Universitat de Girona

ANÀLISI DEL POTENCIAL DE MERCAT DE LA CARN PROCEDENT DE PORCS MASCLES SENCERS COM A ALTERNATIVA A LA CASTRACIÓ QUIRÚRGICA

Francesc Borrisser Pairó

Universitat de Girona

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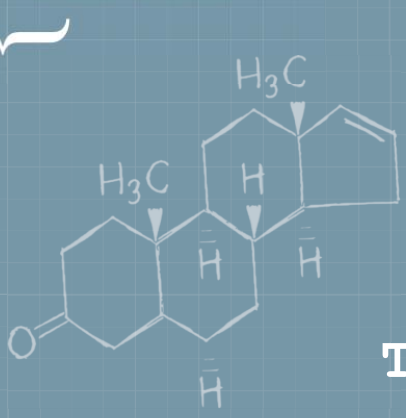
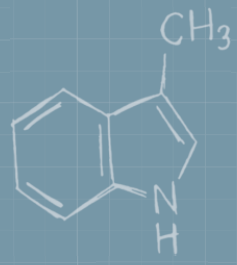
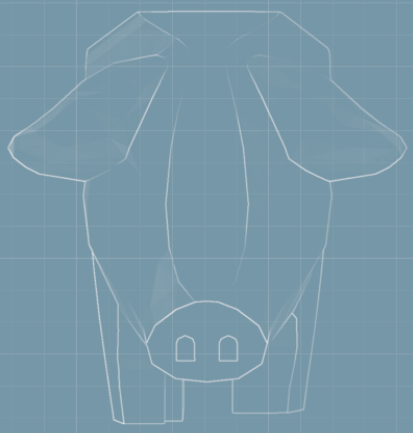
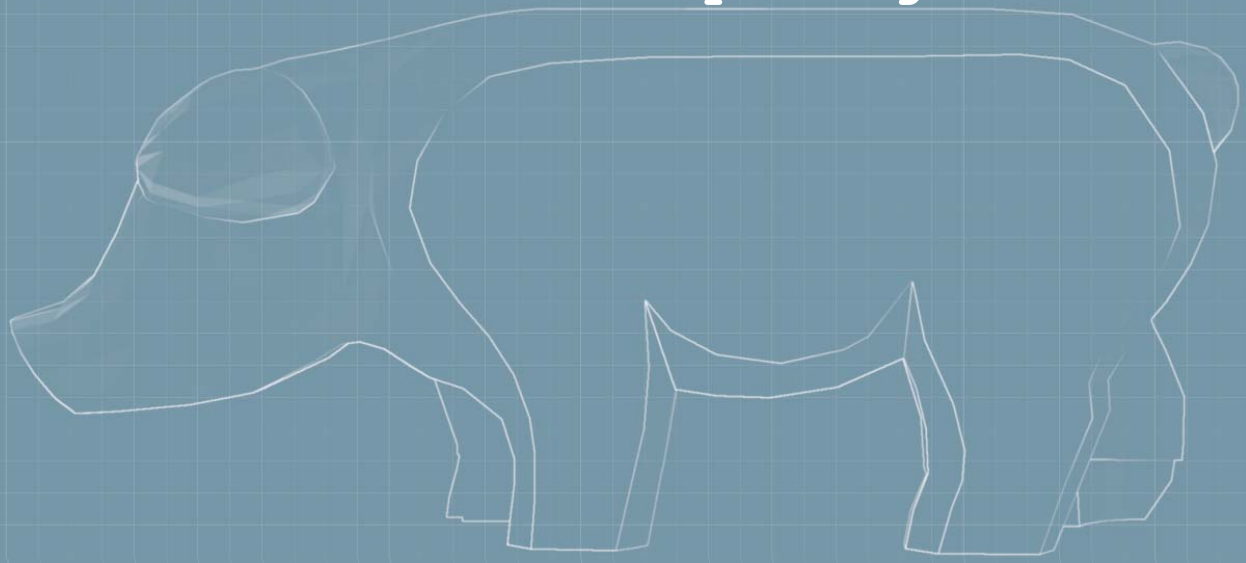






TESI DOCTORAL

Anàlisi del potencial de mercat de la carn procedent de porcs mascles sencers com a alternativa a la castració quirúrgica



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PROGRAMA DE DOCTORAT EN TECNOLOGIA

Dirigida per:

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Memòria presentada per optar al títol de doctor per la Universitat de Girona

La Dra. Mª Àngels Oliver Pratsevall i la Dra. Núria Panella Riera, de l'Institut de Recerca i Tecnologia Agroalimentàries,

DECLAREM:

Que el treball titulat "Anàlisi del potencial de mercat de la carn procedent de porcs mascles sencers com a alternativa a la castració quirúrgica", que presenta Francesc Borrisser Pairó per a l'obtenció del títol de doctor, ha estat realitzat sota la nostra direcció i que compleix els requisits per poder optar a Menció Internacional.

I, perquè així consti i tingui els efectes oportuns, signem aquest document.

Dra. Mª Àngels Oliver Pratsevall **Dra. Núria Panella Riera**

Girona, 2 de setembre de 2016

El treball que es presenta en aquesta memòria s'ha realitzat gràcies a la concessió d'una beca pre-doctoral de l'Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) i s'integra en el marc del projecte de recerca INIA RTA2011-00027-C02-01.

Agraïments

Després d'haver escrit tota la tesi, aquesta hauria de ser la part més fàcil. Dic "hauria" en condicional perquè ara veig que no serà així… Em torno a trobar davant un full en blanc que costa d'emplenar. Revisant altres tesis t'adones que, com en tots els altres apartats, no hi ha disponible cap guia de com ha de ser. Així que l'organitzaré com a mi em sembli, com tot el que faig a la vida, esperant que sigui una bona elecció. Així que, com s'acostuma a dir, "*de perdidos al río*", que vol expressar que un cop començada una acció, especialment si és arriscada, aquesta s'ha de dur a terme acceptar-ne totes les conseqüències (optimot, Fitxa 7225/3). Jo sempre havia traduït a l'anglès aquesta expressió com a "*from lost to the river*", però recentment n'he trobat l'equivalència en català: "*perdut per perdut, m'agafo allà on puc*".

Fa uns quants anys ja que vaig començar el meu camí en aquest món. Concretament va començar un dissabte d'un juliol dels anys 80, concretament un juliol del 84. Un nen dels 80 que no s'espanta, i mira que n'ha fet de voltes pel món com per espantar-se. Però vés per on, aquí em trobo ara, a la Girona on vaig néixer i créixer, presentant aquesta tesi, una tesi que no hauria estat possible sense el suport i ajut incondicional de tota la gent que m'ha acompanyat en aquest llarg camí.

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Toca també fer un petit repàs per la carrera, que van ser molts anys, amb alegries i penes, tot i que potser les alegries van guanyar la partida. En primer lloc, a tots els bons professors i professores que tant m'han ensenyat durant les tantes i tantes hores que he hagut de passar a la facultat. I ja en segon lloc, als companys i companyes que em van acompanyar tots aquells anys. A la **Cuns** per la seva senzillesa, modèstia i humanitat, que ho dóna tot i més. A en **Sergio**, pel seguit de treballs que ens va tocar patir durant la carrera que en el fons m'han anat formant. A en **Pau** perquè també hem hagut de patir vivències similars, tant estudiantils com laborals. A en **Marc** per totes les estones compartides, i per la llarga caminada cap a Santiago, on haurem de tornar per recuperar els cartílags dels nostres genolls. Al grup "Primi-ex" que ens havia de treure de la pobresa i al final només ens va donar un "ens ha tocat!", que ens va permetre pagar 4 tapes del Txapela. A la **Natàlia**, per ser així, tan natural ella, com la vida mateixa. A la **Lídia**, perquè tot i l'inici "bordil" que vam tenir ha estat allà dia rere dia compartint molts moments bons i moments dolents, però bons sobretot. A la **Mentxu**, per la seva generositat i perquè hem compartit, compartim i compartirem tantes coses.

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imaginativa en fer coses. **Palmi**, pels teus "palmimentos" que han fet superar els dies més durs. **Siscu** per la teva sinceritat, les coses clares, la xocolata espessa. **Maria**, per la teva motivació. **Nekane**, perquè no entenc com pots ser tan artista. I per no deixar-me a ningú: **Mireia U**, **Oriol**, **Ester S**, **Laura**, **Mariona**, **Ingrid**, **Gemma S**, **Àlex**, per tots els bons moments de la meva etapa post-Clínic. En resum, a tota la gent del Clínic, perquè entre tots m'heu fet veure que una tesi pot ser divertida a la par que instructiva.

Aquí ja arribo a l'etapa IRTA. A la **M. Àngels** per confiar en mi per realitzar aquest treball. A la **Núria** també per confiar en mi i per la seva ajuda i proximitat en tot moment. A la **Marta** per la seva organització i discreció, ja saps que sóc molt fan teu. A en **Dario** per ensenyar-me i formar-me en la metodologia laboratorial d'aquest treball. A la **Mª José** per les hores que dedicaves a avaluar sensorialment les agradables mostres de la tesi, suposo que el preu que he pagat és haver-te de fer de secretari contestant el telèfon. A l'**Agustí** també per la seva avaluació sensorial de les agradables mostres i per la seva energia. A la **Marina A**, per haver patit les grans transcripcions dels grups de discussió d'aquesta tesi. A la **Lídia** (sí, un altre cop, no és culpa meva que estiguis en tantes etapes de la meva vida) per la seva voluntat i immesurable ajuda en unes quantes activitats d'aquesta tesi mentre jo era fora, ja saps que part d'aquesta tesi és teva també. A en **Mauro** per les hores de cotxe que hem compartit recollint estadants desemparats d'altres països. A en **Joel** per la seva tranquil·litat i paciència. A l'**Albert B** pels dubtes que m'ha solucionat. A l'**Anna** per totes les estones polivalents i els contrastos tèrmics viscuts, i també per tota la confiança has posat en mi, tot i que segueixo pensant que em sobrevalores :P. A l'**Albert R** per les estones de laboratori que hem compartit. A en **Narcís** per la seva inestimable ajuda en tots els dubtes del laboratori que m'han anat sorgint durant aquests anys. A en **José Antonio** pel seu assessorament cromatogràfic. A la **Carme** per la seva ajuda en la cerca de material per poder treballar. A la **Carmen** pel seu sentit de l'humor en els moments de laboratori compartits. A la **Maria** per estar sempre amb la porta oberta per als dubtes estadístics que he tingut durant aquests anys. A la **Marina** per totes les consultes productives que m'ha resolt. A en **Xin**, per la simpatia i tranquil·litat que transmet. A l'**Aurora**, l'**Elena** i la **Mireia** per tot el suport en els temes administratius

que m'han solucionat. I també a tota la gent de Monells en general que m'han acollit i ajudat en el que he necessitat.

Els becaris/precaris mereixen un paràgraf a part. Així que gràcies **Anna** (sí, jo també et repeteixo :P), **Tiago**, **Nicu** i **Ricard** per tots aquells moments de teràpia grupal, suposo que si la pena és conjunta passa millor, no?

Durant aquests anys de doctorat he tingut l'oportunitat de treballar amb gent d'altres grups de recerca i realitzar diferents estades, així que també toca agrair a la gent que m'ha acollit i m'ha ajudat a seguir formant-me. Així que els dos paràgrafs que segueixen van per la gent que m'ha acollit i també format.

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Moltes gràcies a tota la gent que m'ha ajudat, aguantat i suportat en les penes i alegries durant la confecció d'aquesta tesi!

Als meus pares,
al meu germà,
a en Tuki i en Min

Llista de publicacions derivades de la tesi

Aquesta tesi doctoral es presenta com a compendi de les publicacions següents:

Article 1

Borrisser-Pairó, F., Panella-Riera, N., Zammerini, D., Olivares, A., Garrido, M. D., Martínez, B., Gil, M., García-Regueiro, J. A. i Oliver, M. A. (2016). *Prevalence of boar taint in commercial pigs from Spanish farms*. Meat Science 111: 177-182.

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 2,801

20/124 (1r quartil) a la categoria Food Science & Technology

Article 2

Borrisser-Pairó, F., Kallas, Z., Panella-Riera, N., Avena, M., Ibáñez, M., Olivares, A., Gil, J. M. i Oliver, M. A. (2016). *Towards entire male pigs in Europe: a perspective from the Spanish supply chain*. Research in Veterinary Science 107: 20-29.

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 1.504

31/138 (1r quartil) a la categoria Veterinary Sciences

Article 3

Borrisser-Pairó, F., Panella-Riera, N., Gil, M., Kallas, Z., Linares, M.B., Egea, M., Garrido, M.D., i Oliver, M.A. *Consumers' sensitivity to androstenone and evaluation of diferent cooking methods to mask boar taint*. Meat Science (enviat el 13 d'abril de 2016).

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 2,801

20/124 (1r quartil) a la categoria Food Science & Technology

Índex de figures

Índex de taules

Índex

Resum

La producció porcina és una de les majors indústries del món, d'Europa, d'Espanya i de Catalunya. Una gran quantitat de porcs es castren per motius de qualitat i de maneig. La carn dels porcs mascles sencers pot presentar una olor coneguda com a olor sexual i la castració evita aquest defecte sensorial. L'olor sexual és deguda a la presència d'almenys un dels dos compostos responsables: l'androstenona (una feromona sexual) o l'escatol (un producte de degradació del triptòfan). La presència d'aquesta olor sexual pot produir rebuig per part d'alguns consumidors.

En els darrers anys, la castració s'ha qüestionat per considerar que pot comprometre el benestar dels animal, i, per aquest motiu, la Comissió Europea planeja prohibir la castració quirúrgica a partir de l'any 2018 i promoure la producció de porcs mascles sencers a través de la *European Declaration on alternatives to surgical castration of pigs*. L'objectiu d'aquesta tesi ha sigut estudiar l'impacte que la possible prohibició de la castració quirúrgica pot tenir sobre el sector porcí espanyol. Per aconseguir aquest objectiu principal, es van plantejar 3 objectius específics:

El primer objectiu consistia en analitzar la prevalença d'olor sexual en canals porcines espanyoles. Per assolir-lo, es van agafar a nivell d'escorxador 903 mostres de greix subcutani de porcs mascles sencers comercials provinents 5 comunitats autònomes (Aragó, Catalunya, Castella i Lleó, Madrid/Castella-La Manxa i Múrcia). Es van mostrejar un total de 30 granges, 6 per cada comunitat i 30 mostres per granja. La presència d'olor sexual es va avaluar en primer lloc per un panel entrenat amb la metodologia coneguda com a *Human Nose* mitjançant un soldador elèctric. Posteriorment es va determinar la concentració d'androstenona i escatol mitjançant cromatografia de gasos i cromatografia líquida, respectivament. Segons els resultats obtinguts, un 10,2% de les canals presentaven nivells alts d'almenys un dels dos compostos estudiats (nivells alts d'androstenona: >1 µg/g de teixit adipós; nivells alts d'escatol: >0,2µg/g de teixit adipós).

El segon objectiu es centrava en analitzar l'opinió dels diferents agents de la cadena de producció porcina (des de productors fins a consumidors) sobre l'impacte que tindria una possible prohibició de la castració. Es van utilitzar grups

de discussió (*focus groups*) amb els diferents representants del sector porcí. Segons els resultats obtinguts, els productors i la indústria creuen que la prohibició de la castració no seria un problema en un futur proper ja que gran part de la producció espanyola és de mascles sencers. Els productors, la indústria i els minoristes consideren que els productes d'alta qualitat com ho són els de qualitat diferenciada o la producció d'Ibèric s'haurien d'incloure en una llista d'excepcions a la declaració. L'estudi es va completar realitzant enquestes cara a cara a carnissers per analitzar la seva actitud de compra. El que més valoren els carnissers a l'hora de comprar carn de porc és el greix intramuscular i conèixer el sexe de l'animal: busquen una quantitat moderada de greix intramuscular i principalment compren carn procedent de femelles.

El tercer objectiu anava enfocat en estudiar diferents estratègies de cuinat i d'emmascarament de la carn amb olor sexual, i comparar l'acceptabilitat de la carn fresca de mascles sencers i mascles castrats cuinats amb les estratègies proposades. Els mètodes de cuinat van ser cuinat al buit (en una bossa fins aconseguir una temperatura interna de 72ºC) i fregit (arrebossat amb all i julivert en oli d'oliva). Es van utilitzar dos tipus de carn: llom de mascles sencers amb nivells alts d'androstenona (2,3 µg/g de teixit adipós) i nivells baixos/mitjos d'escatol (0,1 µg/g de teixit adipós); i lloms provinents de mascles castrats sense nivells detectables d'androstenona i escatol. Un total de 150 consumidors van avaluar les mostres, els quals, també van realitzar un test de sensibilitat a l'androstenona. Els resultats de l'estudi demostren que les estratègies proposades van emmascarar la percepció de l'androstenona. En les comparacions del mètode de cuinat al buit, els consumidors altament sensibles que no els agradava l'androstenona preferien l'olor de la mostra de castrat, però en l'acceptabilitat global no es van trobar diferències entre les dues mostres. Segons els resultats de sensibilitat a l'androstenona, un 28,0% dels consumidors eren altament sensibles i no els agradava l'olor a androstenona, aquests consumidors són més propensos a rebutjar la carn amb olor sexual.

En general, aquesta investigació proporciona una informació actualitzada i objectiva sobre l'olor sexual i sobre l'impacte que tindria sobre la producció porcina espanyola una possible prohibició de la castració a Europa. La producció

convencional no es trobaria afectada per aquesta prohibició, tot i que es recomana la implantació d'un sistema de classificació de canals en funció de la presència d'olor sexual per tal de dirigir aquesta carn cap a aquells productes on la percepció d'aquesta olor es pugui minimitzar. Per altra banda, els productes tradicionals i d'alta qualitat es veurien afectats per una prohibició de la castració. Així doncs, caldria confeccionar una llista d'aquells productes on la castració és inevitable per tal d'incloure'ls com a excepcions a la *European Declaration on alternatives to surgical castration of pigs*.

Resumen

La producción porcina es una de las mayores industrias a nivel mundial, europeo, español y catalán. Una gran cantidad de cerdos se castran por motivos de calidad y de manejo. La carne de cerdos machos enteros puede presentar un olor conocido como olor sexual y la castración evita este defecto sensorial. El olor sexual se debe a la presencia de al menos uno de los dos compuestos responsables: la androstenona (una feromona sexual) o el escatol (un producto de degradación del triptófano). La presencia de olor sexual puede causar rechazo por parte de algunos consumidores.

En los últimos años la castración se ha convertido en un asunto de bienestar animal, por este motivo la Comisión Europea planea prohibir la castración quirúrgica a partir del año 2018 y a su vez fomentar la producción de cerdos machos enteros a través de la *European Declaration on alternatives to surgical castration of pigs*. El objetivo de esta tesis ha sido estudiar el impacto de la prohibición de la castración quirúrgica sobre el sector porcino español. Para conseguir este objetivo principal, se plantearon 3 objetivos específicos:

El primer objetivo consistió en analizar la prevalencia de olor sexual en canales porcinas españolas. Para conseguirlo, se tomaron a nivel de matadero 903 muestras de grasa subcutánea de cerdos machos enteros comerciales, procedentes de 5 comunidades autónomas (Aragón, Cataluña, Castilla y León, Madrid/Castilla-La Mancha y Murcia). Se muestrearon un total de 30 granjas, 6 por comunidad y 30 muestras por granja. La presencia de olor sexual se evaluó en primer lugar mediante un panel entrenado con la metodología conocida como *Human Nose* utilizando un soldador eléctrico. Posteriormente se determinó la concentración de androstenona y escatol mediante cromatografía de gases y cromatografía líquida, respectivamente. Según los resultados obtenidos, un 10,2% de las canales presentaban niveles altos de al menos uno de los dos compuestos estudiados (niveles altos de androstenona: >1 μg/g de tejido adiposo; niveles altos de escatol: >0,2 μg/g de tejido adiposo).

El segundo objetivo se centró en analizar la opinión de los diferentes agentes de la cadena de producción porcina (desde productores hasta consumidores) sobre

el impacto que tendría una posible prohibición de la castración. Se utilizaron grupos de discusión (*focus groups*) con diferentes representantes del sector porcino. Según los resultados obtenidos, los productores y la industria creen que la prohibición de la castración no sería un problema en un futuro cercano ya que gran parte de la producción española es de machos enteros. Los productores, la industria y los minoristas consideran que los productos de alta calidad, como son los de calidad diferenciada o la producción de Ibérico, deberían incluirse en una lista de excepciones a la declaración. El estudio se completó realizando encuestas a carniceros cara a cara para analizar su actitud de compra. Lo que más valoran a la hora de comprar carne de cerdo es la grasa intramuscular y conocer el sexo del animal: buscan una cantidad moderada de grasa intramuscular y principalmente compran carne procedente de hembras.

El tercer objetivo estaba enfocado en estudiar distintas estrategias de cocinado y enmascaramiento de la carne con olor sexual, y comparar la aceptabilidad de la carne fresca de machos enteros y machos castrados cocinados con las estrategias propuestas. Los métodos de cocinado fueron cocinado al vacío (en una bolsa hasta conseguir una temperatura interna de 72ºC) y frito (rebozado con ajo y perejil en aceite de oliva). Se utilizaron dos tipos de carne: lomo de machos enteros con niveles altos de androstenona (2,3 μg/g de tejido adiposo) y niveles bajos/medios de escatol (0,1 μg/g de tejido adiposo), y lomos procedentes de cerdos castrados sin niveles detectables de androstenona y escatol. Un total de 150 consumidores evaluaron las muestras. Los consumidores también realizaron un test de sensibilidad a la androstenona. Los resultados del estudio demuestran que las estrategias propuestas fueron útiles para enmascarar la percepción de la androstenona. En las comparaciones del método de cocinado al vacío, los consumidores altamente sensibles que no les gustaba la androstenona preferían el olor de la muestra de castrado, pero en la aceptabilidad global no se encontraron diferencias significativas entre las dos muestras. Según los resultados de sensibilidad a androstenona, un 28,0% de los consumidores eran altamente sensibles y no les gustaba el olor a androstenona, estos consumidores son los más propensos a rechazar la carne con olor sexual.

En general, esta investigación proporciona una información actualizada y objetiva sobre el olor sexual y sobre el impacto que tendría sobre la producción porcina española una posible prohibición de la castración en Europa. La producción convencional no se vería afectada por esta prohibición, pero de todos modos se recomienda la implantación de un sistema de clasificación de canales en función de la presencia de olor sexual para poder dirigir esta carne hacia productos donde la percepción de este olor se pueda minimizar. Por otro lado, los productos tradicionales y de alta calidad sí se verían afectados por una prohibición de la castración. Por tanto, se recomienda confeccionar una lista de aquellos productos donde la castración es inevitables para poder incluirlos como excepciones a la *European Declaration on alternatives to surgical castration of pigs*.

Abstract

Pig production is one of the major industries in the world, Europe, Spain and Catalonia. Many pigs are castrated for quality and management purposes. Meat from entire male pigs may present an odour called boar taint, and castration may prevent this sensory trait. Boar taint is caused by the presence of at least one of the two compounds responsible of it: androstenone (a sexual pheromone) or skatole (a breakdown product of tryptophan). The presence of boar taint may produce rejection of some consumers.

Pig castration has become an animal welfare concern in recent years, because of this, European Commission is planning to ban surgical castration by 2018 and promote entire male pig production by the *European Declaration on alternative to surgical castration of pigs*. The objective of this thesis was to study the impact that the banning of castration could have on the Spanish pig sector. To achieve this main objective, three specific aims were set:

The first aim was to analyse the prevalence of boar taint in Spanish pig carcasses. To achieve it, 903 subcutaneous fat samples from commercial entire male pigs were collected at slaughterhouses from 5 Autonomous Regions (Aragón, Catalunya, Castilla y León, Madrid/Castilla-La Mancha and Murcia). Thirty Spanish farms were screened, 6 from each region and 30 samples per farm. The presence of boar taint was firstly scored by a trained panel with the methodology known as *Human Nose* using a soldering iron. Secondly androstenone and skatole concentration were determined by gas chromatography-mass spectrometry and high-performance liquid chromatography, respectively. Results showed that 10.2% of the carcasses had high levels of at least one of the two boar taint compounds studied (high levels of androstenone: >1 µg/g of fat tissue; high levels of skatole: >0.2µg/g of fat tissue).

The second aim was to analyse stakeholder's opinion from the pig meat chain supply (from farmers to consumers) concerning the potential impact of banning castration. Focus group methodology was used with different stakeholders from the pig chain supply involved. Results showed that the stakeholders from farms and industry believe that ending castration might not be a problem in a near future

because production of entire male pigs in Spain is already high. Farmers, industry and retailers think that exceptions to the declaration for high quality products such as those from differentiated quality and Iberian pig production have to be listed. Face-to-face surveys at butchers were also performed to analyse their purchasing attitudes. For butchers the most important thing when purchasing pig meat was intramuscular fat content and the fact of knowing sex of the pig: they look for an average intramuscular fat and they buy mainly meat from female pigs.

The third aim was to study different cooking and masking strategies for tainted meat, and to compare the acceptability of fresh pig meat from entire males and castrated males cooked with the proposed strategies. The cooking methods used were vacuum cooking (in a bath until a core temperature of 72ºC) and fried (breaded with garlic and parsley in olive oil). Two types of meat were used: loins from boars with high levels of androstenone (2.3 µg/g of fat tissue) and low/medium levels of skatole (0.1 µg/g in fat); and loins from castrated male pigs with no detectable levels of androstenone and skatole. One hundred and fifty consumers evaluated the samples. Consumers were also tested for their sensitivity to androstenone. Results of this study showed that the proposed cooking methods were useful to mask the perception of androstenone. In the vacuum cooking method comparison, high sensitive consumers that dislike androstenone preferred the smell of the castrated meat sample, but in the overall liking no differences were found. The results of androstenone sensitivity showed that 28.0% of consumers were high sensitive and disliked androstenone smell, these consumers were more likely to reject tainted meat.

In general, this research provides updated and objective information about boar taint and about the impact that a possible banning of castration in Europe would have in Spanish pig production. Conventional pig production would not be affected by this banning, although an on-line system to classify carcasses according to the presence of boar taint is highly recommended to allocate tainted meat to products where boar taint perception could be minimised. High quality and traditional production would be affected by a banning of castration. Therefore, a list of these products where castration is unavoidable should be done to be

included as exceptions to the *European Declaration on alternatives to surgical castration of pigs*.

Llista de publicacions derivades de la tesi

Aquesta tesi doctoral es presenta com a compendi de les publicacions següents:

Article 1

Borrisser-Pairó, F., Panella-Riera, N., Zammerini, D., Olivares, A., Garrido, M. D., Martínez, B., Gil, M., García-Regueiro, J. A. i Oliver, M. A. (2016). *Prevalence of boar taint in commercial pigs from Spanish farms*. Meat Science 111: 177-182.

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 2,801

20/124 (1r quartil) a la categoria Food Science & Technology

Article 2

Borrisser-Pairó, F., Kallas, Z., Panella-Riera, N., Avena, M., Ibáñez, M., Olivares, A., Gil, J. M. i Oliver, M. A. (2016). *Towards entire male pigs in Europe: a perspective from the Spanish supply chain*. Research in Veterinary Science 107: 20-29.

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 1.504

31/138 (1r quartil) a la categoria Veterinary Sciences

Article 3

Borrisser-Pairó, F., Panella-Riera, N., Gil, M., Kallas, Z., Linares, M.B., Egea, M., Garrido, M.D., i Oliver, M.A. *Consumers' sensitivity to androstenone and evaluation of diferent cooking methods to mask boar taint*. Meat Science (enviat el 13 d'abril de 2016).

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 2,801

20/124 (1r quartil) a la categoria Food Science & Technology

Altres publicacions elaborades durant el període pre-doctoral

Durant l'etapa pre-doctoral es va realitzar una estada científica a la Swedish University of Agricultural Sciences (SLU), on es va elaborar un article científic Aquest article no està inclòs el compendi d'articles que conformen aquesta tesi doctoral però s'adjunta a l'**Annex 1**.

Borrisser-Pairó, F., Rasmussen, M. K., Ekstrand, B. i Zamaratskaia, G. (2014). *Gender-related differences in the formation of skatole metabolites by specific CYP450 in porcine hepatic S9 fractions*. Animal. 9(4): 635-642.

Índex de qualitat de la revista d'acord amb el JCR 2015:

Índex d'impacte: 1,508

12/58 (1r quartil) a la categoria Agriculture, Dairy & Animal Science

30/138 (1r quartil) a la categoria Veterinary Sciences

Capítol I

Introducció general

I.1 La producció porcina

La producció porcina és una de les activitats econòmiques més potents a nivell mundial. L'any 2013 la producció mundial de carn de porcí representava el 36,4% del total de carn produïda, amb 113,0 milions de tones (FAOSTAT, 2014). La **Figura 1** mostra la producció de carn de les diferents especies animals a nivell mundial l'any 2013. La carn de pollastre és la segona més produïda amb 96,1 milions de tones seguida per la de boví amb 64,0 milions de tones.

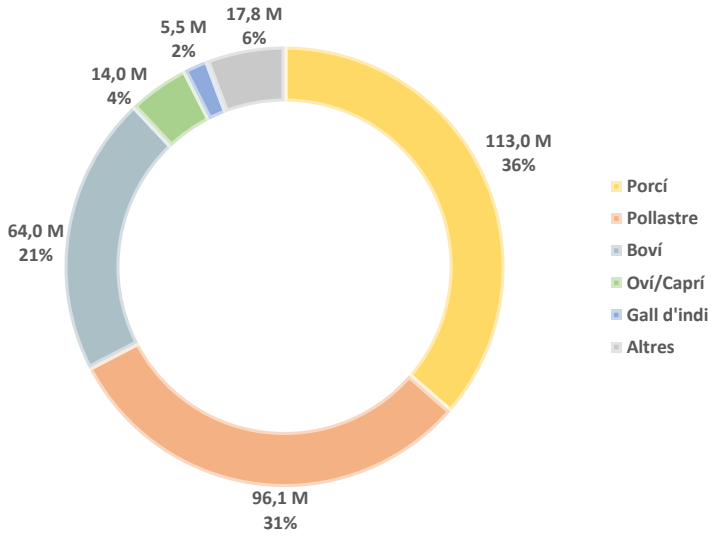


Figura 1. Producció mundial de carn per espècies de l'any 2013 (en milions de tones) (FAOSTAT, 2014).

Pel que fa al rànquing de països productors de carn de porcí del 2013, el primer lloc l'ocupa la Xina amb 52,7 milions de tones de carn produïdes, seguida pels Estats Units amb 10,5 milions, Alemanya amb 5,5 milions i el quart lloc l'ocupa Espanya amb 3,4 milions, just per davant de Brasil que va tenir una producció de 3,3 milions de tones (FAOSTAT, 2014).

A escala europea la **Figura 2** mostra els 5 principals països productors de carn de porcí de la Unió Europea del 2013, essent Espanya el segon país productor amb un 15% de la producció de la Unió Europea. Aquestes dades constaten la importància del sector porcí a l'estat espanyol i el potencial que té l'estat dins la Unió Europea.

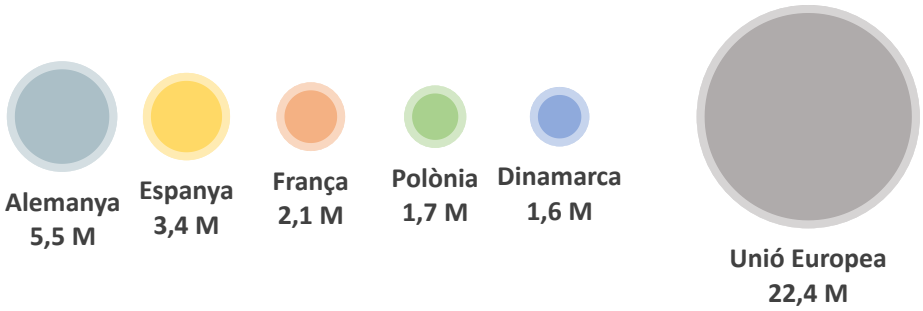


Figura 2. Principals països productors de carn de porcí de la Unió Europea l'any 2013 (en milions de tones) (FAOSTAT, 2014).

I.1.1 Producció porcina a l'estat espanyol i Catalunya

La producció porcina és una de les activitats ramaderes més importants a Espanya i Catalunya. A més, el clúster agroalimentari espanyol i català és un dels més importants d'Europa. Per altra banda, es tracta d'un sector que, tot i la crisi, ha fet un paper extraordinari en el manteniment de llocs de treball (Babot*, et al.*, 2014).

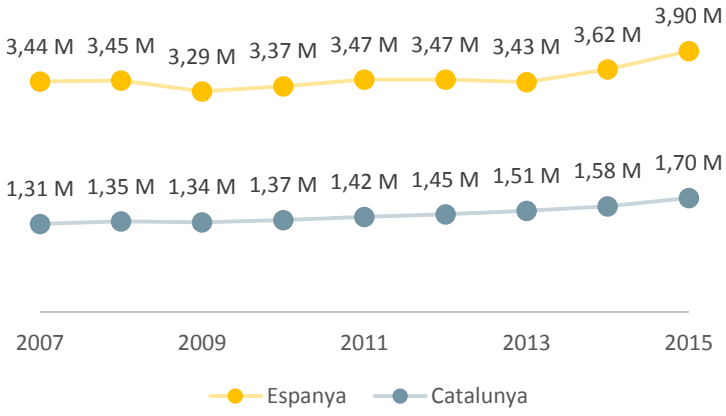


Figura 3. Evolució de la producció de carn de porcí a Espanya i Catalunya de l'any 2007 al 2015 (en milions de tones) (MAGRAMA, 2015).

Segons dades provisionals de 2015 de l'enquesta anual de sacrifici de bestiar del MAGRAMA (Ministerio de Agricultura, Alimentación y Medio Ambiente), a tot l'estat el cens de caps sacrificats de porcí va ser de 46,4 milions d'animals. A Catalunya es van sacrificar 20,1 milions de porcs (43,3% del total), essent aquesta comunitat la que més porcs va sacrificar en el conjunt de l'estat.

A la **Figura 3** es mostra l'evolució de la producció de carn de porcí a Espanya i a Catalunya des de l'any 2007 al 2015 segons les enquestes anuals de sacrifici de bestiar (MAGRAMA, 2015). Aquestes dades reflecteixen, malgrat la crisi, l'increment gradual que hi ha en la producció de carn de porcí a l'estat, i també s'aprecia la importància que té la producció catalana sobre el conjunt de l'estat, ja que un 43,6% de la carn produïda és de Catalunya.

