

ASSOCIATION BETWEEN GENETIC MARKERS AND PROCESSING TRAITS OF BAYONNE DRY CURED HAM

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Abstract— In dry cured ham production, salt counteracts the negative effects of high proteolysis on the sensory and processing quality of ham. Our objective is to identify suitable genetic markers to facilitate rapid selection of pigs with low protease activity for reduced salt dry ham production without any detrimental quality trait, especially those related to slicing ability. In this experiment 3 SNPs (single nucleotide polymorphism) were studied: two from the gene CAST at the loci 249 and 638, and one from the gene PRKAG3 at the locus 199. 120 hams were selected, half of them was submitted to normal processing while the other half was less salted (- 20%). Hams were genotyped for the previous genes. Green hams traits (weight, fat cover, semimembranosus pH and colour) were determined. Compositional analysis were conducted on *Biceps femoris* : moisture, NaCl, protein content, non protein nitrogen or proteolysis index, and TBARS. Processing and slicing yields were monitored. The moisture content was affected by the PRKAG3 gene. The homozygotes Ile/Ile presented higher moisture content compared to the homozygotes Arg/Arg. The processing traits were affected by gene CAST at the locus 249, the homozygotes Lys/Lys presenting lower losses after the salting period and higher processing yields. For CAST gene at the locus 638, similar results for processing traits were found except for the salting losses. Taking all the above points into consideration, our results suggest that the salt reduction tested in this study did not impair the final product and the CAST gene at the loci 249 and 638 affected positively the processing traits.

Index Terms—dry cured ham, CAST gene, PRKAG3 gene, salt, processing yield.

I. INTRODUCTION

Bayonna ham enjoys EU Protected Geographical Indication (PGI) status. This certification requires professional processors to comply with a set of specifications that provide the consumer with a finished product of optimal quality, in particular as regards texture. Nowadays we assist to an increase demand for low salt food. In dry cured ham production, it is admitted that salt counteracts the negative effects of high proteolysis on the sensory (texture, flavour) and processing (slicing ability) quality of ham. In 2003, Garnier *et al.* made an inventory of candidate genes associated with certain quality traits of fresh meat. DNA markers for meat quality have been already identified (Stefanon *et al.*, 2004; Plastow *et al.*, 2005) but research is needed for specifically dry cured products. In 2005, Stalder *et al.* evaluated the effect of calpastatine gene polymorphism on US dry cured ham. The US processing is shorter than those generally used in European countries. The polymorphism concerns a nucleotide mutation (Ser 638 Arg) of calpastatine which is associated with pork meat texture (Ciobanu *et al.*, 2004a). The authors demonstrated that the CAST gene marker influenced significantly cured ham moisture content leading to the conclusion that selection for this CAST genotype would produce cured hams having more efficient moisture loss, then requiring less processing time. In this experiment 3 SNPs (single nucleotide polymorphism) were studied: two from the gene CAST at the loci 249 and 638, and one from the gene PRKAG3 at the locus 199. The gene CAST codes for calpastatine, which is an inhibitor of calpains. The gene PRKAG3 codes for a sub unit of the AMP kinase, involved in the glycolytic pathway and affecting ultimate pH in muscle. Indeed, among several factors, meat pH has been reported to affect proteolysis (Buscailhon *et al.*, 1994; Arnau *et al.*, 1998; Garcia-Garrido *et al.*, 1999; Tabilo *et al.*, 1999). Our objective is to identify suitable genetic markers to facilitate rapid selection of pigs with low protease activity for reduced salt dry ham production without any detrimental quality trait, especially those related to slicing ability.

II. MATERIALS AND METHODS

Origin of hams and sampling. The study was based on a total of 60 pigs (PIC synthetic line: PIC410 x C25) that had been fed a cereal-based diet (60-80%), slaughtered at the Lahontan abattoir, and selected to meet the processing specifications of PGI Bayonna ham for both legs. The processing of Bayonne hams was carried out at the Pyragena experimental station using the following steps : salting, settling, air drying, grease covering and ripening for a total duration of 12 months. In order to control raw ham heterogeneity, from the selected 60 carcass, one leg was submitted to normal processing (group 1: salting of 11-13 days) while the opposite leg was salted 2.5 days less (group 2) corresponding to expected 20% less salt. Green hams traits (weight, fat cover, semimembranosus pH and colour) were determined according to Robert et al (2005). Compositional analysis were conducted on *Biceps femoris* : moisture, NaCl (NF V04-401), protein content (NF V04-407), non protein nitrogen or proteolysis index (Kjeldahl), and lipid content (NF V04-403). Processing yields were monitored after salting, resting and ripening periods. Slicing ability and total amount of prepacked slices of Bayonna ham were determined for each ham. Small pieces of skin tissue were taken from each animal for genotyping. After the final selection of hams, the samples were genotyped for PRKAG3 Ile199Val polymorphism according to Milan et al. (2000) and for both CAST polymorphisms according to Ciobanu et al. (2004a).

