an impact on meat tenderization. The objective of this investigation was to study the effect of kiwifruit protease enzyme on spent hen meat tenderization.

Materials and methods

Procurement of raw material

The raw material i.e. kiwifruit (*Actinidia deliciosa)* was procured from Kiwifruit Orchard, Department of Fruit Science, YSP University of Horticulture and Forestry, Nauni-Solan (India), while spent hen chicken from Modern Abattoir, Tatta Pani, Shimla H.P. (India). However other materials used for tenderizing spent hen chicken viz. salt, soya sauce, garlic-ginger paste, turmeric powder and red chilli powder etc. were obtained from the local market of Nauni-Solan, Himachal Pradesh (India).

Extraction, partial purification and characterization of kiwifruit enzyme

Protein concentration and extraction of crude enzyme from kiwifruit was determined at different stages of maturity by using procedure followed by Lawrie (1998) [13] and Thimmaiah (2006) [20]. The procedure followed by Sadasivam and Manickam (1998) was employed for partial purification of enzyme. The crude extract of kiwifruit was precipitated by ammonium sulphate using different concentrations (0-90 per cent). The characterization of the enzyme include the effects of pH, temperature and time of incubation on enzyme activity. The procedure of Thimmaiah (2006) [20] was employed for assessment of enzyme assay.

Optimization of enzyme concentration

Kiwifruit enzyme concentration was optimized on the basis of texture and sensory qualities. For this the enzyme concentration ranges from 0 to 15 per cent was taken. Juice was injected in the perpendicular of muscle fibers of 2x2 cm^2 pieces of spent hen chicken.

For texture analysis, the modified method of (Li, 2006) [15] was used. The textural properties of raw breast piece of spent hen chicken sample was measured by using Texture Analyzer, TAXT2i (Stable 70 Microsystems, UK) using P/75 cylindrical probe. Texture parameters included firmness (negative area under the curve). Force calibration of the instrument was done prior to start of the experiment to minimize measurement error. The instrument was operated at test mode= compression, pre-test speed = 1.00 mm/s, test speed = 2.00 mm/s, post test speed = 10 mm/s, strain rate = 40%, trigger force = 5 gm, force and data acquisition rate of 100 pps.

Sensory analysis was done after seasoning with spices and then grilling in microwave at 200^0C for 20 min. For sensory analysis the products were served in cleaned white plate to panelist of all age groups of both sexes at room temperature (25^0C)and then analysis was done by using Hedonic scale where 1 = Dislike extremely and 9 = Like extremely.

Effect of tenderization on proximate composition

Proximate composition of raw as well as tenderized spent hen chicken was evaluated. Moisture was determined by oven method, protein was determined by Kjeldahl method, fat was analyzed by the Soxtec apparatus method and ash was determined with a furnace 600 ^{o}C (AOAC, 1984) [1].

Statistical analysis (Cochran and Cox, 1967) [5]

The data pertaining to the sensory evaluation of spent hen chicken were analyzed according to Randomized Block Design (RBD), while the data on chemical characteristics of different products were analyzed statistically by following Completely Randomized Design (CRD).

Results and discussion

The aim of the present study was to assess the effect of kiwifruit enzyme on tenderization of spent hen chicken. In this study, first kiwifruit enzyme was extracted at various stages of fruit maturity followed by their partial purification and characterization. Partially purified kiwifruit enzyme was employed for tenderization of spent hen chicken. Finally the quality attributes of tenderized spent hen chicken were compared with raw chicken.

Extraction of kiwifruit enzyme

The data in Table 1 represents the protein content and enzyme activity of kiwifruit at various stages of fruit maturity. The maximum protein content and enzyme activity (0.42±0.20 mg/gm and 200.32±0.20 µg/gm) was observed in immature stage of fruit maturity followed by mature (0.28±0.10 mg/gm and 131.50±0.20 µg/gm) and ripened (0.25±0.20 mg/gm and 130.25±0.20 µg/gm) stage respectively. This was in agreement with Whitaker (1958) [21] observations that the enzyme activity of the fruits (*Ficus carica*) was highest when they were unripened green.

Table 1: Protein content and enzyme activity of kiwifruit (Mean±SE)

Stage	Protein (mg/gm)	Enzyme activity (µg/gm)
Immature (TSS<6.5 ^{0}B)	0.42 ± 0.20	200.32 ± 0.20
Mature (TSS=6.5 ^{0}B)	0.28 ± 0.10	131.50 ± 0.20
Ripened (TSS<14 ^{0}B)	0.25 ± 0.20	130.25 ± 0.20

