Universitat Autònoma de Barcelona

ALTA PRESSIÓ ISOSTÀTICA APLICADA A CARN RECUPERADA MECÀNICAMENT D'AVIRAM

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ALTA PRESSIÓ ISOSTÀTICA APLICADA A CARN RECUPERADA MECÀNICAMENT D'AVIRAM

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AL GRAU DE DOCTOR EN
CIÈNCIA I TECNOLOGIA DELS ALIMENTS

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XARXA DE CENTRES
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A LA INNOVACIÓ
TECNOLÒGICA

MONTSERRAT MOR-MUR FRANCESCH, Professora Titular de Tecnologia dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona

FA CONSTAR: que el llicenciat en Veterinària Josep Yuste Puigvert ha dut a terme, sota la seva direcció, a l'Àrea de Tecnologia dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona, el treball titulat: "Alta pressió isostàtica aplicada a carn recuperada mecànicament d'aviram", que presenta per optar al grau de Doctor.

I perquè així consti, signo el present document a Bellaterra (Cerdanyola del Vallès) a 16 de desembre de 1999

Montserrat Mor-Mur Francesch

NEU

També la neu passarà i tornarem
als dies clars i oberts.
Fonda, la vida
farà el seu curs immutable i el temps
transcorrerà sense fer gens de cas
dels desficis que ens xuclen i ens exalten.

I també passaran els dies clars
i tornarà la neu, tancant un cicle,
o obrint-lo, tant se val.
Només nosaltres
desapareixerem, i potser tot
per uns instants serà quasi perfecte.

Miquel Martí i Pol ("Els bells camins")

Per al meu pare,

qui primer em va suggerir fer el doctorat,
qui em va animar i aconsellar durant tots aquests anys;
la seva absència fa que tot perdi una mica de sentit...
ara, de segur, estaria orgullós de tot això;
ell, més que ningú altre, es mereixia poder-ho gaudir.

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D'una manera molt especial, a la Montse, la meva directora, «per tot en general i per res en particular». Una fora de sèrie tant en el terreny professional -intel·ligència, coratge i decisió- com, principalment, en el terreny personal -sinceritat, sensibilitat i comprensió-; «deixa'm que remarqui la confiança i la paciència que has tingut i, per damunt de tot, la teva amistat».

A la resta del personal i els companys de la 'Unitat', els quals m'han hagut de suportar i m'han facilitat la feina en molt moments.

Als meus amics, uns més prop meu i uns altres no tant, «heu continuat fent-me costat malgrat els innombrables "no puc", "no em va bé" o "tinc feina"».

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I. INTRODUCCIÓ

1. ALTA PRESSIÓ ISOSTÀTICA

1.1. Definició, història i situació actual

Entenem per alta pressió (AP) la tecnologia en què es tracta un producte a 100 MPa o més. Tradicionalment, l'AP s'ha utilitzat en materials inorgànics, per exemple en la producció de ceràmiques, acers, superaliatges de metalls i materials sintètics (Hoover i col., 1989). Des de començaments de la dècada dels 80, aquesta tecnologia està sent estudiada a fons en sistemes biològics i alimentaris.

Ara fa cent anys que per primera vegada es va aplicar pressió per a la conservació d'aliments: el 1899, Bert H. Hite va intentar millorar l'estabilitat de la llet mitjançant AP, però el seu treball no cridà gaire l'atenció (Butz i Ludwig, 1986). El 1924, Cruess menciona un altre experiment de Hite al seu llibre sobre productes vegetals i afirma que l'AP pot ser emprada com a sistema de tractament de sucs de fruites. Tenint en compte els beneficis d'aquesta tecnologia, sorprèn que hagin hagut de passar quasi setanta anys fins l'aparició al mercat japonès (1990) del primer producte alimentós tractat per AP -melmelada- (Hayashi, 1992; Knorr, 1993; Mertens, 1993).

Posteriorment als primers experiments, la major part de la investigació dels efectes sobre les cèl·lules s'ha fet en microorganismes a pressions que es donen de manera natural a la biosfera. ZoBell i altres autors han estudiat l'efecte de la pressió existent al fons del mar sobre els bacteris baroresistents, que poden créixer i multiplicar-se a les profunditats dels oceans, on les pressions arriben fins i tot a 100 MPa (Butz i Ludwig, 1986; Hoover i col., 1989).

En els darrers anys, la creixent demanda de productes segurs, mínimament tractats (semblants, tant com es pugui, als frescos), nutritius i amb noves presentacions, impulsa la recerca en tecnologies alternatives al tractament per calor, entre elles l'AP. Actualment ja es produeixen a petita escala aliments tractats per pressió al Japó -melmelades, gelatines, salses i sucs de fruites, vi i pastís d'arròs-, els EUA -crema d'alvocat- i França -suc de taronja- (Cheftel, 1995; Hayashi, 1997; Cheftel i Culioli, 1997).

En aquesta última dècada s'han celebrat nombrosos seminaris i congressos internacionals. Al congrés anual del Grup Europeu de Recerca en Alta Pressió, EHPRG (*European High Pressure Research Group*), ja s'inclou un apartat que tracta d'aliments. La UE, d'altra banda, ha finançat diversos projectes de recerca, en dos dels quals ha participat el nostre grup de treball.

1.2. Fonaments

La unitat de pressió del sistema internacional és el pascal (Pa). En aquest treball es fa servir el megapascal (MPa) i es transformen les unitats utilitzades en altres estudis per poder establir comparacions amb més facilitat. Les unitats de pressió més freqüentment emprades són:

$$1\ \text{MPa} = 9{,}869\ \text{atm} = 10\ \text{bar} = 10{,}197\ \text{kg F/cm}^2$$

La pressió afecta la conformació espacial (les estructures secundària, terciària, quaternària i supramolecular) de les macromolècules (els àcids nucleics, els polisacàrids i les proteïnes), la temperatura dels canvis de fase de l'aigua i els lípids i nombroses reaccions químiques (Mozhaev i col., 1994; Cheftel i Culioli, 1997).

El comportament dels sistemes bioquímics sota pressió es regeix pel *principi de Le Chatelier*, segons el qual tot fenomen que implica una disminució de volum és afavorit per un augment de pressió i viceversa (Cheftel, 1992). Així, les interaccions entre les biomolècules es modifiquen d'una o altra manera segons el canvi de volum, positiu o negatiu, que comporti la seva formació.

Enllaços covalents. No s'alteren a pressions inferiors a 1.000 MPa per la seva baixa compressibilitat, excepte en el cas de l'oxidació de grups sulfhidril, que provoca formació de ponts disulfur. D'aquesta manera, l'estructura covalent de les biomolècules de baix pes molecular (els sacàrids, els pèptids i els lípids) i l'estructura primària de les macromolècules no es modifiquen (Cheftel, 1992; Mozhaev i col., 1994; Heremans, 1995).

Interaccions electrostàtiques. L'AP contribueix a la hidratació de grups carregats ja que les molècules d'aigua s'organitzen de manera més compacta al voltant d'aquests

(fenomen anomenat *electrostricció*). En canvi, els parells iònics es dissocien per l'augment de volum que comporten (Gross i Jaenicke, 1994; Mozhaev i col., 1994). Per contra, Timson i Short (1965) afirmen que la pressió, en provocar solvatació d'ions, incrementa la ionització i finalment es formen enllaços iònics perquè els grups estan molt carregats.

Interaccions hidrofòbiques. Mozhaev i col. (1994) afirmen que es desestabilitzen ja que també impliquen augment de volum, mentre que Ohmiya i col. (1989) observen que a determinades pressions ocorre el contrari per la compressibilitat més alta de l'aigua lliure comparada amb la de l'aigua lligada.

Ponts d'hidrogen. Segons Gross i Jaenicke (1994) i Mozhaev i col. (1994), es formen i es destrueixen amb canvis de volum negatius o positius, segons el sistema, però sempre prop de zero; per tant, pràcticament no s'afecten. En canvi, diversos autors afirmen que la formació de ponts d'hidrogen va associada a una petita disminució de volum i, en conseqüència, es veu lleugerament afavorida sota pressió (Morild, 1981; Kunugi, 1992; Wong i Armstrong, 1992; Tauscher, 1995; Smeller i col., 1995); però això no sempre succeeix, perquè la formació d'aquests enllaços pot implicar creació d'estructures cícliques voluminoses o interaccions hidrofòbiques que compensin o superin l'esmentada disminució (Tauscher, 1995).

L'AP també contribueix a la dissociació dels grups àcid de les cadenes laterals dels aminoàcids i el trencament de ponts salins intramoleculars (Cheftel, 1992; Heremans, 1995).

L'altre principi científic que cal considerar per a l'aplicació d'AP en el camp de l'alimentació és el de la *tranmissió isostàtica*, és a dir, la transmissió uniforme de la pressió a tots els punts de l'aliment. Això evita la deformació del producte a pesar d'estar sotmès a tan altes pressions. A més, la pressió es transmet quasi instantàniament i, una vegada s'assoleix el valor desitjat, no hi ha pèrdues d'energia ni, conseqüentment, requeriments energètics addicionals (Cheftel, 1992; Mertens, 1993; Heremans, 1995). En ser un procés uniforme i instantani, independentment de la mida i la forma de l'aliment (Knorr, 1993), es poden obtenir productes molt homogenis, sense zones sobretractades. Aquest tipus de tranmissió i l'eficiència energètica són alguns dels avantatges que presenta aquesta tecnologia si la comparem amb el tractament per calor.

Uns altres avantatges són els relacionats amb les característiques originals del producte. L'AP es proposa com a tractament parcialment o totalment alternatiu al convencional per calor quan l'objectiu és la inactivació microbiana. Així, en poder tractar per pressió a temperatures més baixes (ambiental, de refrigeració i de congelació), pràcticament no varien el valor nutritiu i algunes característiques sensorials, com l'aroma i el sabor, de l'aliment (Cheftel, 1992; Hayashi, 1992). Tot això és afavorit pel fet que els enllaços covalents no es modifiquen sota pressió (Knorr, 1993; Mertens, 1993; Heremans, 1995).

1.3. Equips

Un sistema d'AP consta essencialment d'un recipient on es fa el tractament, un sistema de generació de pressió, un medi tranmissor d'aquesta i un sistema de regulació de temperatura.

1.3.1. Recipient de tractament

El recipient on es col·loca el producte i es fa el tractament sol ser cilíndric, d'acer inoxidable i amb una paret molt gruixuda i resistent.

1.3.2. Sistema de generació de pressió

Sistema indirecte. És el més freqüent. El medi transmissor de pressió, generalment aigua o oli, cal que sigui poc compressible. Mitjançant un sistema de bombes (intensificador de pressió), el fluid transmissor de pressió és impulsat des d'un dipòsit fins a l'interior del recipient de tractament (ja tancat). Així, aquest recipient es va omplint fins que l'aire és expulsat totalment (moment en què es tanca la vàlvula de purga) i, finalment, la pressió augmenta fins al valor desitjat (Mertens, 1995).

El sistema indirecte pot ser semicontinu o discontinu. En el sistema semicontinu, el medi transmissor de pressió impulsa un pistó que comprimeix el producte dins del recipient de tractament. S'aplica a aliments líquids que es tracten a granel i ha d'anar seguit d'un envasament asèptic. En el sistema discontinu (Figura 1), el producte es col·loca en el

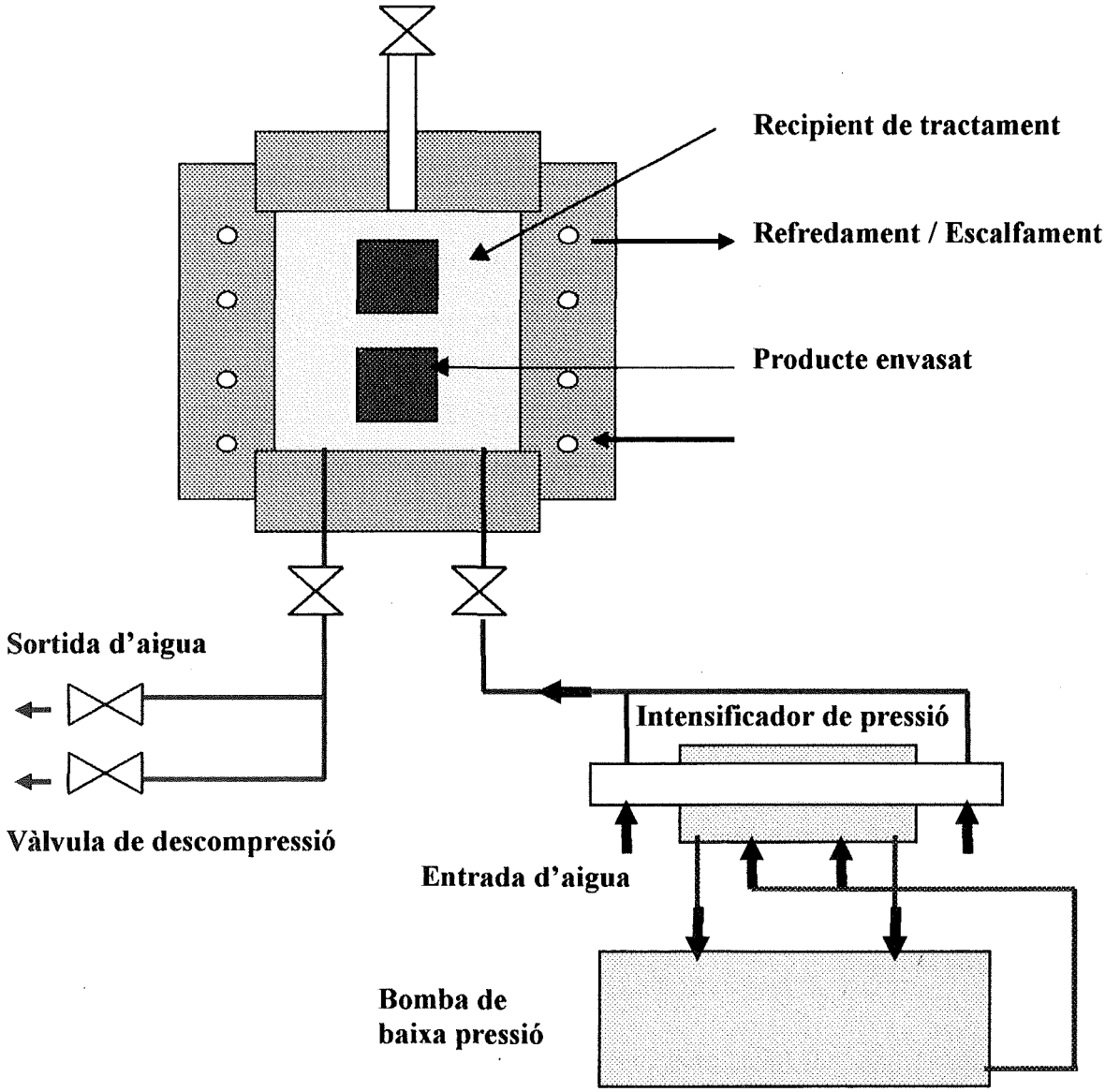

Figura 1. Sistema indirecte discontinu per al tractament per alta pressió d'aliments envasats (per gentilesa d'ALSTOM, Nantes, França).

recipient de tractament, envasat al buit i submergit en el fluid transmissor de pressió. Generalment s'aplica a aliments viscosos, sòlids o amb ingredients sòlids. Els envasos que se sotmeten a AP han de permetre segellaments hermètics i ser flexibles perquè la pressió es pugui transmetre correctament al seu través.

Sistema directe. És menys habitual. L'intensificador de pressió, que en aquest cas és una bomba que impulsa un pistó, es troba en el mateix recipient de tractament.

1.3.3. Sistema de regulació de temperatura

En existir aplicacions de l'AP que requereixen temperatures diferents de l'ambiental, és necessari incorporar als equips un sistema que reguli la temperatura.

Fins ara, les aplicacions comercials més interessants per a la indústria d'aliments s'han assolit combinant pressions de 400 a 600 MPa amb temperatures de 5 a 90 °C durant 10-30 min (Mertens, 1995). S'estan provant tractaments a temperatura més alta (cap a 100 °C) per ampliar les aplicacions al camp de l'esterilització. Els equips industrials utilitzats actualment són sistemes discontinus d'entre 10 i 500 L de capacitat i sistemes semicontinus d'1 a 4 t/h de producció (Hayashi, 1997).

1.4. Costos generals

Per calcular el cost del tractament per AP a una indústria, cal considerar els costos d'amortització (directament relacionats amb la pressió màxima a què pot funcionar l'equip), manteniment i personal i el consum d'energia, tot això en relació amb la producció diària.

En alguns casos, les millores de la tecnologia que permetin incrementar el rendiment o automatitzar el procés possibilitaran una reducció del cost final.

1.5. Efectes sobre els microorganismes

La resistència dels microorganismes a la pressió és molt variable. Generalment, els bacteris Gram-negatius són més sensibles que els Gram-positius (Cheftel, 1995; Earnshaw, 1995). No obstant això, en un estudi més recent, Ludwig i Schreck (1997) no troben cap relació amb el tipus Gram sinó amb la morfologia cel·lular i afirmen que els bacteris més sensibles són els de forma bacil·lar, els més resistents els esfèrics i els mitjanament sensibles corresponen a la varietat de formes entre els bacils curts i els cocs. Diversos autors també observen que diferents gèneres de cocs són molt resistents a l'AP (Shigehisa i col., 1991; Earnshaw, 1995; Patterson i col., 1995; Patterson i Kilpatrick, 1998). Els bacteris en fase estacionària són més baroresistents que els bacteris en fase logarítmica de creixement (Cheftel, 1995; Mackey i col., 1995). Les cèl·lules en fase estacionària són més petites i esfèriques que les que estan en fase de creixement i, a més, l'acumulació de components, com ara les proteïnes i els carbohidrats, pot reduir els efectes de la pressió (Isaacs i Chilton, 1995). Les espores bacterianes són molt resistents (Cheftel, 1995; Gould, 1995).

A grans trets, podem afirmar que com més complex és un organisme més sensibilitat mostra al tractament per pressió, de manera que les cèl·lules eucariotes són més barosensibles que les procariotes (Hoover i col., 1989). Així, els fongs filamentosos i els llevats són molt sensibles. Alguns virus presenten alta resistència mentre que amb els paràsits, sobretot els pluricel·lulars, succeeix el contrari (Cheftel, 1995).

Les condicions del procés i el medi també influeixen en la reducció del nombre de microorganismes quan s'aplica un tractament per AP.

En general, un augment de la pressió o el temps de tractament incrementa la letalitat, però no linealment. Així mateix, alguns autors conclouen que els tractaments de diversos cicles (a pressió alta o combinant pressions moderada i alta) són més eficaços que els continus tant sobre les cèl·lules vegetatives com sobre les espores (Sojka i Ludwig, 1994; Hayakawa i col., 1994a, b; Capellas, 1998; Ponce i col., 1998a i 1999). En la majoria de casos, el tractament per AP a temperatura ambient és el que provoca menys inactivació microbiana, mentre que aquells a temperatures baixes i, sobretot, altes són més efectius (Takahashi, 1992; Smelt i Rijke, 1992; Ludwig i col., 1992; Carlez i col., 1993; Capellas i

col., 1996; Gervilla i col., 1997a, b; Capellas, 1998; Ponce i col., 1998a, b i 1999). Temperatures de 50-70 °C milloren considerablement l'efecte de l'AP (Cheftel i Culioli, 1997).

Diferents ingredients de l'aliment, com ara el clorur sòdic, la glucosa, la fructosa, la sacarosa, el xilitol o el glicerol, poden tenir un paper protector davant del tractament per AP i, per tant, augmentar la proporció de microorganismes supervivents (Takahashi, 1992; Ludwig i col., 1992; Oxen i Knorr, 1993; Mackey i col., 1995). Diversos autors observen un efecte baroprotector de l'aliment globalment i remarquen la importància que pot tenir l'estructura alimentària on es troben els microorganismes en avaluar l'eficàcia dels tractaments per AP (Metrick i col., 1989; Styles i col., 1991; Patterson i col., 1995; Simpson i Gilmour, 1997; Patterson i Kilpatrick, 1998). Alguns d'aquests autors detecten microorganismes danyats subletalment. Per conseqüent, la resistència microbiana a la pressió és més alta en aliments que en solucions tamponades. En general, valors de pH baixos o d'activitat d'aigua alts milloren l'efectivitat del tractament per AP (Cheftel, 1995; Mackey i col., 1995; Roberts i Hoover, 1996; Cheftel i Culioli, 1997).

