

Addendum

PE 1.69 Oxidative environments do not affect shear force tenderness when other tenderness challenges are prominent

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Abstract—Beta agonists used as growth enhancers are known to affect the ageing potential of beef muscle negatively. On the other hand, supplementation of ultra high levels of vitamin D₃ for short periods before slaughter could enhance the ageing potential of beef. High levels of oxygen used in modified atmosphere packaging could act negatively or positively on the aging potential and hence the final tenderness of meat. In this study, ten out of thirty young steers received no beta agonist (C), the remaining twenty steers received a beta agonist (zilpaterol hydrochloride) (Z) the last 30 days on feed. Of these, ten steers also received vitamin D₃ for the last six days before slaughter (7x 10⁶ IU/animal /day) (D). Loin samples were vacuum packed and stored for 14 days when steaks were cut and displayed for seven days under high oxygen conditions (70% O₂, 30%CO₂: MAP). Free thiol (indication of protein oxidation), Warner Bratzler shear force and myofibrillar lengths were measured on loin samples aged for 14 days and 21 days. Free thiol was higher for Z (and D; trend) at 14 days vacuum packed storage, meaning less oxidized. Z showed a higher rate of oxidation under MAP than C and D, while D showed resistance to oxidation. Both Z and D had higher shear values and myofibrillar length than C after 14 and 21 days. MAP did not seem to have a significant effect on tenderness development, while the beta agonist and vitamin D had a major influence on tenderness development over the first 14 and subsequent seven days of aging. Protein oxidation probably play a minor role in tenderness development when other prominent factors influence myofibrillar tenderness and/or when MAP is applied after initial aging under vacuum packed conditions.

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Index Terms - MAP, vitamin D₃, tenderness, zilpaterol

I. INTRODUCTION

The development of meat tenderness is due, in part, to degradation of key muscle proteins [12, 29]. The calpain system is implicated in this process and is composed of calcium-dependent proteases (calpains) and their specific inhibitor, calpastatin [14]. Several factors pre- and post mortem could affect the process of conversion of muscle to meat and subsequent aged or tenderized meat. Beta agonists are known to affect meat tenderness negatively due to an increase in calpastatin activity [15].

Methods to overcome tenderness problems include the supplementation of very high levels of vitamin D₃ over the final days before slaughter [21]. This method is motivated by the suggestion that increased calcium ions stimulated by high vitamin D₃ levels, contributes to meat tenderization by weakening of myofibrillar structures (non-enzymatic; [28] as well as indirect action of calcium ions through activation of u-calpain [14].

Interventions to address other quality characteristics of meat, such as colour shelf life and drip loss, have also proved to interfere with meat tenderness negatively, while other methods may contribute to tenderness development. With modified atmosphere packaging (MAP), high levels of oxygen is intended to extend colour shelf life [20], while supplementation of vitamin D₃ could improve antioxidative capacity of loin muscle [17], thereby extending colour shelf life and support meat and fat quality [17,31]. At the same time increased calcium ion levels could benefit tenderness development as suggested by [17]. In contrast, oxidative environments could decrease tenderization of meat through inactivation of u-calpain followed by decreased amount of myofibrillar proteolyses [24]. Since calpain have an oxidizable cysteine residue at their active site, they require reducing conditions to be active. Thus, oxidizing conditions could negatively influence their activity [10]. In addition,

intermolecular crosslinks and formation of aggregates in muscle proteins under highly oxidative conditions can make proteins less susceptible to enzymatic proteolyses [5].

The objective of this study was to examine the effect of modified atmosphere packaging (MAP) late post mortem in relation to protein oxidation and tenderness of beef steaks.

II. MATERIALS AND METHODS

Thirty young Bonsmara steers (~9 months) were purchased, processed and raised in the research feedlot facilities of the Agricultural Research Council (Irene, Gauteng Province) on a commercial feedlot diet (120 days). The animals were identified and allocated to three treatment groups of 10 animals each so that the average weight and variation for each group was the same. One group (C) receive no beta agonist or vitamin D₃, while the two remaining groups (n=20) were supplemented with the beta agonist, zilpaterol hydrochloride, (Intervet/Schering-Plough Animal Health, South Africa) at 0.15 mg/kg live weight for the last thirty. One group only received zilpaterol (Z), while the other group received zilpaterol and vitamin D₃ at 7x 10⁶ IU/animal /day for six days prior to slaughter (D).

Whole muscles portions of the loin (*M. longissimus lumborum*) were removed the day following slaughter and vacuum packaged and wet aged at 1 - 2°C for 13 days before processing into steaks for a simulated retail display study and samples for Warner Bratzler Shear Force (WBSF) and myofibrillar length (MFL). For the simulated retail display two freshly cut steaks (30 mm) were assigned to a high oxygen MAP (70% O₂, 30% CO₂) treatment displayed for seven days at 1 - 2°C in dark storage. Samples for determination of free thiol groups, MFL and WBSF at 14 days post-mortem were vacuum packed and frozen at -20°C. After the seven day display, one steak was used to measure WBSF and the other was sub-sampled to measure free thiol groups and MFL at 21 days postmortem. Samples were also vacuum packed and frozen at -20°C.

