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Effects of natural plant tenderizers on proteolysis and texture of dry sausages produced with wild boar meat addition

J. Żochowska-Kujawska[1]*, K. Lachowicz[1], M. Sobczak[1], A. Nędzarek[2] and A. Tórz[2]

[1]Department of Meat Science, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, Kazimierza Królewicza St 4, 71-550 Szczecin, Poland.
[2]Department of Water Sozology, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, Kazimierza Królewicza St 4, 71-550 Szczecin, Poland.

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This study was conducted to develop a method for improving tenderness and overall qualities of tough wild boar meat used to dry sausage production with direct addition of raw pineapple (*Ananas comosus*), mango (*Mangifera indica*), kiwifruit - fuzzy kiwi (*Actinidia deliciosa*), or ginger (*Zingiber officinale roscoe* - ginger rhizome) juices contained a plant proteolytic enzyme. Dry-sausages were subjected to various chemical, mechanical and sensory evaluations. An increase in proteolysis was observed in all enzyme-treated samples compared to the control and as a consequence an improvement in juiciness, tenderness and overall acceptability scores were observed. Ginger or kiwifruit juice-treated sausages received better scores for texture, flavor, and overall acceptability. From these results, it is shown that those enzymes as a raw plant juices could be used as tenderizers in dry sausage production.

Key words: Dry sausages, wild boar meat, plant enzymes, proteolysis, texture, sensory properties.

INTRODUCTION

The technology of dry-cured sausages allows many variations as long as the basic concepts (reduction of pH and water activity) are kept in mind (Roca and Incze, 1990). Consequently, these products vary greatly across all of the producer countries, although their manufacture always involves a combination of fermentation and dehydration processes. During the ripening of fermented sausages, the proteins and lipids experience great changes. Proteolysis influences both texture and flavor development due to the formation of several low-molecular-mass compounds, including peptides, amino acids, aldehydes, organic acids, and amines. All of them are important flavor compounds or precursors of flavor compounds (Demeyer et al., 1995; Fadda et al., 2001). Lipolysis plays an essential role in the development of dry-sausage flavor. Lipids are hydrolyzed by enzymes, generating free fatty acids, which are substrates for the oxidative changes that are responsible for flavor compounds (Samelis et al., 1993; Stahnke, 1995; Verplaetse, 1994).

Fermented sausages are usually prepared from seasoned raw meat stuffed in casings and allowed to ferment and mature (Moretti et al., 2004; Živković et al. 2012). The meat of different species of adult, well-fed animals is preferred in raw sausage production: Pork, beef, poultry but also goat, lamb and venison, or combinations thereof. The use of game in meat technology is worth emphasizing, especially since in recent

*Corresponding author. E-mail: Joanna.Zochowska-Kujawska@zut.edu.pl. Tel.:+ 48 091 449 6582. Fax: +48 091 4233448.

years consumer interest in game meat as an alternative for pork and beef is now increasing. The reason for the increase in consumption of venison is its high nutritional value. Thus cured, fermented, and dried products from different game species have appeared on the market (Paleari et al., 2000; Soriano et al., 2006). Despite the increased popularity of game meat, there is a lack of research comparing the nutritional and sensory qualities of meat and meat products, especially fermented sausages, from different game species (Vioque et al., 2003; Soriano et al., 2006; Van Schalkwyk et al., 2011).

It is well known that venison's lack of rich fat, a little thicker connective tissue and/or higher amount of red fibres, compared to for example pork meat can cause it to become tough (Lachowicz et al., 2004; Żochowska-Kujawska et al., 2009, 2010). In the last years different tenderization techniques of beef and sheep meat as well as venison were applied. These techniques include mechanical tenderization, elevated-temperature storage, calcium chloride injection, electrical stimulation, muscle stretching, shock-wave pressure, and enzymatic tenderization (Koohmaraie, 1992; Cheftel and Culioli, 1997; Żochowska-Kujawska et al., 2012).