1.5.1. Cèl·lules vegetatives

L'AP provoca canvis de la morfologia, la paret i la membrana cel·lulars, les reaccions bioquímiques i els mecanismes genètics dels microorganismes (Hoover i col., 1989).

Morfologia cel·lular. Observem allargament de les cèl·lules, separació entre la paret cel·lular i la membrana citoplasmàtica i compressió d'aquesta i també dels vacúols, modificacions dels orgànuls cel·lulars -disminució del nombre de ribosomes, danys a les crestes mitocondrials- i el nucli -porus a la membrana- (Hoover i col., 1989; Osumi i col., 1992; Shimada i col., 1993; Cheftel, 1995; Knorr, 1995).

Paret i membrana cel·lulars. La membrana citoplasmàtica és la principal part del microorganisme danyada per la pressió. S'altera la permeabilitat cel·lular i, per tant, l'intercanvi iònic (Hoover i col., 1989; Macdonald, 1992; Cheftel, 1995). En alguns casos fins i tot poden produir-se trencaments a la paret i la membrana, que comporten la sortida de components intracel·lulars a l'espai extracel·lular i també l'entrada a la cèl·lula de

productes extracel·lulars (Shimada i col., 1993; Isaacs i Chilton, 1995; Hauben i col., 1996; Chilton i col., 1997). La cristal·lització de fosfolípids de membrana produïda per pressió probablement contribueix a la inactivació de diversos microorganismes (Cheftel, 1995).

Russell i col. (1995) observen com la composició lipídica de la membrana cel·lular pot condicionar la resistència als tractaments per AP. Atès que l'augment del contingut de la membrana en difosfatidilglicerol li causa pèrdua de fluïdesa i que, generalment, una disminució de la fluïdesa de la membrana incrementa la sensibilitat a l'AP, les cèl·lules més riques en difosfatidilglicerol són més susceptibles a les alteracions produïdes per pressió.

És probable que l'alteració de la membrana cel·lular sigui la principal causa de l'aparició de microorganismes danyats subletalment després del tractament per AP, fenomen descrit per diversos autors i causant que aquestes cèl·lules supervivents no puguin créixer en medi de cultiu selectiu i necessitin un període de recobrament (Metrick i col., 1989; Styles i col., 1991; Carlez i col., 1994; Raffalli i col., 1994; Patterson i col., 1995; Capellas i col., 1996; Carballo i col., 1997; Capellas, 1998; Patterson i Kilpatrick, 1998; Ponce i col., 1998a, c i 1999).

Reaccions bioquímiques. La inactivació d'enzims clau per a la funció cel·lular pot explicar una part dels efectes inhibitoris de l'AP sobre els microorganismes (Hoover i col., 1989). Smelt i col. (1995) afirmen que la inactivació de la Na^+-K^+-ATPasa de la membrana cel·lular, ja sigui per dislocació o per desnaturalització, és un dels mecanismes que provoca inhibició de microorganismes perquè s'interromp la hidròlisi de l'ATP necessària per mantenir el transport actiu de protons cap a l'exterior de la cèl·lula que garanteixi l'homeostasi i, en conseqüència, disminueix el pH cel·lular. Chong i col. (1985) i Fortes i col. (1995) igualment observen inactivació irreversible d'aquest enzim sota pressió. La Ca^{2+}-ATPasa de la membrana també s'inactiva per AP (Heremans i Wuytack, 1980).

Mecanismes genètics. Els àcids nucleics són més resistents a la desnaturalització per AP que les proteïnes. Aquesta resistència més alta, en el cas de l'estructura de doble hèlix de l'ADN, pot ser deguda a la gran quantitat de ponts d'hidrogen intramoleculars (Hoover i col., 1989; Heremans, 1995). Malgrat l'estabilitat dels àcids nucleics sota pressió -àdhuc a 1.000 MPa- (Hoover i col., 1989; Mozhaev i col., 1994; Cheftel, 1995), Chilton i col.

(1997) observen que *in vivo* l'ADN i l'ARN ribosòmic sí es poden veure afectats (trencament de cadenes), la qual cosa suggereix que es tracta d'un efecte indirecte en què està implicada l'activitat de l'ADNasa i la ribonucleasa.

Els enzims relacionats amb els mecanismes de replicació i transcripció de l'ADN i traducció a proteïnes també s'afecten per AP i, així, aquests processos resten inhibits (Hoover i col., 1989; Cheftel, 1995). En la inhibició de la síntesi proteica també influeix la dissociació dels ribosomes produïda per pressió (Gross i col., 1992).

1.5.2. Espores bacterianes

Així com les cèl·lules vegetatives generalment s'inactiven entre 400 i 600 MPa, les espores resisteixen més de 1.000 MPa si no s'augmenta la temperatura per damunt dels 45-75 °C segons el cas (Cheftel, 1995; Gould, 1995).

Diversos autors han estudiat les causes de la resistència de les espores a l'AP. Gould (1986) l'atribueix a l'estat viscós existent al seu interior i Timson i Short (1965) a la presència d'àcid dipicolínic, que les protegeix de la solvatació i ionització excessives, causants de l'efecte letal sobre les cèl·lules vegetatives.

S'ha observat que la pressió pot provocar germinació d'espores bacterianes. Sale i col. (1970) suggereixen que l'AP produeix inactivació d'espores perquè primer inicia la germinació d'aquestes i després inactiva les cèl·lules germinades. En general, combinant pressions de 50 a 250 MPa amb temperatures de 40 °C o més, es pot iniciar la germinació sobretot per activació d'algunes reaccions enzimàtiques, més que per distorsió física de l'espora (Clouston i Wills, 1969; Gould i Sale, 1970; Nishi i col., 1994; Heinz i Knorr, 1998). Temperatures baixes i valors extrems de pH minimitzen la germinació iniciada per pressió (Gould i Sale, 1970). Craven (1988) observa que la germinació de les espores millora amb diversos tractaments que trenquen les interaccions hidrofòbiques. D'acord amb això, i tenint en compte que l'AP desestabilitza les esmentades interaccions, aquest podria ser el mecanisme pel qual s'afavoreix la germinació.

Per reduir el nombre d'espores viables i, fins i tot, per aconseguir solucions estèrils mitjançant AP, es pot aplicar un tractament de germinació (a pressió moderada) seguit d'un d'inactivació (a pressió alta) una o més vegades (Sojka i Ludwig, 1994; Capellas, 1998). La inactivació d'espores mitjançant diversos cicles a pressió alta és deguda, bàsicament, a alteracions a la paret -canvis de permeabilitat i també trencaments- (Hayakawa i col., 1994a, b). Alguns autors proposen el tractament per AP a temperatures d'entre 60 i 80 °C, prescindint de la germinació prèvia, per inactivar espores.

1.5.3. Virus i paràsits

S'ha estudiat l'efecte inhibitori de l'AP sobre el poder infectant del virus de la immunodeficiència humana (Otake i col., 1997). Diferents soques del mateix virus tenen desigual sensibilitat a la pressió, que ha de ser sempre superior a 400 MPa per aconseguir la inactivació. Alguns autors han comprovat que els virus inactivats per AP conserven les seves propietats immunogèniques, cosa que possibilitaria l'obtenció de vacunes amb aquest tractament (Silva i col., 1992; Pontes i col., 1997).

S'ha observat que diversos paràsits presents a vegetals, peix o carn, entre ells triquinela, poden inactivar-se amb tractaments per AP (Ohnishi i col., 1993; Butz i Tauscher, 1995).

1.5.4. Tractaments combinats

Es proposa la combinació d'AP amb altres tractaments (químics o físics) per augmentar la letalitat dels microorganismes. Aquesta combinació de processos permet suavitzar les condicions aplicades en cada tractament individualment i, així, aconseguir aliments mínimament tractats que presentin, alhora, una qualitat microbiològica òptima.

D'una banda, s'ha estudiat l'addició de bacteriocines (nisina i pediocina), peròxid d'hidrogen, lisozima, xitosan, monoterpens d'olis essencials, agents acidificants, butilhidroxianisol, sorbat potàssic, etanol o sulfit sòdic, combinada amb AP (Papineau i col., 1991; Kalchayanand i col., 1994; Knorr, 1995; Mackey i col., 1995; Roberts i Hoover,

1996; Hauben i col., 1996; Marquis, 1997; Adegoke i col., 1997; Stewart i col., 1997; Capellas, 1998; Ponce i col., 1998c).

De l'altra, se suggereix l'aplicació d'altes o baixes temperatures, radiacions ultraviolades o ionitzants, polsos elèctrics d'alt voltatge o ultrasons, en combinació amb el tractament per AP (Shimada i Shimahara, 1991; Shimada, 1992; Knorr, 1995; Roberts i Hoover, 1996; Capellas i col., 1996; Crawford i col., 1996; Bolder, 1997; Gervilla i col., 1997a, b; Stewart i col., 1997; Capellas, 1998; Patterson i Kilpatrick, 1998; Ponce i col., 1998a, b i 1999).

1.6. Efectes sobre els components dels aliments

1.6.1. Aigua

A temperatura ambient, l'aigua experimenta una disminució de volum d'aproximadament 4% quan se sotmet a 100 MPa i 15% a 600 MPa. Els aliments amb molta aigua i poc gas presenten una compressibilitat semblant a la de l'aigua. Aquesta disminució de volum implica un augment de densitat i, en conseqüència, els coeficients de difusió de soluts disminueixen (Cheftel, 1992).

La compressió de l'aigua provoca un augment de temperatura, de 2-3 °C per cada 100 MPa, que depèn de la temperatura inicial de l'aigua i de la velocitat de compressió. Aquest canvi és reversible en fer-se la descompressió, ja que es produeix una disminució de la temperatura de la mateixa magnitud (Cheftel, 1992).

La pressió afavoreix la dissociació iònica de diversos àcids i bases dèbils i de l'aigua. Per tant, en tractar per AP sistemes biològics no tamponats, poden produir-se canvis dràstics de pH (Lüdemann, 1992). En el cas de l'aigua, a 100 MPa i temperatura ambient, el pH disminueix en 0,73 unitats (Kunugi, 1991).

Els canvis de fase de l'aigua es veuen afectats de manera reversible. En augmentar la pressió, primer disminueix el punt de fusió del gel i després augmenta. A 210 MPa s'assoleix el valor mínim: l'aigua és manté en estat líquid fins a –22 °C, ja que la pressió

s'oposa a l'augment de volum causat per la formació de cristalls de gel de tipus I (Deuchi i Hayashi, 1991). Segons la pressió, es poden formar cristalls a més de 0 °C (Cheftel, 1992). Els cristalls de tipus I són els únics que causen un augment de volum, mentre que els altres tipus (II a VIII) impliquen absència de canvi o una petita disminució de volum amb relació a l'estat líquid, la qual cosa comporta menys dany en els teixits que no pas el que produeixen els cristalls de tipus I. Aquests fenòmens tenen importants aplicacions pràctiques (Kalichevsky i col., 1995):

a) Acceleració de la descongelació: els productes poden ser descongelats entre 0 i –22 °C. La descongelació és ràpida (no instantània) i molt uniforme ja que es dóna de manera simultània en tots els punts del producte. Això pot ser útil principalment en els camps mèdic i alimentari, sobretot en aquells casos en què les mostres s'alteren de manera considerable durant la descongelació.
b) Emmagatzematge de productes a baixes temperatures, entre 0 i –22 °C, sense formació de cristalls de gel. El temps d'emmagatzematge pot ser limitat per l'activitat enzimàtica, que no es veu tan reduïda com en la congelació.
c) Congelació ultraràpida i uniforme, en fer la descompressió en productes que, entre 0 i –22 °C, no estan congelats. En general, els cristalls de gel que es formen són de mida inferior a la dels formats convencionalment; en descongelar, doncs, hi ha menys pèrdua d'aigua i una estructura més homogènia, i s'obté un producte amb millor textura (Deuchi i Hayashi, 1991 i 1992; Cheftel i Culioli, 1997; Fuchigami i Teramoto, 1997; Fuchigami i col., 1997 i 1998).

1.6.2. Hidrats de carboni

La transició sol-gel dels polisacàrids s'afecta per AP; sovint es formen gels diferents dels obtinguts mitjançant calor (Gekko, 1992; Mozhaev i col., 1994).

La pressió provoca gelatinització dels gränuls de midó, també a temperatura més baixa que l'habitual (Hayashi i Hayashida, 1989); Rubens i col. (1997) observen que aquells s'inflen, però la seva estructura no es veu alterada. La pressió a la qual ocorren aquests canvis depèn de l'origen del midó.

1.6.3. Proteïnes

L'AP causa desnaturalització (dissociació i desplegament) de proteïnes, la qual cosa comporta una disminució de volum principalment deguda a un augment de la superfície d'hidratació (Balny i col., 1989; Masson, 1992).

Les interaccions hidrofòbiques intervenen en les estructures terciària i quaternària. La desestabilització d'aquestes interaccions explica el fet que les proteïnes oligomèriques i polimèriques siguin molt sensibles a l'AP i es dissociïn. En general, els efectes de la pressió sobre les estructures terciària i quaternària són reversibles, però després de la descompressió el replegament de la proteïna pot ser molt lent (Masson, 1992; Balny i Masson, 1993; Heremans, 1995).

Els ponts d'hidrogen intervenen en l'estructura secundària. Generalment, aquests enllaços són estables sota pressió; però a valors molt alts pot canviar la conformació, sobretot l'estructura α-hèlix. Els efectes de l'AP sobre l'estructura secundària solen ser irreversibles (Masson, 1992; Wong i Armstrong, 1992; Balny i Masson, 1993; Heremans, 1995).

Timson i Short (1965) afirmen que la reducció del nombre de grups hidrofílics lliures de les proteïnes, derivada de la formació d'enllaços iònics posterior a la ionització, disminueix la solubilitat d'aquestes, de manera que s'acaben formant complexos que precipiten. Suggereixen que aquest mecanisme de desnaturalització pot tenir un paper important en la inactivació de sistemes biològics mitjançant AP.

La desnaturalització proteica causada per pressió, combinada amb canvis de pH i temperatura, ofereix aplicacions importants.

Enzims. Els efectes de l'AP sobre els enzims van des de canvis de la velocitat de les reaccions que catalitzen, fins a canvis estructurals (Balny i Masson, 1993). Així, la funció dels enzims es pot veure afectada de maneres molt diverses, segons el tipus d'enzim, les condicions de tractament (pressió, temps i temperatura) o el pH del medi. Alguns enzims augmenten la seva activitat catalítica mentre que altres, a causa de la desnaturalització provocada per pressió, perden activitat o, fins i tot, s'inactiven reversiblement o

irreversible, segons cada cas (Matthews, Jr. i col., 1940; Heremans i Heremans, 1989; Hara i col., 1990; Ogawa i col., 1990; Ohmori i col., 1991; Mozhaev i col., 1996; Kunugi i Tanaka, 1997; Hayashi i col., 1998). Alguns enzims són baroresistents i la seva funció no es veu alterada ni en un sentit ni en l'altre (Asaka i Hayashi, 1991). Ko i col. (1990) no observen desnaturalització per calor de la Ca^{2+}-ATPasa de l'actomiosina tractada per AP. Hayakawa i col. (1988 i 1992) i Mori i col. (1991) descriuen la reactivació per pressió de l'α-amilasa inactivada per calor. Els canvis estructurals de l'enzim també poden afectar la relació enzim-substrat (Balny i Masson, 1993). Sovint les reaccions enzimàtiques són realçades o inhibides pel tractament per AP segons comportin un canvi de volum negatiu o positiu (Asaka i Hayashi, 1991; Cheftel, 1992). Ko i col. (1991) i Seyderhelm i col. (1996) observen un efecte baroprotector tant de l'aliment com d'alguns ingredients sobre diversos enzims, similar al descrit en el cas dels microorganismes.

El midó i les proteïnes són més sensibles als enzims una vegada han estat, respectivament, gelatinitzat o desplegades per AP: per exemple, s'observa més sensibilitat a les hidròlisis, la qual cosa en millora la digestibilitat i la disponibilitat (Cheftel, 1992; Mertens, 1993). L'augment de la susceptibilitat del midó d'arròs a l'amilasa s'aprofita en la fabricació de *sake* (Miyama i col., 1992). Les proteïnes són més sensibles a les proteases (Ohmori i col., 1991); Okamoto i col. (1991) afirmen que la hidròlisi de la β-lactoglobulina pot disminuir el poder al·lergen de la llet.

Transició sol-gel. L'AP, mitjançant reaccions d'agregació posteriors a la desnaturalització, provoca gelificació de nombroses suspensions de proteïnes (igual que succeeix amb els polisacàrids) i dóna lloc a gels diferents dels que es fomen per calor (Suzuki i Macfarlane, 1984; Ikeuchi i col., 1992). Els gels proteics obtinguts per pressió són tous i brillants i, com més pressió, més fermesa i menys adhesivitat presenten (Tauscher, 1995). Sovint, en augmentar la pressió es redueix la temperatura de transició sol-gel, de manera que es poden produir gels a temperatura ambient (Okamoto i col., 1990; Cheftel, 1992; Mozhaev i col., 1994). També s'ha aconseguit la gelificació a temperatura de refrigeració i, fins i tot, de congelació (Ko i col., 1990; Ponce i col., 1996). Una vegada més, segons si implica un canvi de volum negatiu o positiu, el mecanisme de gelificació es veurà afavorit o no per AP. La pressió necessària perquè es produeixi agregació depèn del tipus i la concentració de proteïna, el pH i la força iònica del medi i la temperatura de tractament (Cheftel, 1992).

Propietats funcionals. L'AP, a pH i temperatura adequats, pot causar desnaturalització parcial de proteïnes, la qual cosa modifica positivament algunes propietats funcionals (Macfarlane, 1974; Suzuki i Macfarlane, 1984; Denda i Hayashi, 1992; Ikeuchi i col., 1992; Mandava i col. 1994; Fernández i col., 1998). Però en altres casos, aquestes propietats es poden veure afectades negativament (Elgasim i col., 1982; Carballo i col., 1996; Jiménez Colmenero i col., 1997).

1.6.4. Lípids

El punt de fusió augmenta, de manera reversible, en més de 10 °C per cada 100 MPa. Així, els lípids que a temperatura ambient i pressió atmosfèrica es troben en estat líquid, cristal·litzen sota pressió; els cristalls que es formen són més densos i estables (Cheftel, 1992 y 1995).

Heremans (1992) afirma que les modificacions dels canvis de fase dels lípids poden ser la causa de la destrucció per AP de membranes biològiques.

1.6.5. Vitamines

Al contrari del que succeeix en el tractament per calor, l'AP altera poc les vitamines (Horie i col., 1991; Cheftel, 1992; Mertens, 1993; Bognar i col., 1993). No obstant això, en estudis més recents, Kübel i col. (1997) i Taoukis i col. (1998) observen una disminució considerable del contingut en vitamines A i C, respectivament, en solucions i sucs de fruites tractats per pressió.

Principalment per l'efecte sobre aquests nutrients, parlem de menys afectació del valor nutritiu per AP que per alta temperatura. Per tant, aquesta és una de les diferències més importants en comparar ambdós tractaments.

1.7. Altres aplicacions generals

El tractament per AP produeix una gran varietat d'efectes, positius i negatius, que poden manifestar-se sobre qualsevol característica de l'aliment. Hi ha moltes aplicacions potencials d'aquesta tecnologia a més de les que s'han anat comentant anteriorment.

És possible pasteuritzar aliments a temperatura baixa o moderada i aconseguir productes de qualitat òptima, segurs (inactivació de microorganismes patògens) i de llarga vida útil en refrigeració o, fins i tot, a temperatura ambient (inactivació de microorganismes causants de deteriorament). També es parla d'esterilització si es tracta per pressió a temperatures altes. És important l'ús d'AP, a menys temperatura que la d'una cocció convencional, per obtenir plats preparats de gran qualitat microbiològica, útil principalment en aquells amb ingredients que es modifiquen de manera considerable si són tractats per calor -carn, peix, vegetals o bolets- (Cheftel, 1995).