I.1.2 Exportacions de carn de porcí

Les dades d'exportació de carn de porcí són un indicador que la producció porcina estatal i catalana són un important motor econòmic del país. L'estat espanyol és un gran exportador pel que fa a la carn de porcí. En termes econòmics l'exportació de carn de porcí l'any 2015 va representar 2.700 milions d'euros. Es van exportar 1,3 milions de tones, essent un 32% del volum total de la producció (DataComex, 2015). Particularment a Catalunya aquest percentatge va arribar gairebé al 50% de la producció (**Figura 4**).

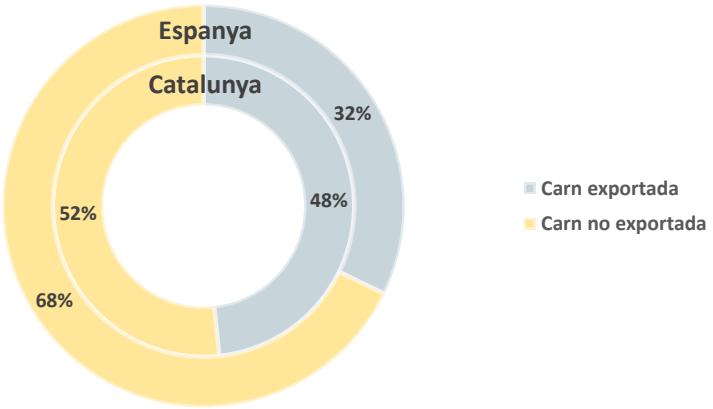


Figura 4. Percentatge de carn de porcí exportada l'any 2015 a Espanya i Catalunya (DataComex, 2015).

Pel que fa a l'exportació de carn per comunitats autònomes, Catalunya és la comunitat més exportadora amb 822 mil tones l'any 2015, seguida per Aragó amb 160 mil tones. Malgrat la crisi econòmica, l'evolució de l'exportació de carn de porcí de les comunitats autònomes més exportadores de l'estat durant els últims anys ha estat positiva (**Figura 5**).

En referència al destí de les exportacions de carn de porcí de l'estat de l'any 2015, un 72% (892 mil tones) es va exportar a països de la Unió Europea. Un 25% (312 mil tones) de les exportacions van a Àsia. La resta de continents (Àfrica, Amèrica i Oceania) també reben carn però de manera molt minoritària. El país que importa més carn procedent de l'estat és França, que l'any 2015 va rebre 271 mil tones de carn de porc. L'any 2015, la meitat del volum exportat es va concentrar només en 4 països: França, Itàlia, Xina i Portugal (DataComex, 2015).

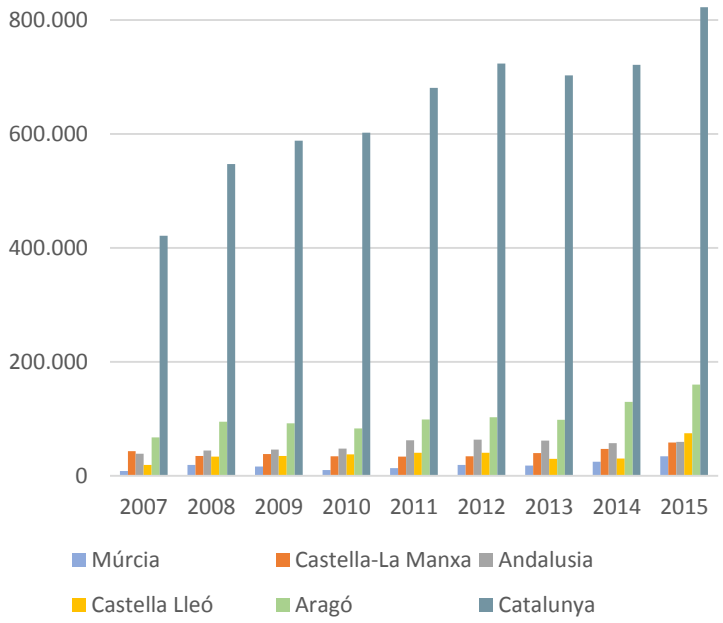


Figura 5. Evolució de l'exportació de carn de porcí (en tones) per comunitats autònomes de l'any 2007 al 2015 (DataComex, 2015).

I.1.3 Consum de carn

El consum de carn de porc en els últims anys ha patit certa davallada en favor d'altres espècies (**Figura 6**). De totes maneres, malgrat la crisi, s'observa certa recuperació, almenys pel que fa a Catalunya (MAGRAMA, 2014).

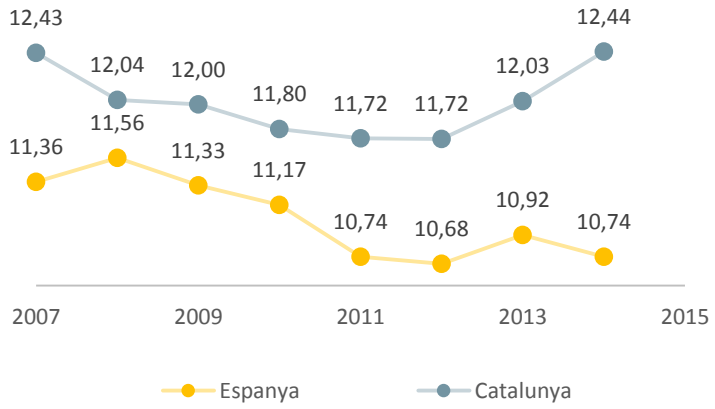


Figura 6. Evolució del consum per capita de carn fresca de porcí a Espanya i Catalunya de l'any 2007 al 2014 (en kg/capita/dia) (MAGRAMA, 2014).

I.2 Pràctiques productives a nivell de la Unió Europea

I.2.1 La pràctica de la castració

A Europa, la castració dels garrins és una pràctica que s'utilitza i s'ha utilitzat per impedir el desenvolupament de conductes sexuals o agressives indesitjables i evitar l'aparició de l'olor sexual (**secció I.3**) a més d'incrementar el greix intramuscular (Lundström*, et al.*, 2009). Es tracta d'una elecció motivada pel mercat, ja que la castració influeix en el tipus, la qualitat i la quantitat de greix i magre (adequat per a productes de qualitat diferenciada). Així que la castració pretén evitar el rebuig per part dels consumidors d'aquesta carn sobretot degut a la presència d'olor sexual (Fredriksen*, et al.*, 2009; Font i Furnols, 2012).

La pràctica de la castració està regulada actualment per la Directiva 2008/120/CE relativa a les normes mínimes per a la protecció dels porcs. Aquesta Directiva codifica la Directiva 91/630/CEE que va ser modificada per la Directiva 2001/93/CE. Aquesta normativa estipula que s'han d'establir normes per garantir unes millors pràctiques. Segons el punt 8 del capítol I d'aquesta Directiva la castració de porcs mascles s'ha de realitzar per mitjans que no comportin esquinçament de teixits i que només la podrà realitzar per part d'un veterinari o una persona formada amb experiència en l'execució de les tècniques, mitjançant els medis adequats i en condicions higièniques; i en el cas que la castració es realitzi

a partir del setè dia de vida, s'ha de dur a terme únicament mitjançant anestèsia i analgèsia prolongada practicada per un veterinari.

L'any 2009 la castració es realitzava en la majoria de països europeus, afectant un 83% dels porcs mascles, uns 100 milions a tot Europa (Fredriksen*, et al.*, 2009). En el cas de Regne Unit i Irlanda gairebé no es castren els animals, o d'altres països com Espanya, Portugal i Xipre on la major part de la producció es destina a mascles sencers (Fredriksen*, et al.*, 2009). A l'estat espanyol l'any 2009 es va estimar que la castració es realitzava al 33% dels garrins. Tot i que actualment no hi ha dades oficials, s'estima que a l'estat espanyol aquest percentatge ha disminuït en els darrers anys (Backus*, et al.*, 2014). Altres països com Alemanya, França i Països baixos han disminuït també el percentatge de garrins castrats (Backus*, et al.*, 2014; Bee*, et al.*, 2015).

I.2.2 Declaració de Brussel·les sobre alternatives a la castració

Com que la castració té un efecte negatiu sobre el benestar animal (Taylor*, et al.*, 2001; AHAW, 2004; Prunier*, et al.*, 2006), hi ha hagut una pressió social per tal d'abandonar aquesta pràctica i trobar altres alternatives per evitar l'aparició d'olor sexual (Tuyttens*, et al.*, 2011). L'any 2010, representants europeus de ramaders, minoristes, membres de la indústria càrnia, ONG de benestar animal i científics van ser convidats per la Comissió Europea i la presidència belga per abordar el tema de la castració a Europa (Brussel·les, 2 de setembre, 13 d'octubre i 19 de novembre de 2010). D'aquesta reunió se'n deriva el que es coneix com la Declaració de Brussel·les: *European Declaration on alternatives to surgical castration of pigs* (DG-SANCO, 2010).

I.2.2.1 Origen de la Declaració de Brussel·les

Des del punt de vista productiu la pràctica de la castració en porcs mascles té diferents efectes sobre els animals: impedeix les conductes sexuals o agressives, evita l'aparició de l'olor sexual, influeix en el tipus, la qualitat i la quantitat de carn i greix (Lundström*, et al.*, 2009). La castració sovint no és una elecció del propi

ramader sinó que és una elecció que ha estat motivada pel mercat. Malgrat els beneficis productius que té la castració, hi ha altres efectes que poden ser considerats negatius, com ho és l'efecte que té sobre el benestar animal.

Tot i que la directiva europea 2001/93/CE permet legalment la castració quirúrgica sense anestèsia abans dels 7 dies de vida, existeixen diversos estudis que han demostrat que l'animal pot patir dolor durant la castració independentment de l'edat (Taylor*, et al.*, 2001; Prunier*, et al.*, 2006). Per tant, aquesta pràctica pot ser perjudicial pel benestar animal, i és aquest fet el que ha portat a les institucions europees al debat sobre l'abandonament de la castració dels garrins.

En alguns països europeus ja es practicaven alternatives a la castració quirúrgica sense anestèsia i analgèsia. A Noruega la castració ja es realitzava amb anestèsia i analgèsia des del 2002, i Suïssa va aprovar la immunocastració el gener de 2007. Als Països Baixos es va promoure amb la Declaració de Noordwijk de 2007 que a partir de l'1 de gener de 2009 els supermercats deixessin de vendre carn de porcs castrats sense anestèsia i analgèsia, amb l'objectiu d'abandonar completament la castració quirúrgica l'any 2015. Alemanya va redactar la Declaració de Düsseldorf de 2008 per utilitzar mitjans per minimitzar el dolor dels animals durant la castració. La Declaració d'Àustria i la Declaració de Bèlgica també van anar en la mateixa línia per tal de potenciar l'ús d'analgèsia en el moment de la castració. Totes aquestes actuacions a diferents països europeus són les predecessores de la Declaració de Brussel·les de 2010 que vol promoure l'eliminació de la castració quirúrgica dels porcs a Europa.

I.2.2.2 Contingut de la Declaració de Brussel·les

La Declaració de Brussel·les estableix dues fases o etapes en les que, de manera voluntària, s'hauria d'abandonar progressivament la castració quirúrgica. Com a una primera fase, a partir de l'1 de gener de 2012, si es castra quirúrgicament, es farà amb anestèsia i analgèsia prolongades. En la segona fase i més a llarg termini, la castració quirúrgica s'hauria de deixar de practicar a partir del dia 1 de gener de 2018.

Abandonar la castració quirúrgica suposa un repte pels productors, per això la Declaració de Brussel·les inclou un seguit de mitjans que haurien de facilitar aquest canvi i garantir la desaparició de la castració dels porcs (**Taula 1**).

Taula 1. Eines que haurien d'estar disponibles per facilitar l'abandonament de la castració segons la Declaració de Brussel·les (DG-SANCO, 2010).

a)	Mètodes mútuament reconeguts per avaluar l'olor sexual
b)	Mètodes de referència reconeguts a Europa per mesurar els compostos responsables de l'olor sexual
c)	Mètodes de detecció ràpida de l'olor sexual als escorxadors
d)	Reducció dels compostos que causen olor sexual mitjançant pràctiques relacionades amb la selecció genètica, el maneig i l'alimentació dels porcs
e)	Sistemes de producció i maneig dels mascles sencers duran la cria, el transport i el sacrifici orientats a minimitzar les conductes sexuals i agressives
f)	Alternatives a la castració quirúrgica en el cas dels productes tradicionals d'alta qualitat

Per altra banda el punt 6 de la Declaració de Brussel·les contempla la creació d'una llista de productes tradicionals que serien una excepció a la Declaració i que en els que es podria seguir utilitzant la castració amb analgèsia i/o anestèsia. Aquests productes serien el cas de carn registrada sota Denominació d'Origen Protegida (DOP), Indicació Geogràfica Protegida (IGP), Especialitat Tradicional Garantida (ETG) o carn produïda per productes tradicionals d'alta qualitat. Actualment un projecte europeu anomenat CASTRUM (*Pig castration: methods of anaesthesia and analgesia for all pigs and other alternatives for pigs used in traditional products***,** contract number SANTE/2015/G3/SI2.723717) està treballant per identificar quins productes podrien incloure's en aquesta llista.

I.2.3 Actuacions a diferent països per canviar el sistema de producció

Diferents països europeus han començat a aplicar accions per deixar de castrar els porcs seguint les indicacions de la Declaració de Brussel·les (**Figura 7**).

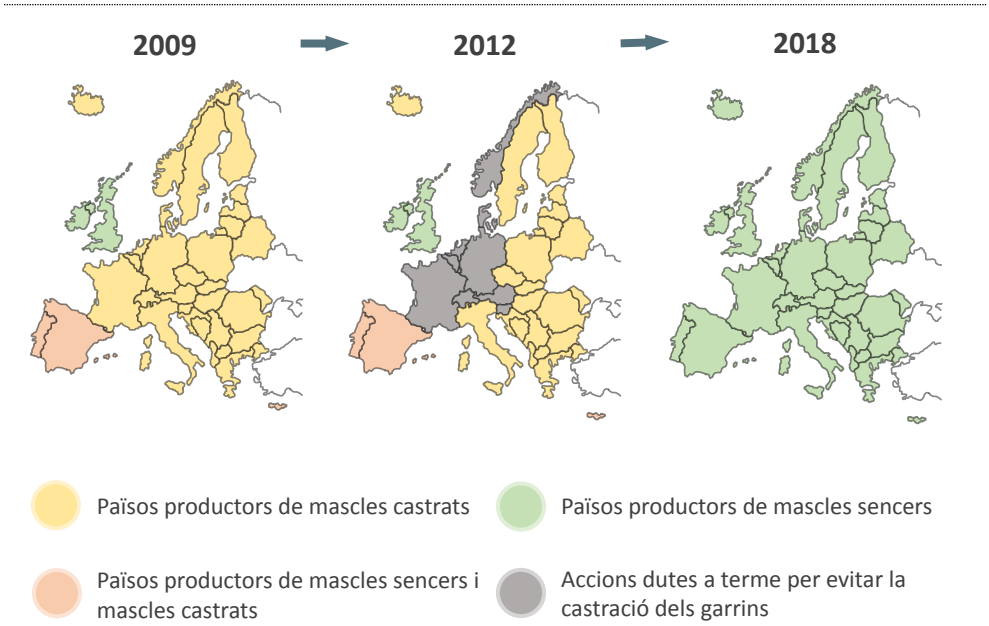


Figura 7. Pràctiques relatives a la producció porcina en diferents països europeus.

Algunes cadenes de supermercats s'estan enfocant també en la venda de productes més respectuosos amb el benestar animal. A Bèlgica alguns supermercats ja no venen carn procedent de mascles castrats i a Alemanya alguns supermercats ho deixaran de fer a partir de l'any 2017. De fet, en el primer informe sobre el progrés de la declaració de Brussel·les ja s'observa que la producció de mascles sencers ha incrementat a Alemanya, Bèlgica, Espanya, França i Països Baixos (Backus*, et al.*, 2014).

Així doncs, l'objectiu principal de la Declaració de Brussel·les és l'abandonament de la castració el 2018. Amb això, la Unió Europea vol potenciar, en últim terme, la producció de porcs mascles sencers. De totes maneres, actualment s'està treballant per identificar possibles excepcions per a productes on es permetria seguir castrant els animals (*Pig castration: methods of anaesthesia and analgesia for all pigs and other alternatives for pigs used in traditional products***,** n. SANTE/2015/G3/SI2.723717, DG SANTE).

I.3 Olor i gust de la carn de porc: olor sexual

Un dels principals problemes que podrien trobar-se els productors a l'hora de produir porcs mascles sencers, és el que es coneix com olor sexual. L'olor sexual es defineix com una olor forta i desagradable que pot estar present en algunes canals porcines, especialment en aquelles procedents de mascles sencers. Es tracta d'un defecte en la qualitat sensorial de la carn que pot ser percebut per part dels consumidors. Aquesta olor és deguda principalment a dos compostos: androstenona i escatol (Bonneau, 1982; Bonneau*, et al.*, 1992; Andresen, 2006; Bonneau i Chevillon, 2012; Font i Furnols, 2012; Haugen*, et al.*, 2012), tot i que també s'han descrit altres compostos com l'indol o androstenols però amb molta menys importància (García-Regueiro i Diaz, 1989). La presència d'olor sexual a la canal és deguda a una excessiva acumulació d'almenys un d'aquests dos compostos en el greix de l'animal (Claus*, et al.*, 1994). L'androstenona i l'escatol són compostos volàtils (Denhard*, et al.*, 1995; Rius*, et al.*, 2005), és per això que aquesta olor es detecta especialment a l'hora de cuinar la carn, degut a que les substàncies responsables s'alliberen en augmentar la temperatura.

En funció de la concentració de cada un dels compostos en el greix, s'han definit uns llindars de rebuig sobre els quals alguns consumidors podrien percebre l'olor i rebutjar el producte (Font i Furnols*, et al.*, 2000). Diferents estudis han definit llindars per l'androstenona en 0,5 i 1,0 µg/g de greix (Rhodes, 1971; Claus*, et al.*, 1994; Font i Furnols*, et al.*, 2003; Allison*, et al.*, 2011) i per l'escatol en 0,10 i 0,20 µg/g de greix (Desmoulin*, et al.*, 1982; Bonneau*, et al.*, 1992; Claus*, et al.*, 1994; Walstra*, et al.*, 1999; Font i Furnols*, et al.*, 2003; Allison*, et al.*, 2011). La **Taula 2** mostra una classificació en nivells d'olor sexual segons els llindars definits a partir de la reacció dels consumidors per androstenona i escatol.

Pel que fa a la percepció per part dels consumidors dels compostos responsables de l'olor sexual, diversos estudis han demostrat que les dones són més sensibles que els homes (Wysocki i Beauchamp, 1984; Weiler*, et al.*, 2000; Bremner*, et al.*, 2003; Lunde*, et al.*, 2009; Blanch*, et al.*, 2012). La problemàtica de l'olor es relaciona principalment amb nivells alts d'escatol, mentre que el flavor està relacionat també amb l'androstenona (Matthews*, et al.*, 2000; Aluwé*, et al.*, 2015).

Taula 2. Nivells d'olor sexual segons la concentració llindar d'androstenona i escatol (Font i Furnols*, et al.*, 2000).

Nivell	Androstenona (µg/g de greix)	Escatol (µg/g de greix)
Alt	>1,0	>0,20
Mig	0,5 - 1,0	0,10 - 0,20
Baix	<0,5	<0,10

I.3.1 Androstenona

Un dels principals compostos responsables de l'olor sexual va ser aïllat i identificat per Patterson (1968) per espectrometria de masses com a 5α-androst-16-ene-3-one (androstenona). Es tracta d'una feromona sexual produïda als testicles que indueix el zel de les femelles, així que en últim terme té una funció reproductora (Melrose*, et al.*, 1971; Claus*, et al.*, 1981).

L'androstenona es sintetitza a les cèl·lules de Leydig dels testicles dels porcs mascles juntament amb la testosterona (Zamaratskaia i Squires, 2009) i també, tot i que en menys mesura, a les glàndules adrenals (Ahmad i Gower, 1968). Després de ser sintetitzada s'allibera cap a la circulació a través de la vena espermàtica (Gower*, et al.*, 1970). Com que l'androstenona és un compost hidrofòbic i lipofílic es transporta a través de la sang i s'acumula al teixit adipós (Bonneau, 1982; Brooks i Pearson, 1986; Claus*, et al.*, 1994). Aquesta acumulació és, però, reversible i l'androstenona pot retornar al sistema circulatori. Una de les vies que té l'organisme per eliminar l'androstenona és el catabolisme hepàtic a través de l'enzim porcí 3ß-HSD (Chen*, et al.*, 2015). També hi ha una via d'eliminació a través de l'orina (Gower i Patterson, 1970) o a través de les glàndules salivals com a feromona sexual (Claus*, et al.*, 1981). La **Figura 8** mostra un esquema de la distribució de l'androstenona des de la seva síntesi fins a la seva eliminació (Xue i Dial, 1997).

La problemàtica de la presència d'androstenona en la carn fresca ve precisament per aquesta acumulació d'androstenona a nivell de greix. Es presenta com una intensa olor a orina (Blanch*, et al.*, 2012) i es detecta principalment en la carn de mascles sencers, de manera que no es troba en mascles castrats o femelles (Patterson, 1968; Bonneau, 1982).

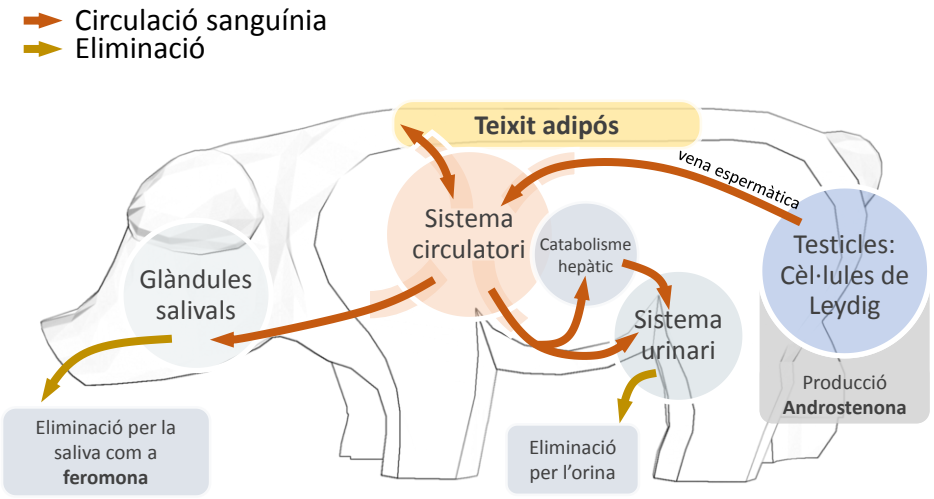


Figura 8. Diagrama de producció, eliminació i acumulació de l'androstenona.

L'androstenona és una substància que no és percebuda per tots els consumidors. Aquesta incapacitat de certs individus per percebre una substància és el que es coneix com a anòsmia. La percepció de l'androstenona està determinada genèticament (Wysocki i Beauchamp, 1984) i està relacionada amb l'expressió genètica del receptor olfactiu humà OR7D4 (Keller, *et al.*, 2007). Diversos estudis han avaluat la sensibilitat a l'androstenona i s'ha vist que al voltant d'un 40-50% de la població és anòsmica a aquest compost (Weiler, *et al.*, 2000; Font i Furnols, *et al.*, 2003; Blanch, *et al.*, 2012; Razafindrazaka, *et al.*, 2015). L'androstenona s'ha descrit amb atributs molt diversos, però els més freqüents són: suor, dolç, químic i brut (Annor-Frempong, *et al.*, 1997; Font i Furnols, *et al.*, 2000; Blanch, *et al.*, 2012). Un estudi de Panella-Riera, *et al.* (2016) classifica els consumidors segons si els agrada la carn de porc, els agrada l'androstenona o bé la rebutgen.

I.3.2 Escatol

Un altre dels principals compostos responsables de l'olor sexual és el 3-metilindol (escatol), que va ser aïllat per Vold (1970). Es tracta d'un producte de degradació de l'aminoàcid triptòfan produït per la flora microbiana al tracte digestiu de remugants (Yokoyama i Carlson, 1979), humans (Fordtran, *et al.*, 1964), porcs

(Jensen*, et al.*, 1995) i altres monogàstrics (Deslandes*, et al.*, 2001). Es troba present en les femtes d'aquestes espècies (Dehnhard*, et al.*, 1991) i la quantitat que no s'excreta s'acumula al greix (Zamaratskaia*, et al.*, 2006).

L'origen del triptòfan és controvertit. Per una banda, pot procedir directament de la dieta (Claus*, et al.*, 1994), tot i que diferents estudis han demostrat que l'increment de triptòfan a la dieta no té un efecte directe en la concentració d'escatol (Claus*, et al.*, 1994; Wesoly i Weiler, 2012). Per altra banda, les restes cel·lulars degudes a la proliferació i apoptosi de les cèl·lules de la mucosa de l'intestí prim que arriben al còlon són provablement la principal font de triptòfan (Claus*, et al.*, 1994; Raab*, et al.*, 1998; Claus i Raab, 1999; Wesoly i Weiler, 2012). En arribar a còlon, el triptòfan és degradat per microorganismes anaerobis d'una manera similar a la que es produeix en el rumen dels remugants (Chung*, et al.*, 1975; Yokoyama i Carlson, 1979; Jensen*, et al.*, 1995). El metabolisme del triptòfan per part dels microorganismes intestinals porta a la producció de dos compostos volàtils: indol i escatol (Deslandes*, et al.*, 2001). Una vegada produït, l'escatol pot quedar-se a la llum intestinal i ser excretat amb les femtes, però prop d'un 87% és absorbit per la mucosa intestinal (Agergaard i Laue, 1993; Xue i Dial, 1997; Deslandes*, et al.*, 2001). L'escatol és transportat a través de la vena porta al fetge, on és metabolitzat principalment per l'enzim hepàtic citocrom P450IIE1 (Babol*, et al.*, 1998). S'ha descrit que l'androstenona pot tenir un efecte inhibidor sobre l'enzim que catabolitza l'escatol (Doran*, et al.*, 2002; Tambyrajah*, et al.*, 2004), així que la presència d'androstenona també pot portar a una major acumulació d'escatol. És per això que la carn de castrat i femella té menys acumulació d'aquest compost. Els metabòlits de la degradació de l'escatol són eliminats a través de l'orina (Friis, 1993), mentre que l'escatol que no s'ha metabolitzat s'acumula al teixit adipós (Claus*, et al.*, 1994). La **Figura 9** mostra un esquema de la síntesi d'escatol des del seu origen fins a la seva acumulació i eliminació.

La problemàtica de la presència d'escatol en la carn fresca ve, com en el cas de l'androstenona, de la seva acumulació al teixit adipós sobretot en les canals de porcs mascles sencers. L'escatol es percep com una olor a femta o naftalina (Patterson, 1968; Bonneau, 1982).

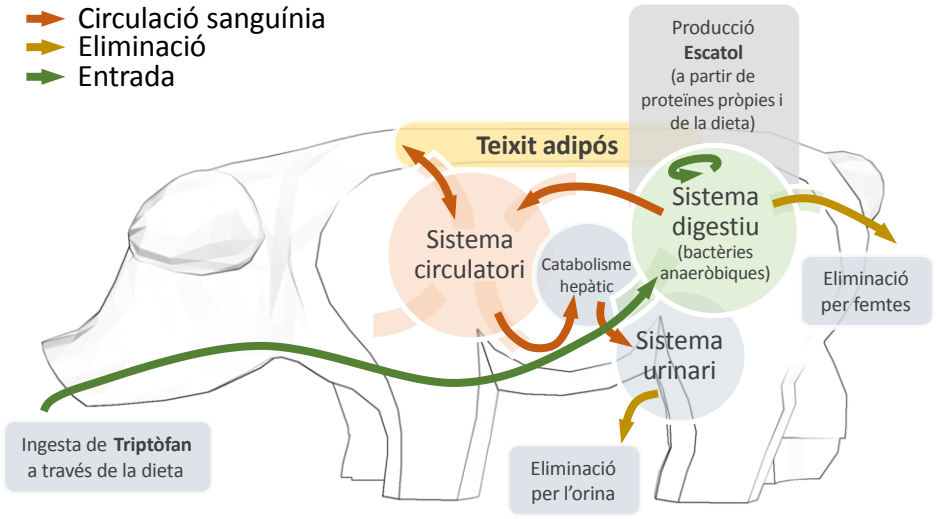


Figura 9. Diagrama de producció, eliminació i acumulació de l'escatol.

A diferència de l'androstenona, l'escatol és percebut per un 99% dels consumidors (Weiler*, et al.*, 1997). Al ser un compost que es volatilitza abans que l'androstenona (Rius*, et al.*, 2005), alguns estudis li posen més pes com a responsable de l'olor sexual (Font i Furnols, 2012). L'escatol s'ha descrit amb atributs també molt diversos: porc, estable, resclosit, naftalina i dolç (Dijksterhuis*, et al.*, 2000).

I.3.3 Prevalença d'olor sexual

L'olor sexual té una prevalença que pot variar en funció de la genètica i el maneig dels animals. Per tal d'estudiar quin és l'abast d'aquesta qüestió a nivell productiu, diversos estudis s'han centrat en la prevalença d'olor sexual en poblacions porcines. Malmfors i Hansson (1974) van analitzar canals porcines de mascles sencers per determinar la prevalença d'olor sexual a Suècia i van trobar que un 20% dels animals presentava aquesta olor. En un estudi realitzat en 6 països europeus (Dinamarca, Espanya, França, Països Baixos, Regne Unit, Suècia), Walstra*, et al.* (1999) van trobar que un 29% de les canals analitzades presentaven nivells alts d'androstenona (>1,0 µg/g de greix) i un 15% presentaven nivells alts d'escatol (>0,2 µg/g de greix). Allison*, et al.* (2011) va estudiar la prevalença d'androstenona i escatol en 8 països europeus (Alemanya, Dinamarca, Espanya, França, Hongria,

Països Baixos, Regne Unit i República Txeca) i va trobar que un 9,1% presentava nivells alts d'androstenona i escatol. Prusa*, et al.* (2011) va fer un estudi similar als Estats Units trobant que un 55,8% de les canals tenien nivells alts d'androstenona i un 34,2% d'escatol.

Si bé és cert que aquests estudis han analitzat les mostres amb metodologies diferents, si que permeten una aproximació a quina és la prevalença d'olor sexual en les canals porcines. De totes maneres és necessari un estudi amb més profunditat a nivell de l'estat espanyol que permeti classificar les canals porcines en funció dels diferents llindars d'olor sexual (baix, mig i alt). Això permetria determinar més acuradament quin és el percentatge de canals en presenten nivells alts, ja que són aquestes les que podrien ser rebutjades per part dels consumidors.

I.3.4 Legislació sobre l'olor sexual

El Reglament (CE) Nº 854/2004 del Parlament Europeu i del Consell de 29 d'abril de 2004 estableix les normes específiques per a la organització de controls oficials dels productes d'origen animal destinats a consum humà. En el capítol V de l'annex 1 recull que la carn serà declarada no apte pel consum humà si presenta anomalies organolèptiques, en particular, un olor sexual fort. Encara que existeixi una regulació a nivell europeu d'aquest defecte sensorial vigent des de 2004, el fet que no existeixi actualment cap mesura eficaç i ràpida per controlar si la carn presenta aquesta olor anòmala dificulta molt l'aplicació d'aquesta normativa en aquest sentit, i, per tant, no s'aplica.

I.4 Alternatives a la castració

La pràctica de la castració evita la presència d'olor sexual produïda per l'acumulació d'androstenona i escatol a la carn (Bonneau, 1982; Bonneau*, et al.*, 2000). Si la Declaració de Brussel·les pretén eliminar la castració quirúrgica en un futur, és evident que s'han de trobar alternatives viables i aplicables per part del sector perquè la producció porcina no se'n vegi afectada. Hi ha diferents alternatives a la

castració quirúrgica viables actualment: la immunocastració, la selecció espermàtica i la producció de mascles sencers.

La immunocastració és un mètode aplicable a la granja que permet que els animals tinguin un creixement més magre (Pauly*, et al.*, 2009; Fàbrega*, et al.*, 2010). L'objectiu és inhibir el desenvolupament testicular mitjançant la neutralització amb anticossos de l'hormona alliberadora de gonadotropina (Gn-RH). Per tal d'assegurar l'efectivitat de la immunocastració és necessari l'aplicació de dues vacunes separades de 4 a 6 setmanes; i després de l'última injecció és necessari un temps d'espera de 4 o 5 setmanes abans del sacrifici. Aquesta tècnica es va aprovar a Europa l'any 2009.

La selecció espermàtica per a la producció de femelles podria ser també una alternativa a la castració quirúrgica i a la producció de mascles sencers (Vazquez*, et al.*, 2009). La tècnica consisteix en seleccionar, mitjançant citometria de flux, els espermatozoides que contenen el cromosoma X i utilitzar només aquests espermatozoides per a la inseminació de les truges. Aquesta opció encara no està disponible a nivell comercial ja que el nombre d'espermatozous que es poden arribar a separar per sexe és baix, a més de tenir un cost econòmic elevat.

Finalment, l'alternativa que des del punt de vista de benestar animal està més ben acceptada és la producció de porcs mascles sencers, ja que els animals no pateixen cap intervenció quirúrgica ni tampoc cap actuació farmacològica. Aquests animals produeixen canals més magres (Pauly, et al., 2009) però poden presentar olor sexual (Font i Furnols, et al., 2000). L'inconvenient de la producció de mascles sencers és la sortida de mercat que pot tenir aquesta carn, ja que si presenta defectes sensorials com és l'olor sexual pot no ser acceptada pels consumidors.

I.4.1 Diferències en la qualitat de la carn segons el sexe de l'animal

La pràctica de la castració té un altre objectiu a banda d'evitar l'aparició d'olor sexual en la carn. La carn de porcs mascles castrats presenta més greix intramuscular i, per tant, té una qualitat sensorial diferent que n'afecta l'acceptabilitat (Bañón*, et al.*, 2004; Gispert*, et al.*, 2010; Aluwé*, et al.*, 2013). Si bé

és cert que els mascles sencers són més eficients des del punt de vista productiu, ja que presenten un major índex de conversió (Fàbrega*, et al.*, 2010), les canals produïdes són més magres i més dures que les que procedeixen de mascles castrats, que tenen més greix infiltrat i més saturat (Pauly*, et al.*, 2009; Gispert*, et al.*, 2010). Aquesta major infiltració de greix és el que busquen certes empreses per a l'elaboració dels seus productes.

