PE4.08 Effects of modified atmosphere packaging on protein profile and quality of pork meat 27.00

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Abstract— **The aim of this study was to evaluate by proteomics analysis, the effects of modified atmosphere (MA) on the quality of pork meat conditioned on masterpack and under refrigeration. Steaks of *longissimus dorsi* were packaged on expanded polystyrene trays and PVC films. Afterwards, trays were placed in a second masterpack containing three gas mixtures (O_2:CO_2): 75:25, 50:50 and 0:100, six trays in each masterpack, and stored under refrigeration (2.0±1.0ºC) for 22 days. Sampling was carried out at the 1st, 8th, 15th and 22th days for analysis of the proteins and meat quality. The meat quality measurements were pH, Drip Loss and Shear Force. The proteins were extracted from meat and analyzed by Two-Dimensional Electrophoresis (2DE). The effects of gas composition and storage time in meat quality parameter and protein expression level were analyzed using the program PROC GLM (SAS, 9.1). It was observed significative effect of gas composition and storage time in the number of spots. The 2-DE images showed 159 to 311 spots and 8 matches in common. It was observed a significative effect (P<0.05) of treatment and storage time and gas composition (P<0.08) in the expression values of two spots. In general, there was an increase in the number of spots from 1st to 22nd days. The correlation coefficients for pH to all spots varied from -0.20 (pH *vs* Spot 3) to 0.18 (pH *vs* Spot 7). The correlation coefficients for Drip Loss ranged to 0.19 (Drip Loss *vs* Spot 7) to 0.55 (Drip Loss *vs* Spot 3). For Shear Force, the correlations coefficients oscillated from -0.08 (Shear Force *vs* Spot 6) to 0.34 (Shear Force *vs* Spot 8). In conclusion, high concentrations of O_2 did not affect the protein profiles of pork meat. However, high concentrations of CO_2 affected the protein profiles of pork meat.**

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I. INTRODUCTION

THE modified atmosphere (MA) can be used to extend the shelf life of a great variety of foods, among them, fresh meat cuts [1,2]. In the last years, much research has focused on the influence of MA on meat quality attributes and the purchasing preferences of consumers [3,4]. These packs usually contain mistures of O_2 (to enhance color stability) and CO_2 (to inhibit microbiological growth) [5]. It's already know that high O_2 concentrations may impact negatively the oxidative stability of muscle lipids, cause a rapid development of meat rancidity, and consequently affect meat quality attributes. Protein oxidation can affect the quality of meat and meat products by decreasing of enzyme activity and solubility and through the formation of protein complexes and non-enzymatic browning products [6].

The proteomic analysis technique has been successfully applied to describe *post mortem* modifications of pig muscle protein [7,8], or characterize PSE (pale, soft and exsudative) in pig muscle [9]. Other studies have investigated the correlation between proteins and their fragments abundance with meat quality traits such as texture [10], L* and Drip Loss [11]. Therefore, the overall objective of the present investigation was to evaluate, by proteomic tools, the effect of package gas composition on the protein profile of pork meat stored under refrigeration for 22 days, and to correlate protein profile with meat quality traits. For that purpose, a differential proteome analysis was performed by changes in the spot expression of a reference gel of pork meat collected 24 hours *post mortem*.

II. MATERIALS AND METHODS

Muscle Samples and Treatments

Pork steaks taken of the *Longissimus dorsi* (LD) muscle from 16 carcasses were obtained of a local slaughterhouse. Steaks were placed on expanded polystyrene trays (3 steaks per tray) and covered with poly(vinyl chloride) films. The trays were placed in a

second masterpack (78.5 cm x 48.5 cm. 0.35m^2; Cryovac) with high gas barrier property, six trays per masterpack (MP), containing three gas (O2:CO2) compositions: being 75:25 (A); 50:50 (B); and 0:100 (C). After sealing, the atmosphere composition inside the MP was checked using a Dansensor gas analyzer (CheckPoint O2/CO2). No significant variation on the mixture was found during the storage. The MPs were stored in a refrigerated chamber (2.0±1.0ºC) for 22 days. In the 1st; 8th; 15th and 22nd storage days, 4 MP packs containing different gas compositions were removed from the refrigerated chamber and 1g muscle pieces were cut, immediately frozen in liquid N_2 and keep at -80ºC, until protein extraction. Then, the samples were submitted to meat quality evaluations.