Frozen loin samples were prepared as described by [19] using 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) to measure the amount of free thiol groups in proteins determined as µM thiol/mg protein. For WBSF measurement, frozen loin steaks, thawed at 4°C for 24 h and prepared according to an oven-broiling method using direct radiant heat [1] to 70°C internal temperature. Six round cores (12.7 mm diameter) were

removed from the steaks parallel to the long axis of the muscle fibers and sheared perpendicular to the fiber direction, by a Warner Bratzler shear device mounted on an Instron Universal Testing [13]. Myofibril fragment lengths (MFL) were measured by means of a video image analyses (Soft Imaging System, Olympus, Japan). Myofibrils were extracted according to [5] as modified by [11]. Hundred myofibril fragments per sample were examined and measured with an Olympus BX40 system microscope at a 400X magnification.

Data were subjected to analysis of variance for a split-plot design GenStat® [22] with two ageing periods as sub-plots. Means for the interactions between the sub-plot were separated using Fishers' protected t-test least significant difference (LSD) at the 5 % level of significance.

III. RESULTS AND DISCUSSION

WBSF was significantly influenced by treatment (P<0.001), and aging (P<0.001). Z had higher WBSF values than C and ageing for 14 or 21 days did not change the difference between the treatments significantly (Figure 1). Vitamin D failed to improve shear force tenderness of zilpaterol treated loins, which was not expected. In fact, D had higher (P<0.05) WBSF values than Z (and C). Extended ageing decreased WBSF irrespective of treatment.

MFL as indicator of proteolyses was shorter for C compared with Z and D at 14 days ageing and showed marginal change (P>0.05) between 14 and 21 days (Figure 2). However, both D and Z showed significant shorter MFL's after 21 days compared with 14 days (P<0.001).

The free thiol content was significantly higher for Z than C (P<0.01) after 14 days ageing, meaning that Z was less oxidized than C (Figure 3). During MAP packaging for an additional 7 days, the amount of free thiol groups decreased for all treatment groups (P<0.001). However, the decrease was smaller for D than C and Z, respectively. After 21 days ageing, D was significantly less oxidized than C (P<0.05).

Zilpaterol supplementation has previously been shown to decrease the ratio of oxidative fibres (SO) and increase the ratio of fast glycolytic fibres (FG) in comparison to control feeding [27]. Hence, the lower oxidation level seen for the supplementation groups (Z and D) after 14 days in vacuum might be explained by a lower susceptibility to oxidation due to changes in the fibre type composition. After an additional 7 days in MAP, the Z samples were oxidized to a larger extend

than the D samples, suggesting that vitamin D did show an antioxidative effect, which is supported by [17]. The higher oxidation rate exhibited in Z muscle relative to C could be related to both the change in fibre type composition and the effect of the beta agonist on stress susceptibility and consequent effects on the presence of free radicals. [23] reported higher nitric oxide synthase (NOS), the enzyme responsible for formation of nitric oxide (NO), in FG fiber types (like in Z) compared with SO fiber types. Under certain circumstances NO could increase protein oxidation and inhibit proteolytic enzymes, such as calpain, as was reported by [30]. Furthermore, [16] and [18] reported elevated oxidation of mitochondrial proteins in rats under acute stress, in other words in circumstances where stress hormones like adrenaline were implicated. Beta agonists are adrenergic in nature and it is accepted that treated animals are more stress susceptible and show sub-acute indicators of stress [4, 8].

In our study, variation in WBSF among treatments before and after retail display (MAP) was similar and variation in level and rate of oxidation did not seem to have an influence. However, MFL which could be considered as an indication of one dimension of tenderness development showed some variation among treatments during retail display. [24] found that an oxidative environment had a negative effect on the calpain activity, and thus, the tenderization of beef steaks early post mortem. However, μ -calpain is known to autolyze within three days post mortem, which eventually leads to loss of activity [3]. Thus, there seems to be reason to believe that the calpain dependent tenderization was more or less completed before the beef steaks were packed in the oxidative environment, which is supported by [9]. Another possible cause might be found in how proteins change following exposure to oxygen radicals. [6] found that $\cdot\text{OH} + \text{O}_2^- (+\text{O}_2)$, which may mimic biological exposure to oxygen radicals, generally produced a dispersed pattern of protein fragmentation products. Besides, oxidative modification seemed to provide denatured substrates for intracellular proteolysis and thus, increase the proteolytic susceptibility of many proteins [25]. If the latter way of reasoning was considered, the variation in oxidation rate among treatments was not reflected in tenderness development. To some extent the larger decrease in MFL for at least Z but not for D could have been associated with oxidation rate during display, although variation in MFL was not reflected in

WBSF, which is not uncommon according to [7].

Although the effect of oxidation on changes in protein structure can not be ruled out, a more plausible explanation for variation in both WBSF and MFL is probably related to the action of zilpaterol on the proteolytic enzyme system during the initial 14 days and subsequent storage. The difference in WBSF between C and Z was expected [15], even after prolonged aging [2, 26], although the former study showed that prolonged aging decreased the negative effect of Z relative to C, which was indirectly reflected in smaller differences in MFL in the present study but not in WBSF.

IV. CONCLUSION

These results indicate that increased oxidation of muscle proteins late post mortem does not have any significant effect on meat tenderness development (WBSF) when more aggressive factors such as beta agonists impact on meat tenderness.

In addition, it may be that vitamin D₃ supplementation may help protect some proteins from becoming oxidized when the tissue is exposed to a high oxygen atmosphere, even at elevated ageing.

