PROTEOMIC APPROACH TO CHARACTERIZE PALE AND DARK TURKEY BREAST MEAT SORTED BY IMAGE VIDEO

[1]Santé-Lhoutellier Véronique, [2]Le Pottier Gilles; [1]Sayd Thierry & [1]Monin Gabriel

[1]Biochimie et Physiologie du Muscle, Station de Recherches sur la Viande, INRA, 63122 Saint Genès Champanelle, France. [2]CIDEF 35310 Mordelles, France

Background

In the past decade, the turkey meat industry has developed new products based on cured and cooked meat. Meat processors need to adapt the raw material to further processing. The technological quality of the processed meat depends on its major component: the proteins. The state of the proteins will mostly determine the water holding capacity of fresh meat and colour, themselves correlated with pH value. A previous study has shown that early pH determines partly the colour of the fresh meat (Santé *et al*., 1996). Studying proteins requires the use of separating techniques such as 1-D electrophoresis. Utilisation of 2-D electrophoresis is developing since it allows separation of several hundreds of proteins. Further steps includes identification of spots of interest using mass spectrometry. Then, characterisation of meat could be performed through its protein mapping. Besides, Lametsch *et al*. (2003) showed that proteome was correlated to textural trait in pig meat.

Objectives

Predicting the process ability of meat is challenge for meat processors. Even if the turkey BUT9 represents 95% of slaughtered turkey in France and the environmental conditions (slaughter, chilling, etc..) are well defined, the variability still exist in terms of meat quality, i.e. WHC and colour. These traits are influenced by (denaturation of) sarcoplasmic proteins (Sosnicki *et al*., 1998; Wilson & Van Laack ,1999). The aim of the study is firstly to use video image colour on line to sort turkey breast meat according to their potential yield to prevent excess of purge and secondly characterize this meat according to its 1-D and 2-D electrophoretic protein patterns.

Materials and methods

Animals and samples

A total of one hundred and twenty turkey breast muscles were collected in a slaughter plant on the cutting line. The breast meat was obtained from 15-week old male turkeys (line BUT 9) after conventional chilling over a minimum of 8 hours. Firstly selected visually to obtain the largest range of colour, from lightest to darkest, the breast meat colour was measured using a tri-CCD DXC 990 P SONY camera and analysed according to the method developed by CEMAGREF (Marty-Mahé *et al*., 2002). The ultimate pH value (pHu) was determined 24 h post mortem using a portable pH meter equipped with a glass electrode directly inserted into the muscle. The results were expressed as L*, a*, b*, C* and h* coordinates for lightness, redness, yellowness, chroma ($C= (a^{*2}+b^{*2})^{1/2}$) and hue angle (h=arc tang (b*/a*)), respectively. Part of breast meat was cut into scallops, weighted Day1, wrapped and weighted again after 48 h.

The breast meat was subsequently vacuum packaged, immediately frozen and stored at –20°C for further analysis. After thawing, the cooking yield was measured on a muscle sample of standard size (15 x 5 x 2,5 cm; 337 ± 14 g, n=170) after injection of brine (136 g of nitrite salt x l^{-1}) at the rate of 15 %, vacuum packaging and cooking in a water bath until a 68°C internal temperature was reached.

Protein breast samples were frozen in liquid nitrogen and stored at –80°C until analysis.

Protein extraction/fractionation

The extraction buffer consisted of KCl 50mM, Tris 20mM, $MgCl_2$ 4mM, EDTA 2mM, pefabloc 5mM, DTT 2% (w/v) 1% pH 7. 150 mg of muscle were added to 1 ml of extraction buffer in an Eppendorf containing a glass bead. Homogenisation was performed using a Retsch MM2 agitator (Retsch, Haan, Germany) for 1 h at 4°C. Homogenates were centrifuged at 10,000g for 15 min at 10°C. Supernatant was collected, pellet was

washed 3 times using a washing buffer (KCl 75mM, $KH2PO_4$ 10mM, $MgCl_2$ 2 mM, EGTA 2mM, pH 7). Protein content was measured using a Bio-Rad Bradford protein assay kit.

1D eletrophoresis

SDS PAGE was performed on both pellet and supernatant using a Hoefer 250 cell (Amersham) on 11% polyacrylamide gels at 40 volts for 1h then 110 volts for 2 h. Gels were stained with blue coomassie. Gels images were acquired using a GS-800 densitometer and analysed using Sigmagel software (SPSS, Chicago, USA).

2D electrophoresis

2D electrophoresis was performed on 3 samples per group (C1 vs C4). Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (Biorad), using Bio-Rad ready strip , 17 cm, pH 3-10 non linear. 90µg of sarcoplasmic proteins were loaded onto the strips for analytical gels. Proteins loading on strips, IEF and SDS-PAGE were performed according to Morzel *et al*. (2004). Gels were silver stained following the protocol of Yan *et al*. (2000). Gels were produced in triplicate. Gels images were acquired using a GS-800 densitometer and analysed using the PDQuest software (Bio-Rad).