The enzymes of vegetable origin, such as papain from papaya, bromelin obtained from raw pineapple, ficin derived from figs and zingabaine from ginger as well as bacterial collagenase (Foegeding and Larick, 1986; Stanton and Light, 1987; Dransfield and Etherington, 1981; Naveena et al., 2004; Weiss et al., 2010) were often used for postmortem meat tenderization. These enzymes have regulatory approval (U.S.D.A) for meat tenderization and have been used in various forms as marinades, injection in brine, pre-slaughter injection into the animal's vascular system, and incorporation into various spices as meat tenderizers (Dransfield and Etherington, 1981). These exogenous enzymes have very broad specificities and, therefore, generally indiscriminately break down the major muscle proteins (connective tissue/collagen and myofibrillar proteins), sometimes resulting in over- tenderization and a mushy-textured product (Miller et al., 1989).

The proteolytic activity of these enzymes is still a matter of discussion. The same enzyme can show differing results in activity levels and optimal pH and temperature ranges depending upon the substrate (Sullivan and Calkins, 2010). For example Sullivan et al. (2010) studied the tenderization effects of seven enzyme randomized treatments (papain, ficin, bromelain, homogenized fresh ginger, *Bacillus subtilis* protease, and two *Aspergillus oryzae* proteases) in *Triceps brachii* and *Supraspinatus* and they found that except for ginger treatment, all samples treated with enzymes showed improvement in both sensory and instrumental tenderness analysis, however, papain was the enzyme that caused the greatest tenderness in meat. Whereas Ionescu et al. (2008) found a better tenderization for samples treated with bromelin compared to those treated with papain. Kiwifruit has also been studied as a source of actinidin and Han et al. (2009) investigated the ability of prerigor infusion of kiwifruit juice to improve the tenderness of lamb. It was confirmed by Wada et al. (2002) who found that a purified form of actinidain increased the solubility of collagen and thus making it attractive for improving the tenderness of semimembranous muscle.

Addition of enzymes such as lipases (Fernandez and Rodriguez, 1991) and aspartyl-proteinases (Díaz et al., 1993) to dry fermented sausages to enhance flavor has been already considered but with conflicting results. However according to the study of Melendo et al. (1996) plant enzymes such as bromelain have a significant effects on texture of dry sausages, especially with shorten drying period. Despite the increased popularity of natural techniques of improved game meat (Żochowska-Kujawska et al., 2012), there is a lack of research establishing exactly, which enzyme is more suitable to texture and sensory properties of game dry sausages.

The study presented here aimed at evaluating the effects of selected plant enzymes used in our experiment as raw plant juices on the texture and sensory properties of dry sausages produced using traditional process with wild boar meat addition.

MATERIALS AND METHODS

Raw materials

Investigations have been done on dry-sausages produced from pork and wild boar lean meat. Pork was obtain from commercial pigs (about 6 months of age) whereas venison was from a total of 9 male of wild boars hunted during spring in the forest of the Western Pomeranian district in Poland, kept at 4°C for 48 h after shot. The carcass weights of the wild boars were 40±3 kg while their ages were about 3 years. Carcasses were used to obtain hams of pH 5.6 - 5.8. Each ham was deboned, and cleaned of external fat. The lean meat from trimming of biceps femoris semimembranusus and *Quadriceps femoris* muscles was used for sausage production.

Dry-sausages were prepared using a traditional production process as follows: 40% lean pork, 50% lean wild boar meat, 10% pork belly, 3% curing salt, 1% sugar, and spices (red pepper, garlic). Microflora of those traditionally produced fermented sausages originated from the raw material or from the environment in which the sausages were made.

Lean meat and pork belly were minced individually using a 14 mm plate. Sausage mixture was divided into five batches and raw pineapple, mango, kiwifruit, and ginger juices contained enzymes such as bromelain, magneferin, actinidin, and zingabain, respectively, were added to four of them at 5%. Each raw juice was obtained from fresh fruits, purchased at the local market, after peeled, diced and homogenized for 5 min and then centrifuged at 1000 × g for 5 min. The obtained supernatant was used for further study.