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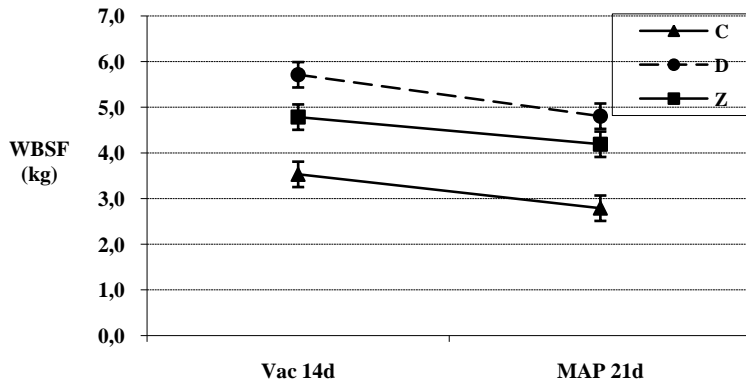


Figure 1: Interaction between treatment and aging for WBSF

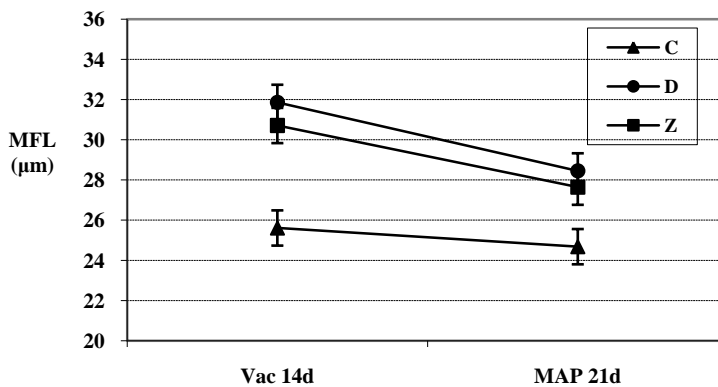


Figure 2: Interaction between treatment and aging for MFL

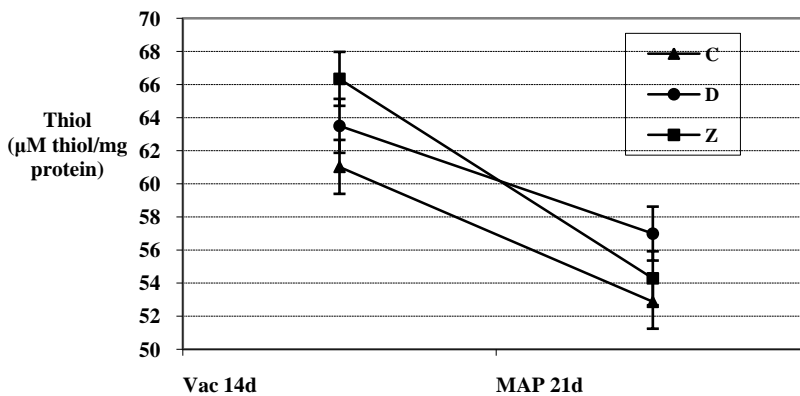


Figure 3: Interaction between treatment and aging for free thiol concentration

PE 4.03 Study on the mechanism of lipid peroxidation initiation in dry-fermented sausages

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Abstract - A study is carried out to determine which of the mechanisms for lipid peroxidation initiation in dry-fermented sausages is dominating. It is estimated that the prooxidative effect of the lipoxygenase complex is 0.21, and that of the metal complex is 0.13 respectively. The results obtained allowed the conclusion to be made that, the prooxidative effect of the lipoxygenase complex is 1.6 times stronger than the one of the concomitant metal complex.

A conclusion is made that the lipid peroxidation processes in the dry-fermented sausages are catalyzed mainly enzyme way – most of all by peroxidases. The catalytic action of the “free” metal ions is more limited but passes in parallel with the enzyme systems.

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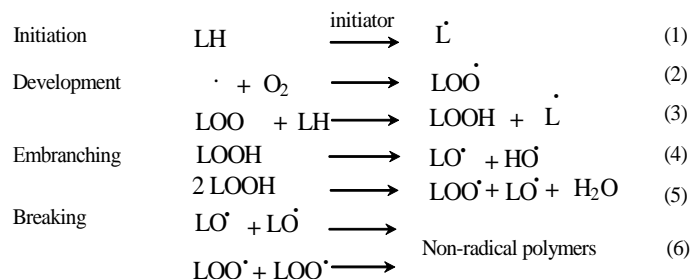
Index Terms: dry-fermented sausages, lipid peroxidation, lipoxygenases, free metal ions.

I. INTRODUCTION

THE dry-fermented sausages are predisposed to lipid peroxidation initiation and development during ripening and drying [19]. The ripening of the dry-fermented sausages is based on biochemical processes. They are connected with the meat proteolytic and lipolytic enzymes action, with the lactic bacteria number increase and their vital activity. The latter is expressed by lactic acid accumulation and medium acidification. During the dry-fermented sausages ripening a row of chemical-physic occurrences is passing as: moisture evaporation, water diffusion from

the center to the surface, congestion and welding together of the filling mass, etc. [6]. The characteristic taste and distinctive flavour of the dry-fermented sausages is due to a bouquet of volatile and non-volatile low molecule chemical compounds, derived: 1) during the fermentation processes, caused by lactic micro flora development [16]; 2) as a result of proteolysis [5]; 3) due to lipolysis and lipids oxidation [2], and 4) from the spices and salting materials [8]. Reactive oxygen species is formed enzymatically, chemically, photochemically in food. It is also formed by the decomposition and the inter-reactions of reactive oxygen species [4].