Gispert*, et al.* (2010) van estudiar diferents característiques de qualitat de la canal i de la carn de porcs mascles sencers, castrats, immunocastrats i femelles. En aquest estudi pel que fa al greix van observar que les canals procedents de mascle sencer tenien un percentatge de magre més elevat que les canals de mascle castrat i immunocastrat. Pel que fa a la carn també van observar que el percentatge de greix infiltrat en el múscul semimembranós era significativament superior en els mascles castrats respecte els sencers (**Taula 3**). Aquest és un dels punts clau a l'hora d'elaborar certs tipus de productes de qualitat diferenciada que busquen una major quantitat de greix intramuscular. La qualitat de la carn procedent de mascle sencer a part de poder presentar olor sexual, té un percentatge de greix intramuscular inferior. Pel que fa a la qualitat sensorial de la carn Font i Furnols*, et al.* (2008) van estudiar l'acceptabilitat de consumidors de carn procedent de mascle sencer, castrat, immunocastrat i femella. Els resultats de l'estudi mostren que la carn procedent de mascle sencer és menys acceptada pel que fa l'olor i el flavor per part dels consumidors respecte els altres tipus de carn.

Així que, per una banda existeix una diferència de qualitat pel que fa a la presència de greix intramuscular que pot tenir un efecte sobre la producció de carn i productes carnis d'alta qualitat. Per altra banda hi ha una menor acceptabilitat de la carn de mascles sencers per part dels consumidors (Font i Furnols, 2012). En aquest sentit s'ha de determinar en quin grau podria afectar al sector una possible prohibició de la castració tenint en compte aquests aspectes de qualitat, però tenint en compte també que la producció de mascles és més eficient degut al major índex de conversió (**Taula 3**).

Taula 3. Comparativa entre els índexs de conversió i el greix intramuscular entre els diferents sexes (Fàbrega*, et al.*, 2010; Gispert*, et al.*, 2010).

	Mascles castrats	Immuno-castrats	Femelles	Mascles sencers
Índex de conversió (pinso/pes)	2.76[a]	2.51[c]	2.61[b]	2.48[c]
Greix intramuscular al múscul semimembranós	2.47[a]	2.07[ab]	1.72[b]	1.84[b]

Diferents superíndexs indiquen diferències significatives entre columnes

I.4.2 Canvi en el sistema productiu cap a producció de mascles sencers

La producció de porcs mascles sencers suposa una millora en l'eficiència productiva respecte la producció de porcs castrats (Lundström*, et al.*, 2009). De fet alguns països europeus ja han fet un canvi en el sistema productiu cap a mascles sencers per aquesta millor eficiència (Bee*, et al.*, 2015). Per altra banda, però, el fet que part d'aquesta carn pugui tenir presència d'olor sexual pot propiciar un rebuig per part d'alguns consumidors (Lundström*, et al.*, 2009).

Per tant, una possible prohibició de la castració portaria a produir animals amb un percentatge de magre més elevat, amb un millor índex de conversió, una menor producció de fems, menor incidència de malaltia i millor benestar (Walstra, 1974; Bonneau, 1998; Lundström*, et al.*, 2009). De totes maneres, s'ha de tenir en compte que aquest canvi de producció comportar la presència de canals i de carn amb olor sexual (Bonneau, 1998).

Així doncs, el fet que incrementi la presència de carn procedent de mascles sencers al mercat pot implicar un major rebuig d'aquesta carn per part d'alguns consumidors. Per tant, és necessari desenvolupar un mètode ràpid i efectiu a nivell d'escorxador per detectar les canals amb presència d'olor sexual (Bonneau, 1998; Lundström*, et al.*, 2009) i també és important trobar un ús adequat per aquesta carn (Bonneau, 1998; Lunde*, et al.*, 2008).

En el cas que hi hagi una possible prohibició de la castració a nivell europeu s'ha d'avaluar en primer lloc si la comercialització de carn procedent de porcs mascles sencers seria un problema pel sector porcí. A més, en aquest cas seria útil

conèixer abans si la indústria, els exportadors, els punts de venda i els consumidors acceptarien aquest canvi en el sistema productiu.

I.5 Actituds dels consumidors i decisió de compra

La producció de porcs mascles sencers suposa una millora en el benestar animal (Prunier*, et al.*, 2006). Diversos estudis han conclòs que els consumidors estan disposats a pagar més per assegurar el benestar animal (Bennett i Larson, 1996; Napolitano*, et al.*, 2010). De totes maneres és necessària una visió més àmplia d'aquests resultats, ja que els consumidors tenen en compte molts més atributs a l'hora de comprar carn de porc.

Kallas*, et al.* (2013) van estudiar, dins el projecte europeu ALCASDE (2008), quina era la importància relativa de la castració respecte altres atributs de la carn per part dels consumidors en 6 països europeus (Alemanya, Espanya, Itàlia, França, Països Baixos i Regne unit). Els atributs de la carn avaluats van ser el sexe de l'animal, l'olor i el sabor, l'origen, i el preu. L'estudi conclou que hi ha un desconeixement dels consumidors pel que fa a la castració dels animals, a més la importància relativa dels atributs en la decisió de compra és diferent segons el país estudiat. Pel que fa al sexe de l'animal, té més importància relativa pels consumidors de Països Baixos i Regne Unit que pels consumidors d'Espanya i França, que consideren que el fet de conèixer el sexe de l'animal és menys important a l'hora de comprar carn fresca de porc. En tots els països estudiats, l'atribut que més tenen en compte els consumidors a l'hora de comprar carn de porc és l'olor i el sabor, representant entre un 40% i un 57% de la decisió de compra. Per tant, si la Unió Europea pretén abandonar la castració quirúrgica, l'increment de carn procedent de porcs mascles sencers al mercat podria afectar en un últim terme l'acceptabilitat dels consumidors més sensibles a l'olor sexual (Lundström*, et al.*, 2009; Panella-Riera*, et al.*, 2016).

I.6 Estudis de consumidors sobre olor sexual

Una de les principals raons per castrar és la presència d'olor sexual en els mascles sencers, fet que pot comportar el rebuig de la carn procedent d'aquests animals per part d'alguns consumidors (Desmoulin*, et al.*, 1982; Diestre*, et al.*, 1990; Font i Furnols*, et al.*, 2000; Bañón*, et al.*, 2004; Meier-Dinkel*, et al.*, 2013).

A l'hora d'estudiar el potencial d'un nou producte, la opinió dels consumidors és bàsica ja que es troben a la part final de la cadena (Amerine*, et al.*, 1965; Font i Furnols, 2012). Per tant, els estudis de consumidors són necessaris per a avaluar l'acceptabilitat sensorial i les actituds sobre els sistemes de producció porcina (Font i Furnols, 2012). S'han realitzat molts estudis de consumidors sobre l'olor sexual però les comparacions són sovint difícils degut a les diferents metodologies emprades en cadascun d'ells (Font i Furnols, 2012). Harmonitzar les metodologies és també una tasca difícil ja que segons l'objectiu de l'estudi una metodologia pot ser més adient que una altra (Aaslyng*, et al.*, 2007). A banda de la metodologia de preparació de la mostra, també és important tenir en compte la influència que pot tenir en la percepció sensorial (Taylor, 1998) el tipus de recipient en el qual es presenta, ja que els compostos responsables de l'olor sexual són compostos volàtils (Denhard, et al., 1995).

Està clar que la presència d'olor sexual en la carn pot afectar la percepció i l'acceptabilitat dels consumidors. De totes maneres cal tenir en compte que l'androstenona només és percebuda per un 40% del consumidors (Panella-Riera*, et al.*, 2010; Blanch*, et al.*, 2012), mentre que l'escatol és percebut per un 99% d'aquests (Weiler*, et al.*, 1997). A més, la sensibilitat d'aquells consumidors que són capaços de detectar l'androstenona és diferent i també ho és la seva acceptabilitat: alguns consumidors la perceben com una olor agradable, mentre que d'altres la perceben com a desagradable o neutre (Blanch*, et al.*, 2012; Panella-Riera*, et al.*, 2016). Per tant, seria convenient harmonitzar els atributs que s'avaluen. Font i Furnols (2012) suggereix incloure sempre l'acceptabilitat de l'olor i del sabor en els estudis de consumidors.

I.7 Mètodes per emmascarar l'olor sexual

La carn de porc es pot cuinar i preparar de maneres molt diverses. Com que els compostos responsables de l'olor sexual són compostos volàtils (Denhard*, et al.*, 1995), està demostrat el mètode de cocció emprat per cuinar la carn té un clar efecte sobre la percepció d'aquesta olor (Agerhem i Tornberg, 1995; Wood*, et al.*, 1995; Prestat*, et al.*, 2002; Font i Furnols, 2012).

Els mètodes de cocció es poden classificar segons el medi utilitzat per transmetre la calor (líquid o no), i segons com s'escalfa (foc directe, superfície calenta o aire calent); a banda hi ha noves tecnologies com el microones o la cuina al buit que també es poden utilitzar (Bello, 1998). La utilització de oli per fregir el producte pot tenir un efecte sobre la percepció sensorial (Prestat*, et al.*, 2002). La cuina al buit prevé l'aparició d'algunes olors anòmales, tot i que també evita la pèrdua dels compostos volàtils i a banda millora la textura del producte (Schafheitle, 1990; Armstrong i McIlveen, 2000).

Existeixen també altres eines i metodologies a l'hora de cuinar la carn on s'afegeixen ingredients durant la cocció. La utilització de plantes i herbes aromàtiques és molt comú en la cuina mediterrània (Bianchi, 2015) i és una estratègia a tenir en compte a l'hora de buscar una fórmula per emmascarar l'olor sexual. Per altra banda l'arrebossat amb pa ratllat també és una estratègia molt utilitzada, que juntament amb el fregit és útil per millorar la palatabilitat de la carn (Antonova*, et al.*, 2003).

La temperatura de consum del producte és també rellevant, ja que un producte que es consumeix fred desprèn menys olor que si es consumeix calent (Lunde*, et al.*, 2008; Martínez*, et al.*, 2016). Lunde*, et al.* (2008) van concloure que el llom adobat era una bona estratègia per disminuir la percepció de l'olor sexual, especialment quan es servia a 15ºC de temperatura. Stolzenbach*, et al.* (2009) van demostrar que el procés de fumat era una bona opció per emmascarar l'olor sexual en salsitxes fermentades. Aaslyng*, et al.* (2015) van estudiar estratègies de fumat i addició d'espècies, les quals van resultar útils per disminuir la percepció d'olor sexual en bacó. Martínez*, et al.* (2016) van estudiar diferents estratègies d'emmascarament en salsitxes Frankfurt i van concloure també que la utilització

d'espècies juntament amb el fumat disminuïa la percepció d'androstenona. Egea*, et al.* (2014) i Linares*, et al.* (enviat per publicar) van estudiar diferents estratègies de cocció mitjançant un panel entrenat i van concloure que el fregit i la cuina al buit eren les estratègies més útils per disminuir la percepció d'olor sexual en llom. Egea*, et al.* (enviat per publicar) van estudiar diferents estratègies d'emmascarament mitjançant un panel entrenat i van concloure que l'arrebossat amb all i julivert va ser la millor opció per disminuir la percepció d'olor sexual en llom. En aquest sentit caldria estudiar si aquestes estratègies aplicades en llom fresc són útils també en consumidors.

Capítol II

Objectius

Aquesta tesi doctoral s'inclou dins del projecte de recerca INIA RTA2011-00027-C02-01 finançat per l'Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) que porta per títol *Potencial* de *mercado y calidad de carne y productos cárnicos procedentes de cerdos machos enteros ante la perspectiva europea de prohibición de la castración (BOARMARKET)*. A més de l'IRTA (on s'ha tutelat i dirigit la present tesi), en aquest projecte hi van participar el Centre de Recerca en Economia i Desenvolupament Agroalimentari (CREDA-UPC-IRTA), la Universidad de Murcia (UMU), la Universidad Complutense de Madrid (UCM) i l'Instituto Tecnológico Agrário de Castilla y León (ITACyL). El plantejament de l'estudi va enfocat en quantificar quina és realment l'abast d'aquesta qüestió i en avaluar quina és l'acceptació de carn procedent de porcs mascles sencers per part del mercat davant una possible prohibició de la castració a nivell europeu. A més, es pretén buscar solucions a la carn de mascle sencer amb olor sexual mitjançant diferents estratègies tecnològiques amb la finalitat d'obtenir-ne una millor acceptació per part del consumidor.

L'objectiu global de la present tesi és analitzar l'estat actual de la producció de mascles sencers a l'estat espanyol com a alternativa al mercat de castrat. Els objectius específics de la tesi són principalment tres:

1. Estudiar quina és la prevalença de canals de porcs mascles sencers amb presència de nivells alts d'olor sexual en aquelles comunitats autònomes de l'estat espanyol que concentren la major part de la producció porcina: Aragó, Catalunya, Castella-La Mancha, Castella i Lleó, Madrid i Múrcia.
2. Conèixer i analitzar l'opinió dels diferents actors de la cadena de producció porcina –des de la granja fins al consumidor– sobre l'impacte que tindria una possible prohibició de la castració. Per altra banda també es vol quantificar l'opinió dels carnissers sobre els diferents aspectes de la producció de mascles sencers com són la qualitat de carn, el benestar animal i les actituds dels consumidors.
3. Comparar l'acceptabilitat per part de consumidors de la carn fresca, procedent de porcs castrats i mascles sencers amb nivells alts d'androstenona, preparada amb diferents estratègies de cocció i emmascarament (cuinat al buit; arrebossat amb all i julivert i fregit). Avaluar

la sensibilitat dels consumidors a l'androstenona. La finalitat última és estudiar quin percentatge de consumidors pot acabar rebutjant la carn amb presència d'androstenona i veure si les estratègies proposades són útils per disminuir la percepció de l'olor sexual en la carn de porcs mascles sencers i millorar-ne l'acceptabilitat.

No existeixen dades publicades de l'estat espanyol sobre els objectius que es plantegen i, en relació als estudis de consumidors, tampoc existeixen resultats recents sobre com pot afectar el sistema de cocció, o l'emmascarament de l'olor sexual en la qualitat i en l'acceptabilitat de la carn de porc. Cal recordar que a l'estat espanyol, tot i que es comercialitza carn de mascle sencer, no està resolt el tema de l'olor sexual. Les investigacions que es plantegen van encaminades a produir dades sobre l'impacte que pot tenir una possible prohibició de la castració quirúrgica a nivell europeu sobre la qualitat sensorial de la carn de porc.

Així doncs, tenint en compte tots els aspectes exposats, aquesta tesi doctoral pretén respondre 3 preguntes:

- Quin percentatge real de canals poden presentar nivells alts d'olor sexual?
- Quina opinió té el sector sobre una possible prohibició de la castració?
- Quin percentatge de consumidors pot rebutjar la carn amb olor sexual, amb especial èmfasi en l'androstenona, i quin destí es pot donar a la carn amb nivells alts d'androstenona?

Capítol III

Metodologia

La present tesi consta de 3 estudis directament relacionats per poder avaluar, en la seva totalitat i en cadascun dels diferents actors implicats, l'efecte d'una possible prohibició de la castració de porcs a Europa. Cada estudi correspon a un article de la tesi i en cada article ja es detallen els mètodes aplicats. La **Figura 10** esquematitza la metodologia del conjunt del treball i els següents apartats d'aquest capítol expliquen breument la metodologia de cada un dels estudis.

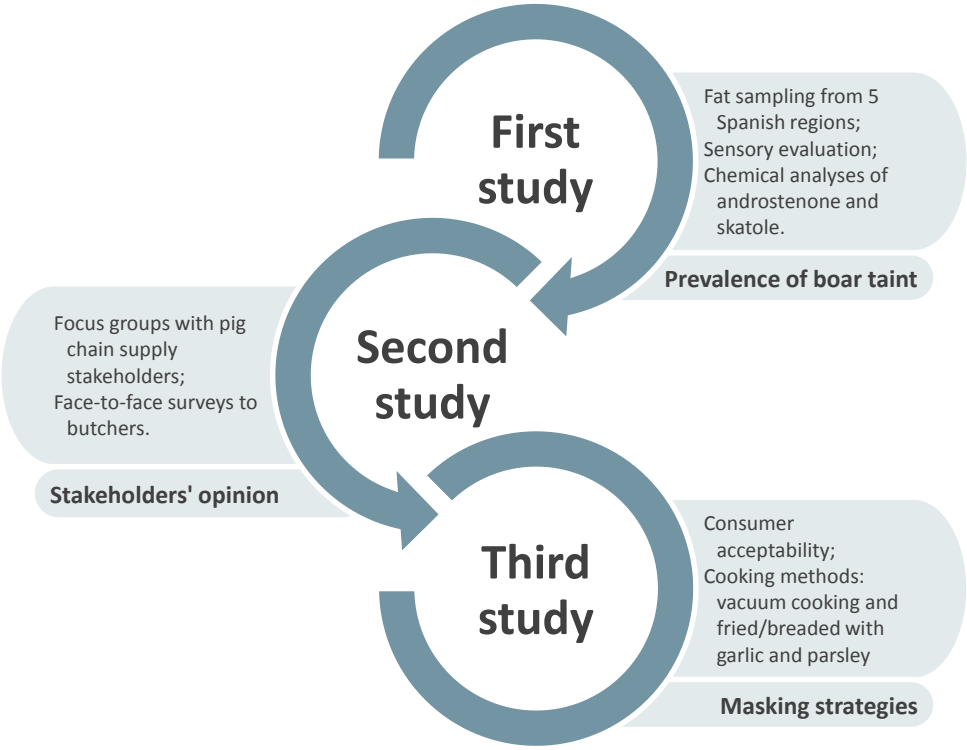


Figura 10. Esquema cronològic dels estudis realitzats per avaluar el potencial de mercat de la carn de mascle sencer.

De manera genèrica, el primer estudi quantifica el percentatge de canals que poden tenir olor sexual a nivell estatal (**Prevalença olor sexual**), el segon estudi avalua amb els diferents actors de la cadena de porcí què implica aquest percentatge i quina és la seva impressió sobre una possible prohibició de la castració a Europa (**Opinió del sector**) i el tercer estudi avalua una possible sortida a la carn de mascle sencer amb presència d'olor sexual (**Emmascarament**).

III.1 Primer estudi – Prevalença d'olor sexual

El primer estudi correspon a l'article 1 *Prevalence of boar taint in commercial pigs from Spanish farms*. La **Figura 11** mostra un esquema de com es va realitzar aquest estudi.

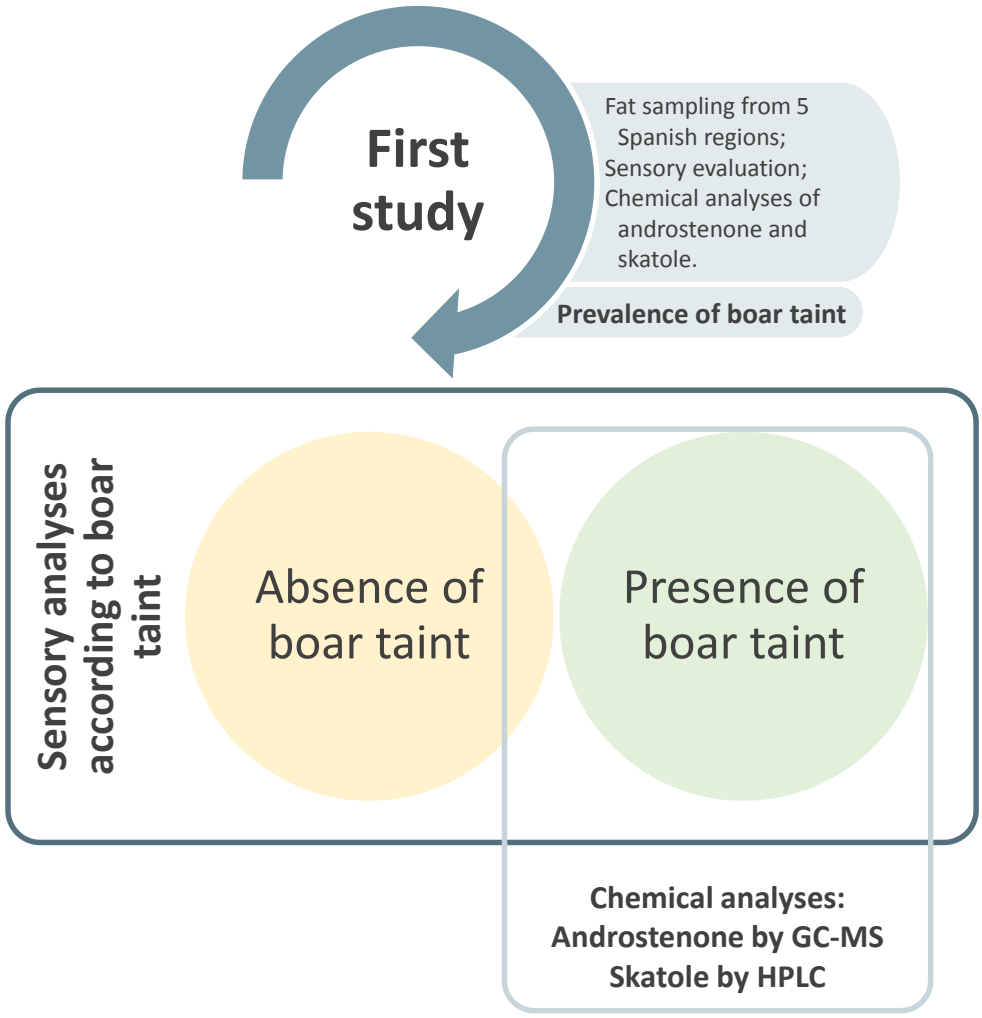


Figura 11. Esquema de la metodologia aplicada en l'estudi 1 per analitzar la prevalença de canals amb presència d'olor sexual.

Aquest estudi estava centrat en estudiar la prevalença de canals de mascles sencers amb presència d'olor sexual en diferents comunitats autònomes (Aragó, Catalunya, Castella i Lleó, Madrid/Castella-La Manxa i Múrcia), l'elecció de les quals és deguda al fet que són les que concentren la major part de la producció porcina de l'estat espanyol. Per dur a terme aquest estudi, es van recollir, a nivell d'escorxador, mostres de greix subcutani d'animals procedents de 6 granges de 2

escorxadors diferents per cada comunitat autònoma. De cada granja es van prendre 30 mostres, i finalment es va obtenir un total 903 mostres de greix subcutani provinents de la regió dorsal el coll de porcs mascles sencers. Les mostres es van recollir entre 30 i 60 minuts *post mortem* i es van etiquetar individualment i es van conservar en congelació a -20ºC per la posterior anàlisi. Es va registrar el pes de la canal, l'espessor de greix a nivell de la última costella, la raça i la granja d'origen per a cada mostra.

Les mostres recollides es van avaluar, en primer lloc, sensorialment per un panel entrenat segons la presència d'olor sexual (Mathur*, et al.*, 2012; Bekaert*, et al.*, 2013). El panel consistia en 2 dones i 1 home, tot i que de cara a l'anàlisi dels resultats no es va tenir en compte ja que els 3 panelistes es van entrenar de la mateixa manera. En primer lloc, els panelistes van fer un test de sensibilitat a l'androstenona i l'escatol i posteriorment es van fer diferents sessions amb mostres de greix per capacitar-los per classificar les mostres segons la presència d'olor sexual. Les mostres que es van classificar com a positives i una submostra de les que es van classificar com a negatives (absència d'olor sexual) es van analitzar químicament, per androstenona mitjançant cromatografia de gasos acoblada a espectrometria de masses (GC-MS) (Rius i García-Regueiro, 1998) i per escatol mitjançant cromatografia líquida d'alta resolució (HPLC) (García-Regueiro i Rius, 1998). Finalment es van classificar els resultats en 3 categories segons el nivell (baix, mig o alt) d'androstenona i d'escatol (**Taula 2**).

III.2 Segon estudi – Opinió del sector

El segon estudi correspon a l'article 2 *Towards entire male pigs in Europe: a perspective from the Spanish supply chain*. En aquest estudi es tractava de conèixer l'opinió del sector porcí –de la granja als consumidors– sobre la prohibició de la castració a Europa. La **Figura 12** esquematitza la metodologia d'aquest segon estudi.

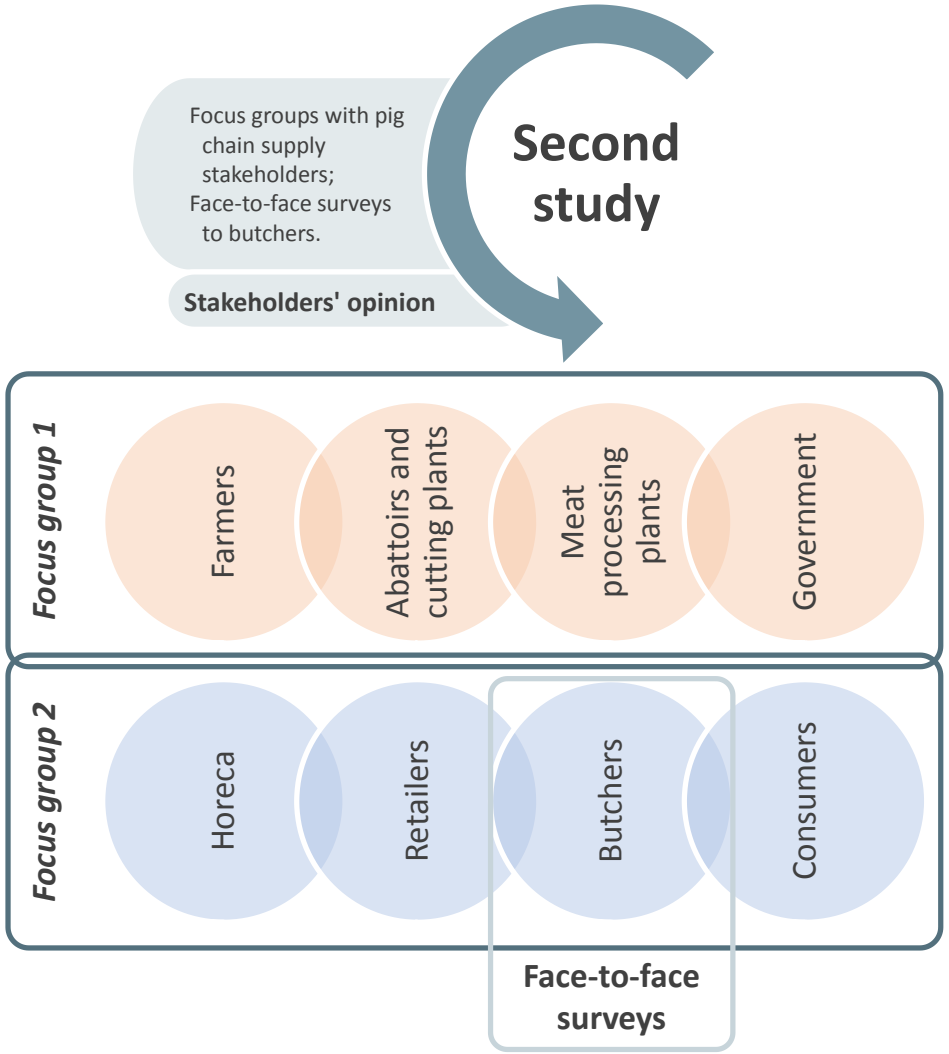


Figura 12. Esquema de la metodologia aplicada en l'estudi 2 per analitzar les opinions i actituds de tota la cadena de producció porcina.

Per conèixer l'opinió del sector sobre la prohibició de la castració es va utilitzar una metodologia coneguda com a *focus group* o discussió de grup. Es tracta d'una tècnica qualitativa on es recullen les opinions dels participants sobre el tema proposat, essent un procediment de recollida de dades en el qual un moderador dirigeix la sessió per tal que els participants vagin parlant dels temes d'interès. Es van dur a terme 2 *focus groups* diferents: el primer (*focus group* 1), enfocat en el sector amb representants de productors, escorxadors i sales de desfer, empreses transformadores i administració; i el segon (*focus group* 2), enfocat en l'últim tram de la cadena, incloent representants de supermercats, carnisseries, horeca i consumidors. Es van realitzar sessions a Barcelona i a Madrid per tal de poder

incloure representants de diferents regions de l'estat. En una altre part d'aquest estudi es van efectuar enquestes a 127 carnisseries de Barcelona, Girona i Madrid per tal de conèixer quina és la importància del problema en els detallistes i quines estratègies comercials tenen en aquest sentit, l'enquesta s'inclou a l'**Annex 2**. Les enquestes es van realitzar a carnisseries perquè són els punts de venda de carn més propers al consumidor i representen un 25% de les vendes dels detallistes de l'estat (MAGRAMA, 2014).

III.3 Tercer estudi – Emmascarament de l'olor sexual

La tercera activitat correspon a l'article 3 *Consumers' sensitivity to androstenone and evaluation of different cooking methods to mask boar taint*. Aquest estudi va consistir en avaluar l'acceptabilitat de carn fresca provinent de porcs mascles sencers amb nivells alts d'androstenona i de mascles castrats, cuinats amb diferents mètodes de cocció i diferents estratègies d'emmascarament. El qüestionari d'aquest estudi s'inclou en l'**Annex 3**. La **Figura 13** mostra l'esquema de la metodologia que s'ha fet servir en aquest estudi.

L'estudi es va centrar en l'emmascarament de l'androstenona. Per fer-ho, es van buscar mostres, a nivell d'escorxador, amb nivells alts d'androstenona i nivells baixos d'escatol a mitjançant un panel entrenat. Es van descartar mostres amb nivells alts d'escatol per minimitzar-ne la seva percepció per part dels consumidors i focalitzar l'estudi en l'androstenona.

Aquest estudi es va realitzar a Madrid amb 150 consumidors. En una primera part van avaluar mostres de llom, cuinat amb diferents mètodes de cocció, procedent de mascle castrat i llom procedent de mascle sencer amb nivells alts d'androstenona i nivells baixos d'escatol. Els dos mètodes de cocció emprats van ser cuinat al buit, i arrebossat amb all i julivert i fregit amb oli d'oliva. I en una segona part d'aquest estudi es va avaluar la sensibilitat dels consumidors a l'androstenona mitjançant la metodologia descrita per Weiler*, et al.* (2000).

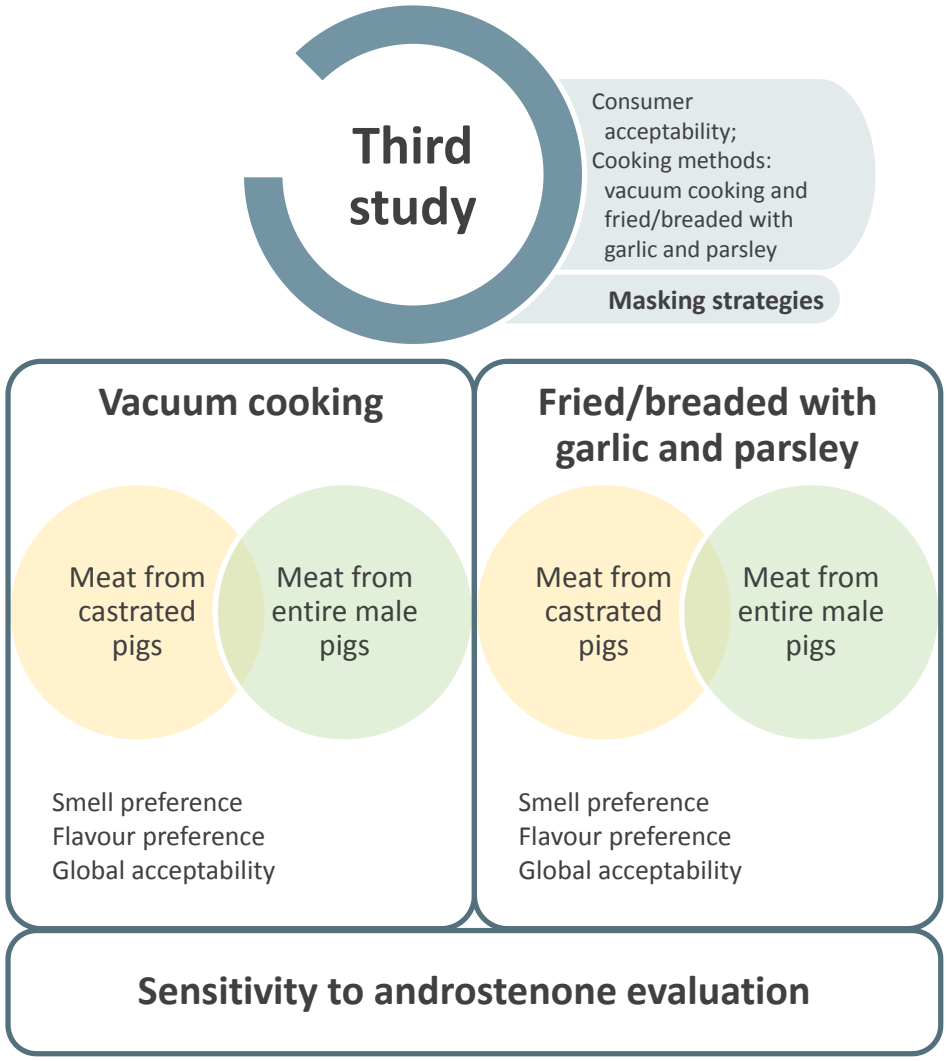


Figura 13. Esquema de la metodologia aplicada en l'estudi 3 per avaluar l'efecte de dos mètodes de cocció com a estratègies per millorar l'acceptabilitat de carn de mascle sencer.