The last batch produced without any plant enzymes addition was the control. All batches were stuffed into 45 mm diameter natural casings (15 sausages were made from each batch) and the sausages were held for 48 h at 22-26°C and 80-85% relative humidity (RH) to allow fermentation. Afterwards, sausages were transferred to a drying chamber at 14-18°C and 70-80% RH and ripened for 14 days. Then sausages were divided into two parts and to counteract a substantial reduction of the sausage weight due to the evaporation, one of them was stuffed in additional fibrous

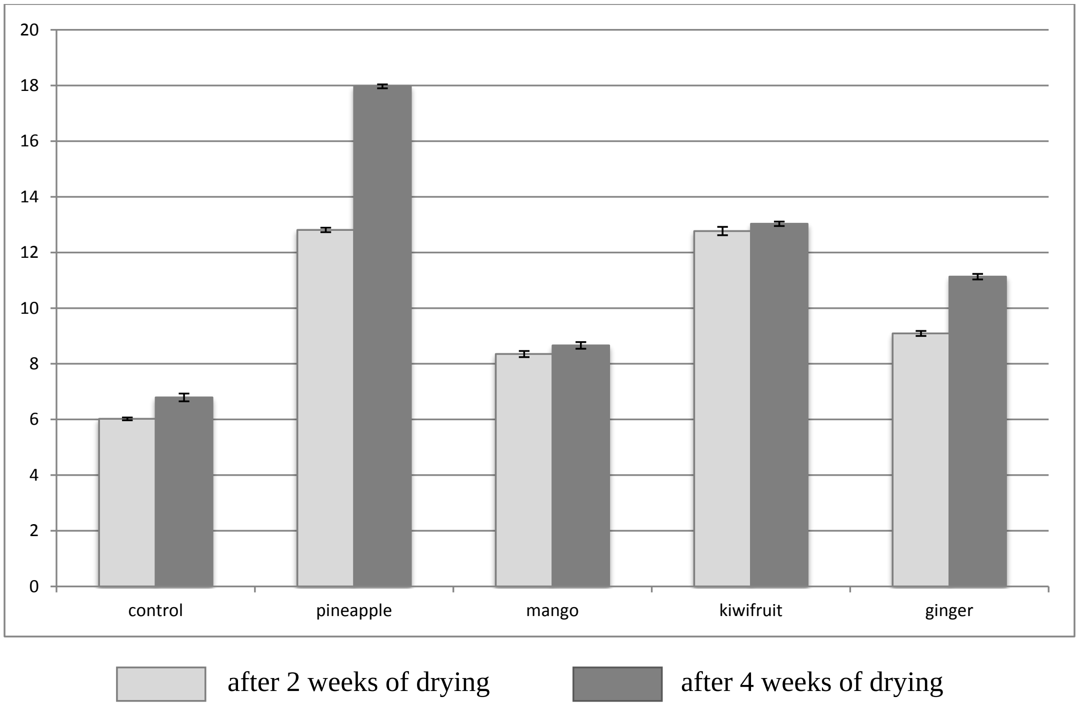

Figure 1. Proteolysis index of dry cured sausages produced with natural plant enzymes addition after two and four weeks of drying.

collagen casings and subjected ripened for another 14 days at 12-14°C and 70-75% RH. Sausages were evaluated at 14 day of ripening (first batch) and at the end of ripening process (28 days, second batch).

Methods

Proteolysis index

The sausages were ground separately in a meat grinder after removing the casing. Proteolysis index were determined as total nitrogen in ca. 2 g of minced sausage by using the Kjeldahl method and calculated as N x 6.25 following the AOAC (2012) method and expressed as percent ratio between the nitrogen soluble in 5% trichloroacetic acid and the total nitrogen (Careri et al., 1993). The proteolysis index was measured 5 times on each batch.