Partial purification of kiwifruit enzyme

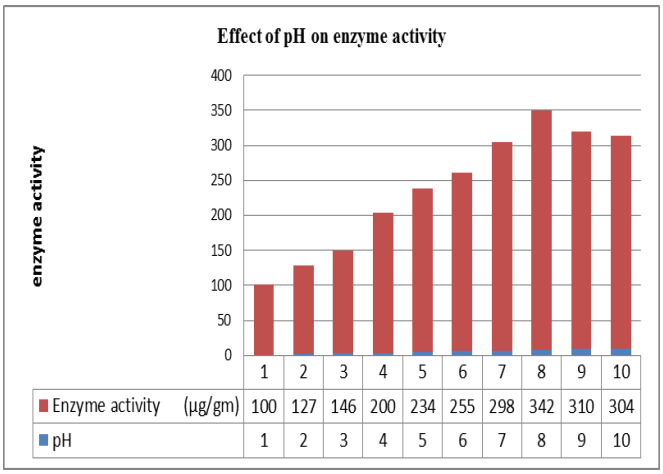
Highest enzyme activity, yield and purification fold was found with 40-60 per cent concentration of ammonium sulphate 86 per cent protease enzyme yield of 1.65 purification fold and 0.86 /mg of protein specific activity (Table 2) whereas Hullikere *et al.* (2014) [8] reported protease enzyme with similar yield at 0.96 purification fold and 1.23 specific activities in latex of papaya (*Carica papaya)*.

Table 2: Purification profile of kiwifruit enzyme

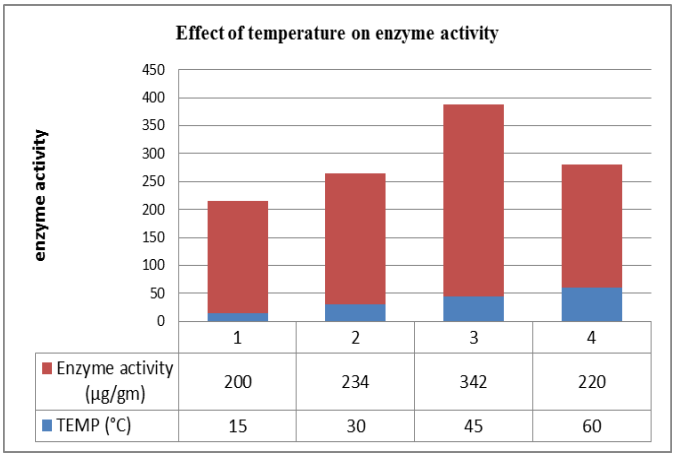
Observations					
Purification step	Protein (mg/gm)	Enzyme activity (µg/gm)	Specific activity (/mg protein)	Purification fold	% Yield
Crude enzyme	0.42	220.00	0.52	1	100
Ammonium sulphate precipitation (40-60%)	0.22	190.00	0.86	1.65	86

Characterization of kiwifruit enzyme

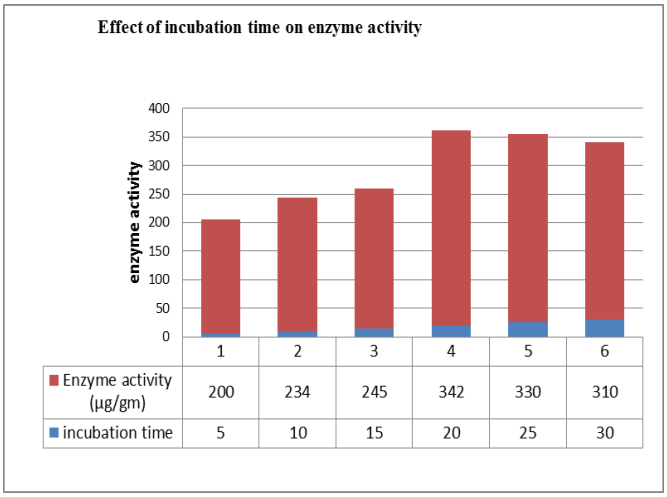
Graphs shown that with increase in pH, temperature and time of incubation up to a certain limit (pH 8.0, 20 min and 45 ^{0}C) significant increase in enzyme activity was observed but after this limit there was considerable decrease in the enzyme activity was noticed whereas Otani *et al*. (1991) [18] characterized the proteolytic enzyme of fig and found optimum temperature and pH 65 ^{0}C and 7.5 respectively and further reported that ficin retained more than 90 per cent of its original activity after a period of 1 hr incubation at 55 ^{0}C.



Graph 1: Effect of pH on enzyme activity



Graph 2: Effect of temperature on enzyme activity



Graph 3: Effect of incubation time on enzyme activity

Optimization of Enzyme Concentration

The concentration of kiwifruit enzyme for tenderization of spent hen chicken was optimized on the basis of texture profile of spent hen chicken and sensory quality of seasoned, grilled chicken. 1 indicates the texture profile of spent hen chicken treated with different concentrations of kiwifruit enzyme. The decrease in firmness value was reported in samples tenderized with kiwifruit enzyme.