Fotografia 1 – Estudi 1

Avaluació sensorial mitjançant un panel entrenat de mostres de greix de porcs mascles sencers

Fotografia 2 – Estudi 1

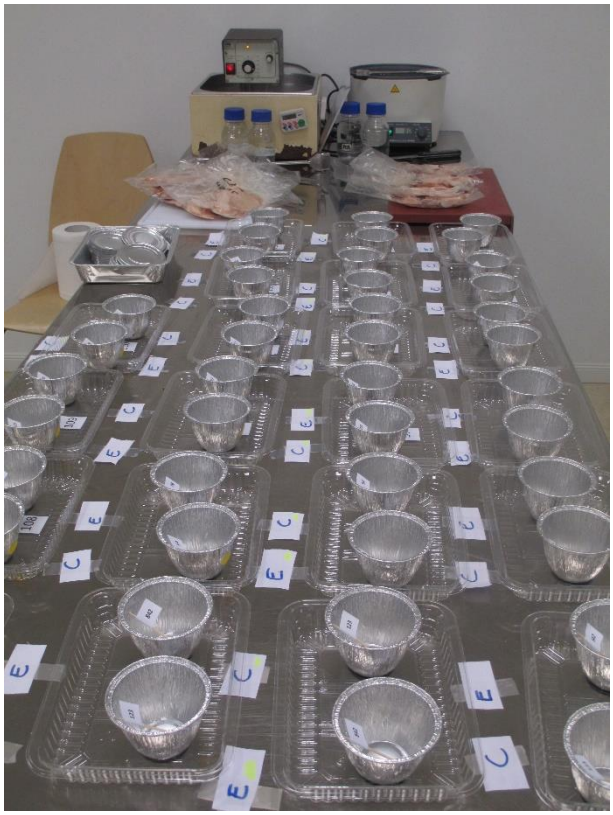
Cromatògraf de gasos acoblat a espectrometria de masses (GC-MS)

Fotografia 3 – Estudi 1

Cromatògraf líquid d'alta resolució (HPLC)

Fotografia 4 – Estudi 3

Mostres de llom procedent de mascle castrat cuinat al buit



Fotografia 5 – Estudi 3

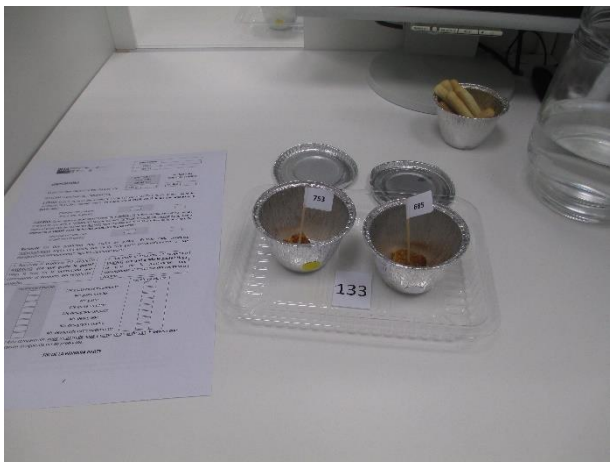
Preparació per a l'avaluació sensorial per part de consumidors de les mostres de llom cuinat al buit

Fotografia 6 – Estudi 3

Preparació del llom arrebossat amb all i julivert

Fotografia 7 – Estudi 3

Fregit del llom arrebossat amb all i julivert amb oli d'oliva



Fotografia 8 – Estudi 3

Cabina preparada per a l'avaluació sensorial de les mostres de llom arrebossat amb all i julivert procedent de porc mascle castrat i mascle sencer

Capítol IV

Compendi d'articles

IV.1 Article 1: Prevalence of boar taint in commercial pigs from Spanish farms

Borrisser-Pairó, F., Panella-Riera, N., Zammerini, D., Olivares, A., Garrido, M. D., Martínez, B., Gil, M., García-Regueiro, J. A. i Oliver, M. A. (2016). Meat Science 111: 177-182.

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Prevalence of boar taint in commercial pigs from Spanish farms

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ABSTRACT

The presence of boar taint can affect the sensory quality of pork because the "off" odours and flavours can be detected by consumers. The aim of this study was to determine the prevalence of boar taint in pig carcasses from 30 Spanish farms located in different regions of the country. Hot carcass weight and subcutaneous fat thickness means were 79.4 ± 8.19 kg and 18.4 ± 5.09 mm, respectively. Subcutaneous fat samples were classified into different levels according to androstenone and skatole concentrations in adipose tissue measured using GC–MS and HPLC. Androstenone results were: 87.4% of the carcasses below 0.50 μg/g, 7.1% from 0.50 to 1.00 μg/g (medium level), and 5.5% ≥1.00 μg/g (high level). Skatole results were: 88.9% of the carcasses below 0.10 μg/g, 4.5% from 0.10 to 0.20 μg/g (medium level), and 6.6% ≥0.20 μg/g (high level). Given these results, a future online method to classify carcasses according to boar taint is strongly recommended.

1. Introduction

Boar taint is a distinctive and unpleasant odour and taste of pork and pork products and is present in some entire male pigs. It is caused mainly by an excessive accumulation of certain volatile compounds linked to the sexual maturity of pigs (Bonneau, 1982). Therefore the rearing of entire male pigs is avoided in most European countries, and the majority of male piglets intended for pork production are surgically castrated to avoid potential consumer dissatisfaction because of boar taint (Fredriksen et al., 2009). However, entire male pig production is more profitable for farmers due to leaner carcasses and a higher protein content, compared to castrated pigs (Lundström, Matthews, & Haugen, 2009). Due to EU legislations, surgical castration has recently been discontinued in an increasing number of European countries, and discussions at European level are aiming to ban surgical castration by 2018 (DG-SANCO, 2010). Because of these reasons, since 2012, some countries such as Germany, The Netherlands and Denmark have already changed their production to entire males.

In Spain, pig production is a major industry and over 40 million pigs are slaughtered per year, representing 16% of European production (FAOSTAT, 2013). In 2009 the practise of castration in Spain was estimated to have been performed on 33% of male pigs (Fredriksen et al., 2009). Currently, as a consequence of the changes in the production model this percentage can be assumed to be lower. Nowadays, castration is approximately 15–20% according to the pig sector (Borrisser-Pairó et al., 2014).

The presence of boar taint is due to high concentrations in the fat of at least one of the two compounds widely accepted to be responsible for tainted pork: androstenone and skatole (Bonneau, 1982). Androstenone (5α-androst-16-ene-3one) is a pheromone produced in the testes and exhibits a urine-like odour, which was isolated from boar fat by Patterson (1968). Skatole (3-methylindole) is a breakdown product of the amino acid tryptophan in the large intestine, exhibiting a faecal-like, naphthalene odour, which was isolated from boar fat by Vold (1970).

Some pigs show levels of these compounds in the adipose tissue over the threshold from which sensory problems can arise (Font i Furnols, Guerrero, Serra, Rius, & Oliver, 2000). The most commonly used cut-off levels for androstenone to categorize tainted meat are 0.5 and 1.0 μg/g of adipose tissue respectively, as suggested in previous studies (Claus, Weiler, & Herzog, 1994; Font i Furnols, Gispert, Diestre, & Oliver, 2003; Rhodes, 1971); whilst for skatole the most commonly used threshold values are 0.10 and 0.20 μg/g of adipose tissue (Bonneau et al., 1992; Claus et al., 1994; Desmoulin, Bonneau, Frouin, & Bidard, 1982; Font i Furnols et al., 2003; Walstra et al., 1999).

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Malmfors and Hansson (1974) studied the prevalence of boar taint in Swedish Landrace and Yorkshire boars by smelling fat samples heated with a soldering iron. They found that 20% of boars presented boar taint. In a European study by Walstra et al. (1999) conducted in six countries, the amount of pigs presenting levels over the thresholds of 1.0 μg/g for androstenone and 0.20 μg/g of adipose tissue for skatole, were approximately 29% and 15% respectively. In Spain particularly, pigs over these thresholds were approximately 42% for androstenone, and 30% for skatole. Prusa et al. (2011) assessed the prevalence of boar taint in commercial abattoirs in different regions of The United States by evaluating fat samples from gilts, sows, barrows and boars with a trained panel and also with chemical analyses. They found that 55.8% and 34.2% of boars had concentrations above the androstenone and skatole thresholds, respectively.

To the best of the authors' knowledge, since Walstra et al. (1999) no updated data has been published in relation to the prevalence of boar taint in the Spanish pig population. Pig production is the most important livestock activity in Europe, particularly in Spain (FAOSTAT, 2013). DG-SANCO is planning to ban surgical castration by 2018 (DG-SANCO, 2010), so Europe is moving towards entire male production. It is relevant to know, as a first step, the percentage of entire male pigs with high levels of boar taint. Therefore, the objective of the present study, conducted within a national project entitled BOARMARKET, is to know the prevalence of boar taint in Spanish entire male pig production by detecting androstenone and skatole levels in fat. The final aim is to provide objective information about this subject to the pig sector.

2. Material and methods

2.1. Animal and raising conditions

The study was carried out in pigs from commercial farms from 5 Spanish regions (Aragón, Catalonia, Castilla y León, Madrid/Castilla-La Mancha and Murcia). These regions were selected because they are the main pig producing areas in Spain, with 74.7% of the Spanish pig production (MAGRAMA, 2013), and the biggest pig exporters to European and Asian countries (DataComex, 2015). The genetic of the animals were commercial crossbreeds that will be described in the results section. Animals were raised from 30 kg life weight in fattening farms until the slaughtering weight (around 100 kg live weight), during 18 to 20 weeks. The animal density was 10 to 15 pigs/pen.

2.2. Fat sampling

A total of 903 samples of subcutaneous fat were collected in 5 Spanish regions (Aragón, Catalonia, Castilla y León, Madrid/Castilla-La Mancha and Murcia). For each region, samples of subcutaneous fat were obtained from 6 farms, which slaughtered their pigs in commercial abattoirs. A minimum of 30 fat samples was collected from each farm resulting in a minimum of 180 fat samples per region. The sample collected from each pig was taken from the dorsal neck region (referred to as back fat) on the left carcass side. It is important to note that the samples were taken from the cervical region close to the head, and consisted of a maximum area of 15 × 5 cm of skin plus attached fat, all the way down to the intersection with the meat. Samples were individually labelled, vacuum packed and stored at −20 °C before being sent in batches to IRTA. The following data were recorded for each pig: genetic crossbreed, hot carcass weight and mid-line subcutaneous fat thickness at level of the last rib, manually measured with a ruler.

2.3. Selection of samples by human nose methodology

In this study an olfactory scoring system using a soldering iron to heat the samples (Malmfors & Hansson, 1974; Whittington et al., 2011), referred to as human nose methodology (Mathur et al., 2012), was used to select the fat samples to be later analysed for androstenone and skatole levels by chemical analysis. For the various BOARMARKET project experiments, 3 trained assessors who are sensitive to androstenone, two women and one man, were selected and retrained for the sensory assessment of the fat samples using the method described in previous studies (Font i Furnols et al., 2000). In the present study the samples were classified in 2 categories according to the presence of boar taint. Therefore, samples scored as 'no boar taint' by the 3 panellists were classified as 'absence of boar taint'. The samples scored as 'boar taint' by at least one panellist were classified as 'presence of boar taint'. Detection limits of the panellists were 0.20 μg/g of adipose tissue for androstenone and 0.05 μg/g of adipose tissue for skatole, and were determined using decreasing concentrations of androstenone and skatole in sunflower oil (Font i Furnols et al., 2000).

2.4. Chemical analyses of androstenone and skatole

Samples classified as 'presence of boar taint' by human nose methodology were all analysed for androstenone and skatole by chromatographic techniques. In order to check human nose methodology, one sample per farm classified as 'absence of boar taint' was also analysed. The rest of the samples were considered to have concentrations in adipose tissue of androstenone and skatole of <0.20 μg/g and <0.05 μg/g, respectively. Androstenone analysis was performed using the gas chromatography–mass spectrometry (GC–MS) technique (Rius & García-Regueiro, 1998) and skatole and indole analysis by high-performance liquid chromatography (HPLC) (García-Regueiro & Rius, 1998). According to the results from the chemical analysis the samples were classified in different levels (Bonneau & Chevillon, 2012; Claus et al., 1994; Rhodes, 1971) for androstenone as follows: no androstenone <0.2 μg/g, low ≥0.2 to 0.5 μg/g, medium ≥0.5 to 1.0 μg/g and high ≥1.0 μg/g of adipose tissue; and for skatole: no skatole <0.05 μg/g, low ≥0.05 to 0.10 μg/g, medium ≥0.10 to 0.20 μg/g, high ≥0.20 μg/g of adipose tissue. In accordance with this classification, the meat obtained from carcasses with a medium or high concentration of androstenone or skatole can be considered to be an issue for some consumers, whilst low levels may be accepted (Font i Furnols et al., 2003).

2.5. Statistical analyses

The data were analysed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Differences between Spanish regions were studied using the mixed procedure of SAS. Spanish region was considered as fixed effect. Differences were adjusted with Tukey's test, and the significance level was fixed at $P < 0.05$.

3. Results

3.1. Carcass characteristics

A total of 903 back fat samples from entire male pigs were evaluated. Mean and standard error for hot carcass weight was 79.4 ± 8.19 kg, and

Table 1
Least square means and standard error (S.E.) of hot carcass weight and subcutaneous fat thickness measured at the last rib level per region.

Region	Hot carcass weight (kg)[1]	S.E.	Subcutaneous fat thickness (mm)[1, 2]	S.E.
Aragón	80.4[b]	0.56	18.7[a]	0.37
Catalonia	78.1[c]	0.56	16.6[b]	0.37
Castilla y León	85.3[a]	0.56	18.8[a]	0.37
Madrid/Castilla-La Mancha	77.5[cd]	0.56	19.3[a]	0.37
Murcia	75.5[d]	0.56	18.7[a]	0.37

[1] According to variation factor means with different superscripts are significantly different within one column $P < 0.05$.
[2] Mid-line subcutaneous fat thickness was measured at the last rib level.

for mid-line subcutaneous fat thickness 18.4 ± 5.09 mm. Table 1 presents least square means and standard error of carcass characteristics (hot carcass weight and subcutaneous fat thickness) per region. Significant differences were observed in hot carcass weight and subcutaneous fat thickness between regions ($P < 0.001$). Castilla y León showed carcasses with the highest weight (85.3 ± 0.56 kg). Catalonia presented carcasses with significantly less fat thickness (16.6 ± 0.37 mm) than the other regions. Genetic crossbreeds sampled were mainly Pietrain × (Landrace × Large White) (73.4%), other Pietrain crossbreeds (20.0%), and Duroc crossbreeds (6.6%). Duroc crossbreeds were only concentrated in Murcia Region with 33% of the samples.

3.2. Androstenone and skatole concentration in back fat

Mean and standard error for androstenone concentration was 0.20 ± 0.581 μg/g of adipose tissue. For skatole, it was 0.04 ± 0.119 μg/g of adipose tissue. Comparing the 6 regions, significant differences were only observed in skatole concentration (Table 2, $P < 0.05$), Murcia being the region with the highest skatole concentration (0.08 μg/g of adipose tissue), significantly different from Aragón, Castilla y León and Madrid/Castilla-La Mancha.

Results of back fat chemical analysis means, standard errors, minimum and maximum separated in the different levels for androstenone and skatole are shown in Table 3. Regarding the different thresholds for androstenone mentioned before, results showed that 87.4% of samples had levels of androstenone below 0.50 μg/g of adipose tissue, 7.1% presented medium levels and 5.5% had high levels of androstenone. Regarding skatole thresholds, 88.9% of the samples had levels of skatole below 0.10 μg/g of adipose tissue, 4.5% had medium levels, and 6.6% had high levels.

As mentioned in the "Material and methods" section, a representative subsample that includes one sample per farm scored as 'absence of boar taint' by human nose methodology was analysed. The mean concentrations of androstenone and skatole in the fat samples scored as 'absence of boar taint' by the trained panel analysed by chemical analyses were 0.16 ± 0.182 and 0.02 ± 0.020 μg/g of adipose tissue, respectively. No levels of androstenona were detected in 69.0% of the samples, and no levels of skatole were detected in 82.8% of the samples. Low levels of androstenone were found in 24.1%, and low levels of skatole in 17.2%. Moreover, among these samples, 6.9% presented medium levels of androstenone.

Table 4 shows the percentage of samples in each threshold for both androstenone and skatole: 10.2% of the samples showed values over the high level threshold for androstenone and/or for skatole (values with superscript A) and 10.9% of the samples presented medium levels of androstenone and/or skatole (superscript B), whilst 78.9% of the samples showed levels of androstenone and skatole below the low threshold (superscript C). Fig. 1 shows the dispersion of the 903 samples for both androstenone and skatole. A positive correlation between androstenone and skatole was observed ($r = 0.38344$, $P < 0.0001$). Figs. 2 and 3 show the distribution of the hot carcass weight in relation to androstenone ($r = 0.10839$, $P = 0.0011$) and skatole ($r = -0.01953$, $P = 0.558$).

Table 2
Least square means and standard error (S.E.) of androstenone and skatole concentration in fat samples between different regions.

Region	Androstenone (μg/g adipose tissue)[1]	SE	Skatole (μg/g adipose tissue)[2]	SE
Aragón	0.20	0.043	0.04^{bc}	0.009
Catalonia	0.20	0.043	0.06^{ab}	0.009
Castilla y León	0.21	0.043	0.03^{bc}	0.009
Madrid/Castilla-La Mancha	0.13	0.043	0.01^{c}	0.009
Murcia	0.27	0.043	0.08^{a}	0.009

[1] Significance: not significant.
[2] According to variation factor means with different superscripts are significantly different within one column $P < 0.05$.

Table 3
Number of samples, percentage and concentrations of androstenone and skatole in back fat (μg/g of adipose tissue) separated into different levels of boar taint[1].

Androstenone	No of samples	%	Mean (μg/g)	S.E.	Min	Max
Global	903	100	0.20	0.581	0.00	9.29
High (≥1.00 μg/g)	50	5.5	2.05	1.400	–	–
Medium (≥0.50 to 1.00 μg/g)	64	7.1	0.72	0.146	–	–
Low (≥0.20 to 0.50 μg/g)	85	9.4	0.35	0.083	–	–
No boar taint (<0.20 μg/g)	704	78.0	0.01	0.035	–	–
Low + No boar taint	–	87.4	–	–	–	–

Skatole	N° of samples	%	Mean (μg/g)	S.E.	Min	Max
Global	903	100	0.04	0.119	0.00	1.16
High (≥0.20 μg/g)	57	6.6	0.41	0.229	–	–
Medium (≥0.10 to 0.20 μg/g)	41	4.5	0.15	0.028	–	–
Low (≥0.05 to 0.10 μg/g)	88	10.0	0.07	0.014	–	–
No boar taint (<0.05 μg/g)	717	78.9	0.00	0.011	–	–
Low + No boar taint	–	88.9	–	–	–	–

[1] Levels of boar taint according to Bonneau (1982) and Font i Furnols et al. (2003).

4. Discussion

Carcass characteristics varied between the different Spanish regions, Castilla y León being the region with the highest hot carcass weight. Subcutaneous fat thickness was significantly lower in Catalonia with respect to the other regions, which did not show differences between them. These differences in fat thickness and hot carcass weight may be due to the different genetic crossbreeds (Gispert et al., 2007) used in the different regions. Gispert et al. (2000) studied carcass quality in five Spanish commercial abattoirs where hot carcass weight was 78.1 ± 9.91 kg. In the present study higher hot carcass weights were observed (79.3 ± 8.26 kg). These results can be explained because during the last years hot carcass weight has increased (MAGRAMA, 2013).

To the best of the authors' knowledge there have only been few studies published on the levels of androstenone and skatole in Spanish farms (Walstra et al., 1999). In the aforementioned study, Spanish farms showed 42.0% of the carcasses to be above the threshold 1.0 μg/g of adipose tissue for androstenone, whilst in the present study only 5.5% of carcasses had levels above this threshold. In the same way, results for skatole by Walstra et al. (1999) showed that levels of skatole in Spain above the threshold 0.05 μg/g of adipose tissue were 29.9% whilst in our study it is 6.6%. Results by Prusa et al. (2011) also showed higher prevalence of carcasses above androstenone and skatole thresholds. The methods used to analyse androstenone and skatole were different in these studies. Therefore some of the differences between the studies may be explained because there may be a bias in the results between methodologies (Ampuero Kragten et al., 2011; Haugen, Brunius, & Zamaratskaia, 2012). However, these differences may also be an indication that nowadays the European pig sector is slaughtering more immature animals that grow faster than they did 25 years ago, although hot carcass weight is higher (MAGRAMA, 2013). Therefore the decrease of carcasses with high levels of boar taint could be explained by the fact that immature animals and specific selected lines accumulate less androstenone (Brennan, Shand, Fenton, Nicholls, & Aherne, 1986; Zamaratskaia, Babol, Andersson, & Lundström, 2004) and less skatole (Babol, Zamaratskaia, Juneja, & Lundström, 2004; Hansen et al., 1997; Zamaratskaia, Babol, Madej, Squires, & Lundström, 2004) in adipose tissue. Differences in skatole concentration between regions may be due to the different genetic crossbreeds used. Duroc accumulates more skatole than Pietrain (Xargay et al., 2010). Therefore,

Table 4
Percentage of samples in the different androstenone and skatole thresholds.

Androstenone	No (<0.20 μg/g)	Low (≥0.20 to 0.50 μg/g)	Medium (≥0.50 to 1.00 μg/g)	High (≥1.00 μg/g)
Skatole				
High (≥0.20 μg/g)	1.0[A]	1.3[A]	2.4[A]	1.9[A]
Medium (≥0.10 to 0.20 μg/g)	3.7[B]	2.5[B]	2.3[B]	1.4[A]
Low (≥0.05 to 0.10 μg/g)	1.7[C]	1.2[C]	0.8[B]	0.9[A]
No (<0.05 μg/g)	71.7[C]	4.3[C]	1.6[B]	1.3[A]

[A] Levels over the high threshold for androstenone and/or skatole (10.2%).
[B] Levels with medium levels for androstenone and/or skatole (10.9%).
[C] Levels below the low threshold for androstenone and skatole (78.9%).

the region with higher skatole concentration was Murcia, which is the region where 33% of the animals were Duroc crossbreeds.

In the present study androstenone and skatole concentrations were positively correlated ($r = 0.38344$, $P < 0.0001$), this positive correlation has also been seen in previous studies (Walstra et al., 1999). Some studies have concluded that the presence of androstenone inhibits the metabolism of skatole (Babol, Squires, & Lundström, 1999; Panella-Riera et al., 2008), which could explain the positive correlation between the two compounds responsible for boar taint. In the present study a positive correlation between hot carcass weight and androstenone concentration was observed although it was low. Previous studies confirmed also a positive correlation between androstenone and hot carcass weight (Zamaratskaia, Babol, Andersson, Andersson, & Lundström, 2005).

More than 40 million pigs are slaughtered per year in Spain (FAOSTAT, 2013), and 80% of the male pigs are raised entirely (Borrisser-Pairó et al., 2014), therefore 16 million entire male pigs are slaughtered. The percentage of carcasses above the high thresholds of androstenone and/or skatole seem to be low (10.2%), but in numerical terms there are approximately 1.6 million carcasses with high levels boar taint every year.

To date, there is no harmonized online method for the detection of boar taint in slaughterhouses in Europe. During the last thirty years different objective techniques to determine the levels of boar taint compounds in the fat have been developed and improved. Since 2014 a European reference method developed by the Joint Research Centre (JRC) for the determination of boar taint compounds is available (Buttinger & Wenzl, 2014). However, these methods are time consuming and expensive and therefore not applicable for a daily use at slaughterhouses. For these reasons many slaughterhouses in Europe have recently started to select carcasses for boar taint presence by way of a sensory assessment provided by trained people (Bekaert et al., 2013). The main objective is to allocate tainted meat to processed products in which boar taint can be masked (Babol & Squires, 1995; Egea, Díaz, Álvarez, Garrido, & Linares, 2014). Mathur et al. (2012) concluded that human nose can be useful to evaluate large numbers of samples. The percentage of false negatives is not zero by human nose technology. The selection of human nose tester is crucial (Trautmann, Gertheiss, Wicke, & Mörlein, 2014). However, with an objective method, the accuracy and repetitiveness would be higher.

As it has been discussed above, the prevalence of boar taint in carcasses from Spanish farms over the high threshold (1.0 μg/g for androstenone, 0.20 μg/g for skatole, in adipose tissue) in numerical terms may represent an important issue for the pig industry. Consumer studies can provide useful information in order to know the acceptability of this boar meat. Therefore introducing a grading system for boar taint at slaughterhouses would be useful in order to find an adequate final use for tainted meat.

5. Conclusions

Knowing the percentage of carcasses with high levels of androstenone and skatole provides useful and objective information to the sector about boar taint which affects pork sensory quality. As a consequence of this information, actions such as introducing a system to classify carcasses according to the presence of boar taint in slaughterhouses are strongly recommended. Furthermore, consumer acceptability of different pork and pork products would help the sector to make decisions. Further research is needed to find an efficient online

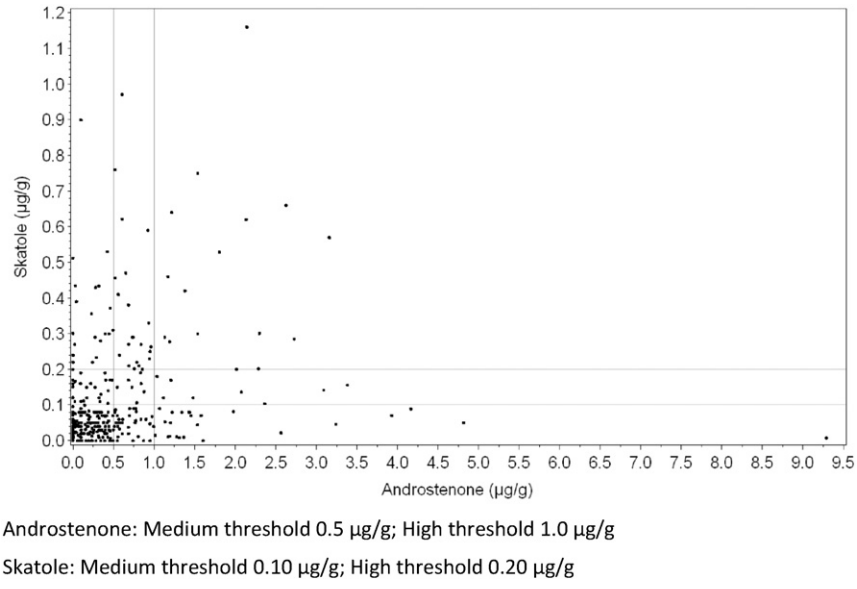

Fig. 1. Distribution of androstenone and skatole levels (μg/g adipose tissue) in pigs from five Spanish regions ($r = 0.38344$, $P < 0.0001$). Androstenone: Medium threshold 0.5 μg/g; High threshold 1.0 μg/g Skatole: Medium threshold 0.10 μg/g; High threshold 0.20 μg/g.

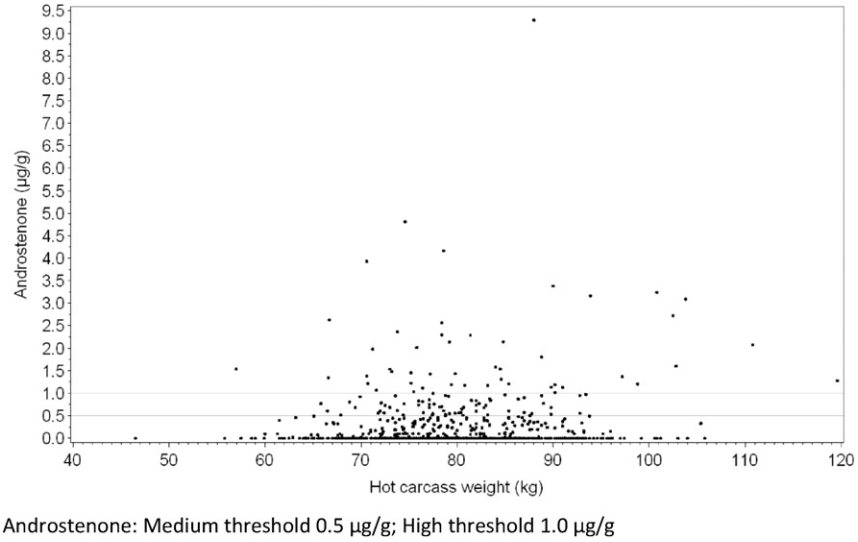

Fig. 2. Distribution of androstenone levels (µg/g adipose tissue) and hot carcass weight (kg) in pigs from five Spanish regions (r = 0.10839, P = 0.0011). Androstenone: Medium threshold 0.5 µg/g; High threshold 1.0 µg/g.

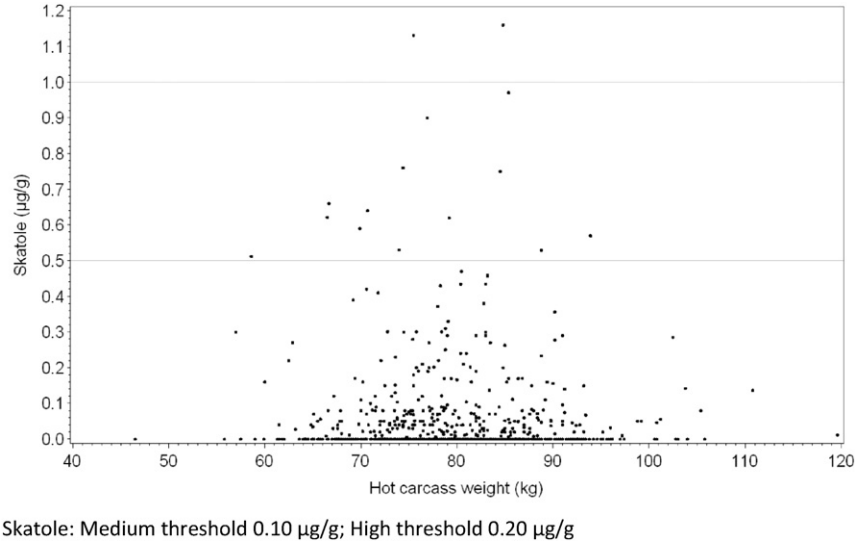

Fig. 3. Distribution of skatole levels (µg/g adipose tissue) and hot carcass weight (kg) in pigs from five Spanish regions (r = −0.01953, P = 0.558). Skatole: Medium threshold 0.10 µg/g; High threshold 0.20 µg/g.

detection method to be used as a tool to avoid pig carcasses with boar taint, to find an adequate use, for example of masking tainted processed products, and to ascertain consumer preferences.

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IV.2 Article 2: Towards entire male pigs in Europe: a perspective from the Spanish supply chain

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Towards entire male pigs in Europe: A perspective from the Spanish supply chain

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ABSTRACT

In pig production, surgical castration is primarily performed to avoid boar taint and for management purposes. The European Commission plans to end surgical piglet castration voluntarily by 2018. The aim of this study was to assess the opinions and attitudes of Spanish stakeholders from the entire pork chain regarding this plan. Two methodologies were used: focus groups with 26 participants (qualitative method) were conducted with representatives of farmers, the meat industry, government institutions, retailers (including butchers), HORECA and consumers, and 127 face-to-face surveys at butchers (quantitative method) were carried out. These include an analytical hierarchical process to assess the determining factors when purchasing fresh pig meat. The results showed that a potential end of pig castration in Europe is not anticipated to affect conventional pig production in Spain. However, butchers are worried of negative effects on high quality meat and meat products, where surgical castration of pigs plays an important role.

1. Introduction

Pig production is the most important livestock activity in Spain; 40 million pigs are slaughtered every year, which represents 16% of European production (FAOSTAT, 2013). A percentage of male pigs are castrated according to legislation (Directive 2001/93/CE), primarily for quality purposes. In 2009, an estimated 79% of male pigs in Europe were castrated, 33% in Spain (Fredriksen et al., 2009). Currently, although no official data are collected, this percentage has dropped to between 15% and 20% in Spain according to the pig industry. In 2013, the exportation rate in Spain was approximately 30%, and the importation rate was approximately 2% (DataComex, 2014). One of the primary retailers of pork meat is butchers, selling 25% of the fresh pork meat in Spain (MAGRAMA, 2013).

The practice of castration avoids the presence of boar taint produced by androstenone and skatole in the meat (Bonneau, 1982; Bonneau et al., 2000). The presence of boar taint in meat may affect the acceptability of pork by consumers (Bonneau and Chevillon, 2012; Desmoulin et al., 1982; Diestre et al., 1990; Font-i-Furnols, 2012; Meier-Dinkel et al., 2013). While skatole is perceived by 99% of consumers (Weiler et al., 1997), approximately 40% of consumers are anosmic for androstenone, which means that they are unable to smell this compound (Blanch et al., 2012; Panella-Riera et al., 2010). Therefore androstenone sensitivity also affects boar meat acceptability (Blanch et al., 2012; Font-i-Furnols et al., 2003; Weiler et al., 2000). A study performed in Switzerland determined that part of the population experienced the presence of boar taint in meat (Huber-Eicher and Spring, 2008). Another reason for castrating piglets is meat quality; meat from castrated males has more intramuscular fat, which affects its acceptability (Aluwé et al., 2013; Bañón et al., 2004; Gispert et al., 2010).

The practice of castration has generated a debate due to its negative impact on animal welfare, resulting in the European Declaration on alternatives to surgical castration of pigs (DG-SANCO, 2010). This declaration was drafted by representatives of European farmers, the meat industry, retailers, scientists, veterinarians and animal welfare NGOs under the management of the European Union. The plan is to end the surgical castration voluntarily (with or without anaesthesia) of pigs in Europe by 1 January 2018.

Several studies in different countries evaluated attitudes and opinions regarding alternatives to surgical castration: for Norwegian consumers castration without anaesthesia was unacceptable (Fredriksen et al., 2011); Swiss consumers did not accept immunocastration as an alternative to surgical castration and preferred castration with anaesthesia (Huber-Eicher and Spring, 2008); and Flemish pig farmers

Abbreviations: AHP, analytical hierarchy process; FG1, focus group 1 with representatives of farmers, the pig meat industry, slaughterhouses and Government; FG2, focus group 2 with representatives of restaurants and caterings, supermarkets, butcheries and consumers associations; NGO, non-governmental organization; PGI, protected geographical indication; PGO, protected designation of origin; TSG, traditional specialities guaranteed.

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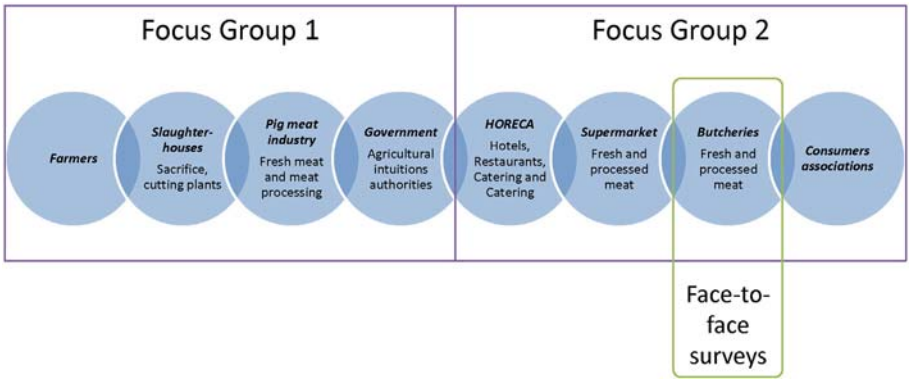

Fig. 1. Methodological approach to analyse the opinions and attitudes of the main stakeholders in the pig supply chain.

considered entire male production as the least profitable strategy while sperm sexing was positively perceived (Tuyttens et al., 2012). Regarding the consumers, (Kallas et al., 2013; Kallas et al., 2012) analysed the relative importance of pig castration in 6 European countries: Spain, United Kingdom, The Netherlands, France, Italy and Germany. These researchers concluded that consumers did not have sufficient information regarding the sex of the pig, and few consumers associated pork with castration. This study also demonstrated that there is heterogeneity between countries: in Spain and France, the sex of the animal was relatively less important, while in the United Kingdom and The Netherlands, sex was relatively more important. To evaluate whether further commercialization of boar meat would be a problem, it would be useful to know if the meat industry, retailers, consumers and exporters were able to accept the change in meat if castrated pigs were replaced by entire males.