Texture measurement

Sausage texture were measured following the texture profile analysis (TPA) procedures (Bourne, 1982), with an Instron 1140. The test involved driving a 0.61 cm diameter shaft twice, into a sample down to 80% of their original height (16 mm), using a crosshead speed of 50 mm min^{-1} and a load cell of 50 N. The force-deformation curve obtained during the TPA test served to calculate meat hardness, cohesiveness, springiness, and chewiness (Bourne, 1982). The TPA test was repeated 7-9 times on each sausages and three sausages were examined in each batch.

Sensory parameters evaluation

The sensory evaluation of the sausage samples was assessed by a trained expert panel of 7 members with, in general, a minimum of four years experience in texture analysis of meat and meat products. The sausage springiness, tenderness, cohesiveness, chewiness, juiciness, palatability, taste: sweet, bitter, sour as well as off-flavor and overall attractiveness were assessed using a 6-points scale as follows: 1 point: The least spring, tender, cohesive and samples assessed as not sweet, bitter, sour, without off-flavor, with the low juiciness, palatability, chewiness and overall attractiveness; 6 points: the most spring, tender, cohesive, juicy, sweet, bitter, sour, with off-flavor and with the highest palatability, chewiness, and overall attractiveness. The data presented in this study was a mean calculated from a set of scores obtained from each member.

Statistical analyses

Statistical analyses of the data involved the calculation of the mean values and standard deviations (SD) for each sample of sausages. The differences in texture, proteolysis index, and sensory properties between the samples were studied using the analysis of covariance. Treatment differences were tested for significance at the 5% level. All the calculations were performed with Statistica® v.7.0 PL software.

RESULTS AND DISCUSSION

Proteolysis

As shown in Figure 1, the addition of plant enzymes regardless of drying time, enhanced proteolysis index (Pi) by about 27-165% compared to the control dry fermented sausages. Of all the sausages tested, the highest values of Pi as an effect of protein degradation were recorded in samples produced with pineapple and kiwifruit juices, lower value of this parameter was typical for ginger addition and the lowest were found in sausages with mango addition. Thus, it can be said that these enzymes can "digest" muscle proteins when they are mixed with meat. As shown in the study of Wada et al. (2002) some fruit proteases affect the structure of myosin and actin filaments. It is widely known that most of the exogenous plant enzymes used to tenderize meat have a good activity in temperature above and close to the environment during the fermentation or drying process. For example, Zhao et al. (2012) reported that almost all of the myofibrillar proteins (MHC and AC) were degraded into fragments with molecular weights lower than 20 kDa when meat was treated with bromelain or papain at 37°C. Thus, a higher proteolysis index recorded in our work can be attributed to dry sausages produced with exogenous enzymes addition compared to the controls. Those enzymes have a strong activity towards all the myofibrillar proteins and in favourable conditions may result in extensive degradation of myofibrillar proteins and meat structure (Zhao et al., 2012).

Another reason for the high proteolysis index could be that some enzymes can also hydrolyze the connective tissue. According to the study of Wada et al. (2002) some plant enzymes can also hydrolyze collagen and elastin, which helps to tenderize meat especially rich in connective tissue like beef or venison meat. Ketnawa et al. (2010) also confirmed that bromelain from pineapple peels can extensively degrade the collagen from beef and giant catfish skin. Also Ketnawa and Rawdkuen (2011) showed that the high TCA-soluble peptides content in bromelain treated samples was due to greater muscle protein hydrolysis. Fragmentation of both myofibrillar proteins and collagen tissue when treated with ammonium hydroxide resulted in the tenderization of buffalo meat (Naveena et al., 2011). The prolongation of enzymatic ageing of dry sausages for 4 weeks, led to the higher changes in proteolysis compared to the samples tested after 2 weeks of drying (Figure 1). For example, due to the 28 days of drying Pi of sausages with pineapple and ginger juices addition had increased about 40.2 and 22.4%, respectively, relative to the sausages dried for 2 weeks. At the same time, corresponding changes in Pi of other sausages were about 2-8%.