Meat quality measurements

Meat pH was measured using a portable pHmeter, with a glass electrode.

The drip loss was calculated by differences in weight between day 1 and each storage time (days 8, 15 and 22), being expressed as percentage.

The tenderness was determined according to the procedure described in the literature [12]. The shear force was measured using a Stable Micro Systems Texture Analyzer (TA.XT2i, SMS) equipped with a Warner-Bratzler shear blade moving with a speed of 500mm/min. The maximum shear force of 6 strips per sample was recorded.

Extraction of Muscle Proteins

Four samples of 1g muscle tissue of each treatment were mixed in glass plate. A 0.5 g sub-sample was homogenized in 5 mL of 8 M Urea, 2 M Thiourea, 65 mM DTT, 2% CHAPS [10] using a Turratec homogenizer, for 60 seconds at 16000 r.p.m.. Crude extracts were transferred to Erlenmeyer's flaks, vigorously shaken for 2 hours, and centrifuged (30 min. at 10 000 x g) in order to remove unextracted cellular components, high molecular weight protein complexes, and insoluble proteins. The protein content was determined with Coomassie protein assay (Pierce).

Two-Dimensional Electrophoresis (2-DE)

A volume of 250 µl of DeStreak rehydration solution (GE Healthcare) premixed with 500 mg of total protein was utilized to passive rehydration 13 cm immobilized pH 3-10 gradient strips (IPG) for 14h at room temperature. Isoelectric focusing was carried out with Multiphor II instrument (GE Healthcare) equipped with a temperature controller run at 20ºC, according to GE Healthcare protocols. The total product time x voltage applied was 17403 Vh. For second dimension, the focused immobilized pH gradient strips were equilibrated for 15 min. in a SDS equilibration buffer solution (GE Healthcare) with DTT. The buffer was poured off and the strips were equilibrated again with the same buffer added iodoacetamide. The equilibrated IPG strips were drained for excess fluid, mounted on the top of 12.5% SDS-PAGE gels and sealed in place with boiling 1% agarose in Laemmli running buffer. The electrophoretic run was performed at a constant current of 25 mA for each gel and 90 V for 30 min, them 250 V until the end of the run. Gels were subjected to Coomassie Brilliant Blue (CBB) staining to visualize proteins.

Image analysis

2-DE maps were obtained by scanning the gels using the Imagescanner (mod. PowerLook 1120; Amersham Biosciences) Analysis of gels was accomplished using the Image Master 2D Platinum software (Amersham Biosciences) including background subtraction, spots detection, volume normalization and the establishment of reference gel. The three treatments of atmosphere composition and different storage time were automatically compared using match set method. For each comparison, a match set was created from muscle extracts obtained 24h *post mortem* (Figure 1). Intensity of each spot was quantified by calculation of spot volume (in percentage) after normalization of the image using the total spot volume normalization method multiplied by the total area of all the spots. The results were evaluated in terms of spot OD.

Statistical analysis

A completely randomized design in a factorial 3 x 4 arrangement of treatments [3 gas compositions (A, B and C) and 4 storage times (1, 8, 15 and 22 days)], with 4 replicates for each gas combination x storage time of analysis, was used for meat quality traits.

The effects of protein expression level on storage time and treatment (gas compositions) were analyzed inside for each individual spot, in volume percentage. In order to evaluate the association between meat quality traits and spots (in volume percentage), correlation analysis was performed. Data statistical analyses were carried out using the GLM and CORR procedures of SAS software [13].

III. RESULTS AND DISCUSSION

Proteomic analysis

In general, the number of spots increased from the 1st to 22nd day of storage, probably by proteolysis effect [10]. Nevertheless, the results of statistical analysis for the number of spots showed interaction ($P<0.01$) between gas composition and storage time (Table 1). The differences among gas composition for each

storage time occurred on 8th day, when the gels of gas composition A had more spots than others gels, and on 15th day, when the gels of gas compositions B and C had more spots than gas composition A. Therefore, the differences among storage time were observed in all gas compositions.