The aim of this study was to assess the opinions of stakeholders in the Spanish pork supply chain –from farmers to consumers– regarding the impact of a potential mandatory banning of pig castration using focus groups. Another objective was to quantify the opinion of butchers using face-to-face surveys concerning different aspects of entire male pig production, including meat quality, animal welfare and consumer attitudes.

2. Material and methods

Our methodological framework was based on two primary approaches to analyse the opinions and attitudes of the primary stakeholders in the pork meat supply chain: farmers, industry, government, retailers and consumers (Fig. 1). Firstly, the focus group as a qualitative methodology was applied to analyse opinions towards the production of entire male pigs and the impact of a potential ban on piglet castration in Spain. Secondly, a face-to-face survey at butchers was employed as a quantitative methodology. These surveys consisted of different questions regarding pig production, meat quality and consumer attitudes. The survey also included an Analytical Hierarchy Process (AHP) to assess the relative importance of pig castration within butchers' decisions.

2.1. Focus groups

Focus group methodology allows the exploration of attitudes and perceptions related to concepts, products, services or programmes by interactions with other people. Focus groups are useful when there are power differences between the participants (Krueger, 1994; Morgan, 1993).

2.1.1. Study design

To obtain a broad overview of the opinions of different stakeholders regarding the production of entire male pigs and the potential ban on piglet castration in Spain, focus groups were organized in two Spanish regions (Barcelona and Madrid). Two focus group sessions–including different parts of the pork supply chain–were organized in each city with the following stakeholders: Focus group 1 (FG1) included representatives of farmers, the pig meat industry, slaughterhouses and the government; and Focus group 2 (FG2) included representatives of HORECA, supermarkets, butchers and consumer associations. Focus groups were performed on 21st November 2012 in Barcelona and 12th February 2013 in Madrid, with 26 participants in total, 5 to 8 in each session, and 1 to 2 participants per stakeholder and session. Number of participants in each session is detailed in Table 1.

2.1.2. Description of focus groups

Focus groups were conducted according to standard procedures (Morgan, 1993). The focus group team consisted of a moderator and an assistant. In all the sessions a moderator led the proceedings and obtained answers to the set of questions from each participant. Each focus group lasted 55–60 min. The Focus group sessions were recorded and field notes were taken during the session to later analyse the data. The

Table 1
Number and type of stakeholder in each session.

	Stakeholder	Barcelona	Madrid	Total
FG1	Farmers	1	1	2
	Slaughterhouses/cutting plants	1	2	3
	Meat processing plants	1	2	3
	Government	2	3	5
	Global	5	8	13
FG2	Retailers	2	1	3
	Butchers	2	2	4
	HORECA (Hotels, Restaurants and Catering)	1	1	2
	Consumers	2	2	4
	Total	7	6	13
	Overall total	12	14	26

Table 2
Focus group (FG) topics.

A[a,b]	Potential impact of banning castration on pork production in Spain – boar taint
B[a]	European Declaration on alternatives to surgical castration of pigs
C[a]	Differences in quality between castrated and entire males
D[a]	Exportation – QS Quality Assurance
E[b]	Quality criteria when purchasing pork meat towards each stakeholder
F[a,b]	Animal welfare
G[a,b]	Standard market (entire males) and high quality products

[a] Topic treated in FG1 (farmers, the pig meat industry, slaughterhouses and government).
[b] Topic treated in FG2 (HORECA, supermarkets, butcheries and consumer associations).

participants were informed that all the data obtained in the focus group were for research purposes only.

2.1.3. Focus groups topics

First, participants were informed about the European Declaration on alternatives to surgical castration of pigs (DG-SANCO, 2010), which plans to ban surgical castration of piglets by 2018. After that, different topics regarding pig castration, pig production, animal welfare and meat quality were discussed in the different focus group sessions. These topics are detailed in Table 2. FG1 and FG2 included stakeholders from different parts of the supply chain, so some of the topics were only introduced in one session.

2.1.4. Data analyses

After each session, audio files were transcribed verbatim to be sure that no important information was lost. The focus group team listened to the audio files several times to avoid errors of transcription that could change the meaning of the comments. Transcriptions and field notes of the focus groups were then analysed and all the data were sorted according to the set of questions.

2.2. Face-to-face surveys

On the basis of the qualitative information gathered from FG1 and FG2, a semi-structured questionnaire was designed with open and open-ended questions. Face-to-face questionnaires with butchers were filled out during the first semester of 2014. The final sample consisted of 127 surveys, 72 of which (response rate 10.2%) were carried out in Catalonia (the regions of Barcelona and Girona) and 55 in Madrid (response rate 6.0%). A list of all of the butchers was provided by the professional association of butchers from each region. The selection of butchers was made randomly using the postal codes of each region as a variable of stratification.

Table 3
Questions on face-to-face surveys at butchers.

Butchers' characteristics	
Age	Household members
Sex	Relation within the business
Education	Years in the butchery
Butchery characteristics	
Years of business	
Surface of the business	
Employees	
Customers	
Meat format when purchasing (whole carcass, primal cuts, pieces)	
SEUROP category of the meat	
Percentage of meat according to the sex of the pig	
Questions regarding opinions and attitudes on pig castration	
Potential impact of banning castration on pig production in Spain – Boar taint	
Differences in quality between castrated and entire males	
Quality criteria when purchasing pork meat	
Animal welfare	
Standard market (entire males) and high quality products	

2.2.1. Questionnaire design

Table 3 shows the different information collected from the face-to-face survey. Butchers were asked about their knowledge of animal welfare and their feelings about animal welfare in Spain using a 9-point scale (1 = very low, 5 = medium, 9 = very high). Regarding pig castration and meat quality, yes-no questions were asked (Table 4). Butchers were also asked about what sex of pig produces meat with better quality. Finally, determining factors when purchasing fresh pig meat were analysed using an Analytical Hierarchy Process (AHP). A copy of the questionnaire is included as Supplementary material.

2.2.2. The Analytical Hierarchy Process (AHP)

AHP is a technique (Saaty, 1977) to support multi-criteria decision-making in discrete environments. The first step of AHP was to clearly define the main attributes that butchers take into consideration when purchasing fresh pig meat. To tackle this issue, we first relied on prior research performed on fresh pig meat (Font-i-Furnols and Guerrero, 2014; Kallas et al., 2012). The attributes identified from the literature were discussed in the applied focus groups. The final set of attributes included was: origin of the meat, external fat content, intramuscular fat content, sex of the pig and colour of the meat. Each attribute in the tree was divided into three different levels to be also valued (Fig. 2).

The relative importance or weights (w_i) of attributes and levels were obtained from paired comparisons determining the intensity of preference for each option on a 1 to 9 scale (1: both attributes are equally

Table 4
Butchers' attitudes and opinions towards banning piglet castration and entire male production.

Topics and questions (N = 127)	Yes	No	DK	P
A Potential impact of banning castration on pig production in Spain – boar taint				
A.1 Banning pig castration would affect your sales? ($\chi^2 = 0.29$; DF = 1)	52.4	47.6		ns
B Differences on quality between castrated and entire males				
B.1 Castration affects quality? ($\chi^2 = 7.8$; DF = 1)	62.6	37.4		**
B.2 Have you had any complain about meat quality? ($\chi^2 = 49.14$; DF = 1)	18.9	81.1		***
B.3 Have you had any complain about a strange odour in the meat? ($\chi^2 = 26.70$; DF = 1)	27	73		***
B.4 Consumers asked about the origin of this abnormal odour? ($\chi^2 = 20.83$; DF = 1)	88.6	11.4		***
E Quality criteria when purchasing pork meat				
E.1 Is it important to distinguish between entire male, castrated male or female? ($\chi^2 = 44.29$; DF = 1)	79.5	20.5		***
F Animal welfare				
F.1 Animal welfare legislation should be more restrictive in Spain? ($\chi^2 = 33.98$; DF = 2)	54.8[a]	33.1[b]	12.1[c]	***
F.2 Castration affects animal welfare? ($\chi^2 = 12.37$; DF = 1)	34.2	65.9		**
F.3 Customers would pay a plus for animal welfare? ($\chi^2 = 49.06$; DF = 1)	18.6	81.5		***
G Standard market (entire males) and high quality products				
G.1 Do you sell different types of meat? ($\chi^2 = 10.78$; DF = 1)	64.6	35.4		**
G.2 Do you sell meat from Duroc? ($\chi^2 = 25.66$; DF = 2)	52.4[a]	32.3[b]	15.3[c]	***
G.3 Customers would pay a plus for quality? ($\chi^2 = 0.20$; DF = 1)	48	52		ns

DK = do not know (only valid for F.1 and G.2); DF = degrees of freedom.
ns: P > 0.1; **: P < 0.001; ***: P < 0.0001.
Different superscripts show significant differences within the same question (P < 0.05).

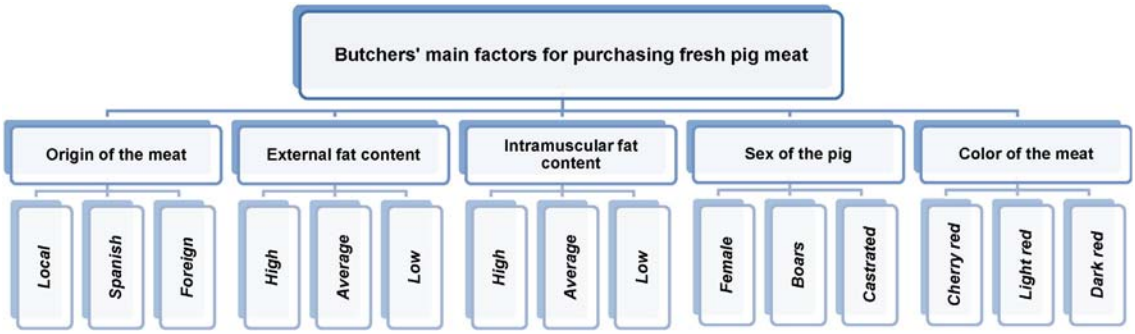

Fig. 2. Hierarchical structure used to value butchers' main factors in purchasing pig meat.

important; 9: very important compared to the other attribute) (Saaty, 1980). The relative importance of each attribute was obtained by comparing this attribute with all other attributes. A matrix of the judgements (a_{ijk}) of the comparison between attribute i and attribute j for each individual k was generated. Once the judgments were obtained, the next step was estimating the attributes' weights or relative importance. In the case of perfect consistency between respondents, n weights (w_{ik}) for each attribute can be easily determined from the $n(n-1)/2$ values for a_{ijk}, where n was the number of attributes. However, perfect consistency is seldom present, and personal subjectivity plays an important role in paired comparisons. Thus, alternative approaches have been proposed, and the row geometric mean is one of the most widely used (Aguarón and Moreno-Jiménez, 2000; Kallas et al., 2007). In this case, the weights assigned by butchers to each attribute are obtained using the following expression:

$$w_{ik} = \sqrt[n]{\prod_{i=1}^{i=n} a_{ijk}} \qquad \forall i,k. \tag{1}$$

The aggregation process of weights across butchers was obtained following Forman and Peniwati (1998), who considered that the most suitable method for aggregating individual weights (w_{ik}) in a social collective decision-making context is the row geometric mean:

$$w_i = \sqrt[m]{\prod_{k=1}^{k=m} w_{ik}} \qquad \forall i \tag{2}$$

where w_i is used to summarize the results of the AHP analysis.

Table 5
Main comments from the different stakeholders regarding focus group 1 questions.

	Farmers	Slaughterhouses	Industry	Government
A	*If only 30% of pigs are castrated, this demonstrates that 70% have no problem at all*	*Genetically, pigs have been selected to avoid boar taint*	*The Iberian pig is an animal with 160 kg live weight, so it would be unthinkable not to castrate them*	*There is still time to react till 2018*
	The only problems for farmers are aggression and strange behaviours of entire male pigs	*Pigs are slaughtered at a young age*	*I'd rather castrate all pigs*	
B	*For farmers who have always castrated, producing entire males may be a big change in handling*	*The farmer will pay in the end*	*We are afraid that another country starts producing Jamón Serrano*	*European Commission won't take any decision in welfare before doing the necessary studies*
	Unfortunately for farmers, slaughterhouses are able to choose other suppliers	*Commercial crossbreeds with large white and landrace have less boar taint than other breeds*	*If immunocastration is expensive they won't do it*	
C	*Originally the reason for giving up castration was productive performance*	*Non castrated animals have higher killing-out percentage because they have less fat*	*Entire males have boar taint*	*From the technological point of view, some products need castration*
D	*We are already exporting entire males to Europe*	*We are exporting for price, not for quality*	*Exportation rate is high*	*Lower weight animals should be produced*
	If you have <2% of carcases with boar taint Human Nose could work [*QS Quality Assurance*]	*I feel that the real problem is that there is no online system for this* [*QS Quality Assurance*]	*The cost of human nose is 2–3€ per carcase* [*QS Quality Assurance*]	*This is a cost for slaughterhouses* [*QS Quality Assurance*]
F	*Intensive production have more economic resources for adapting themselves to animal welfare in a positive way*	*Being so restrictive with animal welfare in Europe may do European market less competitive*	*Entire males are more aggressive between them*	*Some slaughterhouses are not adapted to animal welfare, and this problem goes to transport*
G	*Perhaps we have reached the maximum capacity of entire male production (70–80%)*	*You can't sell meat with boar taint because they will return it*	*Labelling meat from female means that it is not from male, so it won't have boar taint*	*Consumers don't appreciate meat from female pig because they don't know it*
	Farmers producing specific product not included as PSO need to find a way to protect their product		*We don't have any complaints about boar taint*	*We have to join this declaration, but we have some particular products to be included as exceptions*

A: Potential Impact of banning castration on pig production in Spain – Boar taint.
B: European Declaration on alternatives to surgical castration of pigs.
C: Differences in quality between castrated and entire males.
D: Exportation – QS Quality Assurance (classification of fresh meat according to boar taint).
F: Animal welfare.
G: Standard market (entire males) and high quality products.

Table 6
Main comments from the different stakeholders regarding focus group 2 questions.

	Retailers	Butchers	HORECA	Consumers
A	*There is a relationship between castration and boar taint, but percentage of carcasses with boar taint is low*	*Castration is important for us, to avoid boar taint*	*We will not pay to get meat with no boar taint*	*In general, consumers do not have knowledge about boar taint*
E	*Price*	*Food safety*	*Price*	*Quality*
	Colour	*Quality*	*Quality*	*Price*
	Food safety	*Price*	*Texture*	*Origin*
	Quality	*Fat content*	*Fat content*	*Fat content*
	Freshness	*Moisture*	*Age of the animal*	*Colour*
	Fat content	*Colour*	Moisture	Freshness
		Sex		
F	*There is a relationship between castration and animal welfare*	*Castration affects animal welfare*	*We have to discuss if castration is or not an animal welfare issue*	*It will be paid by consumer, but most of them are not willing to pay more for animal welfare*
G	*Iberian pig produce meat with better quality than commercial crossbreeds*	*Our customers look for high quality products*	*We have a low profit margin, so we go for conventional products*	*I prefer to buy in butcheries, mainly because of quality*

A: Potential Impact of banning castration on pig production in Spain – Boar taint.
E: Quality criteria when purchasing pork meat.
F: Animal welfare.
G: Standard market (entire males) and high quality products.

Finally, we followed Chrzan (1994) to mitigate the order effect in applying the AHP by changing the order of the different pairwise comparisons and the order of levels within each pairwise comparison.

Saaty (1980) defined the Consistency Ratio (CR) as a measure of respondent randomness in carrying out the pairwise comparisons. The CR is the quotient of the consistency index (CI) that is obtained from the maximum eigenvalue of the matrix of pairwise comparisons and a Random Index (RI) that denotes the CI for a randomly generated matrix of comparison. Values of CR ≤ 0.1 are acceptable.

2.2.3. Data analyses

The FREQ and MEANS procedures of the SAS software package (version 9.2; SAS Institute Inc., Cary, NC, USA) were used to analyse the questions from the face-to face surveys at butchers. A significance level of <0.05 was used. AHP was analysed by Super Decision Software (Creative Decisions Foundation, Pittsburgh, PA, USA).

3. Results and discussion

3.1. Focus groups

The main comments for each stakeholder are shown in Table 5 for FG1 and in Table 6 for FG2 to summarize the main ideas from the different focus groups.

3.1.1. Potential impact of banning castration on pig production in Spain – boar taint

Representatives of farmers and government agreed that although no official data are known, the castration rate in Spain was between 15% and 20%. All of the participants from FG1 believed that in Spain, there would be no important effects on pig production if castration was banned in the near future because a large part of the market was already based on entire males. However, farmers stated that if the declaration included a prohibition of castration, it would be a problem for the Iberian pig sector. One government representative commented that no clear actions regarding the banning of pig castration were suggested at present. In the same context, a representative from a processing plant stated that they did not castrate, as they had never identified boar taint as a serious problem for their consumers. A farmers' representative commented that the main reason for castrating was animal behaviour. Butchers felt that castration is important for them to produce certain high quality products.

Concerning boar taint, industry stakeholders commented that it was seldom found in fresh meat because animals were being slaughter at an early age (approximately 6 months old) and that genetic types had changed. A meat processing plant stakeholder mentioned that boar taint was more problematic in fresh meat than in processed products because it was easier to mask boar taint in the latter. Previous studies (Blanch et al., 2012; Panella-Riera et al., 2010) showed that approximately 40% of the population was not able to detect androstenone but all the participants from both FG1 and FG2 were uninformed about it. Butchers commented that part of the problem was that their consumers were not familiar with this flavour, as pigs were slaughtered young. Farmers added that in the United Kingdom, where pigs were not castrated and they were slaughtered at heavier weights, people were used to the entire male meat flavour. Participants of the focus group stated that if consumers found meat with boar taint, they did not repurchase from the same retailer and usually changed to another supermarket or butchery. This same conclusion was reached in a previous study testing consumer behaviour (ALCASDE, 2008): 50% of Spanish consumers would not repurchase pork meat, and 50% would not revisit the same shop when they perceived an abnormal odour in the meat. As will be discussed later in the face-to-face butcher results, consumers did not usually complain about abnormal odours in meat.

3.1.2. European Declaration on alternatives to surgical castration of pigs

Stakeholders including farmers, industry and government were aware of this declaration and stated that they knew that it was intended to be voluntary from 2018. However, farmers recognized that it might end up as being passed as a law. This declaration seemed to be a good initiative from the pig supply chain point of view because it was written by farming representatives and other stakeholders in the European pig supply chain as well as NGOs (Non-governmental organizations).

Regarding the potential alternatives to surgical castration without anaesthesia, participants from FG1 talked about castration with

Table 7
Butcher characteristics.

(*n* = 127)	
Age (y; mean ± SD)	48.4 ± 9.14
Sex (% man:% woman)	82:18
Household members (n; mean ± SD)	3.4 ± 1.08
Education (%)	
Primary	3.9
Secondary	50.4
Higher degree/not university	39.4
University	6.3
Relation within the business (%)	
Owner	70.5
Family employee	2.5
Employee	20.5
Other	6.6
Years in the butchery (y; mean ± SD)	17.7 ± 13.65

SD = Standard deviation.

analgesia and anaesthesia, immunocastration and the production of entire males. Participants from FG1 mentioned that it would be important to know if consumers were able to distinguish between meat from a castrated animal and meat from an entire animal. In relation to the cost of castration with anaesthesia and analgesia, the general feeling was that the cost should be the responsibility of the whole supply chain, although in practice, all the participants from FG1 admitted that it would be paid by the farmers. All the participants from FG1 and FG2 agreed that consumers would not accept meat labelled as *meat from immunocastrated animals* as an alternative to surgical castration.

Participants were aware that according to the declaration a list of traditional products–PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) or TSG (Traditional Specialities Guaranteed)–that require castration would be made. The products on this list would be exceptions to the declaration. The government representatives and meat processing plants were concerned about TSGs–they mentioned *Jamón Serrano* as an example–because it was not linked with a territory and a similar product could be produced in a different country with the TSG label. These concerns about traditional products may be because stakeholders were unsure about what exceptions could be included in the declaration.

3.1.3. Differences in quality between castrated and entire males

Regarding production and quality differences between castrated versus non castrated pigs, participants from FG1 knew that entire males had better performance than castrated, which was why certain farmers gave up castration, despite potential boar taint problems. Farmer stakeholders commented that slaughterhouses paid for lean content, and slaughterhouse stakeholders agreed with that. However, meat processing plant stakeholders and butchers said that they looked for meat with more intramuscular fat to produce their high quality products, and meat from entire male pigs sometimes was not suitable for them. All the participants from FG1 and butchers from FG2 agreed that high quality meat needed an adequate level of intramuscular fat to ensure good sensorial quality and consumer acceptability. Previous studies were in concordance with this idea (Cilla et al., 2006). Gispert et al. (2010) concluded that castrated and immunocastrated males had more intramuscular fat than entire males and females. Therefore, the use of immunocastrated animals might be an alternative to surgical castration, and all of the participants from FG1 and butchers agreed with this statement. Most consumer studies showed a high acceptance of immunocastration (Lagerkvist et al., 2006). However, studies in Switzerland and Norway showed that surgical castration with anaesthesia was more accepted than immunocastration. Regarding sensory quality, in a consumer study Font-i-Furnols et al., (2008) concluded that meat from castrated males, immunocastrated males and females had the same acceptability, which was higher than meat from entire males.

The butchers' representative said that some consumers preferred lean meat to fat meat because they did not have information on this subject, and consumers from FG2 thought that leaner meat is healthier. However, FG2 participants said that other customers preferred high quality products with more intramuscular fat content.

In line with the above findings, all of the participants expressed their concerns regarding the consequences of the potential ban on piglet castration on high quality products. The participants believed that for these products, it is necessary to perform surgical castration or immunocastration.

3.1.4. Exportation – QS Quality Assurance

All the participants from FG1 believed that the prohibition of castration would not be a problem for exportation because most pork exportation was performed for profit and not based on high meat quality. Although meat quality is different between castrated and entire males, all the participants from FG1 did not see quality as a problem because the rate of castration in Spain was already low and the exportation rate was high, approximately 30% (DataComex, 2014). Because Spanish farms were producing mostly entire males, all the participants from FG1 commented that Spain might had an advantage with respect to other countries where only castrated pigs were currently produced. However, farmers said that Germany and The Netherlands were starting to produce entire male pigs.

The German QS Quality Assurance scheme is a guideline for slaughtering and deboning. One of its rules states that companies should have a system for classifying fresh meat according to boar taint (https://www.q-s.de/qs-scheme/qs-certification-mark.html). Almost all the participants had notably little knowledge regarding this certification. The general feeling of the stakeholders was that there was a need to have a proper and fast online method available for detecting boar taint to avoid carcasses with boar taint, so that the industry would be able to export products to different markets according to the presence of boar taint. It was mentioned that some European countries were introducing a *human nose scoring system* to classify carcasses with boar taint, but slaughterhouses and pig industry experts thought that more research should be performed because the effectiveness of this method should be improved.

3.1.5. Quality criteria when purchasing pork meat

Participants in FG2 were asked about the purchasing decision from the stakeholder point of view when buying meat. The attributes considered by each stakeholder are detailed in Table 6. It was mentioned that good hygienic conditions were always demanded, which was taken for granted. Regarding the sex of the pig, butchers preferred female pigs, and said that the important thing was not the sex of the pig itself but the fact that the meat did not present boar taint. One of the retailers said that the sex of the animal was not important to them. Retailers added that attributes such as animal welfare and environmental aspects were incipient and currently not relevant. A consumer organization said that it would be important to provide more information in labelling regarding such quality attributes as castrated or non-castrated, type of production and origin although consumers were not able to process this information. Previous studies have reported that the most important attributes for consumers were the price, origin and taste/odour (Kallas et al., 2012; Meuwissen and Van Der Lans, 2005).

Regarding all these opinions, it seems that the primary attributes that consumers looked for were colour, fat content and price. As will be discussed later, this is in agreement with the results obtained in the face-to-face surveys at butchers and is in line with the attributes discussed in the literature (Font-i-Furnols and Guerrero, 2014; Kallas et al., 2012).

3.1.6. Animal welfare

All the participants agreed that currently, in addition to good quality meat, a proportion of consumers demanded products that come from animals with certain welfare standards. All participants agreed that castration was relevant to welfare because animals were castrated with no anaesthesia. This is in agreement with one previous study in Belgium (Heid and Hamm, 2013) in which focus group participants agreed that castration without anaesthesia affects animal welfare. Consumers said that they did not know how castration was performed. All the participants agreed that if pigs were castrated in the same way as veterinarians castrated domestic pets, it would not be a welfare issue. A retailer's representative said that consumers would always say that animals should not be castrated due to welfare issues, but butchers said that consumers did not know that one of the reasons for performing castration was to prevent boar taint. Previous studies also demonstrated that farmers and industry stakeholders agreed that Spanish consumers were not ready to pay for meat coming from animals raised under higher welfare conditions because animal welfare was a comparatively recent issue in Spain. All the participants from FG1 thought that animal welfare during slaughtering was good, but they thought that animal welfare during transportation could be improved.

Table 8
Main features of the butcheries.

(*n* = 127)	
Years of business (y; mean ± SD)	30 ± 20.41
Surface of the business (m^2; mean ± SD)	79 ± 180
Employees (n; mean ± SD)	
Temporary	0.2 ± 0.73
Steady	2.5 ± 2.03
Customers (%; mean ± SD)	
> 60 years old	25 ± 17.38
40–60 years old	49 ± 22.62
25–40 years old	18 ± 13.63
< 25 years old	8.8 ± 8.28
How do they buy meat? (%)[a]	
Whole carcass	28
Primal cuts	26
Pieces	71
What category/ies of SEUROP do they buy? (%)[a]	
S (≥60% lean)	34
E (<60% ≥ 55% lean)	44
U (<55% ≥ 50% lean)	13
R (<50% ≥ 45% lean)	13
O (<45% ≥ 40% lean)	3.1
P (<40% lean)	0
What percentage of meat for each sex of pig do they buy? (%)	
Entire male	7.6
Castrated male	18.0
Female	74.4

SD = Standard deviation.
[a] Multiple choice questions, butchers can choose more than one option.

3.1.7. Standard market (entire males) and high quality products

A representative of a meat processing plant stated that butchers avoided entire males because of boar taint and purchased meat from female pigs. This is in concordance with what butchers revealed about attributes when buying pork meat, and also with the results from the face-to-face surveys at butchers that we will discuss later. It was also mentioned that some retailers labelled pork and pork products as *meat from female*; they explained that with this label, consumers were sure that no boar taint would be present. However, as was said before, most consumers did not know the relationship between male/female meat and the presence of boar taint.

Butchers admitted that producing entire males slaughtered at an earlier age would not solve the problem because the reason for raising heavier animals was to have meat with certain quality characteristics required by the market. To avoid boar taint, farmers and industry stakeholders suggested using tainted meat for products where it was possible to mask this smell, but not for fresh and cured meat.

An industry stakeholder in the Madrid focus group was concerned about the Iberian pig (an autochthonous Spanish pig breed) and thought that it should be included as an exception to the European Declaration on alternatives to surgical castration of pigs (DG-SANCO, 2010).

3.2. Face-to-face surveys

The socio-demographic characteristics of the surveyed butchers and other main features are described in Table 7 and Table 8 respectively.

3.2.1. Potential impact of banning castration on pig production in Spain – Boar taint

Regarding the possible impact of a ban on pig castration on butchers (question A.1, Table 4), results did not show a clear agreement: 52.4% yes and 47.6% no. Because there were no significant differences, we assumed that butchers were unsure about what would happen if castration was banned in the near future. In the focus groups, participants were quite sure that the banning of pig castration would not be a large issue for conventional Spanish pig production, although butchers from the focus group were partly in disagreement with regard to high quality products. These results suggested that if butchers used meat from female pigs, they would avoid boar taint that could be rejected by consumers as seen in previous studies (Bonneau and Chevillon, 2012; Font-i-Furnols et al., 2000). Therefore, they selected female meat because they could not be sure that meat from boars was free from boar taint.

3.2.2. Differences in quality between castrated and entire males

Regarding meat quality, 62.6% of the butchers said that castration affected the meat quality (question B.1, Table 4), and agreed that meat from castrated animals had a better sensory quality than meat from entire males (Table 9). When comparing the different types of meat (entire males, castrated males and females), 74.0% of the butchers thought that meat from females was of better quality, 12.6% said that meat from castrated males was better, and only 4.7% said that meat from entire males was of better quality (Table 9). The results from a consumer study by Font-i-Furnols and Guerrero (2014) confirmed that consumers preferred meat from female or castrated pigs compared to entire male pigs. Focus group participants agreed that meat from castrated males was of better quality than meat from entire males because of intramuscular fat and because castration prevented boar taint. The butchers unanimously agreed that castration affected quality, mainly because of increased intramuscular fat and prevention of boar taint and strong odours.

Regarding complaints about meat quality, 18.9% of butchers admitted some complaints from costumers, among which 27.0% were related to the presence of abnormal odours in the meat (Table 4). Among the customers who complained about it, most of them (88.6%) asked about the origin of this odour, and the answer given by the butchers was that the meat came from entire male pigs. Regarding focus group comments, this low percentage of complaints could be observed because consumers are not used to complaining.

3.2.3. Quality criteria when purchasing pork meat

According to the butchers' opinion, when purchasing meat, 79.5% considered it important to know the pig's sex (Table 4). Bearing this in mind, butchers were asked about the percentage of meat from different sexes that they bought: 74.4% of females, 18.0% of castrated males and only 7.6% of entire males (Table 8). These results confirmed the opinion expressed in the focus groups that butchers preferred the meat from female pigs.

Butchers reported that the main attributes that their customers looked for were (Table 10): colour of the meat (important for 77.2% of consumers), price (72.4%) and fat content (68.5%). Colour of the meat, fat content and price were also the main attributes mentioned in the focus groups, and other attributes such as the origin or odours, although

Table 9
Butcher's opinion about sex of the pig ($\chi^2 = 316.39$; DF = 5; P < 0.0001).

Which sex has the best quality? (*n* = 127)					
EM	FE	CM	FE = CM	EM = FE = CM	DK
4.7[c]	74.0[a]	12.6[b]	5.5[bc]	1.6[c]	1.6[c]

EM = entire male; FE = female; CM = castrated male; DK = do not know.
Different superscripts show significant differences (P < 0.05).
Values with different superscript are significantly different P < 0.05.

Table 10
According to butcher's opinion, what consumers pay attention to, when buying pork meat?

(*n* = 127)	Origin	Price	Brand	Fat content	Colour	Odour
Pay attention (%)	26.8	72.4	15	68.5	77.2	49.6
Indifferent (%)	30.7	11	32.3	15	6.3	14.2
Don't pay attention (%)	42.5	16.5	52.8	16.5	16.5	36.2
χ^2	5.12	87.98	27.28	70.74	111.8	24.39
DF	2	2	2	2	2	2
P	ns	***	***	***	***	***

ns: P > 0.1; **: P < 0.001; ***: P < 0.0001.

mentioned, were ranked with less importance. Previous studies showed similar results (Font-i-Furnols and Guerrero, 2014; Kallas et al., 2012).

3.2.4. AHP Results: The relative importance of the attributes when purchasing fresh pig meat

AHP consistency was verified for each butcher, showing values <0.1 for all the individual pairwise comparison matrices.

The results of the aggregation of weights for the five main attributes (w_{A1}: origin, w_{A2}: external fat, w_{A3}: intramuscular fat, w_{A4}: sex of the pig and w_{A5}: colour) across butchers are shown in Fig. 3.

These results suggested that sex of the pig and intramuscular fat were the most important attributes for the butchers, with aggregate weights of 25.80% and 24.98%, respectively. The colour attribute occupied third position, with an aggregate weight of 21.69%. The origin and the external fat attributes had aggregated weights of 14.89% and 12.65%, respectively.

The global weights represent the total preference score or the total relative importance of each level, taking into consideration all attribute levels. Thus, we found that the most preferred level for butchers was the female level (17.46%), followed by light red colour (15.35%) and an average amount of intramuscular fat (15.25%). The lowest weight was assigned to foreign origin (1.10%) followed by dark red colour (1.96%).

The results from AHP were in accordance with the results from the focus group. Other questions in the survey indicated that butchers preferred and bought mostly meat from female pigs, and that fat content (intramuscular fat content in particular) and colour of the meat were also important attributes for butchers and consumers. Previous studies by Kallas et al. (2012) showed the relative importance of pig meat attributes for Spanish consumers: taste and odour (56.76%), price (20.96%), origin (16.32%) and sex of the pig (5.90%). The relative importance of origin was similar for both butchers (in the present study) and consumers (Kallas et al., 2012), but there was a difference of criteria about sex of the pig. It is important for butchers, while it seems that consumers from Kallas et al. (2012) study did not take sex into account.

3.2.5. Animal welfare

Butchers were asked about their knowledge of animal welfare. The butchers answered that it was 5.3 ± 2.28 on average (9-point scale). Their feeling about the level of animal welfare in Spain was 5.7 ± 2.11 (9-point scale). Table 4 (questions F.1, F.2 and F.3) shows the answers to different questions regarding animal welfare. Although butchers considered that the level of animal welfare was sufficient, 54.8% said that animal welfare legislation should be more restrictive in Spain. 65.9% answered that castration did not have any effect on animal welfare; one

Fig. 3. Relative importance of the main attributes when purchasing fresh pig meat.

explanation for this would be that they may not know how castration is performed. However, 81.5% said that their consumers would not pay a premium for animal welfare. This result confirmed the general focus group opinion that most consumers were not ready to pay a premium for animal welfare.

3.2.6. Standard market (entire males) and high quality products

Regarding the different types of meat, 64.6% of butchers were selling pork meat with different characteristics (different types of meat), and 52.4% were offering meat from Duroc, although they were unable to ensure that consumers would pay more for quality (Table 4). Regarding the types of pork meat that they were offering, the butchers mentioned: meat from Duroc, Iberian, conventional crossbreeds, females and meat from organic production. In line with what was said in the focus groups, Iberian pig breed and Duroc crossbreeds, as well as the sex of the animal, were the two most important factors for high quality products. Therefore, high quality products are linked to specifics breeds and to sex to obtain the appropriate amount of intramuscular fat.