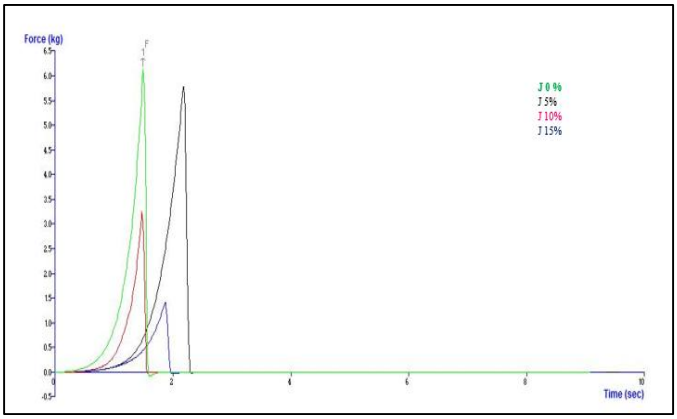
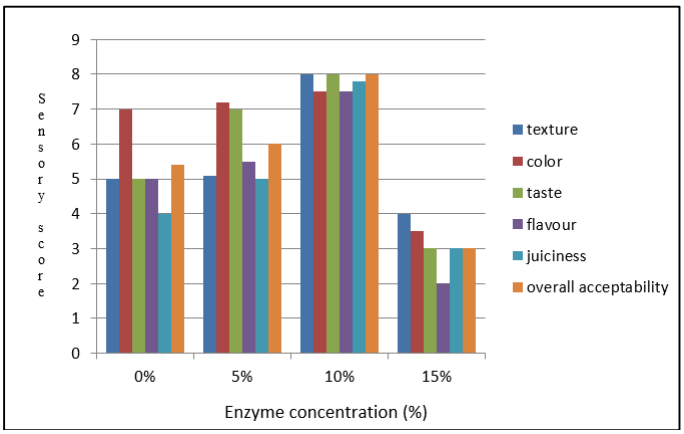


Fig 1: Texture profile of spent hen chicken

Graph4 indicated the sensory characteristics of seasoned and grilled spent hen chicken tenderized with different concentrations of kiwifruit enzyme. On the basis of sensory quality the concentration of 10 per cent kiwifruit enzyme was found optimum for tenderization of on 9- point hedonic scale. The spent hen chicken tenderized with higher concentration was rejected by the panelist because of excessive hydrolysis of muscle proteins. Henceforth 10 per cent of kiwifruit enzyme was found optimum due to moderate tenderization effect on spent hen chicken. Similar results were observed by Ilayabharathi *et al.* (2012). However, Naveena and Mendiratta (2001) [16] reported 3 per cent ginger juice, Rawdkuen *et al.* (2013)[19] observed 0.50 per cent concentration of crude calotropis extract and the findings of Ketnawa *et al.* (2010)[11] concluded 20 percent purified bromelain extract which were found better for desirable tenderization of chicken. Lee *et al.* (1986)[14] explained that below optimum level of enzyme concentration (zingibain) there was less tenderization effect on tough meats of spent hen chicken whereas above the optimum level degradation of muscles occurred.



Graph 4: Effect of different enzyme concentration on sensory quality of spent hen chicken

After the optimization of enzyme concentration, the spent hen chicken tenderized with optimized concentration of enzyme and then evaluated for proximate analysis.

Effect of tenderization on proximate composition

The perusal of data in Table 3 showed the proximate composition of spent hen chicken tenderized with kiwifruit enzyme. The moisture content of spent hen chicken was increased from 71.80± 0.20 to 72.00 ± 0.10 per cent. This may be due to improvement in hydrophilic properties by the enzyme treatment.

Similar trend were recorded by Naveena and Mendiratta (2001) [16] in buffalo meat samples as 77.18% moisture in ginger rhizome extract treated buffalo meat sample as compared with 76.51% in an untreated one. However protein content decreases from 22.04 ± 0.11 to 21.80± 0.12. This might be due to proteolytic nature of kiwifruit enzyme. Similar findings were reported by Ionescu *et al.* (2008) [10] that the decrease in protein content of beef treated with papain and bromelain during storage from 17.81 to 14.67 per cent due to the proteolytic nature of papain and bromelain which hydrolyzed the myofibril and connective tissues of beef during tenderization. Whereas there was no significant difference was seen in case of crude fat and ash (%).

Table 3: Effect of tenderization on proximate composition of spent hen chicken

Sr No.	Attributes	Raw chicken (mean ± SE)	Chicken tenderized with kiwifruit enzyme (mean ± SE)
1.	Moisture (%)	71.80 ± 0.20	72.00 ± 0.10
2.	Crude protein (%)	22.04 ± 0.11	21.80± 0.12
3.	Crude fat (%)	2.36 ± 0.20	2.35± 0.15
4.	Ash (%)	1.46 ± 0.84	1.45± 0.13

Conclusion

Henceforth, it is concluded that kiwifruit enzyme can be a better or effective plant tenderizer in addition to traditional plant tenderizer such as papain, ficin and bromelain as it also imparts characteristics flavor and aroma to the grilled spent hen chicken.

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