Les aplicacions de l'AP van més enllà de la inactivació de microorganismes, segons la combinació de pressió, temps i temperatura que s'utilitzi. Paral·lelament a l'objectiu de reduir la càrrega microbiana inicial, les primeres matèries poden ser tractades per AP a fi de conservar o millorar la seva funcionalitat per a la elaboració de productes. L'efecte sobre les propietats físiques dels aliments també pot donar lloc a nous productes. Per exemple, les interaccions entre els components provocades per pressió originen productes amb textures molt diferents de les que estem acostumats (Yoshioka i col., 1992).

Watanabe i col. (1991) aprofiten la gelatinització del midó causada per pressió per estovar llavors de llegums i grans de cereals (arròs).

Mitjançant pressió es poden inactivar algunes substàncies tòxiques de caràcter proteic (per desnaturalització), com ara els inhibidors de les proteases i els al·lergògens alimentaris (Cheftel, 1992). També es proposa l'AP per escaldar vegetals a baixa temperatura (Cheftel, 1992), per a la qual cosa cal considerar la sensibilitat a la pressió d'enzims com la peroxidasa i la lipooxigenasa (Seyderhelm i col., 1996). Eshtiaghi i Knorr (1993) observen que els escaldats per pressió, a més de reduir la pèrdua de nutrients, són els que millor conserven la textura original d'alguns vegetals.

Yasuda i col. (1991) i Yasuda i Mochizuki (1992) utilitzen AP per solidificar la mantega de cacau i obtenir el mateix efecte que es produeix durant el temperament de la xocolata. La pressió pot millorar l'estructura cristal·lina dels triglicèrids (evita polimorfismes) i, consegüentment, la textura resultant.

L'AP permet aglomerar i compactar productes pulverulents en diferents formes: barres, pastilles, cubs, etc. (Cheftel, 1992).

1.8. Efectes i aplicacions en la carn, la carn d'aviram i els seus derivats

A més de la considerable millora de la qualitat microbiològica, comuna per a la majoria d'aliments tractats per AP, es produeixen altres efectes d'especial interès per a la carn, la carn d'aviram i els seus derivats. Però prèviament cal avaluar tots els pros i els contres per determinar si val la pena fer el tractament o si, per contra, les millores provocades per pressió no compensen les alteracions que pot experimentar el producte durant el procés.

1.8.1. Tendresa

D'una banda, l'AP trenca la membrana dels lisosomes i, per tant, el contingut d'aquests surt al citoplasma (Elgasim i Kennick, 1982; Ohmori i col., 1992; Homma i col., 1994). Els lisosomes contenen proteases que intervenen en la maduració *postmortem* de la carn. A causa de la desnaturalització, les proteïnes són més digeribles per les proteases sense que el seu valor biològic es vegi alterat. A més, es formen quantitats considerables de pèptids solubles, aminoàcids lliures i altres compostos que poden influir en l'aroma i el sabor de la carn (Ohmori i col., 1991; Cheftel, 1992; Mertens, 1993; Suzuki i col., 1994).

De l'altra, el tractament per pressió provoca una sèrie de canvis de l'estructura del múscul: augment dels espais intercel·lular i intermiofibril·lar, separació entre el sarcolemma i les miofibril·les, inflament i, fins i tot, trencament dels mitocondris, inflament del reticle sarcoplasmàtic, modificacions irreparables de l'estructura del sarcòmer (fragmentacions), dissociació de l'actomiosina, desagregació i despolimerització de la miosina i l'actina (Bouton i col., 1977b; Macfarlane i Morton, 1978; Elgasim i

Kennick, 1982; Macfarlane i col., 1982 i 1986; Suzuki i col., 1990); Locker i Wild (1984) i Taylor i col. (1995) suggereixen que, a més de la miosina i l'actina, altres proteïnes del citoesquelet poden estar implicades en el procés. Aquests canvis depenen de si el tractament s'aplica en pre o post-rigor (Cheftel i Culioli, 1997).

No sembla que hi hagi relació directa entre la proteòlisi i els canvis estructurals provocats per pressió i l'entendriment de la carn, però són dos fets que cal tenir en compte ja que, indubtablement, hi influeixen de manera considerable (Macfarlane i col. 1988).

En prerigor, el múscul tractat per AP és molt ferm i està contret, sobretot a causa de la sortida de calci del reticle sarcoplasmàtic (Horgan, 1979). Després de coure'l, és més tendre i menys sucós, a pesar de contenir més aigua, que el múscul no tractat (Macfarlane, 1973; Kennick i col., 1980; Riffero i Holmes, 1983). Bouton i col. (1977b) observen que la tendresa obtinguda està directament relacionada amb la contracció assolida durant el tractament. L'aplicació d'AP en prerigor és poc viable ja que comporta poc temps d'estada a l'escorxador i també un especejament en calent i el tractament per pressió del múscul quan el pH encara és alt, amb el consegüent risc microbiològic (Cheftel i Culioli, 1997).

En post-rigor, el tractament per AP (fins a 150 MPa) a temperatura moderada (55-60 °C) és molt efectiu (Bouton i col., 1977a). El resultat depèn en gran manera del pH de la carn (Bouton i col., 1982). La tendresa s'aconsegueix per modificació de l'estructura miofibril·lar però no del teixit conjuntiu, ja que el col·lagen no es veu afectat per AP excepte en tractaments inusualment llargs (Bouton i col., 1977b, 1978 i 1982; Ratcliff i col., 1977; Robertson i col., 1984; Macfarlane i col., 1988; Beilken i col., 1990; Suzuki i col., 1993). Aquest tractament a temperatura moderada podria ser una solució per a les carns que presenten enduriment per fred (*cold toughening*). El principal inconvenient és l'aspecte, similar al de la carn cuita, que presenta el producte una vegada ha estat tractat (Cheftel i Culioli, 1997). A pressions superiors a 150 MPa, s'aconsegueixen bons resultats, àdhuc sense necessitat d'augmentar la temperatura (Suzuki i col., 1990 i 1992; Cheftel i Culioli, 1997). Però l'aplicació d'una pressió tan alta també provoca canvis de color i la carn perd el seu aspecte fresc (Cheftel i Culioli, 1997).

En estudis recents es parla de l'efecte contrari. Raszl (1998) i Jung i col (1999), aquests últims fins i tot treballant a pressions moderades (130 MPa), observen que la carn tractada per AP és més ferma.

1.8.2. Reestructuració i gelificació

En productes picats o emulsionats, segons les condicions de tractament i el pH i la concentració de clorur sòdic del producte, l'AP augmenta la cohesió entre les partícules per desnaturalització i posterior reagregació de les proteïnes miofibril·lars (Suzuki i Macfarlane, 1984; Macfarlane i col., 1984; Macfarlane, 1985) i també incrementa la solubilitat de les esmentades proteïnes en solució salina (Macfarlane, 1974; Macfarlane i McKenzie, 1976). Aquest tractament pot ser interessant per mantenir o augmentar la funcionalitat proteica quan es vol reduir la quantitat de clorur sòdic de l'aliment. També permet disminuir la dosi de polifosfats afegida (Macfarlane i col., 1984).

En alguns casos, la pressió pot millorar la capacitat de gelificació per calor de les pastes càrnies emulsionades. Les emulsions o/a estabilitzades per proteïnes càrnies poden ser tractades per AP sense que es produeixi separació de fases (Mandava i col., 1994).

Mitjançant AP a temperatura ambient o més baixa, és possible obtenir gels de carn o peix picats, pastes càrnies o surimi. Aquests gels presenten millor aspecte i textura, més uniformitat i menys exsudació que els obtinguts per calor. La miosina, igual que en els tractaments per calor, està implicada en el mecanisme de gelificació (Ko i col., 1990; Okamoto i col., 1990; Yoshioka i col., 1992; Serennes i col., 1996).

1.8.3. Color

Generalment la carn i els productes carnis no curats tractats per AP són més pàl·lids (presenten color rosat). En alguns casos, segons les condicions de tractament, l'aspecte és semblant al de la carn bullida. A més, la proporció de metamioglobina augmenta a costa de la d'oximioglobina. Aquests efectes de la pressió són deguts a la desnaturalització de la globina, la dislocació o pèrdua del grup hemo i l'oxidació de l'àtom de ferro, i estan molt condicionats pels paràmetres de tractament (Carlez i col., 1995; Goutefongea i col., 1995).

Els productes curats (cuits o deshidratats) tenen uns pigments ja fixats i estables, resistents a l'oxidació, i, per tant, el seu color pràcticament no es modifica per pressió (Carlez i col., 1995; Goutefongea i col., 1995; Cheftel i Culioli, 1997).

1.8.4. Oxidació lipídica

El tractament per AP en presència d'oxigen accelera l'oxidació lipídica, tant en la carn com en el peix (Tanaka i col., 1991; Wada, 1992; Cheah i Ledward, 1996). Si els productes es tracten envasats en atmosfera sense oxigen però després s'emmagatzemen en presència d'aquest, l'oxidació també es veu realçada (Cheah i Ledward, 1996).

A més de la presència d'oxigen, l'efecte de l'AP sobre l'estabilitat oxidativa dels lípids del múscul depèn de la presència de teixit muscular -els lípids aïllats i purificats són estables sota pressió- (Tanaka i col., 1991; Ohshima i col., 1993; Cheah i Ledward, 1995) i les condicions de tractament -els canvis que provoquen oxidació lipídica de la carn tractada per AP comencen aproximadament a partir de 300 MPa a temperatura ambient- (Cheftel i Culioli, 1997). Tanaka i col. (1991) afirmen que l'oxidació està relacionada amb la concentració aquosa del medi i, segons el valor d'aquesta, pot ser menys intensa que en productes no tractats per AP. Així mateix, Cheah i Ledward (1995) proposen la seva dependència de l'activitat de l'aigua.

Cheah i Ledward (1996) observen que l'oxidació causada per pressió és comparable a la produïda per calor. En aquest i en altres estudis, se suggereix que, a causa de la desnaturalització proteica provocada per AP, resten lliures ions metàl·lics, que catalitzen l'oxidació (Tanaka i col., 1991; Wada, 1992; Cheah i Ledward, 1995; Angsupanich i Ledward, 1998).

Durant l'emmagatzematge, els productes tractats per AP acumulen menys àcids grassos lliures (que són més susceptibles a l'oxidació que els triglicèrids) ja que la pressió causa inactivació de lipases (Wada, 1992).

1.9. Futures investigacions

L'AP isostàtica és una tecnologia relativament recent en el camp de l'alimentació. És necessari, doncs, continuar investigant i aprofundint en tots els aspectes possibles: nutritiu, microbiològic, fisicoquímic i sensorial.

Fins i tot seria convenient fer estudis toxicològics (toxicitat aguda i crònica) per descartar la formació de productes nocius en els aliments tractats per pressió, que poguessin provocar intoxicacions a curt, mitjà o llarg termini.

2. PRODUCCIÓ I CONSUM DE CARN D'AVIRAM

Es presenten tot seguit algunes dades estadístiques de producció i consum. Cal remarcar les discrepàncies observades en consultar diferents fonts, doncs, malgrat els esforços de les administracions, encara és difícil comptabilitzar els efectius reals sense barrejar en els sumands animals el cicle de producció i consum dels quals es fa per complet a un país amb aquells que tenen diverses zones de producció i/o consum. Aquests fets dificulten principalment l'estimació del consum.

D'altra banda, les tendències de futur s'estableixen partint d'una perspectiva històrica. Tanmateix, els esdeveniments dels últims deu anys que han afectat la producció, la qualitat i, per tant, l'acceptació de la carn de les diferents espècies de consum, fan que les prediccions variïn en un o altre sentit de manera imprevista i amb permanència a més o menys llarg termini.

La producció de carn fresca i els seus derivats augmenta anualment tant a Espanya com a la UE i el món (FAO, 1999), encara que el creixement del consum per capita en els països desenvolupats sembla que s'ha aturat en els darrers anys. Els productes de la indústria càrnia són els més consumits de tot el sector agroalimentari (García Pozo, 1998a i 1999).

2.1. Espanya

Tot i que la producció de carn d'aviram ha experimentat alguns alts i baixos durant l'última dècada, ocupa el segon lloc després de la de carn de porc. El 1998, es van produir 910.000 t de carn d'aviram. Catalunya n'és la principal productora: té més d'un 30% de l'activitat del sector avícola (ANPP, 1997). A Espanya es produeixen un 10,7% de la carn d'aviram de la UE i un 1,5% de la del món (FAO, 1999).

També es registra una producció creixent de salsitxes cuites fabricades parcialment o total amb diferents tipus de carn d'aviram, entre ells la carn recuperada mecànicament. Cap a un 10-12% de les salsitxes cuites que hi ha al mercat contenen carn d'aviram en la seva composició (Ferrer, 1998).

La carn més consumida al nostre país és la d'aviram; el 1995, se'n consumiren 22,1 kg per capita (ANPP, 1997). A més, la carn de pollastre és la més barata (García Pozo, 1998b). El consum d'aquesta carn ha de tendir a l'alça moderada, entre altres causes per la seva composició i la seva bona adaptació als hàbits de consum del ciutadà (ANPP, 1997). Dades de 1996 indiquen que, d'entre els països integrants de la UE, Espanya és el primer consumidor de carn de pollastre i el que presenta els preus mitjans d'aquesta carn més baixos (ANPP, 1997).

2.2. Unió Europea

El mercat de la carn d'aviram ha experimentat un creixement considerable. Entre 1989 i 1998 la producció ha augmentat un 35,6%. Amb 8.524.000 t se situa en segon lloc després de la de carn de porc. La UE és el tercer productor de carn d'aviram, després dels EUA i la Xina (FAO, 1999).

Així mateix, el consum de carn d'aviram ha augmentat considerablement. El 1996, fou de 20,3 kg per capita, el segon després del de carn de porc (CE, 1997). Aquestes xifres demostren que aquell tipus de carn s'ha revalorat en el gust del consumidor europeu (García Pozo, 1998a). Es preveu per als pròxims anys que continuï el creixement del consum de carn d'aviram, afavorit per uns preus molt competititus si es compara amb els d'altres carns i per la creixent preferència del consumidor (CE, 1997).

2.3. Món

La producció de carn d'aviram va ser el 1998 de 60.243.000 t aproximadament. També ocupa el segon lloc després de la de carn de porc (FAO, 1999).

Igual que a la UE i per raons similars, s'espera que augmenti el consum de carn d'aviram, tant en els països en desenvolupament com en els desenvolupats. L'augment mitjà es preveu que sigui un 3,5% anual, inferior al de la dècada dels 80, però força superior a la taxa anual del porquí i, principalment, la del vaquí. Les perspectives per als pròxims anys són una considerable demanda de carn d'aviram i una substitució més gran per part del consumidor de la carn de vaquí per la de porquí i aviram (CE, 1997). Entre els

màxims consumidors de carn de pollastre hi ha gran diversitat de països, de la qual cosa es pot concloure que aquesta és probablement la carn més acceptada internacionalment (ANPP, 1997).

3. CARN RECUPERADA MECÀNICAMENT D'AVIRAM

3.1. Definició, història i situació actual

Carn recuperada mecànicament (CRM) és el producte resultant de la separació mecànica de la carn que resta adherida a ossos després de l'especejament manual o automàtic (Field, 1988; Crosland i col., 1995). No s'han d'utilitzar ni el cap ni les extremitats (potes, en el cas de l'aviram) en la seva fabricació (Anònim, 1997). La carn recuperada mecànicament d'aviram (CRMA) s'obté majoritàriament de carn de les carcasses i altres parts de baix cost, com ara els colls i les ales, dels *broilers*, però també de les gallines ponedores que han acabat el seu cicle de posta, els galls dindi i altres aus (Mountney i Parkhurst, 1995; Lee i col., 1997).

La terminologia d'aquesta carn ha estat debatuda a diversos congressos. El terme usat pels autors en alguns països europeus és el de *carn recuperada mecànicament* (Field, 1988). En aquest treball s'ha optat per utilitzar-lo car és el que millor explica el producte de què es tracta. El terme *carn separada mecànicament* fou aprovat a la 10a sessió de la Comissió del *Codex Alimentarius* sobre Productes carnis i avícoles elaborats, celebrada a Copenhaguen el 1978, i pel *United States Department of Agriculture* (USDA) el 1982. Tanmateix, en el cas de l'aviram, aquest últim l'anomena *carn desossada mecànicament d'aviram.*

La tecnologia de recuperació mecànica de carn va ser adoptada per primera vegada pels japonesos a finals dels anys 40. Ells l'aplicaven al peix i d'aquesta manera podien obtenir i aprofitar carn d'espècies no utilitzades habitualment per al consum humà i també d'espines de peix al qual prèviament se li havia extret la carn. A finals dels 50 començaren a fer-se servir algunes recuperadores mecàniques d'aviram als EUA i el Japó. Però no va ser fins a mitjan la dècada dels 60 que aquestes màquines foren utilitzades a gran escala a la indústria avícola (Froning, 1981; Field, 1988; Mountney i Parkhurst, 1995).

L'augment del consum de carn d'aviram i els seus derivats i la creixent demanda en els darrers anys per part del cosumidor de productes innovadors, han afavorit un augment de la comercialització de carn desossada, cosa que genera quantitats considerables de

primeres matèries per a la recuperació mecànica (Froning, 1981; Jones, 1988; Dawson i col., 1988; Barbut i col., 1990).

Amb aquest procés s'aconsegueixen més rendiment i un ús més eficaç d'aquestes parts com a fonts de carn que, d'altra manera, no seria aprofitada per al consum humà. Separar manualment aquesta carn resultaria una tasca laboriosa, difícil, ineficient i poc rendible (Field, 1976a; Froning, 1976; Froning, 1981; Crosland i col. 1995). A més, Froning (1981) i Barbut i col. (1990) afirmen que les grans quantitats de CRMA que es produeixen anualment comporten una important suma de diners per a la indústria avícola.

Tots aquests fets i altres avantatges, sobretot des dels punts de vista nutritiu i funcional, justifiquen àmpliament la importància que està adquirint en els darrers anys la producció i l'ús de CRM.

3.2. Utilització

La CRMA és una primera matèria útil i molt valuosa que es fa servir, amb molt bons resultats, principalment com a ingredient en productes carnis i avícoles emulsionats tractats per calor (Bijker i col., 1985; Jones, 1988; Dawson i col., 1988). També és incorporada a alguns plats preparats i Knight (1992) fins i tot suggereix fer-ne el mateix tractament i ús que en surimi de peix. Per les seves característiques microbiològiques, és important utilitzar aquesta carn en aliments sotmesos a algun tractament que en disminueixi els recomptes (Anònim, 1997; Nurmi i Ring, 1999). La tecnologia de la recuperació mecànica ha incrementat l'ús de la carn d'aviram en productes elaborats (Froning, 1976).

Segons Field (1988) i Crosland i col. (1995), la CRMA és una primera matèria barata alternativa a altres ingredients carnis. La seva incorporació a diversos productes alimentosos, a més de reduir els costos de producció i els preus, ha obert un bon mercat per a la carn que resta adherida a les carcasses després de l'especejament.

3.3. Equips

Els equips emprats per obtenir CRM funcionen bàsicament comprimint la primera matèria contra les parets d'un cilindre o plaques perforades d'acer inoxidable. La carn passa al seu través i els ossos resten retinguts. Inicialment es feien servir màquines amb un cargol sense fi. Actualment, les més utilitzades són les que funcionen amb un pistó de comandament hidràulic (Field, 1988; Parry, 1989; Lawrie, 1991).

Amb el pas dels anys, la maquinària s'ha millorat i perfeccionat per assolir diversos objectius que permetin obtenir un producte de més qualitat i amb característiques més semblants a les de la carn original (Jiménez Colmenero, 1983; Jones, 1988; Parry, 1989; Lawrie, 1991; Mountney i Parkhurst, 1995):

a) No haver de triturar la primera matèria prèviament a la recuperació mecànica (necessari amb les primeres màquines) i, fins i tot, evitar el trencament dels ossos durant el procés. Així disminueix la quantitat d'os i de medul·la òssia i, en conseqüència, de calci, pigments hemo (ferro), lípids i bases púriques.
b) Evitar, tant com es pugui, el trencament cel·lular per afectar mínimament l'estructura muscular. Això dóna lloc a un producte de millor textura, menys pastós i més semblant a la carn picada. També es produeix menys sortida de components intracel·lulars, com ara els enzims i les bases púriques i pirimidíniques i els seus metabòlits.
c) Evitar el considerable augment de temperatura que es produeix durant la recuperació mecànica. Així és redueix la possibilitat de modificació d'algunes proteïnes, de proliferació microbiana i d'oxidació de lípids i pigments hemo.
d) Minimitzar la quantitat de teixit conjuntiu a la CRM.