4. Conclusions

According to Spanish pig supply chain stakeholders, the potential end of piglet castration in conventional pig production in Europe by 2018 might not be considered a problem because a high percentage of entire males are already currently being produced.

However, pork supply chain stakeholders considered it important to include high quality production, as Iberian breeds, in the list of exceptions to the European Declaration on alternatives to surgical castration of pigs. This is important primarily for butchers, because they produce high quality products that require castration to achieve specific requirements, such as adequate intramuscular fat content and lack of boar taint.

According to the AHP of the face-to-face surveys at butchers, the most important attributes of pig meat are intramuscular fat and sex of the pig. Butchers demand meat with a specific amount of intramuscular fat, which can be obtained from castrated and female pigs.

A list of traditional high quality products (PGI, PGO and TSG) that require castration should be done. These quality schemes must be included as exceptions in the European Declaration on alternatives to the surgical castration of pigs.

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Appendix A. Face-to-face butchery survey

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IV.3 Article 3: Consumers' sensitivity to androstenone and evaluation of different cooking methods to mask boar taint

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Consumers' sensitivity to androstenone and evaluation of different cooking methods to mask boar taint

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Abstract

Boar taint is an unpleasant odour and flavour present in some entire male pigs, produced by the presence of androstenone and skatole. The aim of the study is to assess the sensitivity to androstenone of 150 consumers and to compare the acceptability and liking of meat from castrated and entire pigs, cooked with different cooking methods. Meat samples consisted in loins from castrated (CM) and entire male pigs (EM) with high levels of androstenone cooked by two cooking methods: vacuum cooking and fried/breading with garlic and parsley. Consumers evaluated smell and flavour acceptability, and overall liking of CM and EM for each cooking method. Results of the sensitivity test showed that disliking of androstenone odour increased significantly with the sensitivity. Results of acceptability and overall liking were similar in CM and EM for both cooking methods. Therefore, the two cooking methods used in the study may be useful to mask boar taint in tainted meat.

Key words

Androstenone sensitivity; Boar taint; Cooking method; Consumer; Entire male pig; Masking strategies

1. Introduction

Boar taint is a distinctive odour and flavour that can be evident during the cooking or eating of pork and pork products from some entire male pigs, and it is caused by excessive accumulation of androstenone and skatole (Bonneau, 1982) that are accumulated in fat (Claus, Weiler, & Herzog, 1994). Numerous subsequent studies have confirmed that these two compounds are the main contributors to boar taint (Bonneau et al., 1992; Bonneau & Chevillon, 2012; Font i Furnols, 2012; Haugen, Brunius, & Zamaratskaia, 2012). In Europe, approximately 80% of male piglets are surgically castrated to avoid potential consumer dissatisfaction because of boar taint (Fredriksen et al., 2009). Since surgical castration of piglets has become an animal welfare concern, discussions at European level are aiming to ban surgical castration by 2018 (DG-SANCO, 2010). Therefore, if piglet castration is banned, meat from entire male pigs will increase in the market and therefore, those consumers that dislike boar taint may reject this meat if this taint is perceived.

Androstenone have been described with the attributes sweaty, dirty, silage and parsnips, and skatole have been associated with mothball and musty (Annor-Frempong, Nute, Whittington, & Wood, 1997; Font i Furnols, Guerrero, Serra, Rius, & Oliver, 2000; Blanch et al., 2012). Levels over the threshold of boar taint compounds can arise in some pigs (Font i Furnols et al., 2000). Different thresholds have been suggested, but the most commonly used are 0.5 and 1.0 μg/g of fat tissue for androstenone (Rhodes, 1971; Claus et al., 1994; Font i Furnols, Gispert, Diestre, & Oliver, 2003) and 0.10 and 0.20 μg/g of fat of fat tissue for skatole (Desmoulin, Bonneau, Frouin, & Bidard, 1982; Bonneau et al., 1992; Claus et al., 1994; Walstra et al., 1999; Font i Furnols et al., 2003). While skatole is perceived by 99% of consumers (Weiler, Fischer, Kemmer, Dobrowolski, & Claus, 1997), androstenone is perceived by around 40-50% of consumers (Weiler et al., 2000; Font i Furnols et al., 2003; Blanch et al., 2012; Razafindrazaka et al., 2015) which mean that consumers who are insensitive (anosmics) to this compound cannot smell it. Therefore androstenone sensitivity affects boar taint acceptability of consumers and different

types of consumers are described: pork lovers, boar meat lovers and reject boar taint meat (Panella-Riera et al., 2016). Some studies have also shown that women are more sensitive than men (Wysocki & Beauchamp, 1984; Weiler et al., 2000; Bremner, Mainland, Khan, & Sobel, 2003; Lunde, Skuterud, Nilsen, & Egelandsdal, 2009; Blanch et al., 2012). The prevalence of high levels of boar taint in Spain is 10.2%, but if only high levels of androstenone are considered, the prevalence is 5.5% and for high levels of skatole 6.6% (Borrisser-Pairó et al., 2016).

Since consumers are the last step of the chain production, consumer studies are necessary to assess sensory acceptability and attitudes of consumers towards pork and pork products from boars (Font i Furnols, 2012). Consumers' opinion is important when a new product is released (Amerine, Pangborn, & Roessler, 1965). Font i Furnols (2012) reviewed different consumer studies in boar taint and reported that meat from entire male pigs obtained lower acceptability than meat from castrated male pigs. This study also reported that odour is the main attribute affected by the presence of boar taint.

Previous studies evaluated different cooking methods by a trained panel and they found that the cooking method has an effect on boar taint perception (Agerhem & Tornberg, 1995; Wood, Nute, Fursey, & Cuthbertson, 1995; Prestat, Jensen, McKeith, & Brewer, 2002; Font i Furnols, 2012). Cooking methods can be classified depending on the use or not of a liquid medium, how heat is received (direct fire, hot plate or hot air) and there are also some new technologies such as microwave or vacuum cooking (Bello, 1998). Vacuum cooking prevents the development of some off-flavours and the loss of flavour volatiles (Schafheitle, 1990; Armstrong & McIlveen, 2000). The use of oil as a cooking medium when frying may have an effect on sensory perception due to its aromatic compounds (Prestat et al., 2002). A study from Linares et al. (submitted for publication) performed within the same project as the present study concluded that both vacuum cooking and frying reduced androstenone perception in a trained panel, being the frying method more effective.

The use of some herbs and aromatic plants for cooking is very common (Bianchi, 2015) and it might be considered for masking boar taint in pig fresh meat. Breaded is a common used strategy by consumers, and breaded products are more palatable because of a crispy crust and a soft interior (Antonova, Mallikarjunan, & Duncan, 2003). Mörlein et al. (2015) found that the use of spices or other ingredients mask odour perception of boar taint. Aaslyng, De Lichtenberg Broge, Brockhoff, and Christensen (2015) assessed bacon and pork belly roll and found also that smoked and the different spices used can mask boar taint. A previous study by Egea, Linares, Gil, López, and Garrido (submitted for publication) within the same project as the present study evaluated different masking strategies to reduce androstenone odour and flavour in fresh pig meat in a trained panel. They concluded that breading with garlic and parsley was the best masking strategy.

If castration is prohibited a high percentage of meat from entire male pigs with boar taint will be present in the market (Borrisser-Pairó et al., 2016) and it will be important to find a proper use for this meat (Lunde et al., 2008). The present study had two main objectives: 1) to study consumer sensitivity to androstenone to find how many consumers are high sensitive and may reject tainted meat; 2) to assess different cooking methods (vacuum cooking and fried/breading with garlic and parsley) to mask boar taint, by comparing consumers' acceptability of fresh meat from entire male pigs with high levels of androstenone and from castrated male pigs.

2. Material and methods

2.1. Selection and preparation of meat samples

Loins (*Longissimus thoracis et lumborum*) from castrated pigs were selected to be compared with loins from entire male pigs with boar taint. The human nose methodology (Mathur et al., 2012; Borrisser-Pairó et al., 2016) was used at slaughterhouse to find carcasses with high levels of boar taint. These loins were analysed for androstenone and skatole. Androstenone analysis was performed by gas chromatography-mass spectrometry (Rius & García-Regueiro, 1998) and

skatole analysis by high-performance liquid chromatography (García-Regueiro & Rius, 1998). Loins from entire male pig selected for the study had high levels of androstenone (from 2.02 to 2.30 µg/g in fat tissue) and low/medium levels of skatole (from 0.05 to 0.14 µg/g in fat tissue). After collection, samples were kept in the freezer at -20ºC. Loins were sliced frozen in 1.5 cm loin chops and each loin chop was individually packed, labelled and stored at -20ºC until the time of use.

2.2. Cooking methods

Two different cooking methods were used: 1) vacuum cooking and 2) fried/breading with garlic and parsley. These methods were selected from previous experiments that belong to the same project as the present study, where a trained sensory panel evaluated the effect of different cooking methods (Egea et al., submitted for publication; Linares et al., submitted for publication).

2.2.1 Vacuum cooking

Loin chop samples were thawed for 12 h at 4ºC. The meat samples were vacuum-packaged in polyethylene bags (R-RE; Industrias RAELMA, S.L., Madrid, Spain) and were placed in a water bath at 75ºC until the internal temperature reached 72ºC. Samples were then stored at 4ºC till the next day, when the study was performed. The meat samples were regenerated in a water bath until the internal temperature reached 65-70ºC for the consumers' study.

2.2.2 Fried/breading with garlic and parsley

Fifty grams of breadcrumbs (Aliada, Madrid, Spain), 8 g of fresh white garlic, 8 g of fresh parsley and 1.5 g of salt were chopped and minced in a blender (Classic, Moulinex®, Barcelona, Spain).

Loin chop samples were thawed for 12 h at 4ºC. A 26 cm of diameter pan with 40 ml of olive oil virgin extra (Koipe, Andújar, Spain) was preheated for 2 minutes on an induction cook top until oil temperature reached 150ºC. Each loin chop was flipped 6 times in the mixture to fix the covering before putting it in the pan for frying. Loin chops were turned up-down side after 2, 3, 4 and 5 min (internal temperature 83ºC).

2.3. Experimental design

The consumer study was carried out in Madrid (Spain) during June 2014, in a proper sensory room at Silliker Ibérica S.A. facilities. One hundred and fifty consumers were selected from the region of Madrid following the Spanish population profile over 18 years of age (INE, 2014). Seven sessions were carried out with 20-22 consumers each.

2.3.1 Evaluation of consumer sensitivity to androstenone

A test for androstenone sensitivity was performed to consumers after meat sample evaluation. Following the protocol described by Weiler et al. (2000) and (Blanch et al., 2012), a flask with pure crystals of androstenone was presented to each consumer. Consumers had to answer 3 questions: i) Do you smell anything? (No; Yes); ii) In an 8-point scale, can you score the intensity of this smell? (from 1='extremely weak' to 8='extremely strong'); iii) Do you like the smell? (I like/Neutral or I don't like). Considering the intensity score given, consumers were classified using the classification described by Blanch et al. (2012): 'Insensitive', 'Low sensitive' (1–3), 'Middle sensitive' (4–6) or 'High sensitive' (7–8).

2.3.2 Evaluation of meat samples

After cooking, each loin chop was cut in 8 pieces discarding the edge pieces, so 6 pieces per loin were obtained. Each sample was placed inside a disposable aluminium baking cup to keep it warm for the consumers. Each consumer had to evaluate a sample from castrated male pig and a sample from entire male pig for each cooking method. Consumers always had to evaluate samples starting for the sample from the left side which was changed randomly for each consumer to avoid the first sample effect (Macfie, Bratchell, Greenhoff, & Vallis, 1989), so the position of the castrated and the entire male sample was changing. Consumers were asked to evaluate their acceptability (smell and flavour acceptability): first, they had to smell both samples (boar and castrated) and decide which sample they preferred according to smell; secondly, they were asked to eat both samples and decide which sample they preferred according to flavour. After that, consumers were asked to score each sample individually on a nine-point liking scale where no middle point was given (overall liking: from 1=dislike extremely to 9=like extremely). They had to do it for both cooking methods' comparisons. At the end, consumers were also asked to fill in a questionnaire, with socio-demographic questions.

2.4. Statistical analyses

Data analyses was done using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Descriptive analyses of the parameters was performed using the MEANS and the FREQ procedures. The GENMOD procedure was used for analysing differences in discrete variables. The GLIMMIX procedure was used to analyse consumer acceptability with type of meat, cooking method and position of the sample as fixed effects. Significance level was set at $P<0.05$ for differences and $P<0.10$ for tendencies.

3. Results

3.1. Consumer profile

Socio-demographic characteristics of the 150 consumers are shown in Table 1. Consumer profile was representative of the Spanish population (INE, 2014). The gender ratio of the consumers was balanced. Two thirds of the consumers were regular pork consumers, eating fresh pork at least 2 times per week.

3.2. Consumer sensitivity to androstenone

Results of sensitivity to androstenone are shown in Figure 1. Considering all the consumers, 30.0% of them were insensitive (anosmic) or low sensitive to androstenone, 32.7% middle sensitive and 37.3% high sensitive. No differences between genders were observed when classifying consumers by these 3 sensitivity groups ('insensitive/low sensitive', 'middle sensitive' and 'high sensitive'). However, when consumers were classified in 2 groups ('insensitive/low/middle sensitive' and 'high sensitive') women sensitivity to androstenone tended to be higher than men (P=0.054). Regarding the liking of those consumers that are sensitive to androstenone, 61.5% of consumers disliked the odour and the rest (38.5%) found it neutral or liked it. Results of liking according to the sensitivity to androstenone are presented in Figure 2. The percentage of consumers that disliked androstenone increased with the sensitivity (P=0.016), the highest the sensitivity to androstenone the highest the percentage of consumers that disliked it.

Figure 3 shows the percentage of consumers that may reject tainted meat. Two thresholds of rejection were established: high sensitive consumers that dislike androstenone (Figure 3A); or middle/high sensitive consumers that dislike androstenone (Figure 3B). Depending on the threshold this percentage was between 28.0% and 44.8% for all consumers, 35.6% and 48.0% for women, and 20.8% and 41.6% for men, for high sensitive and middle/high sensitive

211 respectively. In Figure 3A the percentage of women that may reject tainted meat was higher
212 than men (P=0.024). In Figure 3B no differences were observed.
213

214 *3.3. Acceptability of smell and flavour of the meat*

215 Results of percentages of consumers that prefer meat from castrated and meat from entire male
216 pigs for each cooking method are shown in Table 2. Regarding acceptability of smell and flavour,
217 similar results for castrated and entire male pig meat were observed and no significant effect
218 was found for type of meat and cooking method (P>0.05). Table 3 shows the results of smell and
219 flavour acceptability according to consumer sensitivity to androstenone. Regarding smell
220 acceptability for vacuum cooking, the group 'high sensitive consumers to androstenone' showed
221 a lower percentage of acceptability (33.3%) for meat from entire male pigs (P=0.001). Regarding
222 smell acceptability for the fried/breading with garlic and garlic method, the group 'high sensitive
223 consumers to androstenone' showed a higher percentage of acceptability (64.3%) for the meat
224 from entire male pig (P=0.009). No significant differences were found in the flavour acceptability
225 for consumer sensitivity (P>0.05).
226

227 *3.4. Overall liking of meat*

228 Results of liking score means and standard deviation for each individual sample are shown in
229 Table 2. No significant differences were observed in the liking score between samples and
230 methods (P>0.05). Regarding consumers' approval for each sample, results were analysed
231 defining two groups of consumers: those that scored below 5, and those that scored 5 or more
232 in the nine-point liking scale. Therefore the percentage of consumers that approve each sample
233 (giving a score of 5 or more) could be calculated: for vacuum cooking, 73.3% approved castrated
234 pig meat and 76.0% entire male pig meat; for fried/breading with garlic and parsley, 92.0% and
235 95.3% respectively (Figure 4). No differences were observed between type of meat (P>0.05).

Overall liking was also analysed according to gender and according to consumer sensitivity to androstenone but no significant differences were found (P>0.05).

4. Discussion

A lot of consumer studies regarding boar tainted meat have been done, but it is complex to compare between studies because of the utilization of different methodologies (Font i Furnols, 2012). It is difficult to harmonise the methodologies and depending on the aim of the study a specific methodology may be more convenient (Aaslyng et al., 2007). Regarding sensitivity to pure crystals of androstenone, the percentage of insensitive consumers found in the present study (30.0%) was lower than previous studies that found between 41.1% and 69.7% (Weiler et al., 2000; Font i Furnols et al., 2003; Lunde et al., 2009; Blanch et al., 2012; Aluwé et al., 2015; Razafindrazaka et al., 2015). Bremner et al. (2003) reviewed previous studies on sensitivity to androstenone and found that the percentage of insensitive consumers varied from 7.6 to 75.0%. It is important to notice that only Blanch et al. (2012) used the same methodology than the present study to classify consumer sensitivity to androstenone. Mörlein, Meier-Dinkel, Moritz, Sharifi, and Knorr (2013) and Aluwé et al. (2015) used a methodology to evaluate sensitivity to androstenone based on smell strips with a known concentration of androstenone, while the other studies used pure crystals or dissolutions in different medias (Weiler et al., 2000; Font i Furnols et al., 2003; Lunde et al., 2009; Blanch et al., 2012; Razafindrazaka et al., 2015).

Furthermore, when consumers were classified according to gender, previous studies found that women were significantly more sensitive than men (Wysocki & Gilbert, 1989; Weiler et al., 2000; Lunde et al., 2009; Mörlein et al., 2013), though in the study by Blanch et al. (2012) these differences were not found. The present study found no significant differences, although when two groups of sensitivity were done, women tended to have more sensitivity than men (P=0.054) that women had more sensitivity than men. The percentage of consumers that dislike

androstenone odour was similar to the one found in the study by Blanch et al. (2012) but higher than other studies (Font i Furnols et al., 2003; Razafindrazaka et al., 2015). The present study found that high sensitive consumers show higher dislike for androstenone than insensitive consumers (P=0.016). Previous studies had also seen the same relationship between these two parameters (Weiler et al., 2000; Font i Furnols et al., 2003; Blanch et al., 2012; Razafindrazaka et al., 2015).

Consumers may reject tainted meat depending on their sensitivity and their liking of androstenone (Blanch et al., 2012; Font i Furnols, 2012; Panella-Riera et al., 2016). Therefore, high sensitive consumers that dislike androstenone are more likely to reject tainted meat than low sensitive consumers. Blanch et al. (2012) found that between 19.8 and 40.6% of Spanish consumers may reject boar taint. These results do not differ too much from the ones found in the present study (between 28.0 and 44.8%) performed with the same methodology. A review by Font i Furnols (2012) analysed different studies that have shown that boar taint odours are more present in meat from entire male pigs, and the present of these odours reduced the acceptance of this type of meat. Therefore efforts to improve the acceptability of tainted meat should focus on those consumers that are more sensitive to androstenone and dislike the smell.

The presentation of the meat sample is also important. Since boar taint is caused by volatiles compounds, the perception of boar taint may be influenced by the type of recipient used for the study and the serving temperature (Taylor, 1998), so the use of a disposable aluminium baking cup seems to be a good option odours and flavours. All the methods have some limitations but it would be important to harmonize the methodologies to assess consumer sensitivity to androstenone to be able to compare the results of different consumer studies (Font i Furnols, 2012).

In previous experiments from the same project (Egea et al., submitted for publication; Linares et al., submitted for publication), a trained panel compared samples from entire and castrated

male pigs with no masking strategy and they found that meat from entire males had higher scores in boar taint intensity of odour and flavour. When different cooking methods (vacuum cooking and fried/breading with garlic and parsley) were used, the panellists in these studies found no differences between castrated and entire male pig meat. Similar results were found in the present study, in which consumers found no differences between the two types of meat cooked with different cooking methods. Therefore consumers confirmed the results from the previous studies in a trained panel. Regarding smell acceptability, results showed that high sensitive consumers that dislike androstenone preferred castrated meat in the vacuum cooking although in the fried/breading with garlic and parsley the result was the opposite, they preferred the smell of entire male pig meat. Androstenone is a volatile compound so its concentration may be reduced when a cooking method with heat is applied (Denhard, Claus, Herbert, & Hillenbrand, 1995). One explanation of why vacuum cooking entire male pig meat is less preferred might be because the vacuum bags keep the androstenone odour inside, since it is considered as a hermetic packaging that prevents the loss of volatile flavours (Schafheitle, 1990; Armstrong & McIlveen, 2000). High sensitive consumers that dislike this smell may perceive it and may reject this meat. In the case of the fried/breading with garlic and parsley entire male pig meat, the fact that meat is cooked and heated in a pan may reduce the concentration of androstenone that in combination with the other aromatic ingredients (olive oil, garlic and parsley) can increase meat acceptability. Another hypothesis of why fried/breading with garlic and parsley produces less rejection in high sensitive consumers that dislike androstenone may be because of a chemical reaction between components of the masking strategy (breadcrumbs, garlic and/or parsley) or the frying medium with androstenone such as Diels-Alder reaction (Kagan & Riant, 1992; Martínez et al., 2016) or Maillard reaction (Martins, Jongen, & van Boekel, 2000), that may change androstenone structure and consequently reduce its perception. Martínez et al. (2016) concluded that the use of spices with a smoking process reduces androstenone perception in cooked sausages. Therefore the use of some ingredients and

cooking strategies may be useful to reduce boar taint perception and consequently may be a good opportunity for industry to find a market place for tainted meat and increase its acceptability.

5. Conclusions

High sensitive consumers that dislike androstenone odour are more likely to reject tainted meat, consequently actions to improve acceptability and liking of tainted meat have to be focused on these consumers. The cooking methods used (vacuum cooking and fried/breading with garlic and parsley) resulted useful to mask and counteract the negative effect that the presence of androstenone may have in the sensory quality of fresh pig meat. The fried/breading with garlic and parsley method seems to be more effective in this goal as was showed from the evaluation of all the sensory attributes considered, and even improved acceptability in high sensitive consumers. These methods may be a good opportunity for pig industry as a solution for tainted meat, therefore a system to classify tainted meat at slaughterhouse should be considered to orientate these meat to make specific products, commercialized with specific cooking recommendations to mask the off-flavour.

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501 **Tables caption**

502 **Table 1. Description of the consumers that participate in the study (n=150)**

	n	%	Population %[1]		n	%	Population %[1]
Education				**Gender**			
Primary	18	12	17	Women	73	49	49
Secondary	60	40	44	Men	77	51	51
Higher/University	72	48	39				
Employment				**Monthly income**			
Inactive	40	26.7	35	< 1500 €	62	43	46
Unemployed	43	28.7	14	1500 € - 3000 €	66	47	40
Employees	67	44.7	49	> 3000 €	16	11	14
Family Members				**Age**			
1 person/home	5	3.3	26	18-30 years	33	22	20
2 persons /home	25	16.7	30	30-45 years	59	39	35
3 persons /home	61	40.7	20	45-65 years	50	33	34
> 4 persons /home	59	39.3	24	>65 years	8	5.3	11
Frequency of eating fresh pork				**Lived in a rural environment**			
≤ 1 time/week	49	32.7	-	No	111	75	-
2-3 times/week	87	58	-	Yes	38	26	-
≥ 4 times/week	14	9.3	-				

503 [1]Data from Spanish population (INE, 2014)

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505

506 **Table 2. Description of the results from the different cooking methods and type of meat evaluated**

Cooking method	Type of meat	Smell acceptability[1]	Flavour acceptability[1]	Liking score[2]			
				mean	SD	minimum	maximum
Vacuum cooking	Castrated	51	52	5.19	1.3	0	8
	Entire male	49	48	5.31	1.2	2	8
Fried/breading with garlic and parsley	Castrated	49	49	6.03	1.2	2	8
	Entire male	51	51	6.07	1.0	2	8

507 [1]Percentage of consumers preferring castrated or entire male pig meat within the same cooking method
508 for smell and for flavour. No significant effects were found for type of meat and cooking method (P>0.05).
509 [2]Liking score was based on a 1-9 scale (1=dislike extremely to 9=like extremely; the middle point was
510 not given).
511 SD: standard deviation.
512

513

514

Table 3. Percentage of consumers preferring castrated or entire male pig meat within the same cooking method for smell and for flavour according to their sensitivity to pure crystals of androstenone

	Cooking method	Sensitivity to androstenone	castrated male	entire male	Sig*
Smell acceptability	Vacuum cooking	Insensitive and Low and Middle sensitive	45.7	54.3	a
		High sensitive + I like/neutral	35.7	64.3	b
		High sensitive + I don't like	66.7	33.3	b
	Fried/breading with garlic and parsley	Insensitive and Low and Middle sensitive	55.3	44.7	b
		High sensitive + I like/neutral	42.9	57.1	ab
		High sensitive + I don't like	35.7	64.3	a
Flavour acceptability	Vacuum cooking	Insensitive and Low and Middle sensitive	50.5	49.5	-
		High sensitive + I like/neutral	50.0	50.0	-
		High sensitive + I don't like	54.8	45.2	-
	Fried/breading with garlic and parsley	Insensitive and Low and Middle sensitive	47.9	52.1	-
		High sensitive + I like/neutral	42.9	57.1	-
		High sensitive + I don't like	54.8	45.2	-

*Different superscripts show significant differences within the same cooking method ($P<0.05$).

Figures caption

Figure 1. Classification of consumers (%) according to their sensitivity to pure crystals of androstenone and gender (low sensitive: score 0-3; middle sensitive: score 4-6; high sensitive: score 7-8)

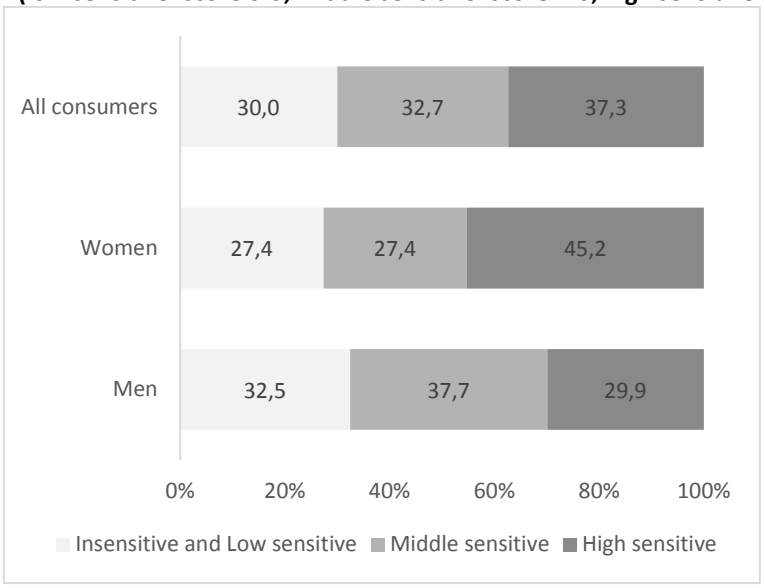
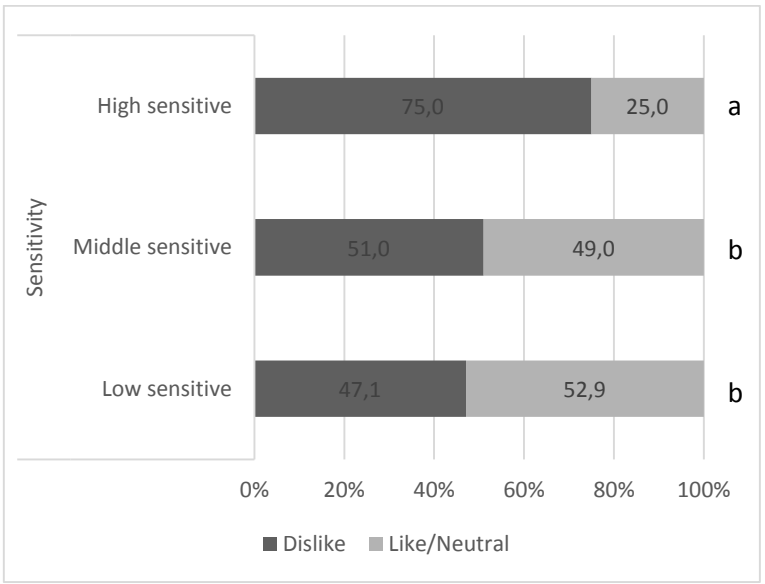
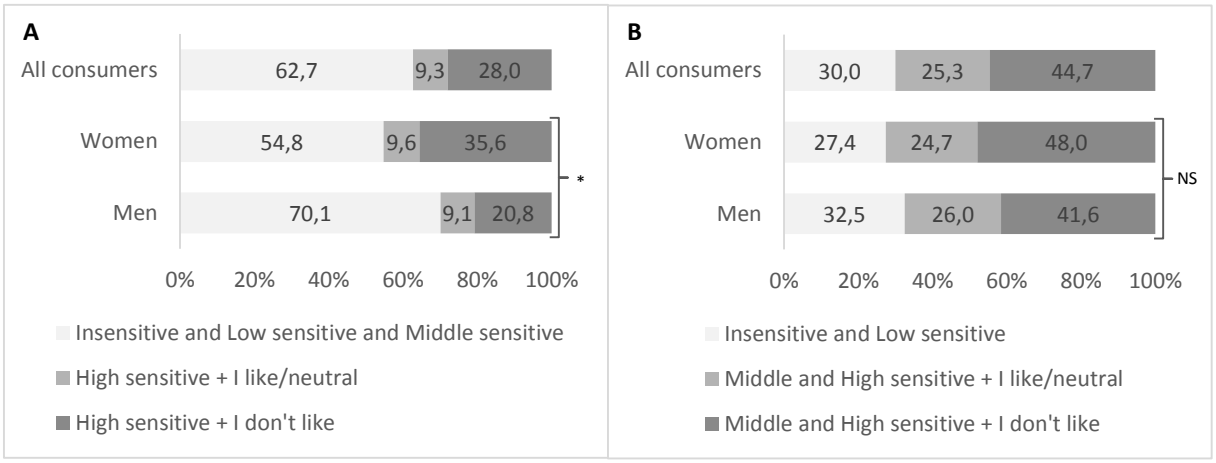


Figure 2. Consumer percentage of liking to androstenone by sensitivity to pure crystals of androstenone (low sensitive: score 0-3; middle sensitive: score 4-6; high sensitive: score 7-8)



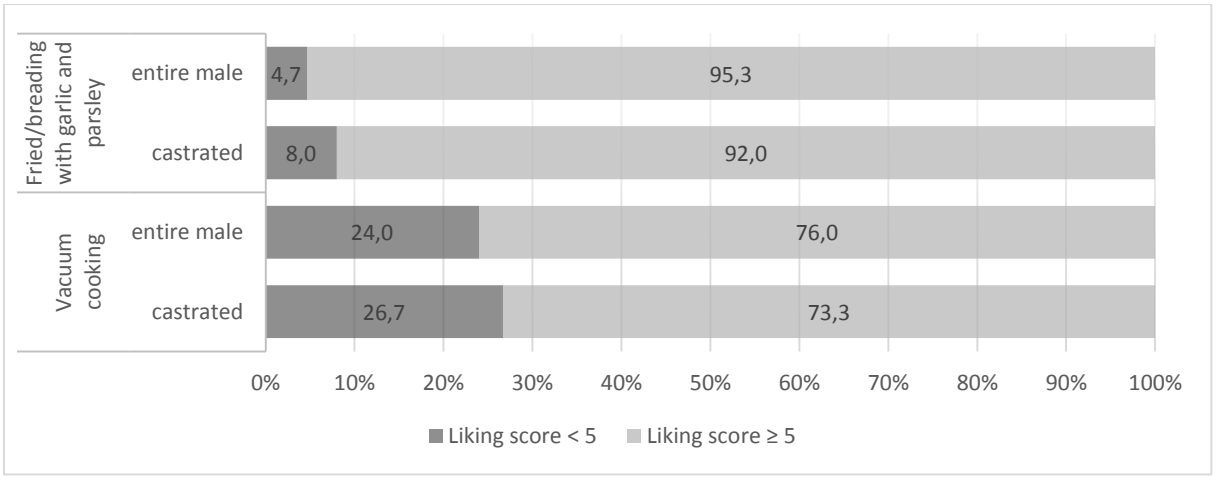
Different letters show significant differences between groups (P<0.05).

Figure 3. Classification (%) of consumers depending on the sensitivity and liking of pure crystals of androstenone, considering that consumers that may reject tainted meat were high sensitive (A) or middle/high sensitive (B)



*Significance: P<0.05; NS: no significance differences.

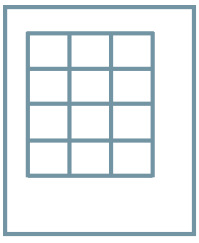
Figure 4. Percentage of consumers that scored 5 or more in a liking scale for entire male and castrated male pig for each cooking method



Liking score was a value in a 9 (step) points scale (1=dislike extremely to 9=like extremely).

Capítol V

Síntesi de resultats i discussió



La producció porcina és un dels sectors més importants de l'estat espanyol, d'Europa i a nivell mundial. La castració quirúrgica abans dels 7 dies de vida en porcs mascles és una pràctica utilitzada de manera rutinària en moltes explotacions, la finalitat de la qual és evitar l'aparició de l'olor sexual en la carn i també aconseguir una adequada proporció de greix intramuscular (Lundström*, et al.*, 2009). De totes maneres en el cas de l'estat espanyol aquesta pràctica no és tant habitual (Fredriksen*, et al.*, 2009). En els darrers anys, a nivell europeu, s'ha qüestionat l'ús de la castració des del punt de vista del benestar animal. Aquesta situació ha portat la Unió Europea a plantejar l'abandonament de la castració quirúrgica dels garrins a partir de l'any 2018 amb la *European Declaration on alternatives to surgical castration of pigs* (DG-SANCO, 2010). Tenint en compte aquest escenari, una de les possibles alternatives a la castració quirúrgica és la producció de porcs mascles sencers, i és en aquest sentit on la present tesi ha enfocat els seus objectius.

La producció de mascles sencers és més eficient que la de castrats (Lundström*, et al.*, 2009) però s'ha de tenir en compte també que existeixen diferències en la qualitat de la carn. Per una banda existeix la possibilitat d'aparició d'olor sexual a la carn i per altra banda la carn provinent de mascles sencers té més contingut en magre i, per tant, menys quantitat de greix intramuscular que la carn provinent de castrat (Fàbrega*, et al.*, 2010; Gispert*, et al.*, 2010).

V.1 Prevalença de canals amb presència d'olor sexual

Un dels primers aspectes a tenir en compte és el percentatge de canals procedents de porcs mascles sencers que poden tenir nivells alts d'olor sexual. La prevalença d'olor sexual en les canals pot variar en funció de la genètica, el pes de l'animal i el maneig (Zamaratskaia*, et al.*, 2005; Aluwé*, et al.*, 2011a; Aluwé*, et al.*, 2011b), a banda també hi ha diferències individuals que fan que animals criats sota les mateixes condicions presentin concentracions diferents d'androstenona i escatol en el greix.