The meat quality data were subjected to analysis of variance using the general linear model procedure of SAS, and the means were separated using the Scheffe's test. 1D and 2 D electrophoresis data were analysed using student test.

Results and discussion

Meat quality

The muscle samples were distributed in 4 groups according to their L* value obtained by image analysis: 45-48 (C 1, n=18), 49-52 (C 2, n=66), 53-56 (C 3, n=58), and 57- 60 (C 4, n=30). Significant differences between C1, C2, C3 and C4 were found for the ultimate pH. Drip loss was higher in lighter meat (C4) but this difference remained not significant. Curing and cooking yield decreased as the lightness increased (Table 1). The Characterisation of turkey breast meat using image video was efficient to sort the meat according to its processing yield.

1D electrophoretic protein pattern

Sarcoplasmic proteins

The gels analysis showed that the density of some proteins increased in C4 group meanwhile others decreased (Table 2, Figure 1). Glycogen phosphorylase density tended to be lower for C4 group (4.7 vs 3.7), as well as creatine kinase (15.2 vs 14.3). In the C1 group, aldolase and enolase had lower density on gels. Glycogen phosphorylase and creatine kinase lower contents in C4 group could be explained by more protein denaturation or / and protein degradation. In unstructured ham, partly characterized by it very pale colour , it was reported that creatine kinase was proteolysed (Sayd *et al*., 2003). In fact, group C4 showed similar traits: pale colour, higher drip loss and lower curing and cooking yield.

Myofibrillar proteins

Small differences on the proteins pattern were found between the two groups C1 and C4 at 24 h. However, in C4 group, we observed the absence of a band at 35-40 kDa approximatly (Figure 2). Again for this group, a 30kDa band was present at a higher density. This band has been well described as a marker of proteolysis in muscle. For turkey, one day ageing was enough to get a 30kDa fragment, which has been well described as a product of troponin-T proteolysis (Ho *et al*., 1996; Negishi *et al*., 1996). 1D electrophoresis did not allow to show more differences as expected in such a group of meat quality.

2D electrophoretic protein pattern

Up to 600 spots were detected per gel. After removal of saturated or poorly reproducible zones, 334 spots were successfully matched across the whole set of the 2 goup (C1 and C4) images. The relative quantities of spots from each group were analysed. Differences were noted when the ratio of the relative quantity was above 2: 158 spots did not differ among the two groups, 26 spots had a higher quantity in the C1 group (darker meat) and 150 spots had a higher quantity in C4 group. 18 spots were only present in the C4 group gels. Among them, one spot is suspected to be a creatine kinase fragement by comparison to results of Sayd *et al*. (2003). With our data, we are not able to evaluate protein denaturation neither its level. However, Van

laack (2000) reported that other factors than protein denaturation are responsible for the low water-holding capacity of pale, soft, exudative chicken breast muscle. So, our results showed higher protein solubilisation, which could indicate either less denaturation or more proteolysis in the paler meat. In further work we will identify spots of interest (either proteins or fragments) by Maldi-tof spectrometry.

Conclusions

The characterisation of turkey breast meat using image video was efficient to sort the meat according to its processing yield : as the lightness increased, curing and cooking yield decreased. In 1 DE gels, proteins pattern differed in a small extent between the paler and darker meat (C4 group *vs* C1 group). Using 2DE gels allows the separation of 10 to 20 more protein bands. The meat with the lower processing yield differed by a higher level of protein solubilisation, indicating either less denaturation or more proteolysis in the paler meat.

References

Ho Y., Stromer M.& Robson R. 1996. Effect of electrical stimulation on post mortem titin, nebulin, desmin and troponin T degradation and ultrastructural changes in bovine Longissimus muscle. J. Animal Science, 74: 1563-1575.

Lametsch R., Karlsson A., Rosenvold K., Andersen H., Roepstorff P. & Bendixen E. 2003. Post mortem proteome changes of porcine muscle related to tenderness. J. Agr. Food Chem., 49: 4351-4357.

Morzel M., Chambon C., Hamelin M., Santé-Lhoutellier V., Sayd T. & Monin G. 2004. Proteome changes during pork ageing following use of two different pre-slaughter handling procedures. Meat Science. 67 : 689-696.

Negishi H., Yamamoto T. & Kuwata T. 1996. The origin of the 30kDa component appearing during post mortem ageing of bovine muscle. Meat Science, 42: 289-303.

Santé V., Lebert A., Le Pottier G & Ouali A. 1996. Comparison between two statistical models for prediction of turkey breast meat colour. Meat Science, 43: 283-290.