Amb tot, quan parlem de CRM, la seva equivalència amb la carn separada manualment i les diferències de composició entre ambdues i, sobretot, el seu etiquetatge especial en ser utilitzada com a ingredient continuen sent qüestions molt debatudes.

3.4. Avantatges

A més del baix preu, la CRMA presenta altres avantatges que la fan ser un ingredient idoni per a l'ús a què principalment es destina.

3.4.1. Propietats nutritives

Field (1988) afirma que la CRMA té un notable valor nutritiu per l'alt contingut en calci i ferro, la qualitat de les proteïnes i l'aportació de vitamines.

Calci. La major part procedeix de les petites partícules d'os que inevitablement hi són presents pels processos de trituració i recuperació mecànica. Koolmees i col. (1986) troben valors mitjans de calci d'entre 0,07 i 0,25%.

Ferro. L'alt contingut és degut principalment a les considerables quantitats de medul·la òssia i, per tant, de pigments hemo. Crosland i col. (1995) parlen d'un valor mitjà de ferro de 0,002% aproximadament. El contingut en medul·la òssia és la diferència més important entre la CRM i la carn separada manualment i, per aquesta raó, és un paràmetre en què es basen alguns mètodes de detecció de CRM (Field, 1988; Crosland i col., 1995; Pickering i col., 1995b; Savage i col., 1995).

Proteïnes. En general, són d'alt valor biològic. A més de les típiques de la carn, hi ha proteïnes de la medul·la òssia, d'entre les quals l'hemoglobina és la més abundant. Tot i així, sovint la quantitat de proteïna és més baixa que la de la carn separada manualment (Froning, 1981; Lawrie, 1991). Froning (1981) indica que la quantitat de mioglobina no es veu modificada pel procés de recuperació mecànica.

Vitamines. L'aportació és similar a la de la carn obtinguda de manera manual. És a dir, les vitamines del grup B són les predominants. A pesar de ser la medul·la òssia rica en vitamines A i, sobretot, C, la quantitat d'aquestes en dietes que inclouen productes carnis o avícoles amb CRMA no sol ser considerable excepte quan el percentatge d'aquesta carn és inusualment alt.

3.4.2. Propietats funcionals

La CRMA té unes propietats funcionals excel·lents, com la capacitat de retenció d'aigua i la formació i l'estabilització d'emulsions (Field, 1988). El pH d'aquesta carn, alt (prop de la neutralitat) sobretot per la presència de medul·la òssia, incrementa l'extracció de les proteïnes musculars, cosa que millora les esmentades propietats funcionals. Aquestes propietats minimitzen les pèrdues per cocció en els productes que contenen CRMA.

3.5. Inconvenients

La susceptibilitat a l'oxidació i la contaminació microbiològica són els desavantatges més importants de la CRMA des d'un punt de vista tecnològic; comporten un seriós problema perquè alteren l'estabilitat durant l'emmagatzematge en refrigeració i, inevitablement, escurcen la vida útil d'aquesta carn (MacNeil i col., 1973; Kumar i col., 1986; Barbut i col., 1990). Per tant, cal prendre mesures preventives ja que condicionen l'ús de la CRMA en els productes carnis i avícoles. Molts estudis sobre CRMA que actualment es fan tracten d'aquests temes i les seves possibles solucions.

3.5.1. Oxidació

El trencament cel·lular, la incorporació excessiva d'aire, el contacte amb estructures metàl·liques i les temperatures altes que es produeixen durant la recuperació mecànica; l'estructura finament picada resultant; la presència de lípids (amb una proporció considerable d'àcids grassos insaturats) i pigments hemo (que tenen propietats catalítiques) de la medul·la òssia i l'alta insaturació dels fosfolípids contribueixen a l'oxidació dels lípids i els pigments hemo i, conseqüentment, al desenvolupament d'aromes desagradables -ranciesa- i al deteriorament del color -enfosquiment- (Froning, 1981; Kolozyn-Krajewska i col., 1983; Field, 1988; Barbut i col., 1990). Froning (1981) afirma que les hemoproteïnes són les principals responsables de l'oxidació dels lípids de la CRMA; la mioglobina té més activitat catalítica que l'hemoglobina.

Per tant, el procés de recuperació mecànica afecta de manera considerable l'estabilitat de la carn durant l'emmagatzematge. És important tenir en compte que les reaccions d'oxidació no es veuen totalment frenades ni tan sols congelant l'aliment i que, conseqüentment, el temps de conservació en aquest estat també és limitat (Jones, 1988).

3.5.2. Contaminació microbiològica

Diversos aspectes de la recuperació mecànica influeixen en la contaminació microbiana de la CRMA. La sortida de fluids intracel·lulars molt rics en nutrients, a causa de l'agitació i el trencament cel·lular que d'ella es deriva; la incorporació d'aire i l'augment de temperatura donen lloc a un medi idoni per al creixement bacterià (Froning, 1981; Kolozyn-Krajewska i col., 1983; Kumar i col., 1986). Froning (1976) i Kolozyn-Krajewska i col. (1983) afirmen que tot això es veu afavorit per la gran superfície que presenta la CRMA, que facilita l'adhesió dels microorganismes, i Chant i col. (1977) remarquen la importància del pH alt d'aquesta carn.

La contaminació de la CRMA depèn dels recomptes microbiològics de la primera matèria i de les condicions higièniques i de temperatura en les fases de producció, emmagatzematge i ús d'aquesta carn (Froning, 1981; Kolozyn-Krajewska i col., 1983; Field, 1988). En general, els microorganismes procedents de l'ambient i el manipulador, que són la principal via de contaminació, poden accedir fàcilment a la CRMA. Es rebutgen quantitats considerables de CRM a causa del deteriorament microbiològic per l'excessiva manipulació o l'abús de temps-temperatura (Gill, 1988).

Per evitar aquesta alta càrrega microbiana, és necessari fer servir primeres matèries amb recomptes baixos i prendre mesures higièniques estrictes durant tot el procés de recuperació mecànica: una correcta manipulació, una temperatura apropiada (prop de la de refrigeració) i un programa de neteja i desinfecció dels equips adequat (Mast i MacNeil, 1975; Froning, 1981; Field, 1988). També és important, com indiquen Ostovar i col. (1971) i Field (1988), fer el procés el més ràpidament possible després de l'especejament manual o automàtic o si no congelar les primeres matèries fins el seu ús. A causa del problema microbiològic, la major part de la CRMA produïda és immediatament congelada

per mètodes ràpids o incorporada a productes que són tractats per calor (Mast i MacNeil, 1975; Field, 1988).

Els principals bacteris causants de deteriorament de la CRMA són psicròtrofs dels gèneres *Pseudomonas* (responsable més freqüent del desenvolupament de ranciesa en aquesta carn), *Acinetobacter*, *Moraxella*, *Psychrobacter* i *Flavobacterium*. També són importants els bacteris acidolàctics i espècies de *Bacillus* i *Micrococcus*. D'entre els patògens cal mencionar *Listeria monocytogenes* i *Yersinia enterocolitica* (ambdós psicròtrofs), espècies de *Campylobacter* i serotips de *Salmonella*. En ser molt manipulada, la CRMA es pot contaminar amb bacteris d'origen humà, com ara *Staphylococcus aureus* enterotoxigen (Ostovar i col., 1971; Maxcy i col., 1973; Gill, 1988; Pascual, 1992; Johnston i Tompkin, 1992; Marth, 1998; Davies i Board, 1998; Nurmi i Ring, 1999).

3.5.3. Altres inconvenients

Greix i colesterol. El contingut és més alt que el de la carn separada manualment. Això és degut a la presència de lípids de la medul·la òssia i també de la pell -sovint són afegides quantitats considerables de pell juntament amb les primeres matèries que cal desossar, la qual cosa influeix negativament en el poder emulsionant de la CRMA- (Froning, 1981; Field, 1988; Jones, 1988; Baker i Bruce, 1989; Lawrie, 1991).

El greix és el més inconstant, des d'un punt de vista quantitatiu, de tots els components de la CRMA. Per conseqüent, la composició d'aquesta carn és molt variable, cosa que comporta un problema quan s'elaboren productes carnis i avícoles (Froning, 1981; Meech i Kirk, 1986; Crosland i col., 1995). Field (1988) i Jones (1988) expliquen que l'espècie, l'edat i el sexe de les aus, el maneig, el mètode de refredament després del sacrifici, el tipus d'os, la quantitat de pell, carn i greix de la primera matèria, la de colls i ales utilitzada per produir la CRM i el tipus i l'ajust de la màquina desossadora influeixen en l'esmentada composició.

Compostos púrics. També es troben, principalment la xantina, en quantitats més altes que en carn obtinguda de manera manual, ja que la medul·la òssia és rica en nucleòtids amb bases púriques. El trencament cel·lular contribueix a aquest augment, perquè implica

la sortida de bases púriques i pirimidíniques i els seus metabòlits a l'espai extracel·lular (Field, 1988; Scarborough i col., 1993). Young (1980) troba valors mitjans de compostos púrics d'entre 0,09 i 0,12%.

Minerals. Segons Field (1988) i Baker i Bruce (1989), el contingut en calci i ferro -que comporta més percentatge de cendres que el de la carn separada manualment (Lawrie, 1991)- no és perillós per a la salut sinó al contrari, excepte en persones que tenen restringida la ingesta d'aquests minerals. Malgrat això, el contingut en calci no hauria d'ultrapassar un 0,25% (Anònim, 1997; Nurmi i Ring, 1999).

El cadmi es troba en quantitats considerables en els ronyons d'aus adultes; Froning (1981) i Baker i Bruce (1989) expliquen que si aquests no són afegits en el procés de fabricació el risc és insignificant. S'ha trobat alta quantitat de fluor a CRM de pollastres adults femella; així mateix, Froning (1981) i Baker i Bruce (1989) afirmen que si la CRMA es limita a un màxim de 20% en els productes a què és incorporada i s'exclou de les dietes de persones pertanyents a poblacions de risc (sobretot nens i malalts), el perill també desapareix.

Partícules d'os. La presència de petits fragments d'os no comporta un risc ni per la mida ni per la duresa perquè es dissolen en els sucs gàstrics. És més correcte parlar de *partícules d'os* ja que són com un material pulverulent -tenen aproximadament la mateixa mida que els cristalls de clorur sòdic- (Froning, 1981; Field, 1988; Baker i Bruce, 1989). D'aquestes partícules, n'hi ha en gran quantitat a la CRMA, però segons el mètode de treball la carn separada manualment en conté més i de mida més gran (Froning, 1979). Segons Field (1988) i Lawrie (1991), són, en general, imperceptibles i, per tant, no provoquen textura granulosa ni arenosa del producte al qual és incorporada la CRMA.

3.6. Altres propietats

La CRMA, per l'aroma i el sabor, el color i la textura que presenta, dóna unes característiques sensorials que poden ser considerades avantatjoses o perjudicials segons el tipus d'aliment que s'elabora. Sovint el color i la textura limiten l'ús d'aquesta carn, quant a la varietat de productes a què és incorporada i la quantitat que cal utilitzar a cadascun

dels productes (Kolozyn-Krajewska i col., 1983; Jones, 1988; Dawson i col., 1988; Lawrie, 1991).

3.6.1. Aroma i sabor

A part de les aromes desagradables derivades de la possible oxidació, la CRMA no presenta problemes seriosos d'aroma o sabor. Carpenter (1975) descriu cert sabor de fetge, atribuït a la medul·la òssia, en alguns productes que contenen aquesta carn.

3.6.2. Color

A causa del contingut en pigments hemo, principalment hemoglobina, el color és intens (Froning, 1976; Jones, 1988). Després de la recuperació mecànica, per la incorporació d'oxigen que comporta, l'hemoglobina i la mioglobina es troben en estat d'oxihemoglobina i oximioglobina, cosa que dóna lloc a una carn de color vermell brillant (Froning, 1981; Field, 1988). Janky i Froning (1975) observen que durant l'emmagatzematge els pigments hemo s'oxiden i llavors el color esdevé vermell fosc. Field (1988) afirma que congelar immediatament després de la recuperació mecànica evita en gran manera aquest problema.

A més, el color de la CRMA depèn de l'edat de l'animal i el tipus d'os, ambdós condicionen la quantitat d'hemoglobina de la medul·la òssia, i del contingut en lípids (per exemple, procedents de la medul·la i de la pell), que poden "diluir" als pigments hemo i donar lloc a una carn més clara (Froning, 1981; Field, 1988).

3.6.3. Textura

La consistència pastosa la fa ser un ingredient interessant principalment per a les pastes càrnies emulsionades (Froning i col., 1971; Froning, 1976). Field i col. (1977) observen que la incorporació de CRMA a un producte dóna lloc a una textura més tova; no obstant això, quantitats excessives fan que la textura sigui generalment massa tova i esponjosa. La sucositat dels productes tampoc se sol veure afectada per la incorporació d'aquesta carn (Field, 1976b), de manera que tenen palatabilitat.

Avaluats els avantatges i els inconvenients, podem concloure, igual que Froning (1970) i Radomyski i Niewiarowicz (1987), que la combinació de CRMA amb carn separada de manera manual és probablement l'opció més encertada des dels punts de vista tecnològic, sensorial i econòmic.

3.7. Legislació i recomanacions

A la UE no existeix legislació específica que reguli les condicions necessàries per a la producció i l'ús de CRM. En algunes directives sobre carns o els seus derivats es mencionen determinats aspectes relacionats amb aquesta carn (BOE, 1993, 1994a, b, c i 1998). Jiménez Colmenero (1983) explica que, en alguns casos, la qualitat microbiològica de la CRMA pot ser indirectament controlada a través de les característiques dictades per les normes de qualitat dels productes a què és incorporada, per bé que la tecnologia d'elaboració d'aquests pot ocultar la qualitat microbiològica inicial d'aquella carn.

La Comissió del *Codex Alimentarius*, dins el programa conjunt FAO/WHO sobre Normes alimentàries, publicà un codi de pràctiques per a la producció, l'emmagatzematge i la composició de carn i carn d'aviram separades mecànicament, destinades a l'elaboració de productes (CCA, 1984).

Cal remarcar que el 1997 un grup d'experts del Comitè Científic Veterinari, el qual assessora a la Comissió de la UE en matèria de salut pública, va presentar uns criteris d'higiene i unes recomanacions bacteriològiques específics per a CRM. Així mateix, també el 1997, l'entitat europea que agrupa les associacions d'indústries que fabriquen productes carnis, CLITRAVI (*Centre de Liaison des Industries Transformatrices de Viandes*), va elaborar un document sobre carn separada mecànicament (Atanassova i Ring, 1998; Nurmi i Ring, 1999).

Als EUA, la CRMA sí que està estrictament regulada (nom, etiquetatge, limitacions, etc.) pel *Food Safety and Inspection Service* (FSIS) del USDA (Froning, 1981; Field, 1988).

3.8. Investigació recent

3.8.1. Mètodes de detecció

Crosland i col. (1995) i Savage i col. (1995) indiquen que la detecció de CRMA en productes alimentosos pot ser útil per evitar fraus, considerant que es tracta d'una primera matèria barata si la comparem amb altres ingredients carnis.

Els mètodes estudiats es basen en l'anàlisi de la composició química (Koolmees i col., 1986; Scarborough i col., 1993; Crosland i col., 1995), l'electroforesi (Savage i col., 1995), la microscòpia òptica (Bijker i col., 1985; Koolmees i col., 1986; Pickering i col., 1995a) o la immunologia (Pickering i col., 1995b), i avaluen components característics de la CRM, com ara les proteïnes de la medul·la òssia, el cartíleg, les partícules d'os o les bases púriques i pirimidíniques lliures i els seus metabòlits, sobretot la xantina. Actualment, el més pràctic i accessible és l'anàlisi de la composició química.

Meech i Kirk (1986) afirmen que algunes propietats de la CRM (per exemple, el color, el pH, la microestructura i el contingut en colesterol, teixit conjuntiu, ferro, calci i partícules d'os) poden ajudar a la seva detecció qualitativa. Aquests autors conclouen que el contingut total en pigments hemo és probablement el paràmetre més útil i fiable per detectar aquesta carn.

3.8.2. Sistemes de conservació

S'han provat diversos sistemes de conservació complementaris al fred per millorar l'estabilitat química i/o microbiològica de la CRMA i allargar, així, la seva vida útil.

Alguns d'aquests sistemes són: tractament per calor (Young i Lyon, 1973; Mast i MacNeil, 1975), envasament en atmosfera modificada (Jurdy i col., 1980), addició de cultius iniciadors (Raccach i col., 1979), agents antioxidants o altres additius (MacNeil i col., 1973) i aplicació de radiacions ionitzants. L'eficàcia d'aquestes per prolongar la vida útil i eliminar microorganismes patògens de la CRMA ha estat àmpliament estudiada (Thayer i Boyd, 1992 i 1994). De fet, la CRMA, per les seves característiques, és un dels

productes alimentosos als quals més s'apliquen radiacions ionitzants; a alguns països europeus (no a Espanya) i als EUA està permesa com a sistema de conservació d'aquesta carn.

3.8.3. Millora de propietats sensorials

També s'han fet diversos estudis per millorar el color i la textura de la CRM i augmentar la varietat de productes a què pot ser incorporada.

Per disminuir la intensitat del color, s'han avaluat, d'una banda, l'addició de proteïna de soia (Lyon i col., 1978) i, de l'altra, tècniques per eliminar pigments hemo (també redueixen el contingut en greix, cosa que contribueix a millorar l'estabilitat de la carn), com la centrifugació (Froning i Johnson, 1973; Dhillon i Maurer, 1975) i el rentat (Miyauchi i col., 1975; Dawson i col., 1988). Per estabilitzar el color, Moledina i col. (1977) afegeixen additius que disminueixen l'oxidació dels pigments hemo.

Perquè sigui més consistent, la textura es pot modificar mitjançant addició de proteïna de soia (Lyon i col., 1978; Barbut i col., 1984), extrusió i posterior assecament en forn (Acton, 1973) o fregida en paella (Maurer, 1979).

II. OBJECTIUS I PLA DE TREBALL

Amb aquest treball experimental es pretén avaluar l'eficàcia del tractament per AP isostàtica en la millora de la qualitat microbiològica, des dels punts de vista sanitari i de conservació, de la CRMA i els productes a què s'incorpora aquesta carn.

Per fer això, és necessari establir combinacions de pressió, temps i temperatura que permetin aconseguir una inactivació bacteriana suficient per donar lloc a un producte alimentós segur i de llarga vida útil en refrigeració.

També cal estudiar condicions de tractament per obtenir productes carnis i avícoles emulsionats en aplicar pressió a temperatura moderada, i avaluar diverses propietats d'aquests productes per determinar-ne la viabilitat.

Per assolir aquest objectius, es tracten per AP (mitjançant combinacions de pressió, temps i temperatura) CRMA i salsitxes cuites l'ingredient majoritari de les quals és aquesta carn.

CRMA:

- determinació de la reducció en les microbiotes mesòfila i psicròtrofa
- avaluació de la combinació amb nisina i glucono-delta-lactona quant a la letalitat de les esmentades poblacions
- comparació de l'eficàcia de tractaments continus i cíclics
- determinació de la inactivació de *Listeria innocua* 910 CECT (*Colección Española de Cultivos Tipo*) prèviament inoculada
- estudi de la conservació durant l'emmagatzematge al buit en refrigeració
- estudi de l'hipotètic efecte baroprotector del greix, sobre els bacteris mesòfils i psicròtrofs.

Salsitxes cuites elaborades a la planta pilot:

- formulació i fabricació amb diferents percentatges de CRMA i carn picada de porc i cocció sota pressió

- avaluació de textura i color i comparació amb salsitxes cuites convencionalment.

Salsitxes cuites elaborades i envasades al buit a la indústria (tractament posterior per AP a temperatura moderada o per calor):

- avaluació del dany subletal en mesòfils i psicròtrofs
- determinació de la letalitat de bacteris acidolàctics, enterobacteris, microbiota Baird-Parker i *Listeria* spp., a més de les dues poblacions anteriorment mencionades
- estudi de la vida útil durant l'emmagatzematge al buit a diferents temperatures de refrigeració.