Els resultats del present estudi mostren que un 10,2% de les canals estudiades presenten nivells alts d'androstenona (>1,0 μg/g de greix), d'escatol (>0,20 μg/g de greix) o ambdós compostos alhora. Aquesta és una informació

objectiva i útil per al sector, que permet actualitzar el percentatge real de canals porcines amb nivells alts d'olor sexual a l'estat espanyol. En total es van mostrejar 903 canals porcines convencionals a nivell d'escorxador, provinents de 30 granges repartides en 5 comunitats autònomes (**Taula 4**). Un 73,4% dels animals classificats eren creuaments comercials de Pietrain × (Landrace×Large White).

Taula 4. Nombre de mostres per comunitat autònoma i per genètica.

	Pietrain × (Landrace × Large White)	Altres creuaments amb Pietrain	Duroc × (Landrace × Large White)	Total per comunitat
Aragó	90	90		180
Catalunya	90	90		180
Castella i Lleó	180			180
Madrid/Castella-La Manxa	180			180
Múrcia	123		60	183
Total per genètica	663	180	60	903

A l'hora de valorar la repercussió d'aquest percentatge, s'ha de tenir en compte que a l'estat espanyol es van sacrificar l'any 2015 uns 46 milions de porcs (MAGRAMA, 2015). Si es contempla que la meitat d'aquests són mascles i no es permetés la utilització de la castració, representaria que aproximadament uns 2,3 milions de porcs mascles sencers podrien presentar nivells alts d'olor sexual. Així que, tot i que el percentatge sembla baix, en nombres absoluts representaria una quantitat important de canals i tones de carn (198 mil tones, considerant un pes canal de 84,48 kg segons dades del MAGRAMA de 2015). En termes econòmics la quantitat de carn amb nivells alts d'olor sexual podria arribar a representar 258 milions d'euros, considerant un preu de 1,28 €/kg segons la llotja de Lleida (carndeporc.cat, 2016).

Com que hi ha aquest 10,2% de canals amb presència d'olor sexual que podrien ser problemàtiques per alguns consumidors, és interessant que la indústria sigui capaç de detectar i separar aquestes canals per destinar-les a altres tipus de productes on s'eviti el rebuig per part dels consumidors (Babol i Squires, 1995; Martínez*, et al.*, 2016; Egea*, et al.*, submitted for publication). Actualment no existeix cap mètode de referència que escorxadors, sales de desfer i indústries càrnies puguin utilitzar per poder seleccionar en mateixa línia de sacrifici aquestes canals. A Europa s'han desenvolupat diferents mètodes per determinar la

concentració d'androstenona i escatol en greix. Entre aquests, hi ha mètodes colorimètrics (Squires, 1990), immunològics (Tuomola*, et al.*, 1997; Claus*, et al.*, 2008), cromatogràfics de gasos (García-Regueiro i Diaz, 1989; Annor-Frempong*, et al.*, 1997) i cromatogràfics de líquids (García-Regueiro i Rius, 1998). El *Joint Research Centre* (JCR) ha desenvolupat un mètode europeu de laboratori de referència per determinar androstenona i escatol (Buttinger i Wenzl, 2014). El problema de tots aquests mètodes és el cost que tenen i el temps que implica fer els anàlisis, per tant, no són mètodes adequats ni pràctics que es puguin utilitzar a nivell d'escorxador. En aquest sentit i en la recerca de mètodes ràpids i viables, diferents escorxadors europeus han començat a avaluar les canals sensorialment mitjançant panel entrenat (Bekaert*, et al.*, 2013; Trautmann*, et al.*, 2016). Aquest mètode consisteix en escalfar el greix de la canal per tal que s'alliberin els vapors que panelistes entrenats oloraran i avaluaran segons la presència d'olor sexual, es coneix com a *Human Nose Scoring System* (Mathur*, et al.*, 2012; Trautmann*, et al.*, 2016).

V.2 Sensibilitat a l'androstenona

Diversos estudis han demostrat que, com que la percepció d'androstenona està relacionada amb el receptor olfactiu OR7D4 (Keller*, et al.*, 2007) i està determinada genèticament (Wysocki i Beauchamp, 1984), existeix un percentatge de consumidors que no són capaços de detectar-la (consumidors anòsmics a la androstenona) i que entre els que la detecten hi ha diversos graus d'acceptabilitat (Blanch*, et al.*, 2012). Els resultats del present estudi mostren que un 30,0% de consumidors avaluats són anòsmics a l'androstenona. Per tant, almenys hi ha aquest percentatge de consumidors pels que la presència d'androstenona no suposaria cap problema. Els consumidors que sí que podrien rebutjar la carn amb presència d'androstenona són aquells consumidors altament sensibles, que han puntuat 7 o 8 d'intensitat d'olor en el test d'anòsmia i que a més no els agrada l'olor (Blanch*, et al.*, 2012; Panella-Riera*, et al.*, 2016). En aquest estudi un 28,0% dels consumidors entraven dins aquesta classificació i, per tant, podrien rebutjar la carn amb presència d'androstenona. En el cas que es tinguessin en compte els consumidors mitjanament sensibles que no els agrada l'androstenona (han puntuat

de 4 a 6 en el test d'anòsmia), aquest percentatge podria augmentar fins al 44,7%. Aquest percentatge pot augmentar si es té en compte també l'escatol, però per tal de minimitzar els factors que poden influenciar la percepció del consumidor, l'estudi va centrar els seus objectius únicament en la percepció de l'androstenona.

Tenint en compte les dades de sensibilitat a l'androstenona juntament amb les dels nivells alts de prevalença d'olor sexual a les canals, el percentatge de canals que podríen ser problemàtiques es redueix ja que el 10.2% de les canals amb nivells alts només podria ser un problema per un 28% de la població segons els resultats obtinguts. A partir d'aquí s'ha de seguir estudiant en aquest sentit per buscar alternatives viables i pràctiques per poder classificar, en la mateixa línia de producció, les canals o la carn amb nivells alts d'olor sexual i poder així evitar el rebuig del consumidor. Per altra banda, és important seguir investigant també en la resposta i la percepció de l'escatol en la carn de porc i la combinació d'androstenona i escatol.

V.3 Estratègies d'emmascarament per a l'androstenona

La qualitat sensorial de la carn de porc procedent de porcs mascles sencers pot veure's disminuïda per la presència d'olor sexual (Lundström, et al., 2009). Tenint en compte els resultats de la sensibilitat a l'andostenona, els estudis d'emmascarament s'havien de focalitzar especialment a aquells consumidors que perceben l'androstenona com una olor desagradable. En aquest cas l'estudi va tenir en compte la percepció de l'androstenona per part dels consumidors i es va enfocar en carn amb nivells alts d'androstenona i nivells baixos d'escatol. El fet de reduir els nivells d'escatol en la carn va resultar útil per minimitzar els factors que poden afectar la percepció dels consumidors i per poder centrar l'estudi en la percepció de l'androstenona i el seu emmascarament.

Els resultats mostren que les estratègies de cuinat utilitzades com el fregit i arrebossat amb all i julivert, i el cuinat al buit contribueixen a disminuir la percepció d'olor i flavor de l'androstenona. No obstant això, en el cas del cuinat al buit la preferència de la carn de mascles sencers era inferior que la de castrat en aquells consumidors que percebien l'androstenona com a una olor desagradable. Egea*, et*

al. (2014) i Linares*, et al.* (enviat per publicar) van demostrar prèviament que aquests dos mètodes de cocció avaluats per un panel entrenat eren efectius per disminuir la percepció d'androstenona. Per tant, els resultats de l'estudi mostren una possible sortida a la carn procedent de porcs mascles sencers amb presència d'androstenona. A partir d'aquí, si des de l'escorxador es poguessin classificar de manera adequada les canals amb nivells alts d'olor sexual, es podrien destinar a productes amb unes indicacions culinàries específiques o a l'elaboració de menjars preparats per a grans superfícies o restauració per tal de minimitzar el risc de rebuig per part dels consumidors. En aquest cas, la indústria hauria d'estudiar els costos que implicaria la producció d'aquests productes i si el mercat els podria absorbir.

El present estudi permet proporcionar eines per emmascarar la presència d'androstenona en la carn, però de cara a futures investigacions és important incloure també l'escatol per trobar estratègies que puguin ser vàlides per tots dos compostos. Dins el marc del projecte BOARMARKET però no inclòs en la present tesi, es va realitzar un estudi de consumidors a Barcelona, seguint la metodologia del projecte europeu CAMPIG (*Consumer acceptance in the European Union and in third countries of pig meat obtained from male pigs not surgically castrated*, contract number SANCO/2012/G3/SI2.639774). En aquest cas es va incloure l'escatol en combinació amb andostenona per tal d'avaluar l'acceptabilitat del consumidor. Es van avaluar 8 tipus d'hamburgueses procedents de carn de mascle sencer amb diferents nivells d'androstenona i escatol. Els resultats inicals van concloure que la carn amb nivells alts d'androstenona i nivells alts d'escatol són menys preferides pels consumidors que la carn de castrat, mentre que no es van observar diferències entre la carn de castrat i la carn amb nivells mitjos i baixos (Borrisser-Pairó*, et al.*, 2015). El projecte europeu CAMPIG va realitzar l'estudi a Dinamarca, França, Itàlia i Polònia, i van observar també que la carn amb nivells alts d'androstenona i escatol tenien menys acceptabilitat, a més d'observar que el compost amb major rebuig era l'escatol (Aluwé*, et al.*, 2015). Dins del mateix projecte també es van realitzar estudis de consumidors a Rússia i Xina amb 3 tipus d'hamburgueses amb diferents nivells d'androstenona i escatol i van concloure també que l'acceptabilitat de la carn de mascle sencer està relacionada amb la sensibilitat a l'escatol, i que l'acceptabilitat de la carn amb nivells baixos d'androstenona i escatol no és significativament diferent que la de la carn

procedent de castrat (Font-i-Furnols*, et al.*, 2016). Així doncs, els resultats d'aquests estudis indiquen que una possible futura classificació de canals a nivell d'escorxador segons la presència d'olor sexual s'hauria d'enfocar en detectar aquelles canals amb nivells alts, que són les que poden ser més problemàtiques de cara el consumidor, deixant de banda els nivells mitjos i baixos. A més a més, com que es demostra que la presència d'escatol té un paper important en la percepció de l'olor sexual, les properes investigacions s'haurien d'enfocar en aquesta línia.

V.4 Opinió del sector porcí sobre una possible prohibició de la castració

Els grups de discussió (*focus groups*) es van realitzar amb representants de tots els agents de la cadena de producció porcina. En el cas dels productors, aquests representaven associacions de productors. Pel que fa a la resta d'agents, en el conjunt de les dues ciutats, hi havia més de dos representants per a cada un.

Els resultats dels *focus groups* mostren una clara separació entre dos tipus productius: la producció convencional i la producció d'alta qualitat. La producció d'alta qualitat es refereix a la producció d'animals que es sacrifiquen a pesos més elevats, més de 120 kg (Schiavon*, et al.*, 2015), en els que es busca certa infiltració de greix per elaborar certs productes amb unes característiques tecnològiques i sensorials diferenciades.

Des del punt de vista del sector, la prohibició de la castració no es percep com un problema en la producció de porc convencional ja que actualment el percentatge de mascles sencers produïts a l'estat és molt elevat. El representants del sector comenten que en aquests moments entre un 15 i un 20% dels porcs mascles es castren, i la resta (75-80%) es deixen sencers, mentre que l'any 2009 estava en un 33% (Fredriksen*, et al.*, 2009). Així doncs, durant els darrers anys hi ha hagut un descens pel que fa a la castració com es conclou també en el primer informe sobre el progrés de la declaració de Brussel·les (Backus*, et al.*, 2014).

Per altra banda si que els preocupa el fet que, si hi ha un canvi normatiu pel que fa a la castració, la producció d'alta qualitat en pugui quedar afectada. En aquest punt els representants del sector en el *focus group* realitzat a Madrid van

mostrar més preocupació, especialment pel que fa al porc Ibèric. De totes maneres el sector és conscient que la Declaració de Brussel·les contempla la creació d'una llista de productes tradicionals o amb Denominació d'Origen Protegida (DOP), Indicació Geogràfica Protegida (IGP) o Especialitat Tradicional Garantida (ETG) que quedarien exempts de la prohibició de la castració. És per això que des del sector es vol insistir molt en la confecció d'aquesta llista, posant especial èmfasi en els productes provinents de porc Ibèric i de raça Duroc, o creuaments amb Duroc, que es sacrifiquen a pesos més elevats i on la castració és necessària per obtenir una carn amb unes característiques òptimes per a l'elaboració de certs productes. En aquest sentit ja s'està duent a terme el projecte europeu CASTRUM (*Pig castration: methods of anaesthesia and analgesia for all pigs and other alternatives for pigs used in traditional products***,** contract number SANTE/2015/G3/SI2.723717) que vol analitzar alternatives a la castració quirúrgica sense anestèsia per a la producció de productes tradicionals. En els casos on la castració és necessària, la Unió Europea podria valorar l'acceptació de la castració quirúrgica sempre i quan vagi acompanyada de l'aplicació d'anestèsia i/o analgèsia.

Pel que fa als carnissers, els resultats de les enquestes estan d'acord amb els dels *focus groups*. Els carnissers acostumen a comprar carn de qualitat diferenciada amb certa quantitat de greix infiltrat per poder elaborar també els seus productes. Així que a l'hora de comprar carn de porc tenen molt en compte la quantitat de greix intramuscular i el sexe de l'animal, essent respectivament un 25,0 i un 25,8% de la seva decisió de compra. Principalment venen carn procedent de femella (74,4%), tot i que també compren carn procedent de mascle castrat (18,0%) i de mascle sencer en menor proporció (7,6%). Una possible prohibició de la castració afectaria aquest 18,0% de carn procedent de mascle castrat, pel que es reforça la idea sorgida als *focus groups*, de la necessitat de confeccionar una llista d'excepcions és important per minimitzar l'efecte sobre les vendes dels carnissers que es focalitzen en productes d'alta qualitat.

En referència als consumidors i a la seva actitud a l'hora de comprar carn de porc, els carnissers participants d'aquest estudi van assegurar que si un consumidor detecta olor sexual en un producte, és molt probable que no torni a comprar-lo. De fet, aquesta va ser una de les conclusions del projecte ALCASDE (2008), on entre un

40 i un 56% dels consumidors no tornarien a comprar carn de porc, i entre un 39 i 78% no tornarien a comprar a la mateixa botiga si es trobessin carn amb mala olor, pel que en aquesta darrer cas en donarien la culpa a l'establiment. Aquest fet també posa de manifest el desconeixement que té una gran part dels consumidors sobre aquest tema, ja que per una banda aposten per millorar el benestar animal, però per l'altra, no són conscients que la producció de mascles sencers pot donar carn amb olor sexual que pot acabar esdevenint un defecte sensorial per alguns consumidors.

Capítol VI

Conclusions generals

(en anglès)

The results of the studies included in this thesis give us an idea of the impact that a possible banning of pig castration in Europe by 2018 could have to Spanish pig production. Considering the objective and current data of boar taint prevalence in Spanish farms, the stakeholder's opinion and the results from the performed consumer studies we can conclude that:

1. The percentage of porcine carcasses with high levels of boar taint is high in numerical terms (representing 195,000 tonnes/year). Therefore, the use of an on-line boar taint detection system at slaughterhouses is highly recommended to ensure a good sensory quality of pig meat, and consequently to divert tainted carcasses to the fabrication of meat products with fewer probabilities to be rejected by consumers. To date there is not an effective and rapid method to detect and classify carcasses according to boar taint, so further research is needed to develop a new European classification system for boar taint.

2. According to stakeholders, a potential banning of pig castration in Europe would not be a problem for Spanish conventional pig production because entire male production is already high and castration rate is low.

3. Traditional and high quality pig production would be affected by a potential banning of castration if they were not included as exceptions to the European Declaration on alternatives to surgical castration of pigs. Therefore, before 2018 –when the banning of castration is supposed to begin–, a list of traditional products should be developed for those products that require heavier pigs where castration is unavoidable (i.e. Iberian or Duroc breeds or crossbreeds). Concurrently, it is also important to work on alternatives to surgical castration without anaesthesia and analgesia to ensure animal welfare.

4. According to butcher's surveys, the most important attributes when purchasing pig meat are intramuscular fat and sex of the pig –to avoid boar taint–, which means meat from castrated male or female pigs. Therefore, butchers would be affected by a possible banning of castration if a list of exceptions to the European Declaration on alternatives to surgical castration of pigs is not done.

5. Regarding the presence of androstenone in meat, high sensitive consumers that dislike this compound are more likely to reject tainted meat. Breaded with garlic

and parsley and fried, and vacuum cooking are useful to mask androstenone in pig meat. Therefore, further research is needed to develop commercial products with specific cooking recommendations to avoid consumer rejection. At the same time, further research is needed on masking strategies for meat with skatole and for meat with skatole and androstenone.

Considering the previous conclusions, we can state that the presence of boar taint in pig meat would not be a big issue for the Spanish conventional pig production. However, it would be a challenge for production orientated to high quality meat products.

Capítol VII

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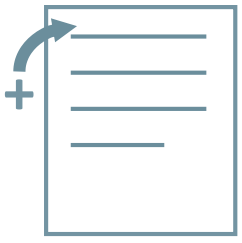
Annex 1



Aquest annex inclou un article que s'ha elaborat en l'etapa pre-doctoral durant l'estada científica a la Swedish University of Agricultural Sciences (SLU), però que no s'inclou en el compendi d'articles d'aquesta tesi.

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Gender-related differences in the formation of skatole metabolites by specific CYP450 in porcine hepatic S9 fractions

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Higher accumulation of skatole in the fat of male pigs compared with female pigs might be due to gender-related differences in the rate of skatole degradation. In the present study, skatole metabolites and cytochrome P450 (CYP450) isoforms involved in skatole metabolism were for the first time investigated in hepatic S9 fractions from six male and four female pigs (crossbred Landrace × Yorkshire dams and Duroc boar). Surprisingly, the rates of production of major skatole metabolites were similar in male and female pigs. The most abundant metabolite of skatole was 3-hydroxy-3-methyloxindole (HMOI) followed by 3-methyloxindole and indole-3-carbinol in both male and female S9 fractions. Concentrations of formed HMOI and 3-methyloxindole did not differ between the genders ($P = 0.124$ *for HMOI, and* $P = 0.575$ *for 3-methyloxindole). Indole-3-carbinol formation was higher in S9 fractions from the females compared with male pigs (*$P = 0.0001$*). Enzyme kinetic parameters were similar for both genders (*$P > 0.05$*). In both male and female pigs, ellipticine, diallyl sulphide (DAS) and quercetin inhibited HMOI formation, confirming the involvement of CYP1A1 and CYP2E1. The formation of 3-methyloxindole was reduced in the presence of the CYP2E1 inhibitor DAS, and formation of indole-3-carbinol was reduced in the presence of CYP1A1 and CYP2A19 inhibitors. We found only minor differences in skatole metabolism between male and female pigs, particularly the involvement of CYP2C and CYP3A in indole-3-carbinol formation in female but not in male pigs. This is a very essential finding, suggesting the involvement of larger number of CYP450 isoforms in female pigs. On the other hand, indole-3-carbinol is a minor skatole metabolite, and the physiological significance of CYP2C and CYP3A involvement in its formation in female pigs, but not in male pigs, needs to be elucidated. Our results, however, should be interpreted with caution because of the low number of animals and possibility of breed and age effects on skatole metabolism.*

Keywords: boar taint, skatole, 3-methyloxindole, indole-3-carbinol, 3-hydroxy-3-methyloxindole, CYP450 isoforms

Implications

We studied skatole metabolism in hepatic S9 fractions from male and female pigs. Generally, skatole levels are higher in entire male than in female pigs. Until recently, we believed that this is because of the differences in skatole metabolism between male and female pigs. The present study suggests that production of skatole metabolites is similar in both male and female pigs. We found only minor differences in skatole metabolism, particularly the involvement of CYP2C and CYP3A in indole-3-carbinol formation. These results contribute to the knowledge on skatole metabolism by CYP450 and are of interest for animal and meat scientists.

Introduction

The sensory quality of meat from some sexually mature male pigs may be negatively affected by high concentrations of androstenone and/or skatole, often referred to as boar taint. Androstenone (5α-androst-16-ene-3one) is a pheromone produced in the testis and exhibits a urine-like and perspiration odour (Patterson, 1968). Skatole (3-methylindole) is synthesised in the large intestine by bacterial degradation of tryptophan, exhibiting a faecal-like and naphthalene odour (Vold, 1970). A part of the skatole is excreted with faeces, whereas the remaining part is absorbed through the gut-wall. Hepatic skatole metabolism is mainly mediated by the cytochrome P450 (CYP450) superfamily of enzymes (Babol *et al*., 1998; Diaz and Squires, 2000). The activities of CYP1A, 2A and 2E1 are known to be of importance in the metabolism of skatole, although other CYP450 isoforms might also be involved (Terner *et al*., 2006; Matal *et al*., 2009; Zamaratskaia and Squires, 2009). Pigs with high rate of skatole synthesis and low activity of CYP450 enzymes may accumulate high skatole in the fat due to decreased hepatic clearance. In the pigs with low synthesis of skatole, hepatic metabolism is of lower importance.

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Usually, the activities of skatole-metabolising enzymes are higher in female and castrated pigs compared with entire male pigs (Skaanild and Friis, 1999; Zamaratskaia *et al.*, 2009). These differences are suggested to be because of the higher concentrations of sex steroids in male pigs (Skaanild and Friis, 1999; Zamaratskaia *et al.*, 2009; Rasmussen *et al.*, 2011a). Until recently, we believed that this is because of the inhibition of skatole metabolism by high oestrogen levels found in uncastrated male pigs (Zamaratskaia *et al.*, 2007; Rasmussen *et al.*, 2011b). However, a recent *in vivo* study showed no differences in CYP1A, CYP2A and CYP2E1 activities in male pigs with physiological and artificially reduced oestrogen levels (Zamaratskaia and Berger, 2014). It is also believed that androstenone may inhibit skatole metabolism, thus increasing its accumulation in the fat (Doran *et al.*, 2002, Tambyrajah *et al.*, 2004). However, the results on androstenone involvement in skatole metabolism are conflicting, which may be due to the different age and breeds of animals used in the different studies, as well as different concentrations of androstenone used *in vivo* and *in vitro* studies (Doran *et al.*, 2002; Tambyrajah *et al.*, 2004; Zamaratskaia and Squires, 2009). Another possible explanation for gender-related difference in skatole accumulation in the fat might be because of the involvement of different or a larger number of CYP450 isoforms in skatole metabolism in female pigs. Thus, in this study, we determined skatole metabolism in hepatic S9 fractions obtained from male and female pigs in the presence of specific inhibitors of several CYP450 isoforms. We chose S9 fractions, because they contain some cytosolic components that render the metabolism closer to physiological conditions (Brandon *et al.*, 2003). We used known specific inhibitors with documented effect on individual CYP450 isoforms.

Material and methods

Chemicals and reagents

Skatole, 3-methyloxindole (3MOI), indole-3-carbinol (I3C), 2-aminoacetophenone, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), oestradiol, quercetin, ellipticine, furafylline, 8-methoxypsoralen (8-MOP), ketoconazole and diallyl sulphide (DAS) were obtained from Sigma-Aldrich (Steinheim, Germany). 3-hydroxy-3-methyloxindole (HMOI) was synthesised as described by England *et al.* (2007). Methanol and acetonitrile of HPLC grade were obtained from Merck (Darmstadt, Germany).

Animals

A total of six male and four female pigs (Landrace × Yorkshire dam and Duroc boar) were used in this study. All the animals were treated in accordance with the guidelines from the Danish inspectorate of animal experimentation. Animals were raised under the same conditions and slaughtered at the same age. For details about the animals and specific CYP450 expression and activity, see Rasmussen *et al.* (2011b) and Rasmussen *et al.* (2012).

Preparation of S9 fractions and protein measurement

The S9 fractions (supernatants fraction of the 9000 × **g** spin) were prepared. Frozen liver samples, ~1 g, were cut into small pieces and homogenised in 1.2 ml of buffer (50 mM Tris, 150 mM KCl, 2 mM EDTA, pH 7.4). The homogenates were centrifuged at 9000 × **g** for 20 min. The amount of protein in the supernatants was assayed the same day with a commercially available kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer's instructions. The S9 fractions were diluted to a protein concentration of 0.5 mg/ml. Two pooled liver S9 fractions were prepared from entire male pigs, and two were prepared from female pigs. The pools were stored at − 80°C until analysis.

Formation of skatole metabolites

Incubations were performed as described by Diaz *et al.* (1999) with slight modifications. In brief, incubations in a total volume of 0.5 ml contained S9 protein (0.5 mg), 50 mM phosphate buffer, pH 7.4, 38 μM of skatole and 0.5 mM NADPH. After 60 min of incubation at 37°C, reactions were terminated with 0.5 ml of ice-cold acetonitrile. Subsequently, the incubations were centrifuged at 4200 × **g** for 2 min at +4°C, and 100 μl of supernatant was injected into the HPLC. Linearity of metabolite formation from 38 μM of skatole was determined using incubation time from 5 to 90 min, and protein content from 0.2 to 1.6 mg. To determine kinetic parameters, skatole concentrations from 2 to 53 μM were used.

Microsomal incubations with CYP450 isoform inhibitors

To investigate the involvement of the CYP450 isoforms, the following inhibitors were used in the incubations: ellipticine (CYP1A1), furafylline (CYP1A2), 8-MOP (CYP2A19), sulphaphenazole (CYP2C), DAS (CYP2E1), ketoconazole (CYP3A4), quercetin (CYP2E1 in male pigs; CYP3A and CYP1A in pigs of both genders) and oestradiol (E2; CYP2E1 in male pigs). These inhibitors have previously been shown to have an effect on the activities of CYP450 isoforms in microsomal or hepatocyte assays (Zamaratskaia and Zlabek, 2009; Rasmussen *et al.*, 2011b; Zlabek and Zamaratskaia, 2012). The incubations were performed in the presence of 0.125, 1.25 and 12.5 μM of the specific inhibitor, except for E2 (used concentration 0.002, 0.02 and 0.2 μM) and quercetin (16, 32 and 128 μM). The choice of the concentrations of E2 and quercetin in the incubations was based on their inhibitory potency in the microsomes from the same animals (Rasmussen *et al.*, 2011a). In the control incubations, methanol was added in the same volume (5 μl). We have previously shown that this amount of methanol (1%) does not affect CYP450 activity. The mechanism-based inhibitors 8-MOP and furafylline were pre-incubated with S9 protein for 30 min, and E2 for 15 min at 37°C before addition of skatole. The reactions were allowed to proceed as described above, and the concentrations of formed metabolites were measured. The effect of inhibitors on the formation of individual skatole metabolite is expressed as the percentage of metabolite formation remaining in the presence of inhibitor

relative to the metabolite formation in control incubations. The activity was regarded as reduced if it differed from the control activity by at least 30%. No further statistical analyses were performed in order to avoid false results due to intra-assay variations.

Chromatographic conditions

Skatole metabolites were separated by gradient reverse-phase HPLC (Diaz *et al.*, 1999) on Li-Chrospher RP-18 column (Merck KGaA, Darmstadt, Germany) (5 μm) equipped with a guard column. The HPLC equipment consisted of a pump (L-6200A), autosampler (L-7200), fluorescence detector (L-7485), UV detector (L-4000) and D-7000 interface (Merck, Hitachi, Tokyo, Japan). The metabolites were detected with fluorescence (excitation and emission wavelengths of 286 and 350 nm, respectively) and UV (250 nm) detectors.

Statistical analysis

Kinetic parameters (K_m and V_{max} values) were estimated by a GraphPad Prism version 4.0 for Windows, GraphPad Software (San Diego, CA, USA). Student's *t*-test was used to compare K_m and V_{max} values, as well as the quantity of skatole metabolites formed from the S9 fractions from male and female livers. Differences were considered significant when $P < 0.05$.

Results

Skatole metabolites

No metabolites were detected in S9 incubations in the absence of NADPH or skatole, and in the incubations without S9 fractions. In the S9 incubations with skatole and NADPH, three skatole metabolites were detected – 3-hydroxy-3-methyloxindole (HMOI), indole-3-carbinol (I3C) and 3-methyloxindole (3MOI). Skatole and skatole metabolites were detected by the UV detector at the following retention times: skatole 25 min; HMOI 9 min; I3C 12 min; and 3MOI 16 min (Figure 1). In addition, skatole and I3C were detected by the fluorescence detector. HMOI and 3MOI were quantified by UV absorption, and I3C was quantified using data from the fluorescence detector because of its higher sensitivity and accurate estimation of lower concentrations compared with the UV detector.

The formation of HMOI and I3C was linear for 90 min of incubation time, and the formation of 3MOI for 60 min. The formation of all metabolites was linear up to at least 1.6 mg of S9 protein. Incubation time of 60 min and protein content of 0.5 mg were chosen as optimal for subsequent assays.

The effect of skatole concentration on the velocity of metabolite formation is presented in Figure 2. The most abundant metabolite of skatole was HMOI followed by 3MOI and I3C in both male and female S9 fractions. Concentrations of formed HMOI and 3MOI did not differ between the genders ($P = 0.124$ for HMOI, and $P = 0.575$ for 3MOI). Concentrations of formed I3C and V_{max} were higher in S9 fractions from the female compared with male pigs

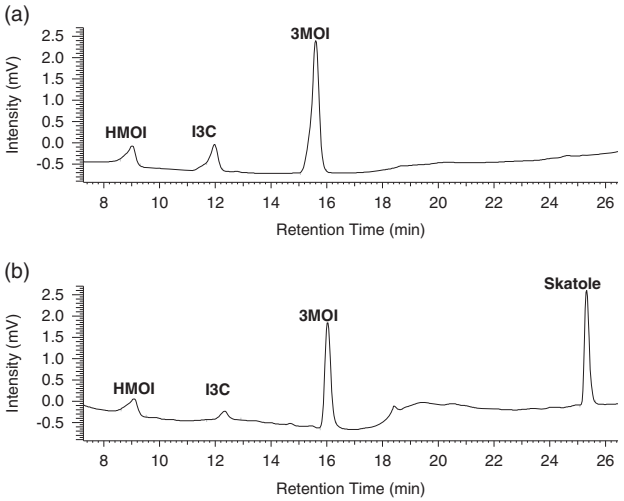

Figure 1 Typical HPLC chromatogram of skatole metabolites. (a) Standard solutions and (b) S9 incubations in the presence of 38 μM of skatole and β-nicotinamide adenine dinucleotide phosphate (NADPH).

($P = 0.0001$ for I3C concentration and $P = 0.008$ for V_{max}). Enzyme kinetic parameters were similar for both genders ($P > 0.05$; Table 1).

Inhibition study

Formation of HMOI (Figure 3) was inhibited by ellipticine (CYP1A), DAS (CYP2E1) and quercetin (CYP1A, 2E1 and 3A). The highest degree of inhibition was observed at high concentration of the inhibitor (remaining activity, mean and standard deviation, 12.9 ± 6.9% for ellipticine; 18.1 ± 9.2% for DAS; and 2.8 ± 2.0 for quercetin). Ketoconazole (CYP3A) at the concentration of 12.5 μM inhibited HMOI formation to a lesser degree, and this inhibition was more pronounced in female pigs (89.2 ± 79.9% in male and 41.3 ± 11.0% in female). Interestingly, at lower concentrations of ketoconazole, an increase in HMOI formation was observed in S9 fractions from female pigs. The rate of HMOI formation also increased in the presence of sulphaphenazole and furafylline.

Formation of 3MOI (Figure 4) was lower in the presence of DAS (CYP2E1; 65.7 ± 9.4% at 12.5 μM of DAS). In the S9 fractions from female pigs, 3MOI formation was inhibited by oestradiol (CYP2E1; 60.7 ± 23.5% at 0.2 μM of oestradiol), although in the male pigs oestradiol did not affect 3MOI formation (89.8 ± 14.0%). Formation of 3MOI increased in the presence of ketoconazole, sulphaphenazole, furafylline and quercetin.

Formation of I3C (Figure 5) was inhibited by ellipticine (CYP1A1; 15.9 ± 7.1% at 12.5 μM of ellipticine, 8-MOP (CYP2A; 15.1 ± 6.2% at 12.5 μM of 8-MOP) and quercetin (CYP1A1, 2E1, 3A; 40.5 ± 18.5% at 128 μM of quercetin). Sulphaphenazole (CYP2C) inhibited formation of I3C only in female pigs (54.3 ± 60.9% at 12.5 μM of sulphaphenazole), but not in male pigs (93.4 ± 21.9% at 12.5 μM of sulphaphenazole). A similar pattern was observed in the presence of ketoconazole (CYP3A): I3C formation decreased in female pigs (57.2 ± 1.5% at 12.5 μM of ketoconazole), but not in male pigs (115.6 ± 70.6% at 12.5 μM of ketoconazole).

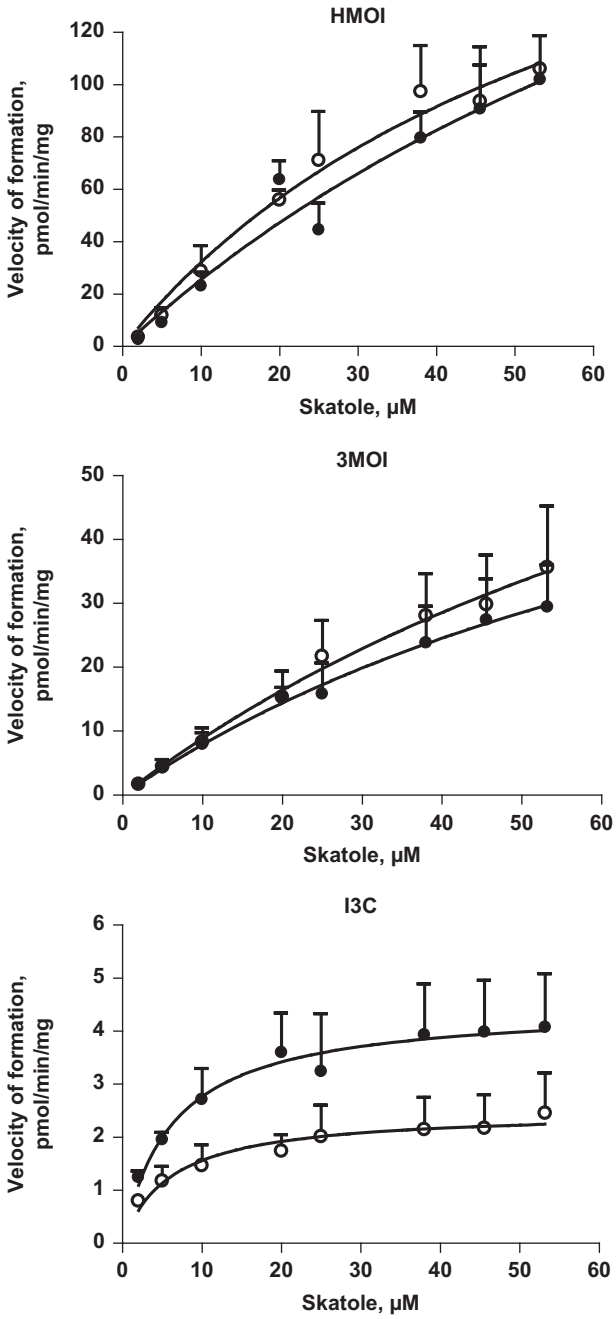

Figure 2 Substrate–velocity plot for skatole metabolism in porcine liver S9 fractions. Data are presented as mean values and standard errors. Open dots represent metabolite formation by S9 fractions from male, and solid dots from female pigs.