Textural properties

Differences in intensity of proteolysis during ripening of dry fermented sausages could be connected with the differences in the textural parameters of these products observed in this study (Figure 2). As suggested in this work, addition of exogenous proteolytic enzymes, by breaking of the protein chains of muscle and collagen fibers and by their structural damage resulted generally in a reduction of muscle hardness, springiness and chewiness compared to dry sausages produced with any enzymes addition.

There were few differences in texture changes among the enzymatic treatments (Figure 2). The sausages with the pineapple or kiwifruit juices were 70-75% or 35-39% less hard than control, respectively, regardless of drying time, while sample with mango juice had the highest numerical value and was tougher than all treatments. As shown in this work, enzymatic activity of enzymes coming from ginger was lower than enzymes from pineapple and kiwi fruit and no significant effect of mango juices on hardness changes was found. Probable reason for small changes in the hardness of the dry sausages produced from the mango juice addition, compared to control, was the fact that in these studies the juice derived directly from the fruit which contains a complex of enzymes as magneferin, catechol oxidase and lactase (Jha et al., 2010) was used, not a proteolytic enzyme in pure form.

The degradation of muscle protein plays a major role in determining the tenderness of meat during post-mortem storage (Koohmaraie et al., 2002). Our results show more pronounced effect of enzymes coming from pineapple and kiwifruit on protein proteolysis and thus texture changes compared with other exogenous proteolytic enzymes. According to the study of Wada et al. (2002), plant thiol proteases have very broad specificities and therefore, they indiscriminately break down the major muscle proteins (connective tissue/collagen and myofibrillar proteins), resulting in over-tenderization to a mushy-textured product.

The level of hydrolysis was more accentuated when longer enzyme action times was used. Regardless of enzymes addition, longer sausage drying was found to induce in general the additional decrease in the hardness, cohesiveness, springiness and chewiness (Figure 2). However changes in textural parameters between sausages after 2 and 4 weeks of drying were dependent on plant enzymes addition. For example, 4 weeks of drying without any enzymes addition induced a 9% and 24% decrease in sausage hardness and chewiness, respectively, and about 8% changes in other textural parameters, compared to sausages after 2 weeks of drying. Whereas the prolongation of enzymatic drying of sausages produced with pineapple, ginger and kiwifruit juices, similar to changes observed in this study in Pi value, led to the higher changes in textural parameters compared to the samples with other exogenous enzymes. For example, due to the 4 weeks of drying, hardness of sausages with those juices addition had decreased about 24, 17 and 13%, relative to the samples dried for 2 weeks. And lower texture changes compared to those observed in control were found in sausages manufactured with mango and vegetables juice addition (by about 5% for hardness and 25% for chewiness).