III. TREBALLS DE RECERCA

Núm. 1:

Microbiological quality of mechanically recovered poultry meat treated with high hydrostatic pressure and nisin (1998)

J. Yuste, M. Mor-Mur, M. Capellas, B. Guamis i R. Pla

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ORIGINAL ARTICLE

Microbiological quality of mechanically recovered poultry meat treated with high hydrostatic pressure and nisin

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The combined effect of high hydrostatic pressure processing, addition of nisin and acidification on aerobic mesophilic and psychrotrophic bacterial populations of mechanically recovered poultry meat (MRPM) kept under refrigeration (2°C) was evaluated 1, 15 and 30 days after pressurization. Nisin (0, 12·5, 100 and 200 ppm) and glucono-delta-lactone (GdL; 0 and 1%) were added to MRPM. Vacuum-packaged samples were treated at 350 or 450 MPa and 2°C for 15 min using both continuous pressurization and three-cycle oscillatory pressurization for 5 min per cycle. In some samples a reduction of mesophile counts between 3·44 and 5·38 log cfu g^{-1} was found. Psychrotrophes seemed to be more sensitive; in samples with 100 ppm of nisin and GdL treated at 450 MPa with cycles they were reduced to undetectable levels (a lethality of approximately 7·5 log units). Cycle pressurization showed slightly better results than continuous pressurization. The combination of 350 MPa, 100 ppm of nisin and 1% of GdL was enough to extend the shelf life of MRPM during the 30 day chilled storage.

Introduction

High hydrostatic pressure processing is an interesting food preservation method, especially for foods whose nutritional, sensory and functional characteristics are thermosensitive (Carlez et al. 1994). One of the most important effects of high pressure is the destruction of micro-organisms (Ludwig et al. 1992, Sojka and Ludwig 1994), so it reduces the number of foodborne pathogens and also delays microbial spoilage of foods to extend their shelf life (Hoover et al. 1989, Shigehisa et al. 1991, Carlez et al. 1994). In addition to microbiological studies, research in high hydrostatic pressure effects on physicochemical, functional, sensory and nutritional properties of foods is being carried out.

Mechanically recovered poultry meat (MRPM) is a raw material with high microbial load as a consequence of the contamination introduced during processing (Gill 1988, Johnston and Tompkin 1992). The small particle size and large surface area, the release of cellular fluids rich in nutrients due to the tissue maceration and the heat generated during mechanical deboning all enhance bac-

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terial growth (Ray et al. 1984, Kumar et al. 1986). Therefore, MRPM is highly perishable and has a short shelf life even under refrigeration (Field 1988); however, it is an excellent ingredient with very good nutritional and functional properties for the formulation of many food products (Froning 1981, Field 1988), being used successfully in some chopped emulsion meat products.

Nisin is a bacteriocin produced by certain strains of *Lactococcus lactis* (Delves-Broughton 1990). It is effective against a wide range of Gram-positive bacteria (Ray 1992), with spores being more sensitive than vegetative cells owing to the mode of action of nisin (Delves-Broughton 1990). Under certain conditions, it is effective against some Gram-negative bacteria (Kalchayanand et al. 1992). Currently, there is an interest to use nisin as a biological food preservative (Daeschel 1989, Kalchayanand et al. 1992) mainly on packaged heat-processed foods, in which the number of bacterial spores may be significant because of the thermal treatment (Delves-Broughton 1990). Nisin involves no sanitary risk since it is a polypeptidic and non-toxic substance (Hurst 1981). At present, the European legislation allows the addition of nisin to just semolina and tapioca desserts and similar products, ripened and processed cheese and clotted cream (DOCE 1995). But nisin has been affirmed as 'generally recognized as safe' (GRAS) for use in cheese and processed cheese products and there are two additional GRAS affirmation petitions which request expanded uses of nisin in food (Fields 1996).

In the present study, combined treatments of high pressure and nisin at two pH values were applied to MRPM to reduce its microbial contamination. The aim of this work was, first, to study the effect of these combined treatments on the lethality of aerobic mesophilic and psychrotrophic bacterial populations of MRPM and evaluate the effectiveness of continuous and cycle pressurization and, second, to follow the microbiological quality of treated MRPM stored in refrigeration to determine the residual effect of the treatments.

Materials and Methods

Physicochemical analyses

The AOAC official methods of analysis were used to determine MRPM percentages of total solids, fat, total nitrogen and ash (McNeal 1990). A potentiometric measurement of pH was done with a penetration pH-meter combination electrode (CRISON, Alella, Spain).

Sample preparation

MRPM, provided by an industrial company, was manufactured from meat remaining on poultry carcasses and kept frozen until use. Throughout the sample preparation rigorous hygiene measures were applied to prevent any further microbial contamination. A commercial nisin preparation containing 1×10^6 IU g^{-1} (Nisaplin™; Aplin & Barrett Ltd., Trowbridge, UK) and glucono-delta-lactone (GdL; Quimidroga S. A., Barcelona, Spain) were mixed with MRPM in an ice-cooled mixer for 2 min in a sterile chamber. Four doses of nisin (0, 12·5, 100 and 200 ppm) combined with 0 or 1% of GdL were tested. Samples of approximately 30 g were vacuum-packaged, kept under refrigeration (2°C) for 24 h to allow additives time to be effective and, finally, treated with high pressure.

High-pressure treatment

The equipment used was a discontinuous isostatic press (ACB, Nantes, France). The time needed to achieve the treatment pressure was between 1 and 2 min, depending on the required pressure, and the decompression time was between 30 and 45 s. The pressure chamber and the liquid inside (ethanol–water, 1:1) were cooled to the treatment temperature with a constant flow of an ethanol-water mixture (3:1). High-pressure processing was carried out at 350 or 450 MPa and 2°C for a total of 15 min. In a first experiment, continuous pressurization for 15 min or three-cycle oscillatory pressurization for 5 min per cycle was used. In a second experiment, only cycle pressurization was used and each treatment was applied to three identical samples at the same time; these samples

were used to follow the bacterial population changes 1, 15 and 30 days after treatment. Each pressurization treatment was performed twice. All samples were stored at 2°C.

Microbiological analyses

Twenty-five grams of MRPM were homogenized in 225 ml of peptone water (Oxoid, Basingstoke, UK) for 1·5 min in an electromechanical blender and decimal dilutions were also prepared with peptone water (Pascual 1992). Plate count agar (PCA; Oxoid) was used to enumerate aerobic mesophilic and psychrotrophic bacteria; duplicate plates were incubated at 30°C for 72 h (Pascual 1992) and at 7°C for 10 days (Cousin et al. 1992), respectively. The Most Probable Number (MPN) procedure was also used to determine bacterial counts when negligible growth in PCA plates was expected. For this purpose, tryptone soya broth (Oxoid) was used; tubes were incubated at 30°C for 48 h for mesophiles and at 7°C for 10 days for psychrotrophes. To confirm microbial growth, a loopful of the medium was transferred to tryptone soya agar (Oxoid) plates, which were incubated at 30°C for 48 h for both bacterial populations (ICMSF 1983, Peeler et al. 1992). In the first experiment, samples were analysed the day after high-pressure processing and, in the second experiment, the microbial quality of treated MRPM was assessed 1, 15 and 30 days after pressurization.

Statistical analysis of data

All pressurization treatments were carried out twice. For each treatment, the sample was plated in duplicate both for mesophiles and for psychrotrophes. Data were analysed by using analysis of variance of the General Linear Models procedure of SAS® software (the SAS® System for Windows™, release 6·11, 1989, SAS Institute, Inc., Cary, North Carolina). Level of significance was set for $P < 0·05$. Differences among means from each variable (treatment pressure, type of pressurization—continuous or cycle treatments, nisin dose and addition of GdL) were determined using Duncan's multiple range test. Interaction among the four variables was tested.

Results and Discussion

Characteristics of MRPM

Table 1 shows that the composition of MRPM used in this study was typical for this kind of meat. According to Froning (1981) and Jones (1988) MRPM can present a very variable composition. Fat is the most changeable component: Jones (1988) reported that it can range between 4·5 and 29·3% and varies depending on factors such as the age and sex of the bird, the amount of skin and meat left on the starting material, the ratio of backs and necks used to produce the MRPM and the type and setting of the deboning machine. In general, MRPM has high percentages of fat (Froning 1981, Lawrie 1991). This is very important because fat and, in general, food constituents appear to protect micro-organisms against high pressure (Carlez et al. 1993, Patterson et al. 1995, Gervilla et al. 1997).

In general, the pH of MRPM is relatively high and allows most micro-organisms to grow. In this study, the addition of 1% of the acidulant agent GdL decreased pH to 5·42 (standard deviation = 0·059). At more acidic pH, some micro-organisms will be inhibited and nisin is more stable, which makes it a more effective preservative agent (Delves-Broughton 1990, Ray 1992).

Comparison between continuous and cycle pressurization

Results from the first experiment are shown

Table 1. Proximate composition and pH of mechanically recovered poultry meat

	Mean ± s.d.[a]
Total solids (%)	28·76 ± 0·260
Fat (%)	11·55 ± 0·469
Total nitrogen (%)	2·55 ± 0·061
Ash (%)	1·03 ± 0·018
pH	6·43 ± 0·076

[a] Mean from four replications ± standard deviation.

Table 2. Bacterial counts of non-pressurized and pressurized mechanically recovered poultry meat with or without additives.

Bacteria	Pressurization	Without additives	12·5 ppm nisin		100 ppm nisin		200 ppm nisin	
			no GdL	1% GdL	no GdL	1% GdL	no GdL	1% GdL
Mesophiles	Non-pressurized	$7{\cdot}33^{a,x}$	$6{\cdot}90^{b,w}$	$6{\cdot}59^{b,x}$	$6{\cdot}61^{b,x}$	$6{\cdot}04^{c,x}$	$6{\cdot}49^{b,x}$	$5{\cdot}84^{c,x}$
	350 MPa (co)	$5{\cdot}48^{a,y}$	$5{\cdot}28^{a,x}$	$4{\cdot}74^{b,y}$	$4{\cdot}19^{c,y}$	$3{\cdot}85^{c,y}$	$3{\cdot}89^{c,y}$	$2{\cdot}77^{d,y}$
	350 MPa (cy)	$5{\cdot}35^{a,yz}$	$4{\cdot}84^{b,y}$	$4{\cdot}37^{c,yz}$	$4{\cdot}10^{cd,y}$	$3{\cdot}89^{d,y}$	$3{\cdot}86^{d,y}$	$3{\cdot}00^{e,y}$
	450 MPa (co)	$5{\cdot}02^{a,z}$	$4{\cdot}58^{b,yz}$	$4{\cdot}34^{b,z}$	$3{\cdot}77^{c,yz}$	$1{\cdot}95^{e,z}$	$3{\cdot}23^{d,z}$	$2{\cdot}18^{e,z}$
	450 MPa (cy)	$5{\cdot}02^{a,z}$	$4{\cdot}37^{b,z}$	$4{\cdot}31^{b,z}$	$3{\cdot}60^{c,z}$	$1{\cdot}95^{d,z}$	$3{\cdot}31^{c,z}$	$1{\cdot}95^{d,z}$
Psychrotrophes	Non-pressurized	$7{\cdot}41^{a,w}$	$7{\cdot}12^{ab,x}$	$6{\cdot}46^{c,x}$	$6{\cdot}74^{bc,x}$	$5{\cdot}77^{d,w}$	$6{\cdot}82^{bc,x}$	$5{\cdot}85^{d,x}$
	350 MPa (co)	$3{\cdot}57^{a,x}$	$3{\cdot}13^{ab,y}$	$3{\cdot}04^{b,y}$	$1{\cdot}95^{c,y}$	$0{\cdot}95^{d,y}$	$3{\cdot}15^{ab,y}$	$0{\cdot}60^{d,z}$
	350 MPa (cy)	$3{\cdot}49^{a,xy}$	$2{\cdot}84^{b,y}$	$3{\cdot}04^{ab,y}$	$1{\cdot}60^{c,yz}$	$1{\cdot}95^{c,x}$	$2{\cdot}70^{b,yz}$	$1{\cdot}60^{c,y}$
	450 MPa (co)	$3{\cdot}04^{a,y}$	$2{\cdot}70^{ab,y}$	$2{\cdot}89^{ab,y}$	$1{\cdot}36^{c,z}$	$0{\cdot}60^{d,y}$	$2{\cdot}42^{b,z}$	$0{\cdot}60^{d,z}$
	450 MPa (cy)	$2{\cdot}30^{a,z}$	$2{\cdot}70^{a,y}$	$2{\cdot}63^{a,y}$	$1{\cdot}60^{b,yz}$	n.d.^{d,z}	$2{\cdot}30^{a,z}$	$0{\cdot}95^{c,z}$

Counts are given as means from four replications, in log cfu g^{-1}. Pressurizations: continuous (co, 15 min) or cycle (cy, 3 × 5 min) treatments at 2°C.
$^{a-e}$ Means within a row lacking a common superscript differ significantly ($P < 0{\cdot}05$).
$^{w-z}$ For each bacterial population, means within a column lacking a common superscript differ significantly ($P < 0{\cdot}05$).
GdL, glucono-delta-lactone; n.d., non-detected growth (< 3 cfu g^{-1}).

in Table 2. Initial aerobic mesophile and psychrotrophe counts of MRPM were above 10^7 cfu g^{-1}. In nisin-added samples a reduction between 0·29 and 0·84 log units was observed. The acidification enhanced the plate count reduction up to 1·64 log units. All non-pressurized samples without GdL, and those containing 12·5 ppm of nisin and GdL, presented counts significantly greater than the rest. The greater effectiveness of nisin in the more acidic environment must be the main explanation of the better results achieved, in general, in non-pressurized and, as set out below, pressurized samples with nisin plus GdL.

In general, the lethality of microbial populations increased as the conditions applied (treatment pressure, nisin dose and addition of GdL) were more severe. Samples containing 100 or 200 ppm of nisin and GdL treated at 450 MPa showed the greatest reductions of mesophile numbers, up to 5·38 log units. Pressurization treatments assayed were very effective on psychrotrophic bacteria. In acidified samples with 100 ppm of nisin treated at 450 MPa with cycles, no psychrotrophe growth was detected, indicating a reduction of almost 7·5 log units. From these results it can be stated that psychrotrophic bacteria are more sensitive to high hydrostatic pressure than mesophiles. Carlez et al. (1993) obtained similar results in inoculated minced beef muscle. One possible explanation is that most psychrotrophes when subjected to high pressure lose the ability to grow at low temperatures. This pressure sensitivity of psychrotrophic bacteria is very beneficial as they are the most common spoilage organisms in chilled poultry-derived ingredients and food products.

Hauben et al. (1996) and Roberts and Hoover (1996) achieved a great bacterial inactivation in inoculated buffered solutions after combined processes of high hydrostatic pressure, nisin and other agents or treatments. They used much lower doses of nisin but, in working with buffers, the likely baroprotective effect of some meat constituents is avoided. Taylor et al. (1985) studied the antibotulinal effectiveness of nisin–nitrite combinations in chicken frankfurter emulsions and had to use doses of nisin similar to this work to get satisfactory results.

Cycle pressurization showed results significantly better than continuous pressurization (Table 2). However, type of pressurization was not the more weighty variable and, moreover, it was influenced by the effect of interaction among variables. Thus, cycle oscillatory pressurization was chosen in the second experiment to monitor the microbiological quality of treated MRPM. Hayakawa

et al. (1994) and Sojka and Ludwig (1994) found that cycle treatments are more effective against bacterial spores suspended in saline solutions than continuous ones. Mor-Mur et al. (1998) came to the same conclusion working with *Salmonella enteritidis*-inoculated liquid whole egg. In the present study, the better results obtained with cycles were not so clear. This could be due to the microbial profile of the MRPM used and the baroprotective effect of the food matrix itself.

Refrigerated storage

Results from the second experiment are shown in Figs. 1 to 4.

All non-pressurized samples had a very short shelf life due to their high initial contamination. After 15 days of storage, counts were already between 10^8 and 10^9 cfu g^{-1}. So, addition of nisin at any dose was not enough for preserving MRPM, even in samples containing GdL. Pressurized samples without additives did not have extended refrigerated shelf life either, even those treated at 450 MPa reached approximately 10^6 cfu g^{-1} 30 days after high-pressure processing (Fig. 1).

Treatments combining high pressure and additives (Figs. 2–4) gave better results, offering greater possibilities for preservation. At any pressure, addition of 100 ppm of nisin and GdL, or 200 ppm of nisin with or without GdL, gave the significantly lowest counts. There was one exception: the most severe combined treatment decreased psychrotrophe numbers below 1 log unit [Figs. 3(b) and 4]. After 30 days, samples treated with the same combinations still presented very low bacterial numbers, from 1·44 to 2·92 log units, and, again, with the same exception for the psychrotrophe population.

These results indicate that high-pressure processing at low temperature combined with nisin, especially at more acidic pH, can im-

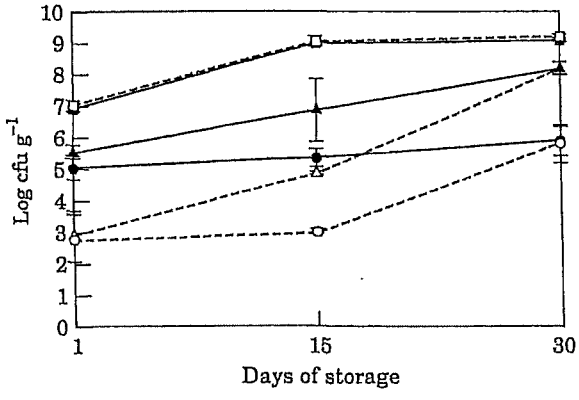

Figure 1. Plate counts of mesophilic (m) and psychrotrophic (p) bacteria during 30 days of chilled storage (2°C) of mechanically recovered poultry meat treated with high hydrostatic pressure (HHP) at 2°C for 15 min of total time (three cycles of 5 min each). (■), no HHP (m); (□), no HHP (p); (▲), 350 MPa (m); (△), 350 MPa (p); (●), 450 MPa (m); (○), 450 MPa (p).

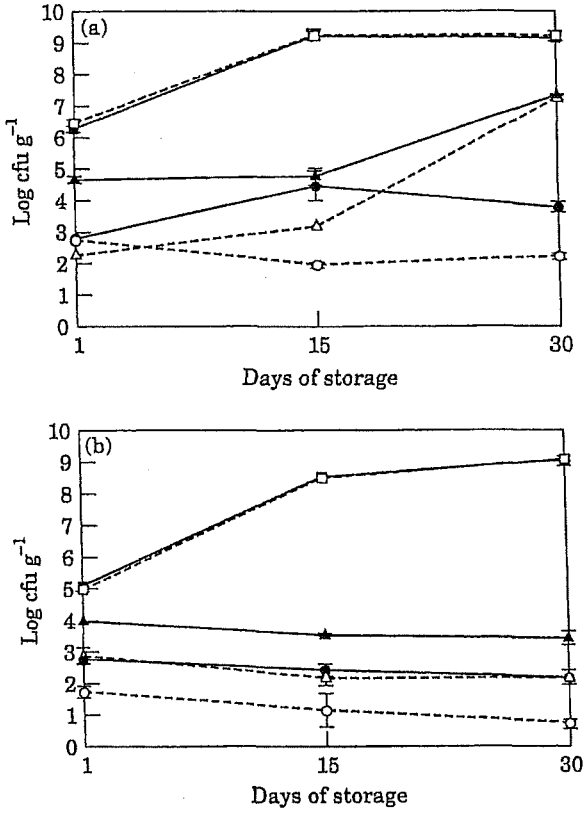

Figure 2. Plate counts of mesophilic (m) and psychrotrophic (p) bacteria during 30 days of chilled storage (2°C) of mechanically recovered poultry meat containing 12·5 ppm of nisin treated with high hydrostatic pressure (HHP) at 2°C for 15 min of total time (three cycles of 5 min each). (a) Without glucono-delta-lactone (GdL); (b) with 1% GdL. (■), no HHP (m); (□), no HHP (p); (▲), 350 MPa (m); (△), 350 MPa (p); (●), 450 MPa (m); (○), 450 MPa (p).