Table 1 – Number of spots detected in gels for three gas composition and four storage periods.

Storage Time (days)	Gas Composition (O_2:CO_2)					
	A (75:25)		B (50:50)		C (0:100)	
	Mean*	S.E	Mean*	S.E	Mean*	S.E
1	263 aA	1 1.5	235 aB	1 0.8	252 aAB	1 1.2
8	300 aA	1 2.2	229 bB	1 0.7	215 bB	1 0.4
15	159 bB	8 .9	276 aAB	1 1.8	296 aA	1 2.2
22	311 aA	1 2.5	306 aA	1 2.4	290 aA	1 2.1

S.E = Standard error * Means in the same line (column) sharing a common minuscule (capital) superscript letter are significantly different by Turkey's test (P<0.05).

Duplicated gels of each gas composition and storage time (24 gels) were matched with a reference gel of the 24 h *post mortem* pork muscle protein, with 360 spots detected (Figure 1). Eight spots were found in common among all treatments and storage time (identified by arrows in the gel), but only spots 1 (P<0.05) and 8 (P<0.08) showed a significant differential in spot intensity (Figures 2 and 3, respectively).

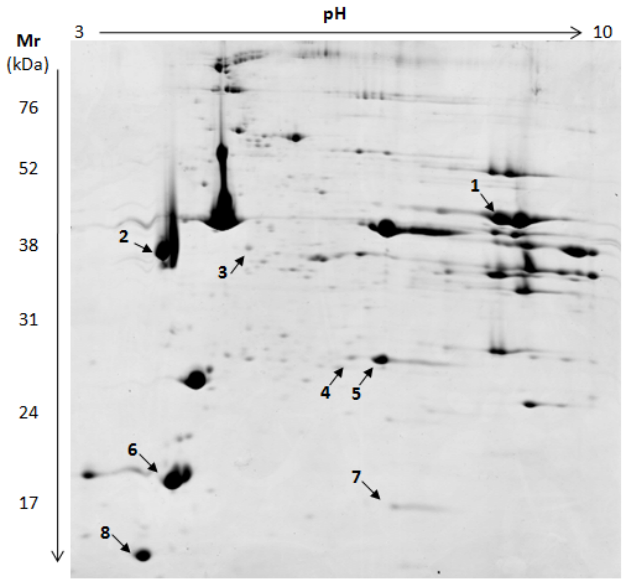

Figure 1 – 2DE gel of pork *Longissimus dorsi* muscle proteins collected 24 hours *post mortem*. Arrows show the identified proteins that were differentially express between gas composition and storage time.

There was a decrease in spot 1 intensity during storage period of gas composition C (Figure 2) and no differences were observed in the spot intensity of other gas compositions for the other storage period. Also, it was observed differences in the spot intensity on the storage time for gas compositions A, between 8th and 15th and C, between 1st and 22nd storage days.

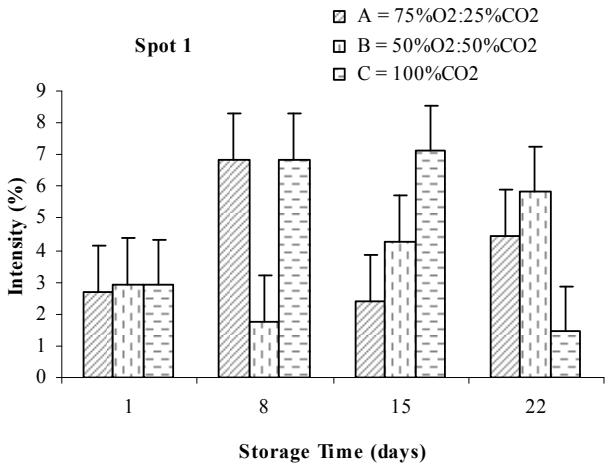

Figure 2 – Spot intensity for each gas composition and storage time (Spot 1).

On the other hand, the values observed on spot 8 for gas composition C (Figure 3), were higher than others gas compositions.

The results of the spots 1 and 8 suggested that somehow it is possible that high concentration of CO_2 (gas composition C) affected the protein expression.

According to literature [10], the differences observed in the spot intensity may be a result of either decreases in protein degradation or changes in protein expression.