As shown in this work, enzymatic activity of pineapple

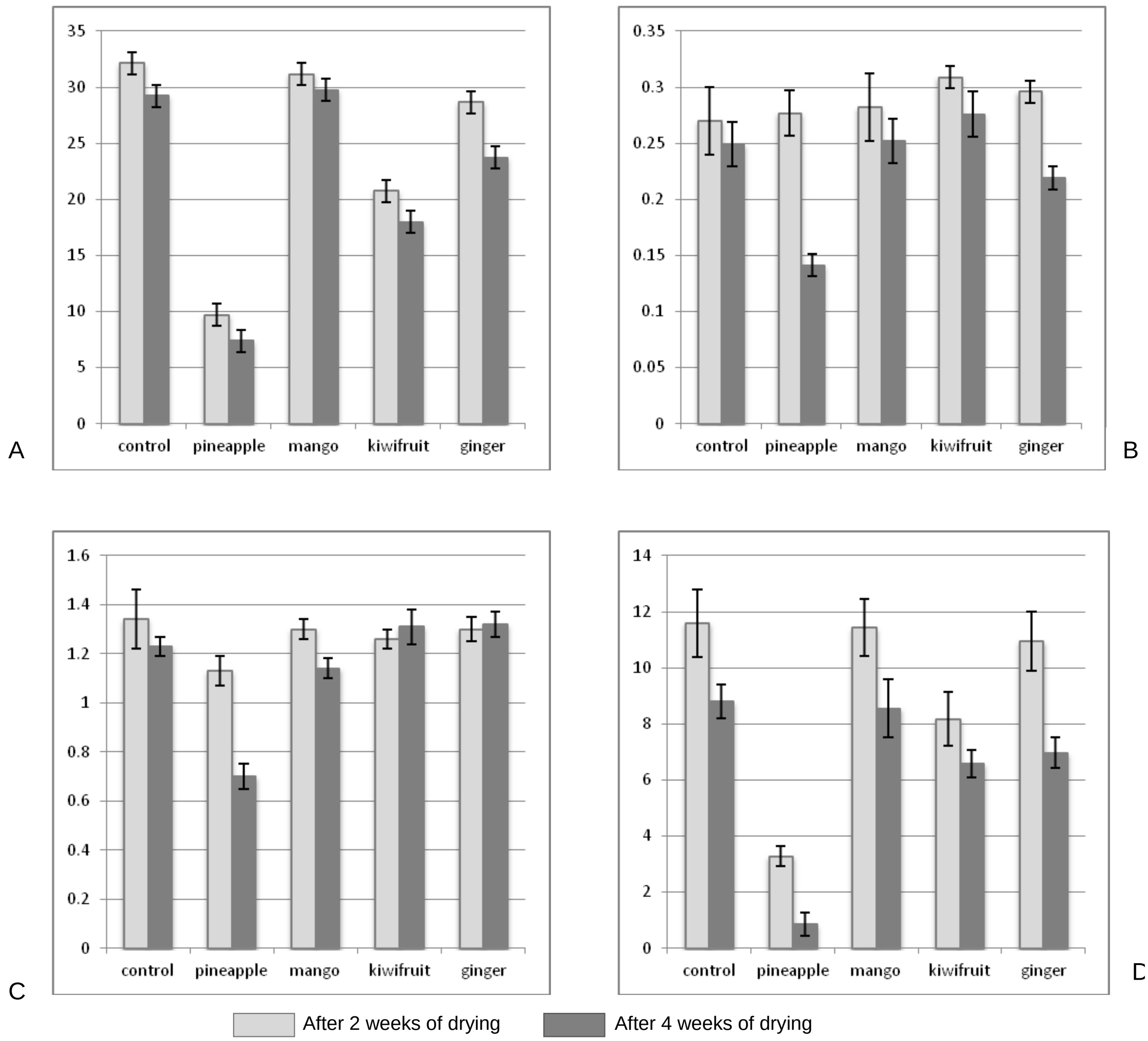

Figure 2. Texture of dry cured sausages produced with natural plant enzymes addition after two and four weeks of drying. A, Hardness (N); B, cohesiveness (-); C, springiness (cm); D, chewiness (Nxcm).

and kiwifruit juices were higher than other fruit enzymes activity. According to the study of Ionescu et al. (2008), the bromelain exhibits a more accentuated hydrolytic action on collagen than on myofibrillar proteins. As can be seen in other research, the activity of these enzymes strongly depends on pH. Kim and Taub (1991) found that pH 5.0 which is slightly similar for environment occurred in fermented sausages, is the optimal pH for bromelain activity. Thus probably, the conditions occurred in our work during dry sausages production were optimal for bromelaine activity and for this reason the highest texture changes were observed when the enzyme from pineapple juice affected on connective tissue from wild boar meat.

Also McKeith et al. (1994) found a significant increase in tenderness when a bromelaine solution was injected into muscle versus dipping or tumbling in brine.

When the effect of kiwi juice addition was considered, it was found that similar results obtained in this study were also achieved by Samejima et al. (1991) who demonstrated that actinidin could degrade the insoluble collagen under unheated conditions, and could also digest elastin into peptide fragments (Wada et al., 2004). They also suggested that kiwifruit tenderizing effects were also partially due to the degradation of the connective tissue in muscle.