The whole lipid peroxidation mechanism is chain radical. It can be described by processes of initiation, development, embranching and breaking of the chain reaction [12]. The initiation starts with hydrogen reduction and alkyl radical (L^{\bullet}) formation (reaction 1). This radical reacts with oxygen and peroxy radical is formed (LOO^{\bullet}) (reaction 2). The latter takes away hydrogen from the fatty acids and forms hydroperoxide ($LOOH$), which is basic primary product of the auto oxidation (reaction 3).



where: LH – unsaturated fatty acid; HO^{\bullet} - hydroxyl radical; L^{\bullet} - alkyl radical; LO^{\bullet} - oxyl radical; LOO^{\bullet} - peroxy radical and LOOH-hydroperoxid

Great number of potential initiators and distributors of lipid peroxidation in meat products are known, including hydroxyl radical (HO^{\bullet}), perferil and ferril radical, Fe^{2+} - O_2 -linked radical and porphirine kation-radical ($P-Fe^{4+}=O^{\bullet}$) [12] or enzyme systems as lipoxigenases, cyclogenases, dependent on Nicotineamide-Adenine-Dinucleotide-Phosphate (NADPH), Adenosine-Di-Phosphate (ADP)- Fe^{3+} and O_2 enzymes [13, 18].

It is not completely clarified which of the various ferric forms: free or linked, chemic or non-chemic, oxidized or reduced has the ability to oxidize the poly unsaturated fatty acids in the meat and meat products [11]. One of the most important questions connected with the lipid peroxidation, concerns the primary catalysts source, which initiate and develop the process and its cycle character. Several mechanisms for free radicals initiation from the muscle tissue lipids are known (fig1). Main initiators for the poly unsaturated fatty acids oxidation on the three described reaction routes appear the oxygen species [4] and the activated catalysts [10, 20].

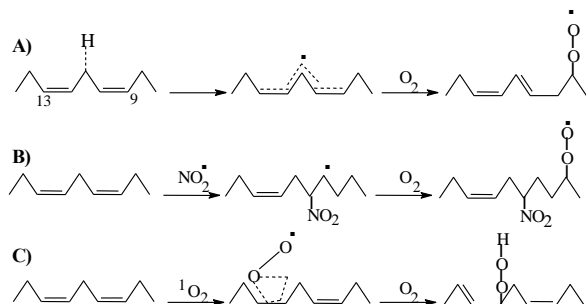


Figure 1 Lipid peroxidation initiation:

- A) by reduction of hydrogen;
B) by double linkage attacking of free radicals;
C) by singlet oxygen on so called “en“ reaction

As it is seen from the references review, the lipid peroxidation processes in the meat products can be initiated and spread on non-enzyme catalyzed routes, also [7].

The aim of the current study is to determine which one of the two mechanisms dominates and thus to recommend more effective methods for lipid peroxidation inhibition during the production and storage of dry-fermented sausages.

II. MATERIALS AND METHODS

A. Materials

The samples dry-fermented sausage “Manastirska lukanka” type are prepared from: 550 g/kg chilled beef type CL 95 stored 72 h *post mortem* at 0 - 4°C, frozen to -5°C, 6 h prior its grinding; 250 g/kg chilled semi-fat pork - type 50/50, stored 72 h *post mortem* at 0 - 4°C and frozen to -10°C, 6 h prior its cutting; 200 g/kg frozen to -10°C hard dorsal bacon, stored 24 h *post mortem*, prior its use; 22 g/kg salt; 0.4 g/kg potassium nitrate; 3 g/kg black pepper; 2 g/kg cumin, and 2 g/kg sweet red pepper.

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To carry out the analyses distilled clear chloroform and methanol were purchased (Fluka Chemie AG, Buchs, Switzerland). The rest of the reagents were AP or GPL quality and were supplied by Aldrich Chemical Co (Steinheim, Germany).

B. Sample preparation

The setting of the experiment for investigation the mechanism for lipid peroxidation initiation in dry-fermented sausages is presented at fig2. To clarify the mechanism for lipid peroxidation initiation, the method for accelerated determination of the pro-oxidative

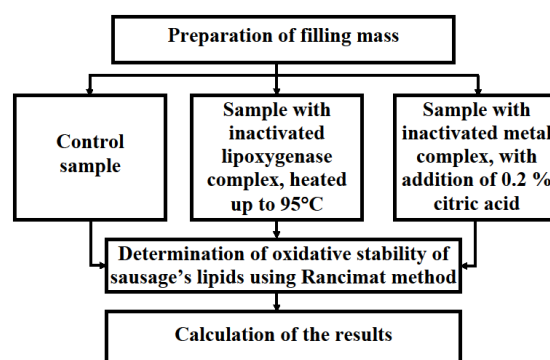


Figure 2 Setting of the experiment for study of lipid peroxidation initiation mechanism in dry-fermented sausages

effect of both the lipoxygenase and concomitant metal complex was used. For the purpose two samples for each of the methods were extracted. According the recommendations of Ivanov [9], for lipoxygenase complex inactivation, heating up to 95°C was applied, while for the metal complex inactivation to the samples 0.2 % citric acid solution was added. After the lipid extraction from the sausage samples according Bligh and Dyer [3], the oxidative stability was determined according Rancimat method [17]. The pro-oxidative effect of the lipoxygenase complex was calculated as ratio between the thermally inactivated sample stability and the stability of the unheated sample.