Discussion

The risk of boar taint in meat from intact male pigs is mainly associated with skatole and androstenone accumulation in the fat. High skatole levels in the fat might be due to insufficient skatole hepatic metabolism, which prevents appropriate elimination of skatole from the body. Several skatole metabolites were detected in pig plasma and urine (Bæk *et al.*, 1997). Major insights into skatole metabolism came from *in vitro* microsomal studies (Babol *et al.*, 1998; Diaz *et al.*, 1999; Diaz and Squires, 2000). In these studies, CYP2A and CYP2E1 were identified as major isoforms involved in skatole metabolism. Further *in vitro* studies on primary cultured pig hepatocytes (Terner *et al.*, 2006), individual purified porcine enzymes (Matal *et al.*, 2009) and individually expressed enzymes (Wiercinska *et al.*, 2012) confirmed an involvement of CYP2A and CYP2E1, and revealed that other isoforms (CYP1A, CYP2C, CYP3A) are also involved in skatole metabolism. Moreover, gender-related differences in the rate of skatole metabolism are frequently suggested as a cause of the higher levels of skatole in male pigs compared with the female pigs. Indeed, the expression and activities of skatole-metabolising enzymes were higher in females compared with male pigs (Skaanild and Friis, 1999; Zamaratskaia *et al.*, 2006; Rasmussen *et al.*, 2011a). Interestingly, no data are available on the skatole metabolite formation in both male and female pigs.

The aim of the present study was to evaluate gender-related differences in skatole metabolism. We utilised S9 fractions, which contain a wide variety of Phase I and II metabolising enzymes, and more closely represent a more complete physiological environment compared with microsomes (Zhang *et al.*, 2012). S9 fractions from both male and female pig livers were included. The following three skatole metabolites were identified: HMOI, I3C and 3MOI. Interestingly, in our pilot study performed on the porcine microsomes, two additional unidentified peaks that might be related to skatole metabolites were detected by fluorescence (unpublished data); however, these peaks were not detected when we used S9 fractions. We speculated that these two unidentified peaks produced by the microsomes were 6- and 5-OH-3-methylindole. In the S9 fractions, these metabolites may immediately undergo sulpho-conjugation by phase II enzymes and cannot be detected. Similarly, no 6- and 5-OH-3-methylindole formation was observed in the study with hepatocyte preparations (Terner *et al.*, 2006).

Surprisingly, the metabolic rates of production of the three skatole metabolites and kinetic parameters were similar in both male and female pigs. The activities of skatole-metabolising enzymes were previously measured in the liver samples from the same animals, and significantly higher activities of CYP1A and CYP2A in female pigs compared with male pigs were found (Rasmussen *et al.*, 2011a). Obviously, these differences in the activities were not reflected in the rate of skatole metabolism. This might be due to the high substrate concentration used in the present *in vitro* study. Skatole concentrations in the range from 2 to 53 μM were used, which correspond to the high physiological concentrations of skatole in fat. It is likely that skatole levels in liver tissue are lower.

Ellipticine, DAS and quercetin inhibited HMOI formation, confirming the involvement of CYP1A1 and CYP2E1. In addition, HMOI formation in male pigs was inhibited by oestradiol. We have previously shown that oestradiol inhibits CYP2E1 activities in male pigs, but not in female pigs (Zamaratskaia *et al.*, 2007; Rasmussen *et al.*, 2011b). This explains why HMOI formation was not decreased by

Table 1 *Enzyme kinetic parameters for the formation of skatole metabolites by hepatic S9 fractions from male and female pigs*

	K_m (μM)			V_{max} (pmol/min per mg)		
Metabolite	Male	Female	*P*-value	Male	Female	*P*-value
HMOI	62.9 ± 111.5	120.2 ± 96.6	0.698	160.5 ± 177.7	302.3 ± 182.1	0.592
3MOI	114.3 ± 126.2	94.8 ± 88.9	0.902	110.2 ± 90.2	82.7 ± 54.8	0.799
IC3	6.0 ± 3.5	6.2 ± 3.2	0.969	2.5 ± 0.3	4.5 ± 0.5	0.008

HMOI = 3-hydroxy-3-methyloxindole; 3MOI = 3-methyloxindole; IC3 = indole-3-carbinol.
Kinetic parameters were calculated from data obtained separately for six male and four female pigs. Incubations were conducted using substrate (skatole) concentrations from 2 to 53 μM. Results are presented as best-fit values ± standard errors from nonlinear regression analysis (GraphPad Prism program 4.0 kinetic software). Student's *t*-test was used to compare K_m and V_{max} values in the incubations with male and female S9 fractions.

oestradiol in female pigs in the present study, and confirms the involvement of CYP2E1 in HMOI formation. CYP2C (sulphaphenazole) and CYP1A2 (furafylline) were not involved in the formation of HMOI in either male or female pigs. On the contrary, involvement of CYP2C and CYP1A2 was demonstrated by previous *in vitro* studies (Wiercinska *et al*., 2012). The disagreement between these studies might be because of the different *in vitro* systems used. Interestingly, our study suggested that inhibition of CYP2C and CYP1A2 might stimulate other isoforms to produce more HMOI in

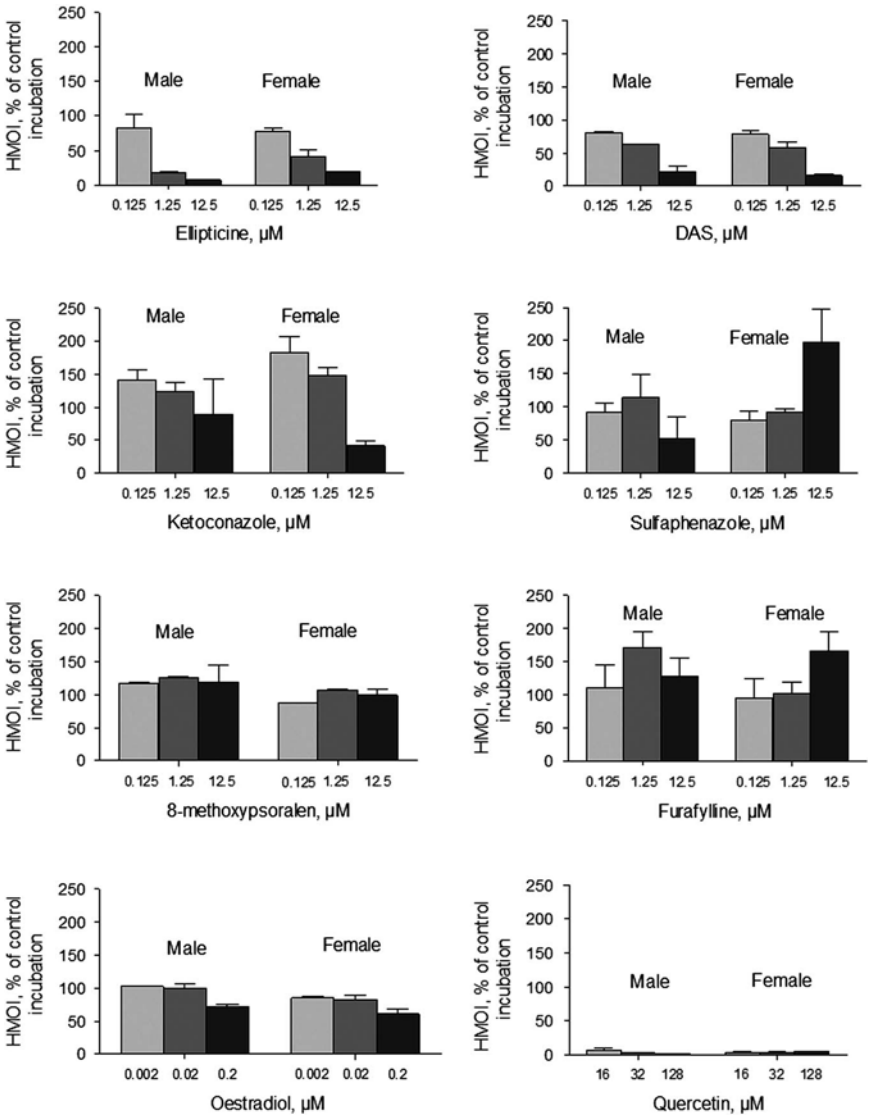

Figure 3 Rate of HMOI (3-hydroxy-3-methyloxindole) formation in hepatic S9 fractions from six male and four female pigs in the presence of various cytochrome P450 (CYP450) inhibitors. Data are presented as mean values and standard errors. The activity was regarded as reduced if it differed from the control activity by at least 30%.

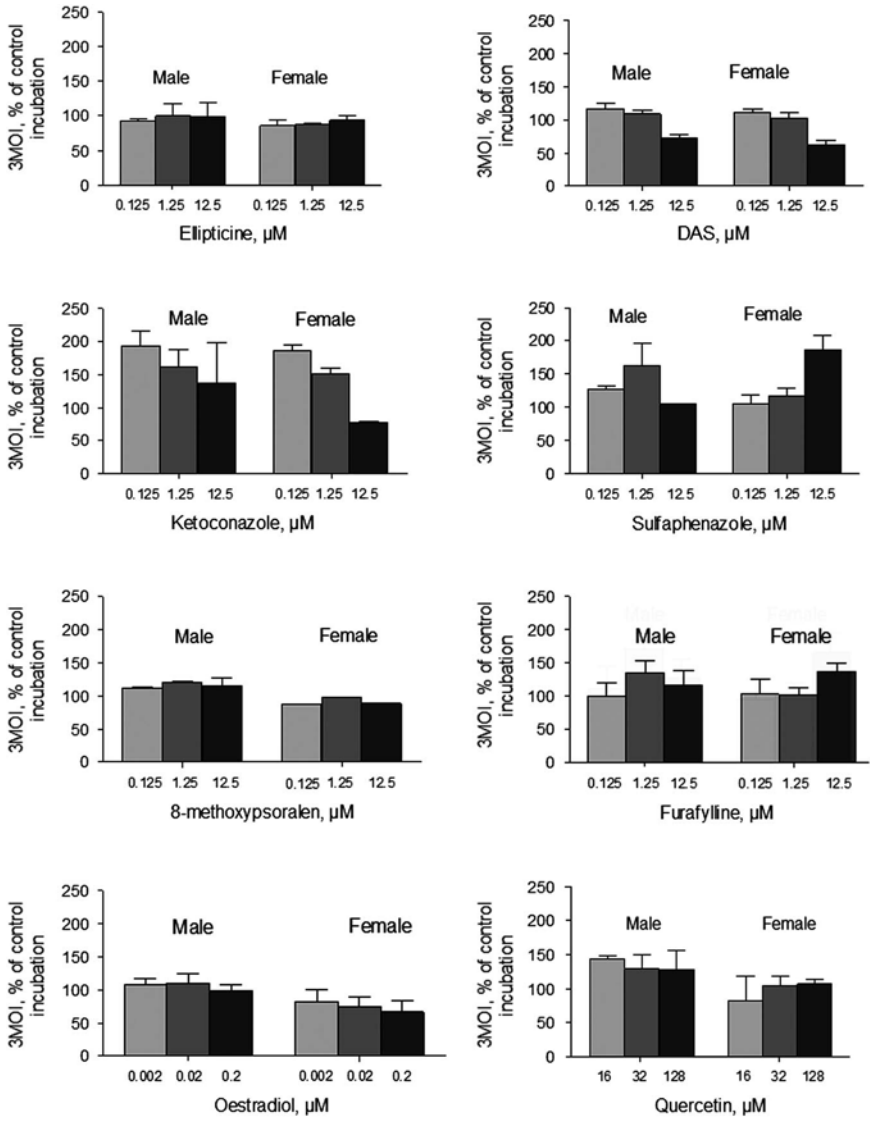

Figure 4 Rate of 3MOI (3-methyloxindole) formation in hepatic S9 fractions from six male and four female pigs in the presence of various cytochrome P450 (CYP450) inhibitors. Data are presented as mean values and standard errors. The activity was regarded as reduced if it differed from the control activity by at least 30%.

female pigs. This, however, needs further investigation. CYP2A19 was not involved in HMOI formation (as supported by Wiercinska *et al.*, 2012).

CYP2E1 was involved in the formation of 3MOI in both male and female pigs. CYP3A and CYP2C were not directly involved in the formation of 3MOI, but probably inhibition of CYP3A stimulated other isoforms to produce more 3MOI.

Our results indicated that CYP1A1 and CYP2A19 were involved in the formation of I3C in both male and female pigs. It is known that specificity of 8-MOP as CYP2A19 inhibitor is low; it inhibits both CYP2A19 and CYP2E1. However, if CYP2E1 is involved in the I3C formation, inhibition would also be expected in the presence of DAS. This was not true, suggesting a minor role, if any, of CYP2E1 in I3C formation. CYP2C and CYP3A were involved in I3C formation in female pigs only, as suggested by inhibition by sulphaphenazole and ketoconazole, respectively. This is a very essential finding supporting our hypothesis that gender-related differences in the rate of skatole metabolite formation might be because of the involvement of larger number of CYP450 isoforms in female pigs. It also explains why I3C formation in the present study was higher in females compared with male pigs. On the other hand, I3C is a minor skatole metabolite, and the physiological significance of CYP2C and CYP3A involvement in its formation in females but not in male pigs needs to be elucidated. CYP2E1, CYP3A and CYP1A2 were not involved in the formation of I3C in neither male nor female pigs.

It should be noted that our results should be interpreted with caution because of the low number of animals and possibility of breed and age effects on skatole metabolism. Thus, more research is needed to elucidate gender-related differences in skatole metabolism. In such research, larger number of animals of different breed and ages should be included.

In summary, skatole metabolites and CYP450 isoforms involved in skatole metabolism were for the first time investigated in hepatic S9 fractions from male and female

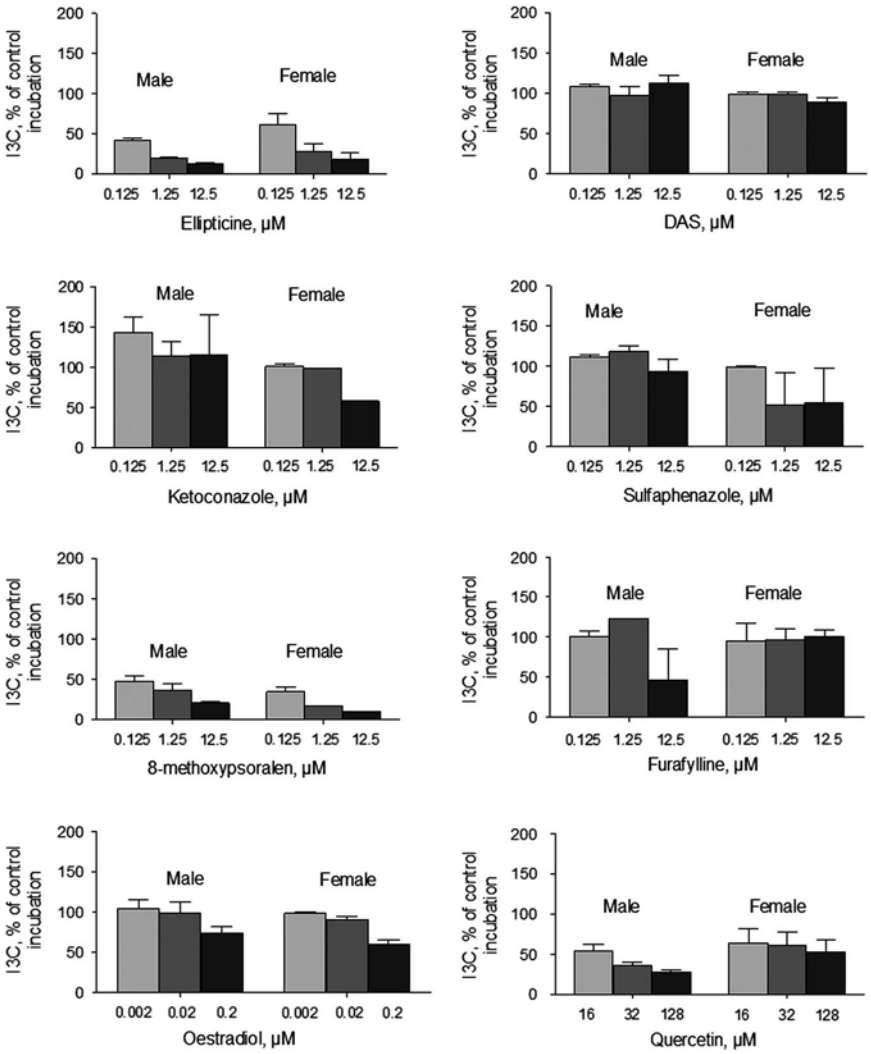

Figure 5 Rate of I3C (indole-3-carbinol) formation in hepatic S9 fractions from six male and four female pigs in the presence of various cytochrome P450 (CYP450) inhibitors. Data are presented as mean values and standard errors. The activity was regarded as reduced if it differed from the control activity by at least 30%.

pigs. Only minor differences in skatole metabolism between male and female pigs were observed, particularly the involvement of CYP2C and CYP3A in I3C formation in females but not in male pigs.

Acknowledgements

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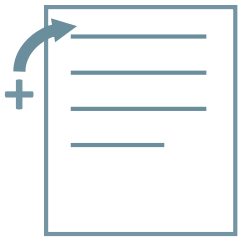
Annex 2



Aquest annex inclou l'enquesta cara a cara realitzada als carnissers en el segon estudi d'aquesta tesi.

The requested information is only for research purposes. We appreciate your cooperation.

A. BUTCHERY CHARACTERISTICS

1. **Name and address of the butchery:**

   _____

2. **Postal code:** _____

3. **Years of business:** __

4. **Surface of the business (in m^2)?** ___

5. **How many temporary and steady employees work in the butchery?**

   __ Temporary __ Steady

6. **According to age, which percentage of customers do you have?**

> 60 years old	%
40-60 years old	%
25-40 years old	%
< 25 years old	%

B. MEAT OFERED IN THE BUTCHERY

7. **How do you buy the meat?**

   ☐ Whole carcass ☐ Primary cuts ☐ Pieces

   7.1. **If you buy whole carcasses, what category/ies of SEUROP do you buy?**

   ☐ S ☐ E ☐ U ☐ R ☐ O ☐ P

8. **Is it important for you to distinguish between entire male, castrated male or female?**

   ☐ Yes ☐ No

   8.1. **According to animal gender, what percentage of meat do you buy?**

Entire male	%
Castrated male	%
Female	%

9. **Do you sell different types of meat?**

   ☐ Yes ☐ No

10. **Do you sell meat from Duroc?**

    ☐ Yes ☐ No ☐ Don't Know

11. **How important is each of the following attributes for your CUSTOMERS?**

0	1	2	3	4	5	6	7	8	9	10
0-4: Not important					5: irrelevant				6-10: Very important	

Origin	
Price	
Brand	
Fat content	
Colour	
Odour	

12. **According to YOUR opinion, what attribute is more important when purchasing fresh pork meat? (9: very important compared to the other attribute; 1: both attributes are equally important)**

A. Origin of the meat

Meat from Catalonia									Meat from other Spanish Regions							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Meat from Catalonia									Meat from other countries							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Meat from other Spanish Regions									Meat from other countries							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

B. External fat content

High amount of external fat									Average amount of external fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

High amount of external fat									Low amount of external fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Average amount of external fat									Low amount of external fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

C. Intramuscular fat content

High amount of intramuscular fat									Average amount of intramuscular fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

High amount of intramuscular fat									Low amount of intramuscular fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Average amount of intramuscular fat									Low amount of intramuscular fat							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

D. Gender of the pig

Meat from female pigs									Meat from castrated male pigs							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Meat from female pigs									Meat from entire male pigs							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Meat from castrated male pigs									Meat from entire male pigs							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

E. Colour of the meat

Cherry red									Light red							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Cherry red									Dark red							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Light red									Dark red							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Comparing different attributes

Origin of the meat									*External fat content*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Origin of the meat									*Intramuscular fat content*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Origin of the meat									*Gender of the pig*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Origin of the meat									*Colour of the meat*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

External fat content									*Intramuscular fat content*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

External fat content									*Gender of the pig*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

External fat content									*Colour of the meat*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Intramuscular fat content									*Gender of the pig*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Intramuscular fat content									*Colour of the meat*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

Gender of the pig									*Colour of the meat*							
9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9

C. ANIMAL WELFARE AND PIG CASTRATION

13. Your knowledge of animal welfare is:

0	1	2	3	4	5	6	7	8	9	10
0: Very low					5: Medium					10: Very high

14. According to your opinion, the level of animal welfare in Spain is:

0	1	2	3	4	5	6	7	8	9	10
0: Very low					5: Medium					10: Very high

15. Do you think that animal welfare legislation should be more restrictive in Spain?

☐ Yes ☐ No ☐ Don't Know

16. What do you think is the aim of castrating piglets?

_____
_____
_____

17. Do you think that castration affects animal welfare?

☐ Yes ☐ No

18. Do you think that castration affects meat quality?

☐ Yes ☐ No

18.1. How does castration affect meat quality?

_____
_____
_____

19. According to your opinion, which gender of pig produces better quality?

_____

20. Have you had any complain about meat quality?

☐ Yes ☐ No

21. Have you had any complain from your customers about a strange odour in the meat?

☐ Yes ☐ No

21.1. Did these customers ask about the origin of this abnormal odour?

☐ Yes ☐ No

21.2. What did you answer?

_____
_____
_____

22. Do you think that customers would pay a plus for meat from animals with better level of animal welfare?

☐ Yes ☐ No

23. Do you think that customers would pay a plus for meat with better quality?

☐ Yes ☐ No

24. European Union I planning to ban castration in piglets. Banning pig castration would affect your sales?

☐ Yes ☐ No

D. BUTCHER CHARACTERISTICS

25. Age: __

26. Gender:

☐ Man ☐ Woman

27. Household members: __

28. Education:

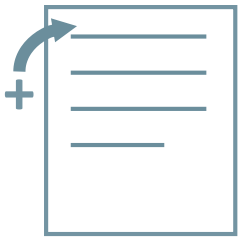
☐ Primary ☐ Secondary
☐ Higher degree/not university ☐ University

29. Years in the butchery: __

30. Relation within the business:

☐ Owner ☐ Family employee
☐ Employee ☐ Other

Annex 3



Aquest annex inclou el qüestionari de l'estudi de consumidors del tercer estudi d'aquesta tesi.

CONSUMIDOR	
FECHA	
SESIÓN	

EVALUACIÓN DE CARNE

Bienvenido/da. Hoy nos gustaría saber su opinión sobre lomos de cerdo. Serviremos dos porciones cada vez. Queremos que las compare y nos puntúe cuál de ellas prefiere en referencia al olor y al sabor. En total hará 2 comparaciones. Nos interesa su punto de vista, no hay ninguna respuesta incorrecta. ¡¡Muchas gracias!!

COMPARACIÓN 1

	producto del recipiente AMARILLO	producto del recipiente BLANCO
En primer lugar anote el código del producto	Código ☐	Código ☐

NO COMA el producto aún (SÓLO HUELA)

1.OLOR: Coja la muestra del recipiente AMARILLO con el palillo y huela. Ahora coja la del recipiente BLANCO y también huela. **En referencia al OLOR, ¿cuál de los dos productos le gusta más?**

(marque con una cruz el que más le guste)	☐	☐

2.SABOR: Coma ahora el producto del recipiente AMARILLO (utilice el palillo y coma al menos la mitad de la porción). MEMORICE las impresiones. Tome un poco de agua y coma pan. Coma ahora el producto del recipiente BLANCO (con el palillo y al menos la mitad de la porción). **En referencia al SABOR, ¿cuál de los dos productos le gusta más?**

(marque con una cruz el que más le guste)	☐	☐

Recuerde los dos productos que acaba de probar. Puntúe cada producto individualmente según una escala que va de "Me gusta extremadamente" a "Me desagrada extremadamente". Siga los siguientes pasos:

3. Recuerde el producto del recipiente AMARILLO. **¿En qué grado le gusta?** Haga la cruz en la puntuación que corresponda al producto del recipiente amarillo.

4. Recuerde el producto del recipiente BLANCO. **¿En qué grado le gusta?** Haga la cruz en la puntuación que corresponda al producto del recipiente blanco.

recipiente amarillo		recipiente blanco
☐	Me gusta extremadamente	☐
☐	Me gusta mucho	☐
☐	Me gusta	☐
☐	Me gusta un poco	☐
☐	Me desagrada un poco	☐
☐	Me desagrada	☐
☐	Me desagrada mucho	☐
☐	Me desagrada extremadamente	☐

Para terminar ésta comparación, beba un poco de agua y coma un poco de pan. Espérese que enseguida le traerán el siguiente par de productos.

CONSUMIDOR	
FECHA	
SESIÓN	

COMPARACIÓN 2

	producto del recipiente AMARILLO	producto del recipiente BLANCO
En primer lugar anote el código del producto	Código	Código

NO COMA el producto aún (SÓLO HUELA)

1.OLOR: Coja la muestra del recipiente AMARILLO con el palillo y huela. Ahora coja la del recipiente BLANCO y también huela. **En referencia al OLOR, ¿cuál de los dos productos le gusta más?**

(marque con una cruz el que más le guste)	☐	☐

2.SABOR: Coma ahora el producto del recipiente AMARILLO (utilice el palillo y coma al menos la mitad de la porción). MEMORICE las impresiones. Tome un poco de agua y coma pan. Coma ahora el producto del recipiente BLANCO (con el palillo y al menos la mitad de la porción). **En referencia al SABOR, ¿cuál de los dos productos le gusta más?**

(marque con una cruz el que más le guste)	☐	☐

Recuerde los dos productos que acaba de probar. Puntúe cada producto individualmente según una escala que va de "Me gusta extremadamente" a "Me desagrada extremadamente". Siga los siguientes pasos:

3. Recuerde el producto del recipiente AMARILLO. **¿En qué grado le gusta?** Haga la cruz en la puntuación que corresponda al producto del recipiente amarillo.

4. Recuerde el producto del recipiente BLANCO. **¿En qué grado le gusta?** Haga la cruz en la puntuación que corresponda al producto del recipiente blanco.

recipiente amarillo		recipiente blanco
☐	Me gusta extremadamente	☐
☐	Me gusta mucho	☐
☐	Me gusta	☐
☐	Me gusta un poco	☐
☐	Me desagrada un poco	☐
☐	Me desagrada	☐
☐	Me desagrada mucho	☐
☐	Me desagrada extremadamente	☐

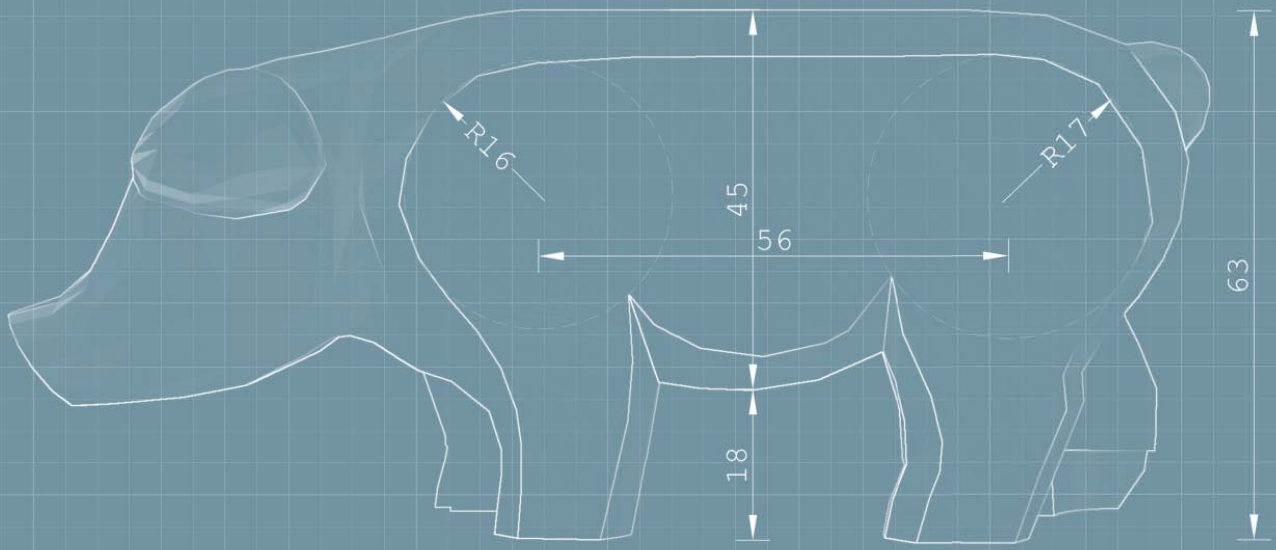
Para terminar ésta comparación, beba un poco de agua y coma un poco de pan. Espérese que enseguida le traerán el siguiente par de productos.

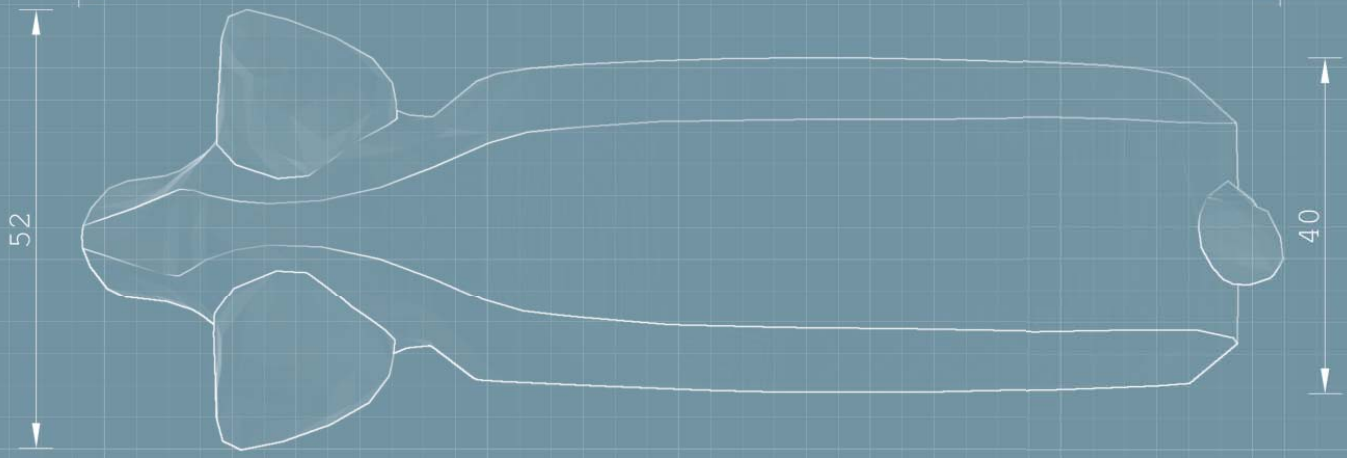
FIN DE LA PRIMERA PARTE

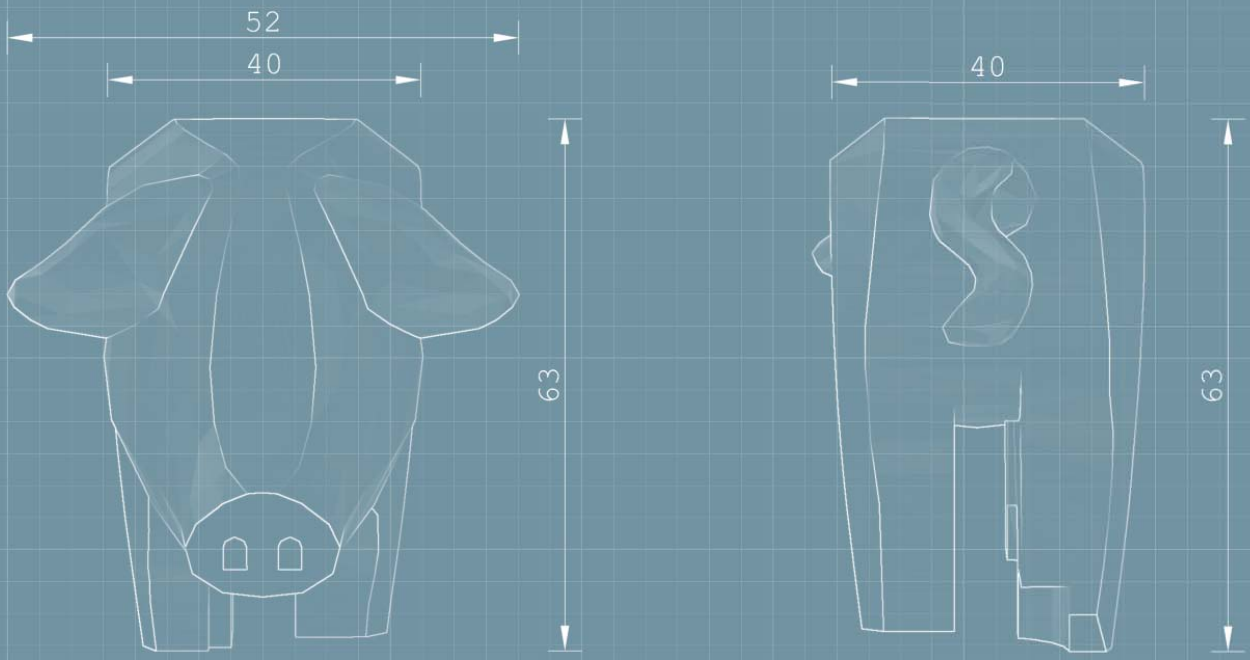
2016

Tesi doctoral
Francesc Borrisser Pairó

Anàlisi del potencial de mercat de la carn procedent de porcs mascles sencers com a alternativa a la castració quirúrgica







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