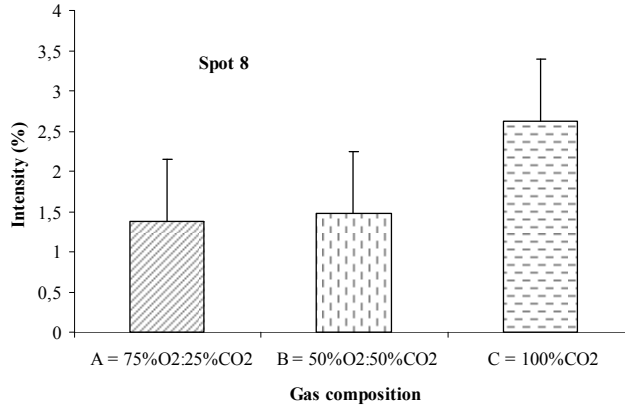

Figure 3 – Spot intensity by gas composition (Spot 8).

Meat quality traits

Pork meat quality measurements were carried out on samples taken from each gas composition and storage time. It was observed effect of gas composition and storage time in pH values ($P<0.05$), and the values varied from 5.1 to 5.7. Although some differences among pH values were observed, these values were very smaller and do not affected overall meat quality. For Drip Loss, it was observed effect of gas composition ($P<0.05$). The meat stored with gas composition B had lower values of drip loss (3.5%) than others compositions: 5.3% and 4.8% for meats conditioned under gas composition A and C, respectively, being that no differences was observed between these last values. On the other hand, no differences were observed in Shear Force values, and the overall mean was 3.9 kgf.

Correlations between spot intensity and meat quality traits

The meat quality data were correlated to the spot intensity. In Table 2, it was presented estimated Pearson correlations coefficients between the individual spot intensity and meat quality traits. The correlation coefficients for pH to all spots varied from -0.20 (pH *vs* Spot 3) to 0.18 (pH *vs* Spot 7). The correlation coefficients for Drip Loss ranged from 0.19 (Drip Loss *vs* Spot 7) to 0.55 (Drip Loss *vs* Spot 3). For Shear Force, the correlations coefficients oscillated from -0.08 (Shear Force *vs* Spot 6) to 0.34 (Shear Force *vs* Spot 8). Thus, the best correlation coefficient (0.55) was found between Drip Loss and Spot 3. But, in overall, only the spot 4 had good correlations (modulus > 0.2) with all studied properties.

Table 2 – Pearson correlations coefficients for spot intensity and meat quality measurements.

spot	pH	Drip Loss	Shear Force
1	- 0.18	0.37	0.28
2	- 0.20	0.35	0.10
3	0.04	0.55	0.11
4	- 0.21	0.28	0.34
5	- 0.13	0.27	0.28
6	- 0.09	0.23	- 0.08
7	0.18	0.19	0.28
8	0.14	0.32	0.34

Lametsch et al. [10] studied postmortem proteome changes in porcine M. *longissimus dorsi* related to tenderness (shear force values) and reported significant correlations between the *post mortem* degradation of actin and myosin heavy chain and tenderness. However, the coefficients correlations found by Lametsch et al. [10] ranged from -0.55 (actin 6 *vs* Shear Force Day 4) to 0.32 (CapZ *vs* Shear Force Day 4). These authors suggested that *post mortem* degradation of these proteins changed meat texture. Thus, it could be suggested that the spots 4 and/or 8 were related to the myofibrillar proteins responsible by the texture and water hold capacity of meats, but more studies are need to confirm that statement.

On the other hand, Morzel et al. [14] studied the meat texture of "Blonde D`Aquitane" cattle and related 11 spots strong correlated with initial or overall tenderness scores ($r > 0.75$). In addition, the better correlation coefficients were 0.76 and 0.82. These spots were considered potential predictors of tenderness.

IV. Conclusion

The results in this experiment allowed concluding that high concentrations of O_2 did not affect the protein profiles of pork meat. However, high concentrations of CO_2 affected the protein profiles of pork meat.

The correlations coefficients between spot intensity and meat quality measurements had lower intensity.

Finally, it is necessary more studies to elucidate the relationships between gas composition and meat quality.

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