C. Determination of oxidative stability of extracted lipids

The oxidative stability of extracted lipids from samples was presented by induction period. The induction period was determined with the Rancimat model 679 (Metrohm AG, Switzerland) [17] at 100°C and the air flow of 20 L/h. The oxidative stability was calculated from the measured induction periods of samples.

D. Statistical analysis

Data were analyzed using SPSS for Windows, version 10.0.1 (SPSS Inc., Chicago, IL, USA). All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance (ANOVA) was made with the General Linear Models (GLM) with a significant level of $P \leq 0.05$. The Tukey's test with significant difference at $P \leq 0.05$ was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

On fig. 3 the results from the experiments with "Manastirska" lukanka samples for determination of the pro-oxidative effect of both lipoxygenase and metal complex are presented. It is estimated that after inhibition and deactivation of the lipoxygenase

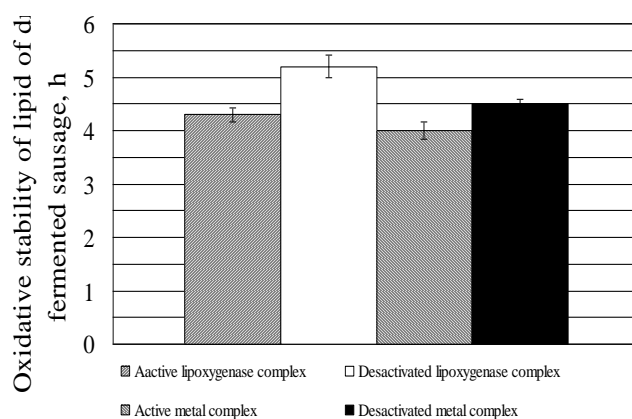


Figure 3 Oxidative stability of the lipids in dry-fermented "Manastirska" lukanka sausage samples with active and inhibited lipoxygenase and metal complexes, h

complex, the oxidative stability of the lipids, extracted from the "Manastirska" lukanka samples, increases with 20.9 %, or the lipoxygenase complex pro-oxidative effect is 0.21 (table.1). In comparison with these results, after the metal complex inhibition, the oxidative stability of the lipids, extracted from the "Manastirska" lukanka samples is increased only with 12.5 %. The pro-oxidative effect of the metal complex is estimated as 0.13, respectively. (table1).

The results obtained in this way allowed to make the conclusion that the pro-oxidative effect of the lipoxygenase complex is 1.6 times stronger than the concomitant metal complex. Therefore, in the dry-fermented sausages the lipid peroxidation processes

Table 1 Pro-oxidative effect of the lipoxygenase and metal complexes in dry-fermented sausage "Manastirska" lukanka

Pro-oxidative effect of the lipoxygenase complex	0.21
Pro-oxidative effect of the metal complex	0.13

are catalyzed mainly on enzyme route. As we know, the dry-fermented sausages are not treated thermally, but are dried and ripened [7]. Due to this reason the enzyme complexes are not desactivated and continue their action during the sausage technological treatment [5]. The dry-fermented lukanka type sausages are produced from beef and pork. According Turubatović et al. [20] an imbalance between oxidative stress and the cell's anti-oxidant defense system may have effects on cell membranes through the indiscriminate oxidation of susceptible molecules such as polyunsaturated fatty acids, the main substrates for lipid peroxidation. Those investigators have suggested that the alteration in the CuZn-superoxide dismutase/selenium dependant glutathione peroxidase plus cytosolic antioxidative defense enzymes – catalase ratio correlate well with increases in lipid damage. The catalytic action of the "free" metal ions is more limited, but it occurs in parallel with the enzyme systems hemoprotein and non-heme iron components are active catalysts of lipid peroxidation. In raw meat, lipid oxidation is inhibited at high pH because of removal of oxygen by enzymatic reducing systems. Both heme and non-heme iron were active at lower pH values [14] such as in the beginning of the ripening of dry-fermented sausages. In raw meat systems heme pigments catalyze oxidation of tissue lipids causing a stale or rancid odor and flavor. Free radicals from lipid oxidation can oxidize and decompose the red ferrous hemes [10]. In order the enzyme complex to be desactivated and as far as possible, the "free Fe^{2+} " ions disintegration processes to be inhibited, it is necessary the natural anti oxidative factors of muscle tissue to be preserved as active as possible [20]. That is why, the lipid peroxidation inhibition in the dry-fermented sausages must start with the pre-slaughtering treatment of the animals and to proceed during their technological processing and storage [13]. During the ripening and drying of the dry-fermented sausages, as during their consequent storage, it is recommended, as far as possible, the access of oxygen and light to be limited, and the temperature to be the lowest possible for the technology applied [19].