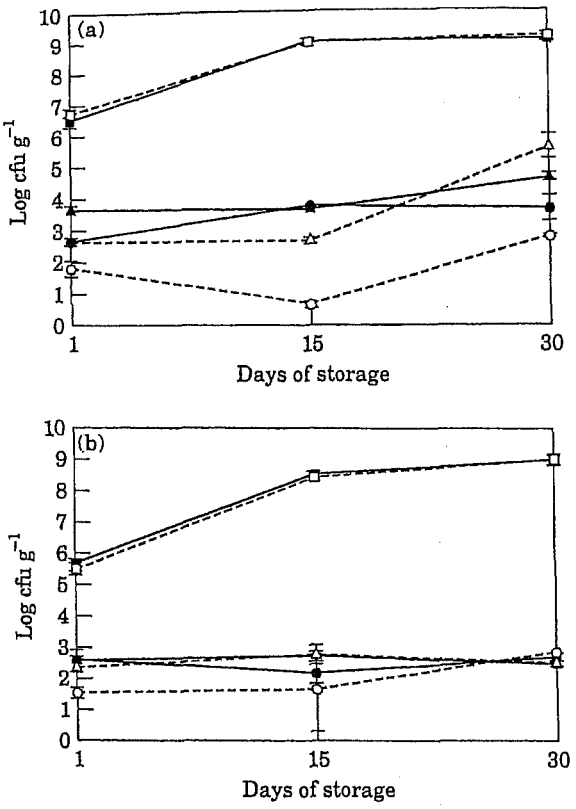

Figure 3. Plate counts of mesophilic (m) and psychrotrophic (p) bacteria during 30 days of chilled storage (2°C) of mechanically recovered poultry meat containing 100 ppm of nisin treated with high hydrostatic pressure (HHP) at 2°C for 15 min of total time (three cycles of 5 min each). (a) Without glucono-delta-lactone (GdL); (b) with 1% GdL. (■), no HHP (m); (□), no HHP (p); (▲), 350 MPa (m); (△), 350 MPa (p); (●), 450 MPa (m); (○), 450 MPa (p).

prove the microbiological quality of MRPM during 1-month storage under refrigeration. Carlez et al. (1994) and O'Brien and Marshall (1996) worked with pressurized raw ground beef muscle and chicken, respectively, without any other treatment and found that shelf life could be improved, but not to the same extent as reported here. Cutter and Siragusa (1996) stated that a combination of nisin spray treatments and vacuum-packaging may also be useful to improve the microbial stability of red meat.

Moreover, in some cases, a reduction of surviving mesophilic and psychrotrophic bacteria was observed during the 30 day chilled storage. This suggested that high-pressure processing sublethally injured some cells and afterwards they were not able to survive refrigeration. Capellas et al. (1996) and Ponce et al. (1998) studied the behaviour of several micro-organisms surviving high pressure during storage of fresh goat's milk cheese and liquid whole egg, respectively, and came to similar conclusions.

Results obtained from this study suggest a synergistic effect between high-pressure processing and nisin. Roberts and Hoover (1996) stated the same. Separately, high pressure or nisin did not enhance the shelf life of MRPM; however, the combination of nisin and pres-

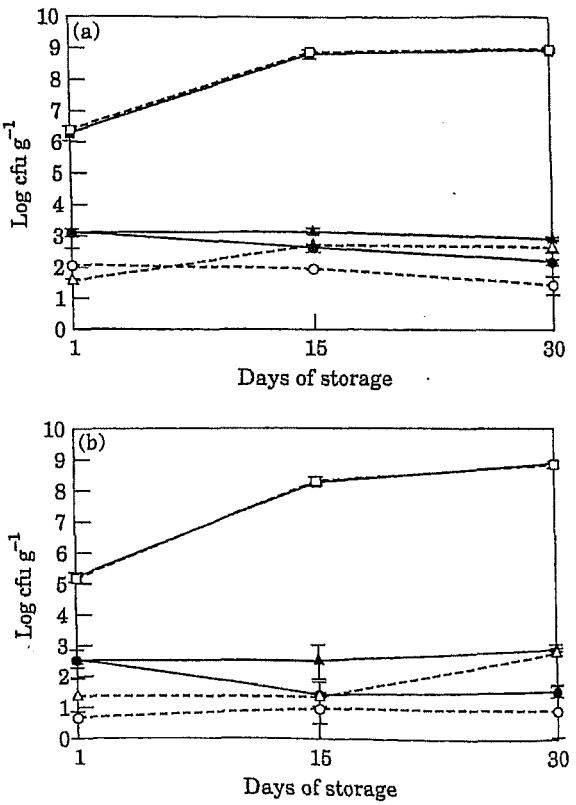

Figure 4. Plate counts of mesophilic (m) and psychrotrophic (p) bacteria during 30 days of chilled storage (2°C) of mechanically recovered poultry meat containing 200 ppm of nisin treated with high hydrostatic pressure (HHP) at 2°C for 15 min of total time (three cycles of 5 min each). (a) Without glucono-delta-lactone (GdL); (b) with 1% GdL. (■), no HHP (m); (□), no HHP (p); (▲), 350 MPa (m); (△), 350 MPa (p); (●), 450 MPa (m); (○), 450 MPa (p).

sure promoted a large reduction in MRPM plate counts, especially for psychrotrophe populations, and extended the shelf life of MRPM during all the study. This combination was expected to work well because nisin is more effective against Gram-positive bacteria and high-pressure processing against Gram-negative ones (Cheftel 1992); moreover, high-pressure damages and makes bacterial cells more sensitive to nisin (Kalchayanand et al. 1992, 1994).

In conclusion, if nisin is allowed to be used in MRPM, its combination with high-pressure processing will extend the shelf life of this raw material and improve the quality of products formulated with this kind of meat. Studies on physicochemical, functional and sensory characteristics are being undertaken to obtain more information about the effects of high hydrostatic pressure on MRPM.

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Núm. 2:

Listeria innocua and aerobic mesophiles during chill storage of inoculated mechanically recovered poultry meat treated with high hydrostatic pressure (1999)

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Listeria innocua and aerobic mesophiles during chill storage of inoculated mechanically recovered poultry meat treated with high hydrostatic pressure

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Abstract

Mechanically recovered poultry meat (MRPM) was inoculated with *Listeria innocua* 910 CECT at a level of approximately 10^8 CFU g^{-1}. Vacuum-packaged samples were treated by combinations of pressure (350, 400, 450 and 500 MPa), time (5, 10, 15 and 30 min) and temperature (2, 10 and 20°C) and later stored at 2°C for 2 months. Counts of *L. innocua* and aerobic mesophilic bacteria were determined 1, 4, 7, 15, 30 and 60 days after pressurisation. For mesophiles, in most treatments, pressurization at 2°C gave the significantly best results. High pressure caused a marked bactericidal effect on *L. innocua*: reductions higher than 7.5 log units were achieved in several cases. Some cells were just sublethally injured by pressure. Samples treated at 500 MPa for 30 min at 2°C had counts of only 2.3 log units after 60 days of chill storage. Noninoculated pressurised MRPM did not show *Listeria* growth throughout storage. These results suggest that high pressure processing can enhance the microbiological quality of MRPM.

Keywords: Poultry meat; High hydrostatic pressure; *Listeria* spp.; Chill storage

1. Introduction

Production of poultry meat and the sale of derived products has grown considerably during the last few years (EC, 1997). Consumer preference is one of the reasons for this as people demand products containing this kind of meat which, at the same time, are different from the traditional ones. Therefore, novel food products with poultry meat as an ingredient are being manufactured.

Poultry processors produced more than 180 million kg of mechanically recovered poultry meat (MRPM) in 1985 (Dawson, Sheldon & Ball, Jr., 1988). These quantities contribute considerably to the economy of the poultry industry (Froning, 1981). MRPM is a raw material with excellent nutritional and functional properties used for the formulation of many foods (Field, 1988; Froning, 1981), being successfully used in a wide variety of emulsified products. However, it is usually heavily contaminated with microorganisms, which are introduced during processing, mainly by extensive handling, and this makes MRPM highly perishable (Gill, 1988; Johnston & Tompkin, 1992). Moreover, some pathogenic bacteria may be present in MRPM if strict sanitary measures are not taken. It is a major concern in the poultry industry to improve the microbiological quality and safety of MRPM and so there is a need to evaluate the use of any new technology which may help achieve these aims.

High hydrostatic pressure can be applied as an alternative to conventional food processing and preservation methods. It does not change the wholesomeness of meat and other food products (Shigehisa, Ohmori, Saito, Taji & Hayashi, 1991). High pressure causes microbial inactivation and so can substantially reduce the risk of survival of food-poisoning bacteria (Carlez, Rosec, Richard & Cheftel, 1994; Cheftel & Culioli, 1997; Metrick, Hoover & Farkas, 1989).

Listeria monocytogenes has been implicated as an agent of foodborne disease in several outbreaks. Its ubiquitous character makes it difficult to prevent contamination of many foods, which are the primary means of transmission of this pathogen (Patterson, 1989; Rijpens,

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Jannes & Herman, 1997; Uyttendaele, Neyts, Lips & Debevere, 1997). *Listeria monocytogenes* is a psychrotrophic organism and, therefore, can grow and multiply in chilled food products before consumption. In a review article, Farber and Peterkin (1991) report *L. monocytogenes* contamination rates ranging from 12 to 66% for poultry and poultry products. Cross-contamination from raw meat to other foods and survival of the microorganism in processed poultry are possible causes of this high incidence (Pini & Gilbert, 1988). Wang and Muriana (1994) state that the presence of *Listeria* in retail frankfurters is most likely due to post-process contamination. Glass and Doyle (1989) emphasize the importance of preventing post-processing contamination of ready-to-eat meat products with *L. monocytogenes*. However, poultry and derived products are rarely associated with listeriosis (Johnston & Tompkin, 1992; Uyttendaele et al.), probably because they are usually cooked before being eaten and so the risk considerably decreases (Van Schothorst, 1994). Although *Listeria* spp. other than *L. monocytogenes* are not pathogenic for humans, their presence in foods can indicate unsanitary conditions (Rijpens et al.).

In the present study, high pressure processing was applied to MRPM inoculated with *Listeria innocua*, a nonpathogenic indicator microorganism for *L. monocytogenes* (Fairchild & Foegeding, 1993). The objectives of this work were to study the lethal effect of pressurisation on *L. innocua* and aerobic mesophilic bacteria of MRPM and, also, to follow the microbiological quality of treated MRPM stored under refrigeration (2°C).

2. Materials and methods

2.1. Inoculum preparation

Listeria innocua 910 CECT (*Colección Española de Cultivos Tipo*) was the test microorganism used. For revival, a freeze-dried culture was inoculated into 10 ml of brain heart infusion (BHI; Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. Revived cells were streaked onto BHI agar (BHIA; Oxoid) tubes and incubated at 37°C for 24 h. Stock cultures of *L. innocua* strain were maintained on these tubes at 2°C and transferred monthly. Five millilitres of BHI were inoculated with *L. innocua* colonies from a BHIA tube and incubated at 37°C for 24 h with shaking. One millilitre of this cultured BHI was transferred to 50 ml of BHI and incubated at 37°C for 24 h with shaking and, later, at 37°C overnight without shaking, to obtain approximately 10^9 cell ml^{-1}. Before the MRPM was inoculated, the BHI culture was left for 4–6 h under refrigeration. The aim was to achieve a culture where most cells were in the late log to early stationary phases of growth.

2.2. Sample preparation and inoculation

Mechanically recovered poultry meat, provided by an industrial company, was manufactured from meat remaining on carcasses and residues from poultry processing and kept frozen until use. It was thawed at 2°C. Portions (190 g) were placed in sterile Stomacher bags, inoculated with 10 ml of cultured BHI to obtain about 10^8 cells g^{-1} and, finally, mixed for 2 min in a Stomacher Lab-Blender 400 (Seward Medical, London, UK). Inoculated samples of approximately 30 g were vacuum-packaged and kept at 2°C until pressurisation. Throughout sample preparation and inoculation, rigorous hygiene measures were applied to prevent further microbial contamination.

2.3. High pressure treatment and chill storage

High pressure processing was performed the day after inoculation as described by Yuste, Mor-Mur, Capellas, Guamis and Pla (1998). When necessary, refrigerated samples were allowed to reach the temperature of pressurisation in the pressure chamber before treatment. Pressurisation was carried out combining different values of pressure (350, 400, 450 and 500 MPa), time (5, 10, 15 and 30 min) and temperature (2, 10 and 20°C). Treatments at 450 and 500 MPa for 10 and 30 min at 2°C were applied to six identical samples at the same time; these samples were used to follow the bacterial population changes during storage at 2°C for 1, 4, 7, 15, 30 and 60 days after processing. Each pressurisation treatment was performed three times.

2.4. Physicochemical and microbiological analyses

The AOAC official methods of analysis were used to determine MRPM percentages of total solids, fat, total nitrogen and ash (McNeal, 1990). Twenty-five grams of inoculated MRPM were homogenised in 225 ml of peptone water (Oxoid) for 1.5 min in an electromechanical blender and decimal dilutions were also prepared with peptone water (Pascual, 1992). PALCAM agar base with added PALCAM Listeria selective supplement (PALCAM; Biokar Diagnostics, Beauvais, France) and plate count agar (Oxoid) were used to enumerate *L. innocua* (Farber & Peterkin, 1991) and aerobic mesophilic bacteria, respectively; duplicate plates were incubated at 37°C for 48 h and 30°C for 72 h (Pascual, 1992), respectively. The *most probable number* (MPN) procedure was also used to determine *L. innocua* counts when negligible growth in PALCAM plates was expected. For this purpose, Listeria enrichment broth base (UVM formulation) with added Listeria primary selective enrichment supplement (Oxoid) was used; tubes were incubated at 37°C for 48 h. To confirm *L. innocua* growth, a loopful of this medium was transferred to PALCAM plates (Peeler, Houghtby & Rainosek, 1992),

which were incubated at 37°C for 48 h. From MPN tubes of samples with no growth, 1 ml of the medium was transferred to BHI tubes and incubated at 37°C to prove complete inactivation. When growth was observed, a loopful of BHI was streaked onto PALCAM and tryptone soya agar (Oxoid).

2.5. Statistical analysis of data

Data were analysed by using analysis of variance of the General Linear Models procedure of SAS® software (the SAS® System for Windows™, release 6.11, SAS Institute, Cary, NC). Level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Characteristics of MRPM

In general, MRPM has very variable composition (Crosland, Patterson, Higman, Stewart & Hargin, 1995; Jones, 1988). It usually has a high percentage of fat, which is the most variable constituent (Jones; Lawrie, 1991). Table 1 shows the composition of the MRPM used in this study. Fat content was relatively low. This favoured the optimum mixing of bacterial inoculum into MRPM samples.

3.2. Counts the day after pressurisation

Initial counts in noninoculated MRPM were, on the average, about 1.6 and 7 log CFU g^{-1} for *Listeria* and mesophiles, respectively. This is in agreement with Sheridan, Duffy, McDowell and Blair, (1994), who stated that raw meats generally contain less than 2 log units of *Listeria*. However, Franco et al. (1995) frequently found *Listeria* counts of 3 log units or higher in chicken muscle. After inoculation, approximately 10^8 CFU g^{-1} were reached in all samples.

Listeria innocua and mesophile numbers after pressurisation are shown in Tables 2 and 3. In noninoculated MRPM, which was pressurised at 500 MPa for 30 min at 2°C, *Listeria* was not detected and mesophiles decreased by 1.7 log CFU g^{-1}.

For mesophiles, in most treatments, pressurisation at 2°C gave significantly better results than at 10 and 20°C

Table 1
Proximate composition of mechanically recovered poultry meat

	Mean ($n=4$) ± s.d.
Total solids (%)	28.76 ± 0.260
Fat (%)	11.55 ± 0.469
Total nitrogen (%)	2.55 ± 0.061
Ash (%)	1.03 ± 0.076

Table 2
Listeria innocua counts (log CFU g^{-1}) of inoculated mechanically recovered poultry meat treated with high pressure at different temperatures[a]

		2°C	10°C	20°C
Raw material		1.38	2.30	1.60
Treated[b] raw material		n.d.[e]		
Inoculated, untreated		8.08r[d]	7.65w	7.96v
MPa	min			
350	5	5.95s	7.36w	7.65vw
	10	5.15st	6.65w	6.50vw
	15	3.70b,tuv[e]	5.77a,w	6.00a,wx
	30	2.30vwxy	2.18xy	2.70yz
400	5	4.70ab,stu	6.49a,w	4.50b,xy
	10	3.00uvw	3.73x	2.73yz
	15	0.60yz	2.30xy	2.30z
	30	0.30b,z[f]	3.46a,xy	1.60ab,z
450	5	2.30vwxy	3.04xy	3.04yz
	10	2.08a,vwxy	0.53b,z[f]	2.30a,z
	15	2.18vwxy	1.85xyz	3.19yz
	30	1.99vwxy	1.60yz	1.60z
500	5	3.04uvw	2.30xy	2.30z
	10	1.45wxyz	2.70xy	1.85z
	15	2.70vwx	1.95xy	3.32yz
	30	0.98xyz	2.70xy	1.95z

[a] Counts are given as means ($n=6$).
[b] 500 MPa/30 min.
[c] n.d.: nondetected growth.
[d] r–z: Means within a column lacking a common letter differ significantly ($p < 0.05$). Least Significant Difference = 1.890.
[e] a,b: Means within a row lacking a common letter differ significantly ($p < 0.05$). Least Significant Difference = 1.890.
[f] Nondetected growth in one replicate.

Table 3
Aerobic mesophile counts (log CFU g^{-1}) of *Listeria innocua*-inoculated mechanically recovered poultry meat treated with high pressure at different temperatures[a]

		2°C	10°C	20°C
Raw material		6.73	7.04	7.60
Treated[b] raw material		5.06		
Inoculated, untreated		8.49a,s[c,d]	7.85b,t	8.27ab,v
MPa	min			
350	5	6.39b,t	7.66a,tu	7.80a,w
	10	5.15b,uvwx	7.01a,vw	7.00a,x
	15	5.19b,uvwx	6.80a,w	6.84a,xy
	30	5.26uvw	5.24yz	5.53z
400	5	5.59c,u	7.27a,uv	6.40b,y
	10	4.88c,wxy	6.29a,x	5.72b,z
	15	5.04vwxy	5.33yz	5.40z
	30	4.98b,vwxy	5.40ab,yz	5.47a,z
450	5	4.30b,z	5.62a,y	5.62a,z
	10	5.39uv	5.25yz	5.63z
	15	5.15b,uvwx	5.28ab,yz	5.67a,z
	30	5.12vwxy	5.16yz	5.50z
500	5	5.34uv	5.41yz	5.56z
	10	5.18uvwx	5.11z	5.50z
	15	4.68b,yz	5.17a,yz	5.41a,z
	30	4.75b,xyz	5.06ab,z	5.42a,z

[a] Counts are given as means ($n=6$).
[b] 500 MPa/30 min.
[c] a–c: Means within a row lacking a common letter differ significantly ($p < 0.05$). Least Significant Difference = 0.456.
[d] s–z: Means within a column lacking a common letter differ significantly ($p < 0.05$). Least Significant Difference = 0.456.

in inoculated samples. Previous studies (unpublished data) had already indicated the greater effectiveness of 2°C treatment on this population. Carlez et al. (1993), Gervilla, Capellas, Ferragut and Guamis (1997), and Ponce, Pla, Mor-Mur, Gervilla and Guamis (1998a) also found that pressurisation at low temperatures was more effective than at room temperature with other inoculated food products.

In general, the bacterial kill increased as pressure and time increased. In most cases, however, no significant differences were found between treatments for 10 and 15 min and, in *L. innocua*, between pressurisation at 450 and 500 MPa. Time seemed to be a more important variable than pressure for microbial inactivation under some conditions. Thus, on some occasions, treatment at 350 MPa for 30 min caused significantly greater microbial reduction than treatment at 400 MPa for 5 or 10 min.

High pressure had a considerable bactericidal effect on *L. innocua*. In samples pressurised at 400 MPa for 30 min at 2°C and at 450 MPa for 10 min at 10°C, one replicate showed absence of growth, indicating an inactivation as high as 8 log units (Table 2). Carlez et al. (1993) and Patterson, Quinn, Simpson and Gilmour (1995), working with inoculated minced meat, observed a reduction of *Listeria* counts of about 6 log units at 350 MPa for 20 min at 4°C and at 375 MPa for 15 min at 20°C, respectively.

For mesophilic bacteria, some treatments at 400, 450 and 500 MPa at 2°C gave the greatest inactivation, up to 4.19 log units (Table 3).

In several treatments, great variability among replicates was observed in *L. innocua* counts, as shown by the standard deviations. The same occurred with several samples during refrigerated storage (see text section). In contrast, mesophile counts were more reproducible. This is related to pressure-induced injury and subsequent recovery and growth, which is different and variable for stressed cells in selective media (Ray, 1989).