In our research the tenderizing effects of ginger juicewas shown but was lower than those occurred when pineapple

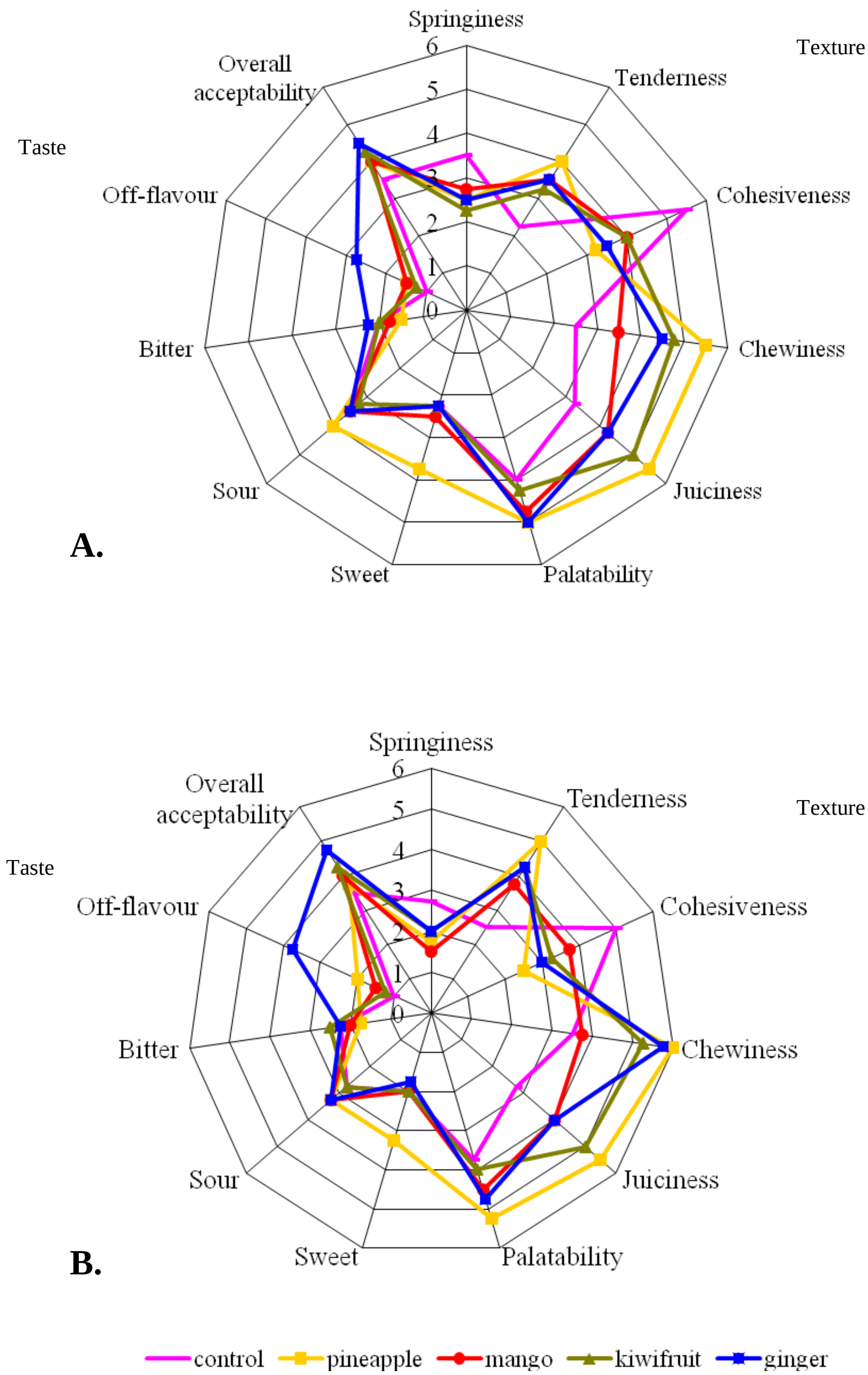

Figure 3. Sensory properties of dry cured sausages produced with natural plant enzymes addition after two (A.) and four (B.) weeks of drying.