The above mentioned methods for the lipid peroxidation inhibition in the dry-fermented sausages production are practically inapplicable, because the contact of the raw materials and the finished dried product with the air oxygen can not be avoided. Insurmountable obstacle also appears the product to be isolated from the light. Thus the technological effects applied are least of all ineffective to inhibit the initiation, development and the spread of the lipid peroxidation. Therefore it is proposed the scientists and technologists' attention to be directed towards easily applicable in the practice methods for oxidative processes inhibition [7]. Such method is the addition of proper antioxidants to the dry-fermented sausages filling mass, which are effective regarding both enzyme and non-enzyme catalytic pro-oxidative factors.

IV. CONCLUSIONS

Practical conclusions for initiating factors inhibition are made. In order to deactivate the enzyme complex and as far as possible to inhibit the processes of Fe²⁺ and Cu²⁺ ions dissociation, it is recommended the natural anti oxidative factors of the muscle tissue to be preserved as more active as possible.

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PE 4.123 Influence of time and pH of acidic marination on the physicochemical and sensory acceptability of poultry meat

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Abstract— The influence of marination time (30, 60, 120 and 180 min) and acidic marinade pH (3.0, 3.2, 3.4, 3.6, 3.8, 4.0 and 4.2) on the instrumental and sensory properties of cooked Chinese-style marinated chicken were investigated. Food grade citric acid solution (1.0 M) was used to modify the corresponding marinade pH which was then placed in an individual tray containing a chicken fillet at 4°C for the respective marination times. The marinated chicken fillets were subsequently dry cooked at 170°C for 15 min to an internal temperature of 73°C. Meat pH, marinade uptake, cook loss, expressible moisture, moisture content, maximum force and surface color (L^* , a^* , b^* values) were measured. Sensory evaluation was also conducted using 25 naïve assessors. With increasing marination time up to 180 min, there was a consistently significant ($p < 0.05$) effect of increasing cooked surface redness (a^* value) and the dark pink sensory attribute, yet decreasing lightness (L^* value) and colour penetration were observed. A clear effect of marination times 30-60min as well as 120-180 min was observed separating the instrumental and sensory qualities of the products studied. However, only marinade uptake, colour L^* (lightness) and a^* (yellowness) value were affected by marinade pH ($P < 0.05$). It was observed that marinade uptake was greater at higher marinade pH levels of 3.8, 4.0 and 4.2, with the highest marinade uptake (3.34%) at pH 4.0. As changes to core meat pH were not observed, the effect of marination time (up to 180 min) and marinade pH on the instrumental and sensory properties of Chinese-style marinated chicken were located principally at the surface of samples.

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Index Terms: Acidic marination, Sensory Evaluation, Marination Time; Marinade pH; Poultry meat

I. INTRODUCTION

Consumers generally incorporate marinades to meat via immersion. This consists of immersing the meat in the marinade and allowing penetration of the meat through diffusion over time. Most commercial marinades essentially are acidic water-oil emulsions containing spices, salt, sugar, functional additives (e.g. xanthan and guar gum), antimicrobial agents (sorbate and/or benzoate) and aroma enhancers, allowing acidic marination. The incorporation of substances such as organic acids (e.g. acetic, lactic and citric acid) and pH reducing adjuncts (e.g. soy sauce) are often employed in the acidic marination process as part of a mix as flavour enhancers as opposed to preservation per se; thus such substances play a major role to tenderize and flavour the meat and to cause the meat fibres to swell [1], [2]. Despite its extensive application, little information is available on the effect of acidic marination to poultry meat. As poultry meat is versatile and does not necessarily have to undergo the process of tenderization, acidic marinades may degrade the texture and deleteriously affect the overall quality of the end product, particularly with respect to sensory acceptability.

In the presented study, multivariate data techniques were employed to study the effect of marination time and acidic marinade pH on the physicochemical properties and sensory acceptability of chicken meat marinated with Chinese-style low pH marinades.

II. MATERIALS AND METHODS

Raw material

Chicken breast fillets, fresh and de-skinned were obtained locally from Shannonvale Foods, Clonakilty, Co. Cork, Ireland. They were received without visible blood splash or bruising and in the range of 130 - 200 g in weight with a pH of 6.0 – 6.2. The remaining surface fat was removed following visual inspection. Meat samples were vacuum-packaged and stored at -18°C until required. The marinades, which were prepared according to the manufacturer guidelines; consists of 20% Chinese-style marinade solution, with the ratio of 20:80 dry mix to distilled water. They were treated with 1M food grade citric acid (Shandong TTCA

Biochemistry Co., Ltd., China) until the targeted pH range of 3.0, 3.2, 3.4, 3.6, 3.8 and 4.0 was achieved for corresponding treatment samples. Small volume differences were compensated for by the addition of distilled water. A Chinese-style marinade (pH 4.2) was used as control because this corresponded to commercial marinades available on the Irish market. All marinades were prepared the day before marination and kept at 4°C until use.

Marination and cooking

Prior to marination, the chicken fillets were thawed to 4°C overnight. Marination was carried out by immersing individually the fillets into the differently-treated marinades in a plastic container, under chill temperature of 4°C for 30, 60, 120 and 180 min. The samples were turned occasionally to ensure even-marination. The samples were subsequently dry cooked at 170°C in a Zanussi convection oven (C. Batassi, Conegliano, Italy) for approximately 15 min to an internal temperature of 73°C, as measured by an internal temperature probe (Testo 110, Lenzkirch, Germany). All test samples were cooked at the same time and segregated to prevent any mixing.