3.3. Refrigerated storage

Results from this part of the study are shown in Figs. 1–3. In noninoculated MRPM, *Listeria* increased by approximately 2 log CFU g^{-1} during storage of nonpressurised samples; numbers were always less than 3.5 log units (Fig. 1). Mesophiles increased by 2.5 log units; at 7 days, counts were already about 10^9 CFU g^{-1}. In pressure-treated samples, no growth of *Listeria* occurred throughout chill storage. Mesophiles increased by 2 log units; at 30 days, these counts were still less than 6 log units.

With the inoculated MRPM, the best results for *L. innocua* were observed in treatments at 2°C. One month after pressurisation, counts remained below 3 log CFU g^{-1} [Fig. 2(a)]. Furthermore, the sample pressurised at 500 MPa for 30 min only had 2.3 log units at 60 days of

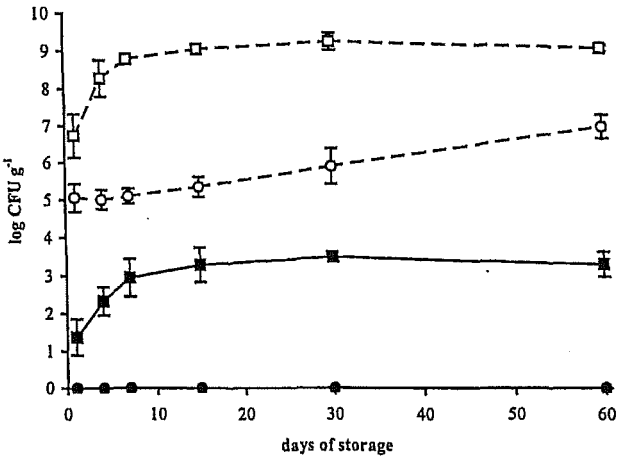

Fig. 1. Counts ($n=6$) during 60 days of chill storage (2°C) of noninoculated mechanically recovered poultry meat. (■), *Listeria*, nonpressurised; (□), aerobic mesophiles, nonpressurised; (●), *Listeria*, pressurised (500 MPa/30 min/2°C); (○), aerobic mesophiles, pressurised (500 MPa/30 min/2°C).

chill storage, which is similar to those from some other samples analysed at 30 days. In samples treated at 10 and 20°C, *L. innocua* numbers began to considerably increase earlier and were, in most cases, between 3 and 4 log units 30 days after pressurisation [Fig. 2(b) and (c)]. At 7 days, in one replicate pressurised at 500 MPa for 30 min at 10°C and one at 450 MPa for 10 min at 20°C, *L. innocua* was not detected.

From the treated samples in which *Listeria* was not found, a few presented growth on BHI tubes. However, these were cocci and sporeforming rods. *Listeria* spp. were not identified in any of these cases; so, complete inactivation was probably achieved in most occasions.

Mesophilic bacteria showed similar behaviour during refrigerated storage, regardless of the treatment temperature. Numbers remained quite stable up to 15 days of storage and then increased slowly (Fig. 3).

Some irregular decreases and increases in counts of surviving microorganisms, mainly in *L. innocua*, were observed during the 60 days of storage. This is probably related to sublethal injury caused by pressure. On the one hand, some stressed bacteria that at first managed to grow, afterwards were unable to survive refrigeration; Capellas, Mor-Mur, Sendra, Pla and Guamis (1996), Ponce, Pla, Sendra, Guamis and Mor-Mur (1998b) and Yuste et al. (1998) observed the same effects when studying the behaviour of several microorganisms following high pressure treatment of various food products. On the other hand, some bacteria that initially were not able to grow, recovered viability at some point, depending on the intensity of damage caused to the cell. This is probably because injured *Listeria* cannot grow in selective media (Palumbo, 1989). Styles, Hoover and Farkas (1991) also state that a selective medium may inhibit the growth of pressure-injured *L. monocytogenes*. Carlez et al. (1994) report that, although *Pseudomonas*

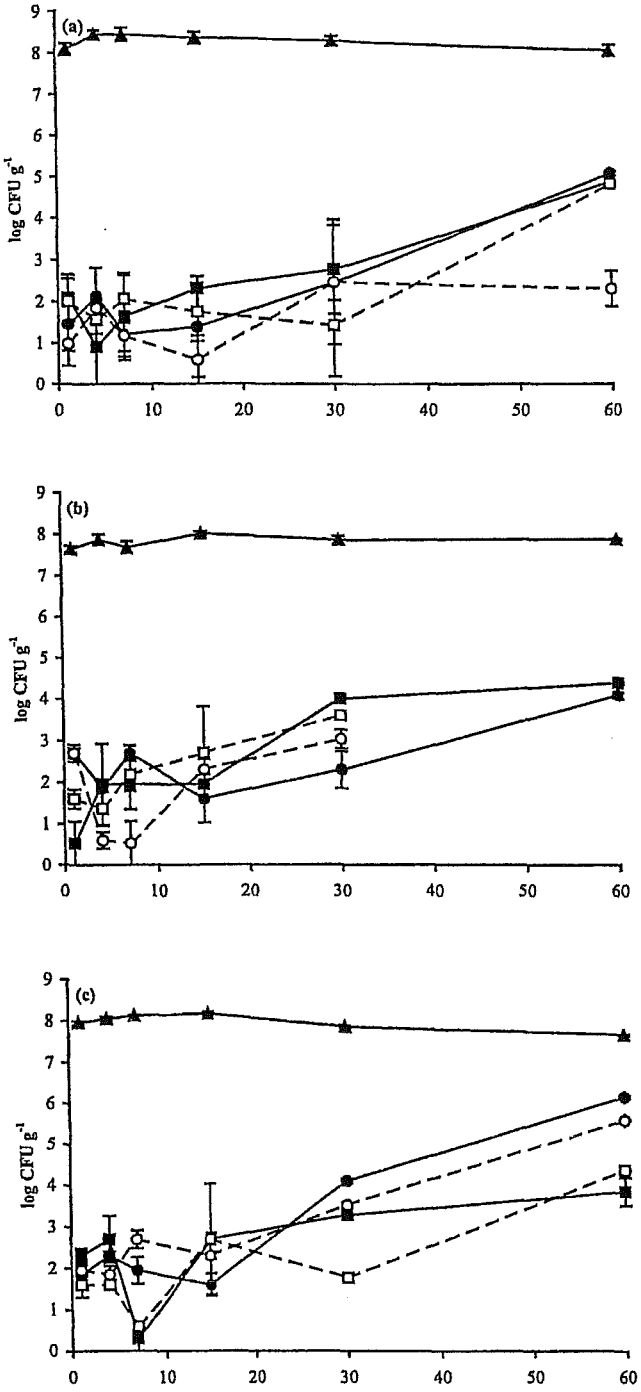

Fig. 2. *Listeria innocua* counts ($n=6$) during 60 days of chill storage (2°C) of inoculated mechanically recovered poultry meat pressurised at (a) 2°C, (b) 10°C and (c) 20°C. (▲), untreated; (■), 450 MPa/10 min; (□), 450 MPa/30 min; (●), 500 MPa/10 min; (○), 500 MPa/30 min.

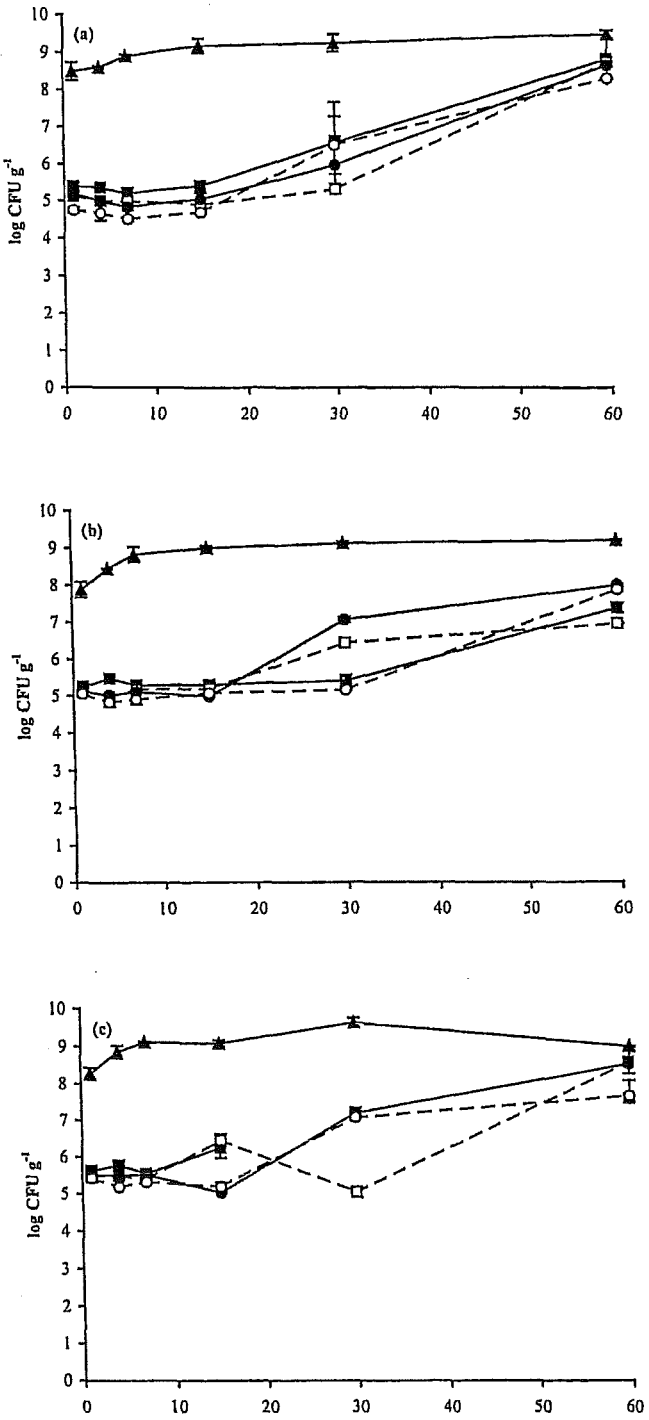

Fig. 3. Aerobic mesophile counts ($n=6$) during 60 days of chilled storage (2°C) of *Listeria innocua*-inoculated mechanically recovered poultry meat pressurised at (a) 2°C, (b) 10°C and (c) 20°C. (▲), non-treated; (■), 450 MPa/10 min; (□), 450 MPa/30 min; (●), 500 MPa/10 min; (○), 500 MPa/30 min.

spp. could not be detected immediately after minced beef was pressurised (450 MPa for 20 min at 2°C), the organisms were detectable after 9 days storage at 3°C. Metrick et al. (1989) report that recovery of *Salmonella* spp. at 37°C, assessed by their ability to grow on a selective medium, is possible in chicken-based baby food but not in phosphate buffer. This is important because it suggests that absence of growth in buffers or media does not necessarily mean no growth in foods. Patterson et al. (1995) and Cheftel and Culioli (1997) emphasize the important implications of microbial injury during storage of pressure-processed foods.

3.4. Summary and conclusions

Pressurisation is a useful technique to inactivate *Listeria* in MRPM. Moreover, in some cases, *Listeria* remained low for a long time under refrigeration (30 and, even, 60 days). High pressure processing, therefore, enhances and extends the safety of MRPM from a microbiological point of view. Styles et al. (1991), Patterson et al. (1995) and Ponce, et al. (1998a) came to the same conclusions working with other raw materials.

On inoculating, a product with an unrealistic high level of *L. innocua* is obtained. As industrial MRPM has much lower counts of *Listeria*, the results are very encouraging no *Listeria* growth is observed in non-inoculated samples during 2 months of storage. Inoculation, however, is necessary so that the effect of pressure on specific microbial populations can be clearly determined.

Yuste et al. (1998) state that most psychrotrophes lose the ability to grow at low temperatures when subjected to high pressure. It is a very outstanding fact since *L. monocytogenes* is one of the most important psychrotrophic foodborne pathogens, and psychrotrophes are the most common spoilage organisms in chilled poultry-derived products.

Further studies on sublethal injury and recovery mechanisms of microorganisms together with their subsequent ability to develop and perform their usual metabolic activity in foods are necessary to further understand the effects of high hydrostatic pressure on MRPM and, in general, on food products.

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Núm. 3:

Bacterial sensitivity to high hydrostatic pressure in mechanically recovered poultry meat. Minor baroprotective role of fat content

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Bacterial sensitivity to high hydrostatic pressure in mechanically recovered poultry meat. Minor baroprotective role of fat content

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CODE WORDS: mechanically recovered poultry meat · high pressure processing · fat content · mesophiles · psychrotrophes

Abstract

The effect of high pressure processing on mesophilic and psychrotrophic bacterial populations of mechanically recovered poultry meat (MRPM) was evaluated. Vacuum-packaged samples from three industrial batches of MRPM containing different fat percentages (11.3%, 22.9% and 36.6%) were treated by combining different values of pressure (300, 350, 400, 450 and 500 MPa), time (5, 10, 15 and 30 min) and temperature (2, 10 and 20°C). Treatment temperature did not greatly determine lethality of mesophiles. In general, the more severe the pressure and time conditions the higher the decreases in counts. Some samples pressurized at 2 or 20°C presented the maximum mesophile lethalities, about 4 log CFU/g. In medium-fat MRPM pressurized at 450 MPa for 15 min at 20°C, a 6 log unit reduction of psychrotrophes was found. High hydrostatic pressure can delay microbiological spoilage of refrigerated MRPM. Fat content does not seem to directly influence microbial inactivation caused by pressurization. Food matrix and structure play a major baroprotective role.

Introduction

Mechanically recovered poultry meat (MRPM) often has considerable microbiological counts, mainly due to the contamination introduced during manufacturing (GILL, 1988). So, it is highly perishable even under refrigeration and, for this reason, a number of methods to delay its microbial spoilage are being tested. In view of the growing consumer demand for poultry products and the excellent nutritional and functional properties of MRPM, it is worth extending the shelf-life of this meat and using it as an ingredient for food products.

High hydrostatic pressure is being investigated as a food processing and preservation

method. It substantially inactivates microorganisms and, therefore, can improve the quality of foods. Pressurization has been studied in model systems but results have to be confirmed in real food systems. Thus, most of the microbiological studies have been done with buffered suspensions (SHIMADA et al., 1993; MACKEY et al., 1994; ROBERTS and HOOVER, 1996), which do not allow interaction between microorganisms and food constituents to be assessed.

It is believed that microbial resistance to pressure is greater in foods than in buffered suspensions. Some authors state that food constituents, especially fat, probably protect microorganisms against pressurization (CARLEZ et al., 1993; RAFFALLI et al., 1994). This baroprotective effect could be important in MRPM, whose composition is highly variable and usually presents considerable percentage of fat (FRONING, 1981).

In the present work, high pressure processing was applied to reduce the microbial contamination of MRPM. The objectives were to study the lethal effect of pressurization at several temperatures on mesophile and psychrotrophe populations of MRPM and to evaluate the influence of fat, from a quantitative point of view, on bacterial inactivation caused by pressure treatment.

Materials and methods

Sample and physicochemical analysis

Mechanically recovered poultry meat, provided by an industrial company, was manufactured from meat remaining on carcasses and left-overs originated in poultry processing and kept frozen until use. Three batches, with low-, medium- and high-fat content (Tab. 1), were used. The AOAC official methods of analysis were used to determine the composition of these batches (McNEAL, 1990). Samples of approximately 30 g were vacuum-packaged and kept under refrigeration (2°C) until treatment. Throughout the sample preparation, rigorous hygiene measures were applied to prevent any further microbial contamination.

High pressure treatment

Samples were pressurized the day after preparation. The equipment used was a discontinuous isostatic press (ALSTOM, Nantes, France). The time needed to achieve the

treatment pressure was between 1 and 2 min, depending on the required pressure, and the decompression time was approximately 30 s. The pressure chamber and the liquid inside were cooled or heated to the appropriate temperature by circulating ethanol-water mixture (3:1) or water, respectively. When necessary, refrigerated samples were allowed to reach the treatment temperature in this chamber before pressurization. First, samples from the low-fat batch were treated by combining different values of pressure (300, 350, 400, 450 and 500 MPa), time (5, 10, 15 and 30 min) and temperature (2, 10 and 20°C). Second, samples from the other two batches were pressurized at 350 or 450 MPa for 5 or 15 min at 20°C. The complete experience was performed twice. All samples were stored at 2°C until analysis.

Microbiological analysis

It was carried out the day after pressurization. Twenty-five grams of MRPM were homogenized in 225 ml of peptone water for 1.5 min in an electromechanical blender and decimal dilutions were also prepared with peptone water (PASCUAL, 1992). Plate count agar (PCA) was used to enumerate aerobic mesophilic and psychrotrophic bacteria; duplicate plates were incubated at 30°C for 72 h (PASCUAL, 1992) and at 7°C for 10 days (COUSIN et al., 1992), respectively. The *most probable number* procedure was also used to determine bacterial counts when negligible growth in PCA plates was expected. For this purpose, tryptone soya broth was used; tubes were incubated at 30°C for 48 h for mesophiles and at 7°C for 10 days for psychrotrophes. To confirm microbial growth, a loopful of the medium was transferred to tryptone soya agar plates (PEELER et al., 1992), which were incubated at 30°C for 48 h for both bacterial populations. Media were purchased from Oxoid (Basingstoke, UK). Lethality is expressed as the difference between logarithms of initial and final counts ($\log_i$ CFU/g – $\log_f$ CFU/g).

Statistical analysis

Numbers of mesophiles and psychrotrophes (n = 4) were subjected to analysis of variance of the General Linear Models procedure of SAS® software (the SAS® System for Windows™, release 6.12, SAS Institute, Inc., Cary, NC) to determine if there were significant differences ($p < 0.05$) among pressure-time combinations or treatment temperatures or fat contents.

Results

Pressurization at several temperatures of low-fat batch

The effect of treatment temperature on mesophile population of 11.3%-fat MRPM is shown in table 2. Temperature did not greatly determine lethality. In some treatments at 400, 450 and 500 MPa, pressurization at 2°C generated the significantly highest decreases in counts, whereas pressurization at 10°C caused the lowest ones.

In general, the higher the pressure and time of treatment the greater the lethality of mesophiles. But neither with pressure values nor with time values, did lethality follow a linear pattern. Highly variable results between replicates were observed in various treatments, regardless of the conditions assayed. Pressures of 400, 450 and 500 MPa combined with times of 15 and 30 min usually induced the highest reductions.

The maximum lethalities, about 4 log CFU/g, were found in samples treated at 500 MPa for 30 min at 2°C and at 450 MPa for 15 or 30 min at 20°C.

Comparison among batches with different fat content

Treatments at 350 and 450 MPa for 5 and 15 min at 20°C were carried out in this part of the study. Results are shown in table 3. The lowest, 0.67 log CFU/g, and the highest, 3.89 log units, lethalities of mesophilic bacteria were found, respectively, for the least and the most severe pressurization conditions assayed in low-fat samples. Comparing among treatments, these samples presented a large range of variation, whereas in medium- and high-fat MRPM lethality values offered less variability. At 350 MPa for 5 and 15 min and at 450 MPa for 5 min, medium-fat MRPM showed significantly greater lethalities than those from the other two batches.

With regard to psychrotrophes, the largest range of variation was observed in medium-fat samples. These samples also presented the significantly highest reductions; in those treated at 450 MPa for 15 min, a 6 log unit lethality was reached.

Both for mesophilic and for psychrotrophic bacteria, in several treatments there were no significant differences between batches containing the maximum and the minimum fat percentages, despite being one more than three times the other.

Discussion

In a previous study (YUSTE et al., 1999), the influence of treatment temperature is more clearly observed than in the present work: pressurization at 2°C of MRPM caused higher inactivation rate of mesophiles and inoculated *Listeria innocua* than treatments at 10 and 20°C. CARLEZ et al. (1993) also find pressurization at low temperature more effective than that at room temperature, working with other food products.

The high variability between replicates confirms that with real food systems a lot of variables that have an impact on microbial baroresistance are implicated. More homogeneous results would be obtained with certainty if working with buffered suspensions.