Analysis of variance was performed using procedure GLM of statistical package SAS (SAS Inst., Inc., Cary, NC).. The linear model included fixed effects of gene polymorphisms and salt. In case of ham weight, carcass weight was included as covariate, while in case of fat thickness, pH and colour measurements, the covariate was green ham weight. When significant effect of gene polymorphism, salt or interaction salt genotype was encountered least squares means were compared using LSMEANS with PDIF option and TUKEY adjustment.

III. RESULTS AND DISCUSSION

1. PRKAG3 gene

The green ham traits, chemical parameters and processing yields analysed for the gene PRKAG3 and salt are in Table 1. The green ham weight was not affected by the gene, neither fat cover or pH of the semimembranosus muscle. Fontanesi et al. (2008) reported similar results for the mutation of the PRKAG3 gene at the locus 199. But they also found that the PRKAG3 affected significantly the rate of pH decline for the genotype Arg/Arg. In general the homozygotes Ile/Ile exhibit slightly higher ultimate pH which is not different from the heterozygotes Ile/Arg or the homozygotes Arg/Arg (Otto et al., 2007; Stalder et al. 2005). Recently Skrlep et al (2010) highlighted the importance of the site of measurement of ultimate pH in the semimembranosus from Slovenian dry cured ham Kraški pršut. The authors demonstrated a PRKAG3 gene effect on pH_u when the measurements were taken at the adjacent part of the femur bone. In this study the gene effect on the Spanish line used for Serrano ham reported that the homozygote Ile/Ile showed a slight but significant higher pH_u (5.61 vs 5.54 for Ile/Arg). The moisture content was affected by the PRKAG3 gene. The homozygotes Ile/Ile presented higher moisture content compared to the homozygotes Arg/Arg. The study of Stalder did not reported any difference in moisture content probably because the processing of country style dry cured ham differed from the Mediterranean dry cured ham style where at least 9 months ripening are required. The loss of weight during processing was not affected by PRKAG3 gene, neither the processing or the slicing yield. Such traits are generally correlated to several fresh pork quality traits (Ramos et al. 2007;) but our purpose was to evaluate the possible use of the PRKAG3 gene polymorphism for dry cured ham production without any detrimental effect on technological traits.

2. CAST gene

The green ham traits, chemical parameters and processing yields analysed for the gene CAST 249 and CAST 638 and salt are in Tables 2 and 3. The green ham weight and the fat cover were not influenced by the gene CAST at the locus 249 but the ultimate pH was affected. The homozygote Lys/Lys has a higher pH than the heterozygote Lys/Arg and the homozygote Arg/Arg. For the gene CAST at the locus 638, ultimate pH was also affected. The highest ultimate pH were found for the homozygotes Arg/Arg and the lowest for the homozygotes Ser/Ser. The pH for heterozygotes Arg/Ser was intermediate. Our results showed some discrepancies in the impact of CAST at the locus 638 with those reported by Stalder et al. (2005). However these authors did not evaluate separately the two loci (ie 249 and 638). They did not demonstrate any effect of CAST 638 on the meat quality traits generally measured but they noticed a lower muscle temperature in the homozygotes Arg/Arg which could slow down and inactivate the post mortem pH decline. This lowering of temperature may be due to the shape of the ham, less rounded or more light. These conformational traits were not measured in our study. Moisture content was affected by gene CAST at the locus 638, the homozygotes Arg/Arg having a higher percentage of moisture at the end of processing. Similar results were shown by Stalder et al (2005). The salting loss was affected by gene CAST at the locus 249, the homozygotes Lys/Lys presenting lower losses after the salting period. This could be explained partly by the higher ultimate pH reported in these hams. This slower water dynamic endured as demonstrated by the lower weight losses found at the resting period and until the end of the processing. Indeed the processing yields were higher for the homozygotes Lys/Lys. However, no differences in the

slicing ability were noted. For CAST at the locus 638, similar results for the processing traits were found except for the salting losses. The CAST gene seems to affect positively the overall processing yield of the dry cured ham without any detrimental effect, such as a reduced slicing ability. In fresh meat, Ciobanu et al (2004b) considered the haplotype CAST 249 Lys/Lys CAST 638 Arg/Arg as the most favorable considering ultimate pH, cooking loss and juiciness scores.

The analysis of the combined effect of the studied genes and salt revealed only one interaction between PRKAG3 and salt for the processing yield. For the other parameters, reducing salt in the dry cured ham decreased the lipids oxidation and the protein content and increased the moisture content and the NPN, in other words the free amino acids. Interestingly, the reduction of salt by almost 20% did not affect negatively the processing traits. As a matter of fact, processing and slicing yields were higher. This increase can be explained by the moisture content. Taking all the above points into consideration, our results suggest that the salt reduction tested in this study did not impair the final product and the CAST gene at the loci 249 and 638 affected positively the processing traits.

IV. CONCLUSION

This work underlined the importance of the gene CAST at the loci 249 and 638 on the several fresh and dry cured ham traits in the pig line used. The PRKAG3 gene effect was almost negligible in our conditions. Moreover our results clearly demonstrated that it is possible to reduce salt content without any deleterious effects on the processing qualities. Further investigations will be provided to evaluate the overall acceptance of the different dry cured ham by the consumer.