Analyses

Muscle pH. The pH values were determined for the individual raw fillets and also after marination, by inserting the probe into the geometric center of the thickest part of the pectoralis muscle (Mettler-Toledo, GmbH, 8603 Schwarzenbach, Switzerland).

Marinade uptake. Sample weights was recorded before and after marination. Excess marinade was carefully drained off by applying paper towel to the sample surfaces. Calculation for marinade uptake was as follows: % marinade uptake = $\frac{\text{marinated weight} - \text{raw weight}}{\text{raw weight}} \times 100$

Cook loss. Samples weights were recorded before and after cooking. Before weighing, the samples were blotted with a paper towel to remove excess surface moisture. Calculation for cook loss was as follows: % cook loss = $\frac{\text{cooked weight} - \text{marinated weight}}{\text{marinated weight}} \times 100$

Expressible moisture (EM) and moisture content. Water holding capacity was determined as expressible moisture and was measured [3] with the modification of using a TA.XT2 Texture Analyser (Stable Micro Systems, England). Samples were cut into approximately 2 cm cubes, and one piece of Whatman Number 1 filter paper was positioned on the top and

one on the bottom of the sample to absorb the expressed moisture. A 10-cm diameter flat disc attachment was lowered onto the sample at a rate of 90 mm/min. A maximum load of 4 kg was applied to the sample and this force was held constant for a total test time of 60 s. Samples typically reached a deformation of 80%. The sample weight before and after compression was recorded. Earlier, the moisture content before and after cooking was determined by using a CEM SMART (moisture) (AOAC PVM 1:2003). Expressible moisture was calculated as % EM = $\frac{W1 - W2}{Wm}$, where W1 and W2 are the sample weights before and after compression and Wm is the total initial moisture in the sample.

Mechanical texture analysis. Each cooked samples was penetrated using a 4mm probe attached to a TA.XT2 Texture Analyser (Stable Micro Systems, England) in compression mode. A cross speed of 100mm/min was applied using the 5kg load cell. Mean values were expressed in terms of peak force (KgF). This was repeated 6 times (n=36) on each breast in the area shown in Fig. 3.2 below.

Colour. Surface colour measurements (Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Osaka, Japan) were taken immediately after the marinated cooked samples were cooled to room temperature. For each samples, measurement of L^* (lightness), a^* (redness) and b^* (yellowness) values were taken at three locations, which included two adjacent locations to the central measurement.

Sensory evaluation. Sensory evaluation was conducted using 25 naive assessors, composed of students and staff of University College Cork, Ireland. The intensity for each attribute was rated on a 10-cm line scale labeled with words; words showing weak intensities on the left and stronger intensities on the right at both ends, respectively. Sensory consumer analysis was undertaken in the panel booths at the university sensory laboratory that conforms to ISO (1988) international standard. Assessors were asked to score the sensory properties of the marinade, using nine terms including; dark pink colour, colour penetration, aroma and flavour liking, toughness, sourness, sweetness, juiciness and overall acceptability.

Statistical analysis. The spatial relationships between sensory attributes and physicochemical properties of the samples were summarized using ANOVA Partial Least Squares Regression (APLSR) employing the software Unscrambler version 9.7 (CAMO ASA, Trondheim, Norway).

III. RESULTS AND DISCUSSION

The APLSR correlation loading plot (Fig. 1) was performed to understand the effect of marination time and pH on Chinese-style marinated chicken quality. The association of groups of sensory attributes and the instrumental properties with the main design indicators, marination time and marinade pH, was clearly observed. The effect of marination time was found to be dividing the plot into two clusters; T30-T60 and T120-T180 (Fig. 1). The T30-T60 group was observed to be highly correlated with colour L^* (lightness), b^* (yellowness) and moisture content whereas the T120-180 group was found to be highly correlated with colour a^* value (redness) and maximum force as well as almost all sensory attributes measured consisting of flavour liking, sweetness, overall acceptability, sourness and aroma liking. Additionally, these attributes were located adjacent to the dark pink and marinade uptake attributes, indicating that a high correlation exists between the dark pink attribute, marinade uptake and the T120-180 marination time group. Additionally, the effect of marinade pH was found to be largely linear in nature, i.e. pH 3.0 to pH 3.2...pH 4.2, spreading along PC 1 (Fig. 1). It can be clearly seen that the sensory quality attributes of Chinese-style marinated chicken including overall acceptability, flavour liking, sweetness, sourness, aroma liking and dark pink was found to be closely located near pH 3.8 and optimally with pH 4.0 and pH 4.2, located a little to the right of the x-axis.

A close correlation relationship was evident between WHC-related attributes including expressible moisture, moisture content, cook loss and juiciness (Fig. 1). More specifically, attributes related to water holding capacity (WHC) namely cook loss, expressible moisture, juiciness and colour penetration can be seen along the axis pH 3.0 to pH 3.6. This result verified the significance of lower acidic marinade pH in determining WHC of meat product, most commonly related to the poor quality of WHC meat described by higher cook loss, expressible moisture and juiciness. This result was also found to be in agreement with the previous study that water-holding capacity is very important in meat quality and is affected by many factors including pH of marinades and meat [4].