and kiwifruit juices were used in dry sausage production. As reported by Naveena et al. (2004), zingabaine could effectively be used for tenderization of tough meat. Also Lee et al. (1986) explained that higher concentration of ginger extract extensively degraded the myofibrils and the degradation appeared to begin at I band of each sarcomere and progressed towards the M line

According to the study of Naveena and Mendiratta (2001), this enzyme has an advantage over other tenderizing agent as a greater proteolytic activity in heated condition, which is desirable. According to the study of Mansour and Khalil (2000) ginger has been shown to have a powerful proteolytic enzyme, which can be used as tenderizing agent for tough meat. Thus, decrease in hardness of sausages produced with pineapple, kiwifruit and also ginger juices observed in this study could be connected with combined proteolysis of two main muscle proteins such as collagen and actomyosin.

Sensory evaluation

Sensory evaluation confirmed that all plant enzymes produced an improvement in tenderness, chewiness juiciness and palatability of dry sausages compared with untreated controls (Figure 3). The pineapple-treated samples received higher scores for those parameters and these sausages were rated as the sweetest, sour and as the least bitter, regardless of the time of drying. Thesensory panel also detected improvements in tenderness and juiciness when the ginger, kiwifruit and mango juices were added. Off-flavours were detected in the ginger-treated samples but those sausages were rated superior and most preferred by the panelists. Also samples produced with pineapple or mango juice addition were characterized by slightly perceptible off-flavours, however, in this case the finished products have been evaluated positively by a panelists. The least perceptible off-flavour was found in kiwifruit-treated sausages, but at the same time they were rated as slightly bitter.

On the other side, the results of our study also showed that dry sausages tenderized with a pineapple juice, especially those after four weeks of drying, despite the high tenderness and juiciness, had the worst general attractiveness which was connected with a slimy texture (Figure 3). The ginger, and kiwifruit juice-treated samples received better scores for overall acceptability compared to others sausages. According to the study of Ionescu et al. (2008) bromelain showed hydrolytic activity on the connective tissue, leading to the better tenderization of the tough meat but sometimes lead to over- tenderization and to a product with a pasty texture (Miller et al., 1995). These results were confirmed by Żochowska-Kujawska et al. (2012), who have found that even very hard wild boar muscles such as biceps femoris and semimembranosus soaked in marinades made from fresh pineapple juice were characterized by the worst sensory properties as a consequence the deepest changes in structure elements. Whereas improvement in flavor, juiciness, tenderness and overall acceptability scores with ginger extract treatment in our experiment is consistent with some earlier reports (Mendiratta et al., 2000; Syed Ziauddin et al., 1995).

Also Lewis and Luh (1988) compared the effect of actinidin on the tenderization of bovine semitendinosus muscles found that this enzyme had a milder protyolytic activity compared with other tested and did not produce off flavors or odors in the meat or excessive surface tenderization.

Conclusions

The results obtained in this experiment indicate the tenderizing effect of pineapple, kiwifruit and ginger, regardless of drying time, even if these enzymes were added directly to the meat during the dry sausages production. In general, there was a significant increase in proteolysis, and a reduction in hardness, chewiness and improvement in sensory quality in all enzyme-treated samples compared to controls. Our results showed more pronounced effect of pineapple and kiwifruit juices on protein proteolysis and thus texture changes compared with other exogenous proteolytic plant enzymes.

In turn, sensory analysis showed that samples treated with ginger and kiwifruit were rated superior and most preferred by the panelists, which can be attributed to the desirable ginger flavor. Pineapple and mango-treated samples scored almost equally, but the first one probably by deepest changes in structure elements resulted in a product with the mashy texture. It follows that, kiwifruit and ginger could be effectively utilized at household or industrial level for tenderization of tough meat such as venison in dry traditional fermented sausages production.

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