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Table 1: Green hams, physico chemical and processing traits affected by PRKAG3 gene and salt content

	PRKAG3			salt			gene effect	salt effect	interaction
	Ile/Ile	Ile/Val	Val/Val	Low	Normal	STD	p	p	
green ham traits									
green ham weight (Kg)	10.34	10.40	10.24	10.49	10.17	0.47	NS	<0.001	
fat cover (mm)	13.34	13.54	13.5	13.17	13.74	3.26	NS	0.046	
pH	5.71	5.66	5.66	5.66	5.68	0.12	NS	0.059	
Physicochemical traits									
TBA RS (mgMDA/Kg n	0.67	0.63	0.66	0.59	0.72	0.40	NS	0.06	
moisture (%)	60.86	60.15	59.82	60.84	59.71	1.47	0.05	<0.001	
Lipid (%)	2.75	3.32	3.29	3.03	3.21	1.45	NS	NS	
Protein	28.98	29.21	29.52	29.01	29.46	1.26	NS	0.011	
NPN (%)	28.44	29.29	28.60	29.39	28.17	2.72	NS	<0.001	
Chloride (%)	5.64	5.64	5.81	5.30	6.10	0.80	NS	<0.001	
Processing traits									
salting loss (%)	4.14	4.21	4.34	3.91	4.56	0.70	NS	<0.001	
resting loss (%)	16.92	17.20	18.02	17.23	17.53	1.74	NS	0.081	
processing yield (%)	69.15	68.91	68.43	68.99	68.66	2.74	NS	0.063	0.020
slicing yield (%)	87.06	86.79	86.18	87.13	86.22	2.74	NS	0.038	

Table 2: Green hams, physico chemical and processing traits affected by CAST gene at the locus 249 and salt content

	CAST 249			salt			gene effect	salt effect
	Lys/Lys	Lys/Arg	Arg/Arg	Low	Normal	STD	ProbF	p
green ham traits								
green ham weight (Kg)	10.25	10.34	10.38	10.48	10.17	0.47	NS	<0.001
fat cover (mm)	13.30	13.75	13.35	13.18	13.75	3.26	NS	0.046
pH	5.71	5.68	5.61	5.66	5.68	0.12	0.04	0.059
Physicochemical traits								
TBA RS (mgMDA/Kg n	0.63	0.66	0.66	0.59	0.71	0.40	NS	0.06
moisture (%)	60.41	60.44	59.67	60.74	59.61	1.47	NS	<0.001
Lipid	3.33	2.89	3.39	3.11	3.29	1.45	NS	NS
Protein	29.06	29.14	29.62	29.05	29.50	1.26	NS	0.011
NPN (%)	28.74	29.04	28.71	29.44	28.22	2.723	NS	<0.001
Chloride (%)	5.52	5.75	5.82	5.30	6.10	0.80	NS	<0.001
Processing traits								
salting loss (%)	3.95	4.33	4.45	3.91	4.57	0.70	0.016	<0.001
resting loss (%)	16.63	17.42	18.433	17.34	17.65	1.74	0.021	0.081
processing yield (%)	70.25	68.67	67.55	69.00	68.66	2.74	0.005	0.063
slicing yield (%)	86.77	86.54	86.55	87.08	86.16	2.74	NS	0.038

Table 3: Green hams, physico chemical and processing traits affected by CAST gene at the locus 638 and salt content

	CAST 638			salt			gene effect	salt effect
	Arg/Arg	Arg/Ser	Ser/Ser	Low	Normal	STD	ProbF	p
green ham traits								
green ham weight (Kg)	10.24	10.44	10.41	10.52	10.21	0.47	NS	<0.001
fat cover (mm)	13.42	13.77	12.92	13.09	13.65	3.26	NS	0.046
pH	5.70	5.64	5.60	5.63	5.65	0.12	0.048	0.059
Physicochemical traits								
TBA RS (mgMDA/Kg n	0.64	0.63	0.7657	0.61	0.74	0.40	NS	0.06
moisture (%)	60.35	60.30	59.01	60.45	59.32	1.47	0.030	<0.001
Lipid	3.26	2.86	3.75	3.20	3.38	1.45	NS	NS
Protein	29.12	29.26	30.08	29.26	29.71	1.26	0.086	0.011
NPN (%)	28.85	28.92	28.57	29.39	28.17	2.723	NS	<0.001
Chloride (%)	5.61	5.74	6.05	5.40	6.20	0.80	NS	<0.001
Processing traits								
salting loss (%)	4.14	4.25	4.74	4.05	4.70	0.70	NS	<0.001
resting loss (%)	17.15	17.47	19.14	17.77	18.07	1.74	0.033	0.075
processing yield (%)	69.28	68.77	66.39	68.31	67.98	2.74	0.031	0.063
slicing yield (%)	86.47	86.75	86.89	87.16	86.25	2.74	NS	0.038