Besides aroma and flavour liking, colour penetration is also considered as one of the factor contributing to the acceptability of Chinese-style marinated chicken [5]. Based on the present study, the result of colour penetration from the APLSR plot might reflect

further/faster penetration of marinade by marinades of lower pH due to their good correlation with the lower marinade pH below 3.6 (Fig. 1). There was a good correlation between colour penetration and shorter marination time (30-60 min) by acidic marinade in which shorter marination time produced a higher colour penetration visible to the consumer's naked eye (Anova result not shown). This possibly due to the pH surface denaturation of meat proteins by the lower pH marinade, inhibiting further marinade penetration after a passage of time. With the increasing popularity of further processed poultry products particularly ethnic meat products, Carmine and Paprika extract often find applications in ethnic chicken dishes as part of cooking sauces or marinades ingredients. Being heat stable, both colourants are retained after the cooking process, yet it is more important that the food colourant be stable during the acidic-marination procedure which usually takes more than an hour.

Marinade uptake was found to be correlated with higher marination times of T120-T180 but the differences were more pronounced in pH levels above 3.6 (Fig. 1). Marinade absorption by chicken fillets was reported before as time-dependent [6]. This might be due to the treatments involved in their studies, e.g. physical process of tumble-marination and the incorporation of phosphate that contribute to these discrepancies. Our results suggest that water/marinade entry into muscle during acidic marination, particularly by immersing/soaking is more a pH-dependant process rather than time-dependant. Marinades must overcome physical barriers in muscle, e.g., sarcolemma and actomyosin cross-linkages, in order to diffuse into the fibers and myofibril matrices [6]; and this was highly depended on the environment provided by the marinade. It is apparent that regardless of time, the acidic pH environment aids in breaking down the meat protein to some degree, causing protein to swell and expand, allowing water/marinade uptake.

Although marinating with acidic solutions might contribute an off-flavour, marinating chicken fillets with different marinade pH did not cause any changes to the sensory acceptability of Chinese-style marinated chicken. The sweet and sourness of the product was found to be more intense after 120-180 min of marination (Fig 1), but the element of sweet and sour is essential and of great importance in determining the acceptability of Chinese-style marinated chicken.

IV. CONCLUSION

Overall, this study suggests that marination time and marinade pH of acidic marination processes could be manipulated to increase the marination performance and sensory acceptability of ethnic meat products. Higher marination time of 120-180 min was found to produce a more acceptable end product with the increase in acceptability of colour, aroma and flavour attributes. It was evident that the differences in marinade pH of 3.0 to 4.2 did affect the physical quality of Chinese-style marinated chicken without causing significant changes in its aroma-flavour liking along with overall acceptability. This indicates that opportunities exist for further improving the physicochemical and sensory characteristics of marinades or cooking sauces for the home and commercial use as a means of imparting flavour and moisture to meat through acidic marination.

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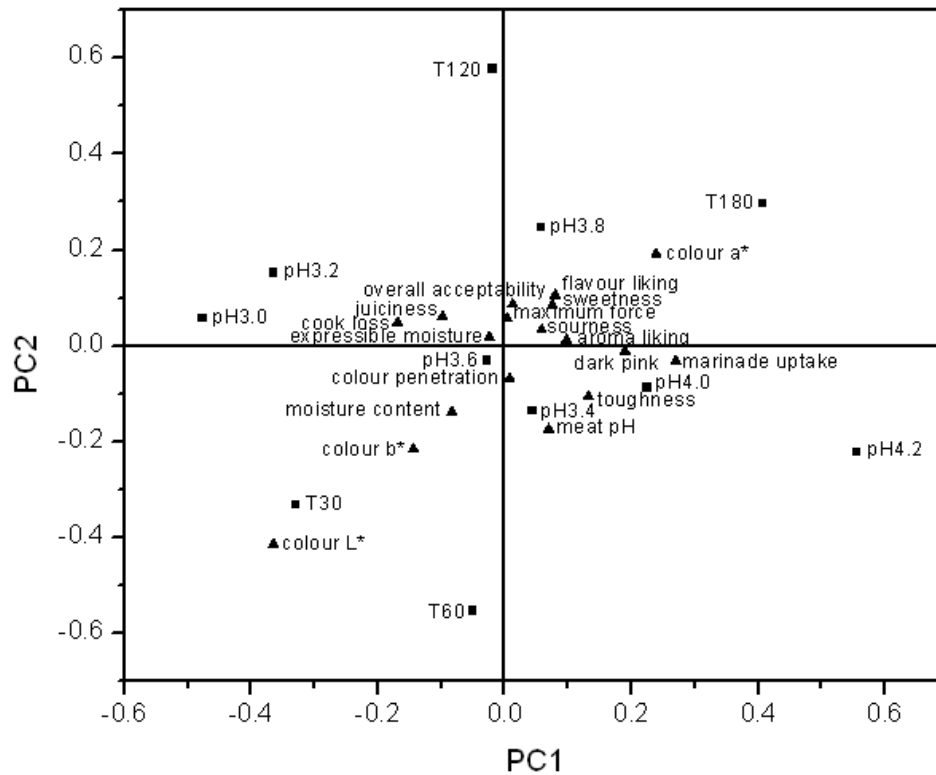


Figure 1 An overview of the variation found in the data from the ANOVA-Partial Least Squares Regression (APLSR) correlation loadings plot for the individual effect of marination time and marinade pH. Shown are the loadings for the X- and Y-variables for PC1 (Principal Component 1) versus PC2 (Principal Component 2). ▲ = Instrumental and sensory descriptor, ■ = Time/pH treatment. The ellipse highlights samples and attributes which are highly correlated to each other.