Sayd T., Santé-Lhoutellier V., Chambon C., Laville E. & Monin G. 2003. Proteomic analysis of destructured pork muscle. In Poster, 20eme Journées Françaises de Spectrométrie de Masse, 16-19 septembre, Toulouse, France.

Sosnicki A.A.// Greaser M.L.// Pospiech E.// Pietrzak M. & Sante V. 1998. PSE like syndrome in breast muscle of domestic turkeys: a review. Journal Muscle Food, 9: 13-23.

Van lack R. & Lane J. 2000. Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. Poultry Science, 79: 101-109.

Wilson G. & Van Laack R. 1999. Sarcoplasmic proteins influence water-holding capacity of pork myofibrils. Journal of the Science of Food and Agriculture, 79: 1939-1942.

Yan J., Wait R., Berkelman T., Harry R., Westbrook J., Wheeler C. & Dunn M. 2000. A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionisation and electrospray ionisation mass spectrometry. Electrophoresis. 21: 3666-3672.

Table 1. Colour, pHu and cooking yield according to lightness (L* value) as measured by image analysis

Group (L* limits)	pH (24 h)	drip loss (%)	Curing and cooking yield
C1 (45-48)	6.01 ± 0.21 [a]	1.92 ± 1.25	93,7 [a]
C2 (49-52)	5.90 ± 0.13 [a]	1.93 ± 0.59	92,6 [a]
C3 (53-56)	5.94 ± 0.22 [a]	1.77 ± 0.57	90,5 [b]
C4 (57-60)	5.77 ± 0.16± [b]	2.48 ± 0.42	89,2 [b]

Table 2: density values of some sarcoplasmic proteins measured on 1 DE gels.

Protein band	C1	C4	anova
B1 phosphorylase	4.7± 1.9	3.7± 1	ns
B5 enolase	13.6± 1.2[a]	15.4± 1.1[ab]	0.04
B6 créatine phosphokinase	15.2± 1 [a]	14.3± 1[b]	0.038
B7 aldolase	13.5±1.5[a]	17.6± 1.6[c]	0.0012

Figure 1

1DE gel : sarcoplasmic proteins from groups C1 and C4

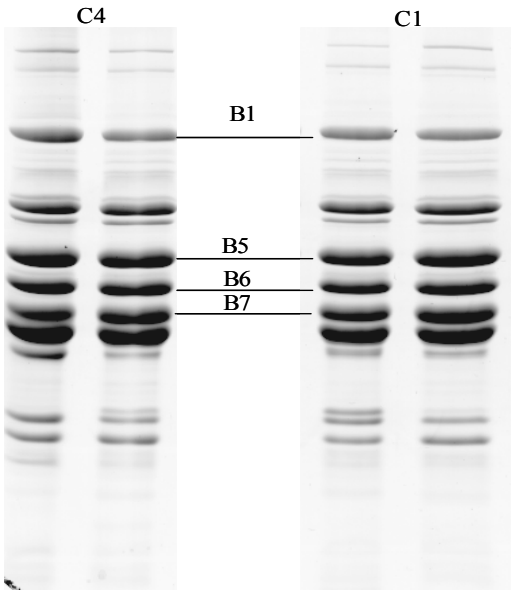

Figure 2

1 DE gel : myofibrillar proteins from groups C1, C2, C3 and C4

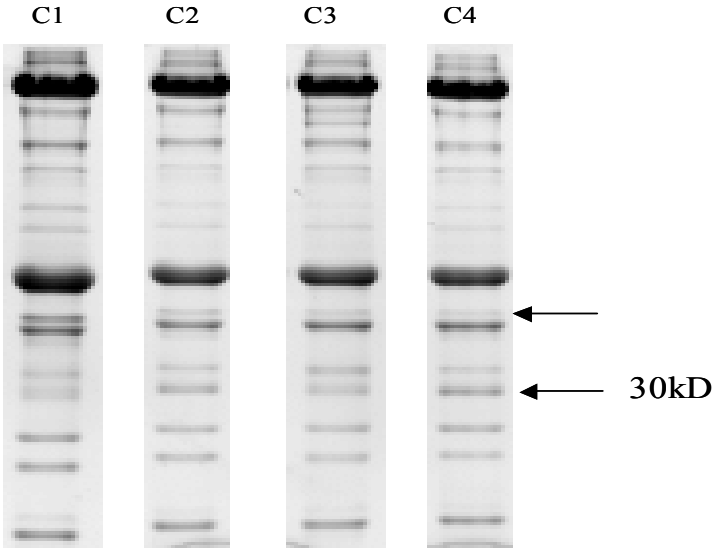

Figure 3 : Computer-generated master gels corresponding to all gels of *Pectoralis superficialis* muscle from C1 (a) and C4 groups (b)