Similar microbiological reductions are reported by other authors on pressurizing at room temperature. Thus, O'BRIEN and MARSHALL (1996) and CAPELLAS et al. (1996) process, respectively, ground chicken and fresh goat's milk cheese and observe mesophile lethalities between 2.7 and 3.6 log CFU/g. CARLEZ et al. (1994) achieve a higher lethality, about 6 log units, in minced beef meat also treated at 20°C.

Concerning the role of fat, some authors compare different foods with a particular fat content each one. But few studies dealing with a certain product containing different fat percentages have been found. In this respect, the most comparable work to the present one is from CARBALLO et al. (1997), who manufacture low- (9.2%) and high-fat (20.3%) beef patties and pressurize them at 5°C. At 300 MPa for 5 and 20 min, they find a significant inactivation rate of mesophiles, psychrotrophes and enterobacteria in all patties, regardless of fat content. In contrast, *Staphylococcus aureus* counts are significantly greater in high-fat samples treated for 5 min.

METRICK et al. (1989), PATTERSON et al. (1995) and SIMPSON and GILMOUR (1997) study the inactivation of various pathogens both in phosphate-buffered saline and in foods to determine the effect of substrate on pressure sensitivity. They report variable results. Milk is found very protective whereas, in some cases, minced poultry proves less protective than buffers.

As PATTERSON et al. (1995), YUSTE et al. (1998) and GERVILLA et al. (1999) have already stated, food matrix and structure play a major role in protecting microorganisms against pressure. Microbial attachment to food constituents obviously influences a lot on this point. All these factors could be the explanation to the high variability observed in microbiological counts among the same or different pressurized

foods.

Thus, regarding fat, amount is a much less weighty factor than fatty acid profile (saturated, mono- or polyunsaturated), location (triglycerides, membrane phospholipids) and composition of raw material (type and percentage of nonlipidic constituents, with which fat can interact). As each food constituent has its own compression rate, under pressure, energy is differently absorbed and the lethal effect of treatment varies according to the composition. Thus, depending on the kind of lipids present in foods, high pressure may affect bacteria in a more or less marked way. Because of the changeable composition of MRPM, usually high in fat, results obtained are often irregular and heterogeneous.

In conclusion, high hydrostatic pressure reduces bacterial counts and, therefore, delays spoilage of MRPM stored under refrigeration. In contrast to what literature states, a relation between fat percentage and bactericidal effectiveness of high pressure can not be established. Fat content does not seem to directly influence microbial inactivation caused by pressurization. It is more a question of food nature itself than of amount of particular food constituents.

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Tab. 1: Composition of mechanically recovered poultry meat batches.

Batch	**Low-fat**	**Medium-fat**	**High-fat**
% Total solids	28.09 ± 0.650	38.34 ± 0.569	49.40 ± 1.070
% Fat	11.25 ± 0.826	22.87 ± 0.684	36.64 ± 1.176
% Total nitrogen	2.58 ± 0.118	1.76 ± 0.778	1.93 ± 0.074
% Ash	1.06 ± 0.036	0.93 ± 0.026	0.71 ± 0.055

Percentages are given as mean ($n = 4$) ± standard deviation.

Tab. 2: Lethality (log_i CFU/g – log_f CFU/g) of mesophilic bacteria from 11.3%-fat mechanically recovered poultry meat pressurized at several temperatures.

		2°C	10°C	20°C
Initial counts		8.02	8.17	8.20
MPa	min			
300	5	0.32^{z}	0.32^{yz}	0.34^{yz}
	10	0.56^{yz}	0.49^{xyz}	0.55^{xyz}
	15	0.82^{xyz}	0.65^{wxyz}	0.15^{z}
	30	1.53^{vwx}	1.08^{wx}	1.04^{wxy}
350	5	0.87^{xyz}	0.22^{z}	0.67^{xyz}
	10	1.14^{wxy}	0.97^{wxy}	1.12^{wx}
	15	1.71^{vw}	1.21^{vw}	1.41^{w}
	30	3.09^{u}	2.62^{t}	2.45^{v}
400	5	$1.19^{ab,wxy}$	$0.62^{b,wxyz}$	$1.63^{a,w}$
	10	2.02^{v}	1.84^{uv}	2.41^{v}
	15	$3.33^{a,stu}$	$2.55^{b,t}$	$3.11^{ab,v}$
	30	$3.75^{a,stu}$	$2.75^{b,t}$	$3.03^{b,v}$
450	5	2.09^{v}	2.24^{tu}	2.70^{v}
	10	3.20^{tu}	2.63^{t}	2.66^{v}
	15	$3.53^{a,stu}$	$2.61^{b,t}$	$3.89^{a,u}$
	30	$3.62^{a,stu}$	$2.72^{b,t}$	$3.90^{a,u}$
500	5	3.15^{tu}	2.47^{tu}	2.73^{v}
	10	3.42^{stu}	2.77^{t}	2.79^{v}
	15	$3.80^{a,st}$	$2.71^{b,t}$	$2.87^{b,v}$
	30	$3.91^{a,s}$	$2.83^{b,t}$	$2.86^{b,v}$

Initial counts and lethalities (log CFU/g) are given as means ($n = 4$).

$^{a-b}$ Means within a row lacking a common superscript differ significantly ($p < 0.05$).

$^{s-z}$ Means within a column lacking a common superscript differ significantly ($p < 0.05$).

Tab. 3: Lethality (log_i CFU/g – log_f CFU/g) of bacteria from mechanically recovered poultry meat batches with different fat content pressurized at 20°C.

			11.3% fat	**22.9% fat**	**36.6% fat**
Mesophiles	Initial counts		7.20	7.51	6.10
	MPa	min			
	350	5	$0.67^{b,z}$	$1.98^{a,y}$	$1.20^{b,z}$
		15	$1.41^{b,y}$	$2.12^{a,xy}$	$1.47^{b,yz}$
	450	5	$2.70^{a,x}$	$2.67^{a,x}$	$1.83^{b,xy}$
		15	$3.89^{a,w}$	$2.59^{b,x}$	$2.32^{b,x}$
Psychrotrophes	Initial counts		6.48	7.60	6.00
	MPa	min			
	350	5	$1.74^{b,z}$	$3.03^{a,z}$	$1.98^{b,z}$
		15	$3.39^{b,y}$	$3.77^{a,y}$	$3.42^{b,x}$
	450	5	$3.44^{b,y}$	$4.23^{a,x}$	$2.96^{c,y}$
		15	$3.78^{b,x}$	$5.97^{a,w}$	$3.70^{b,x}$

Initial counts and lethalities (log CFU/g) are given as means ($n = 4$).
$^{a-c}$ Means within a row lacking a common superscript differ significantly ($p < 0.05$).
$^{w-z}$ For each bacterial population, means within a row lacking a common superscript differ significantly ($p < 0.05$).

Núm. 4:

Mechanically recovered poultry meat sausages manufactured with high hydrostatic pressure (1999)

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Poultry Science, **78**: 914-921

Mechanically Recovered Poultry Meat Sausages Manufactured with High Hydrostatic Pressure

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ABSTRACT The effect of high pressure processing at high temperature on texture and color of frankfurter-type sausages made with different contents of mechanically recovered poultry meat (MRPM) was evaluated and compared with that of a standard cooking process. Five types of sausages containing 100, 75, 50, 25, and 0% MRPM and 0, 25, 50, 75, and 100% of minced pork meat (MPM), respectively, were manufactured. They were pressurized at 500 MPa for 30 min at 50, 60, 70, and 75 C or cooked at 75 C for 30 min. Pressure-treated sausages were less springy and firm, but more cohesive. Moreover, color of pressurized sausages was lighter and more yellow than that of conventionally cooked sausages. Addition of MPM increased cohesiveness, hardness, and force at 80% compression. Minced pork meat also caused the appearance of sausages to be lighter, less red, and less yellow. Cooked sausages made with MRPM can have an attractive appearance and texture via high pressure processing.

(*Key words*: mechanically recovered poultry meat, poultry meat emulsion, high pressure processing, texture, color)

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INTRODUCTION

Consumption of poultry meat and poultry meat products has grown considerably in the last few years (EC, 1997). For several reasons, people prefer this kind of meat to beef or pork. Thus, new meat products containing different contents of poultry meat are being manufactured.

Mechanically recovered poultry meat (MRPM) is a poultry-derived raw material of high quality. Great amounts of this meat are produced yearly (Froning, 1981; Dawson *et al.*, 1988). It is worth using MRPM as an ingredient for some food products, giving MRPM added value. Mechanically recovered poultry meat has very good nutritional and functional properties and is suitable for the formulation of many meat products (Froning, 1981; Field, 1988). It also has some disadvantages, such as color, flavor, and texture (Froning, 1976; Jones, 1988) and the microbial load, which makes it a highly perishable raw material (Gill, 1988). Use of mechanical recovering systems has increased the utilization of poultry meat in further-processed products.

Sensory properties, such as color, flavor, and texture, are very important for consumer acceptance and choice of food products and, consequently, for the manufacturer. For this reason, many studies to optimize and improve these characteristics in various foods are being carried out.

High hydrostatic pressure is an increasingly investigated technology that can be applied as a food processing and preservation method (Hayashi, 1992; Mertens and Knorr, 1992). It does not markedly affect flavor and nutrient content of foods (Cheftel, 1992; Hayashi, 1992; Schöberl *et al.*, 1997), but it can change some other characteristics such as structure of proteins and, therefore, functional properties (Okamoto *et al.*, 1990; Hayakawa *et al.*, 1992; Ikeuchi *et al.*, 1992b; Yamamoto *et al.*, 1992). Thus, gelation and texturization of minced meat or fish, surimi, mechanically recovered meat, and other muscle proteins can be obtained through pressurization (Cheftel, 1992).

Gels made with high pressure from chicken or rabbit pastes, a sheep myosin suspension, or a rabbit actomyosin suspension have been studied and compared to the same gels prepared with heat treatment, and have shown different results depending on the raw material and the treatment conditions (Suzuki and Macfarlane, 1984; Okamoto *et al.*, 1990; Ikeuchi *et al.*, 1992a; Yoshioka *et al.*, 1992).

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Abbreviation Key: MPM = minced pork meat; MRPM = mechanically recovered poultry meat; TPA = texture profile analysis.

In the present study, high pressure processing at high temperature was applied to frankfurter-type sausages containing different percentages of MRPM and minced pork meat (MPM). The objectives of this work were: 1) to evaluate texture and color of sausages generated by pressurization and to compare them with traditionally cooked sausages and 2) to determine whether high pressure is a useful process to overcome the disadvantages of using MRPM in formulated meat products.

MATERIALS AND METHODS

Sausage Preparation

Mechanically recovered poultry meat,[2] manufactured from meat remaining in carcasses and leftovers originated in poultry processing, and commercial left-over MPM[3] were kept frozen until use. Five different batters were prepared, so that the final products contained approximately 15% protein and 14 to 17% fat, combining 100, 75, 50, 25, and 0% MRPM with 0, 25, 50, 75, and 100% MPM, respectively. Other ingredients and additives were incorporated in the formulated batters (Table 2). Batters were left standing overnight at 2 C. The AOAC official methods of analysis were applied to determine total solid, fat, total nitrogen, and ash contents (McNeal, 1990). Sausage batters were stuffed into cellulose casing[4] (22 mm diameter) and kept under refrigeration until processing.

High Pressure and Cooking Treatments

For high pressure processing, the equipment used was a discontinuous isostatic press.[5] The time needed to achieve the treatment pressure was about 120 s and the decompression time was approximately 30 s. The pressure chamber and the water inside were heated to the treatment temperature with a constant flow of hot water. Refrigerated sausages were allowed to reach the temperature in this chamber before treatment. Pressurization was carried out at 500 MPa for 30 min at four different temperatures (50, 60, 70, and 75 C). A standard cooking process (75 C for 30 min) was applied to the nonpressurized sausages in a water bath. Each treatment was performed twice. After processing, sausages were cooled in running tap water for 30 s and stored at 2 C until analyses were performed.

Texture Analyses

A Texture Analyser[6] with a 25-kg (± 1 g) load cell was used to carry out three different tests: texture profile analysis (TPA), force at 80% compression, and force at cutting. For each treatment, four cylindrical replicates (22 mm diameter and 20 mm height) were analyzed when sausages reached room temperature. Crosshead speed was 1 mm/s. Texture profile analysis was performed as described by Bourne (1978) and was carried out with an aluminum compression platen (10 cm diameter). Two 40% compression deformations were done with an interval of 5 s between them. Force at 80% compression was measured using the same probe as in the case of TPA. Force at cutting was measured by a probe consisting of a standard wire (0.3 mm diameter).

Color Analysis

A portable HunterLab spectrocolorimeter[7] was used to evaluate three color parameters: L (lightness), a (redness), and b (yellowness) values. The spectrocolorimeter was standardized before starting, using, in this order, a black glass and a white porcelain calibrated tile (No. M03793; X 79.8 – Y 84.5 – Z 90.5, D65, 10°). For each treatment, three cylindrical replicates (22 mm diameter and approximately 40 mm length) at room temperature were cut longitudinally to analyze the internal color of sausages. Reference (untreated) samples were also tested. Measurements were done with reference to illuminant *Fcw* (cool white fluorescent) and the 10° standard observer and the light reached the internal sides of sausages through a nonreflecting glass container (63 mm diameter, 2 mm thickness) in which samples had been slightly compressed and flattened.

Statistical Analysis

Treatments (four pressurization treatments and one cooking treatment) were carried out twice per each batter. For each treatment, four, in each texture analysis, or three, in color analysis, replicates were performed. Data were analyzed using ANOVA with the General Linear Models procedure of SAS® software.[8] Level of significance was set for $P < 0.05$. Differences among means from each variable [formulation, temperature of treatment, and type of treatment (pressurization or no pressurization)] were determined using Duncan's multiple range test. Interactions among the three variables were tested (SAS Institute, 1990).

RESULTS AND DISCUSSION

Composition of Raw Materials

Proximate composition of MRPM and MPM is shown in Table 1. Due to the great variability of MRPM composition (Froning, 1981; Jones, 1988), this raw material is not always suitable for formulation of any kind of meat

[2]Agropecuària de Guissona, S. Coop. Ltda., 25210 Guissona, Spain.
[3]Arcadíe España, S. A., 08227 Terrassa, Spain.
[4]Nojax®, Viskase, S. A., 93176 Bagnolet, France.
[5]Division Equipements Industriels, GEC ALSTHOM ACB S. A., 44945 Nantes, France.
[6]Model TA-XT2, Stable Micro Systems, Haslemere, Surrey GU27 3AY, U.K.
[7]Model 45/0 LAV, MiniScan XE™, Hunter Associates Laboratory, Inc., Reston, VA 22090.
[8]The SAS® System for Windows™. Release 6.11. 1989–1995. SAS Institute Inc., Cary, NC 27513.

TABLE 1. Proximate composition of MRPM[1] and MPM[2]

Content	MRPM $\bar{x}$[3]	MRPM SD	MPM $\bar{x}$	MPM SD
	(%)		(%)	
Total solids	29.53	0.786	38.25	0.489
Fat	11.82	0.769	20.21	1.006
Total nitrogen	2.61	0.146	2.85	0.054
Ash	1.08	0.026	0.92	0.011

[1]MRPM = mechanically recovered poultry meat.
[2]MPM = minced pork meat.
[3]n = 4.

emulsion. Fat content is important for the batter stability. Initially, a 32% fat MRPM batch was proposed, but it was rejected because the emulsion was not stable and, consequently, substantial amounts of fat were separated despite adding an emulsifier. Thus, another MRPM batch with a lower percentage of fat was used as raw material (Table 1).

Minced pork meat was manufactured from leftovers; therefore, it had a high fat content, almost 8.5% more than MRPM. This difference made necessary, in some cases, the addition of different percentages of lard to adjust fat to approximately 14% (Table 2). Significant differences were caused by the three variables (formulation, temperature of treatment, and type of treatment) and also by the interactions among them.

Texture Analyses

Results are shown in Figures 1 and 2. In general, nonpressurized sausages presented significantly greater springiness than pressurized ones. Okamoto *et al.* (1990) worked with rabbit meat paste and also observed that high pressure reduced springiness compared to a cooking process. In contrast, Carballo *et al.* (1996) and Pérez Mateos *et al.* (1997) stated that this parameter is higher in pressurized pork meat batters and blue whiting muscle protein gels, respectively. Therefore, animal species is a very important factor that, in most cases, determines texture. However, in the present study, similar values of springiness were obtained regardless of the formulation (Figure 1A).

Usually, high pressure processing gave significantly more cohesive sausages than cooking process and, in general, in pressurized samples, the higher the treatment temperature the lesser the cohesiveness. Similarly, Pérez Mateos *et al.* (1997) found higher cohesiveness in pressure- (375 MPa at 38 C for 20 min) than in heat-induced gels. The addition of MPM increased cohesiveness. In this case, the influence of this kind of meat was quite evident: sausages containing 100% MPM generally showed the highest cohesiveness (Figure 1B).

Analysis of adhesiveness presented rather irregular results: neither the type of treatment nor the formulation exerted a clear influence. Samples not pressurized or containing 100% MPM were, in some cases, less adhesive (Figure 1C). Okamoto *et al.* (1990) reported that heat-induced gels from several food proteins are lacking adhesiveness.

Formulation (in particular, absence of MRPM) most influenced hardness; sausages without MRPM were significantly the firmest [(firm is a preferred term to hard) (Jowitt, 1974)], especially those treated at 75 C under pressure or not (2,155.76 and 2,949.93 g × cm/s^2, respectively). Type of treatment was a very important factor. Thus, nonpressurized sausages showed, in general,

TABLE 2. Formulation and composition of batters containing MRPM[1] and MPM[2]

Formulation and composition	100 MRPM: 0 MPM		75 MRPM: 25 MPM		50 MRPM: 50 MPM		25 MRPM: 75 MPM		0 MRPM: 100 MPM	
	(%)									
Formulation										
MRPM	92.02		69.02		46.00		23.01		. . .	
MPM	. . .		21.07		42.10		63.20		84.27	
Lard	3.33		1.79		0.27		. . .		. . .	
Water	. . .		3.47		6.98		9.14		11.08	
Sodium chloride	2.00		2.00		2.00		2.00		2.00	
Emulsifier[3]	1.40		1.40		1.40		1.40		1.40	
Acidificant[4]	1.00		1.00		1.00		1.00		1.00	
Phosphates[5]	0.25		0.25		0.25		0.25		0.25	
	$\bar{x}$[6]	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Composition										
Total solids	34.23	0.079	32.40	0.310	32.54	0.202	32.59	0.120	33.66	0.299
Fat	14.62	0.194	14.70	2.662	12.91	0.062	14.40	0.339	15.26	1.367
Total nitrogen	2.36	0.028	2.36	0.052	2.50	0.080	2.41	0.005	2.50	0.040
Ash	3.73	0.023	3.45	0.006	3.46	0.003	3.35	0.009	3.37	0.056

[1]MRPM = mechanically recovered poultry meat.
[2]MPM = minced pork meat.
[3]Citric acid esters of mono- and diglycerides (E-472c). SKW Bio-Systems, S. A., 08191 Rubí, Spain.
[4]Glucono-delta-lactone (E-575). Quimidroga S. A., 08006 Barcelona, Spain.
[5]Triphosphates (E-451) and polyphosphates (E-452). SKW Bio-Systems, S. A., 08191 Rubí, Spain.
[6]n = 4.

high values of hardness (Figure 1D). Okamoto *et al.* (1990), Yoshioka *et al.* (1992), employing chicken meat paste, and Pérez Mateos *et al.* (1997) came to similar conclusions; all of them pressurized at lower temperatures than in the present study. These results are probably due to the larger cooking losses observed in the firmest sausages, particularly in the case of nonpressurized ones (J. Yuste, unpublished data). Other authors (Macfarlane *et al.*, 1984; Nose *et al.*, 1992; Mandava *et al.*, 1994) also found a relationship between high pressure processing and better cooking yields. The lower cooking losses of pressurized samples result in a considerable improvement in sausage manufacturing yield, which is an important economic issue for manufacturers of cooked meat products. Due to cooking losses, nonpressurized sausages, besides being firmer, were slightly brittle and less juicy; these characteristics led to a poorer texture.

In TPA, gumminess is defined as the product of hardness × cohesiveness and chewiness as the product of hardness × cohesiveness × springiness. Therefore, gumminess and chewiness results followed, obviously, a similar pattern to the hardness ones.

The amount of MRPM played an essential role in the sausage resistance to be 80% compressed. In general, the lower the content of MRPM the higher the force required for the compression (Figure 2A).

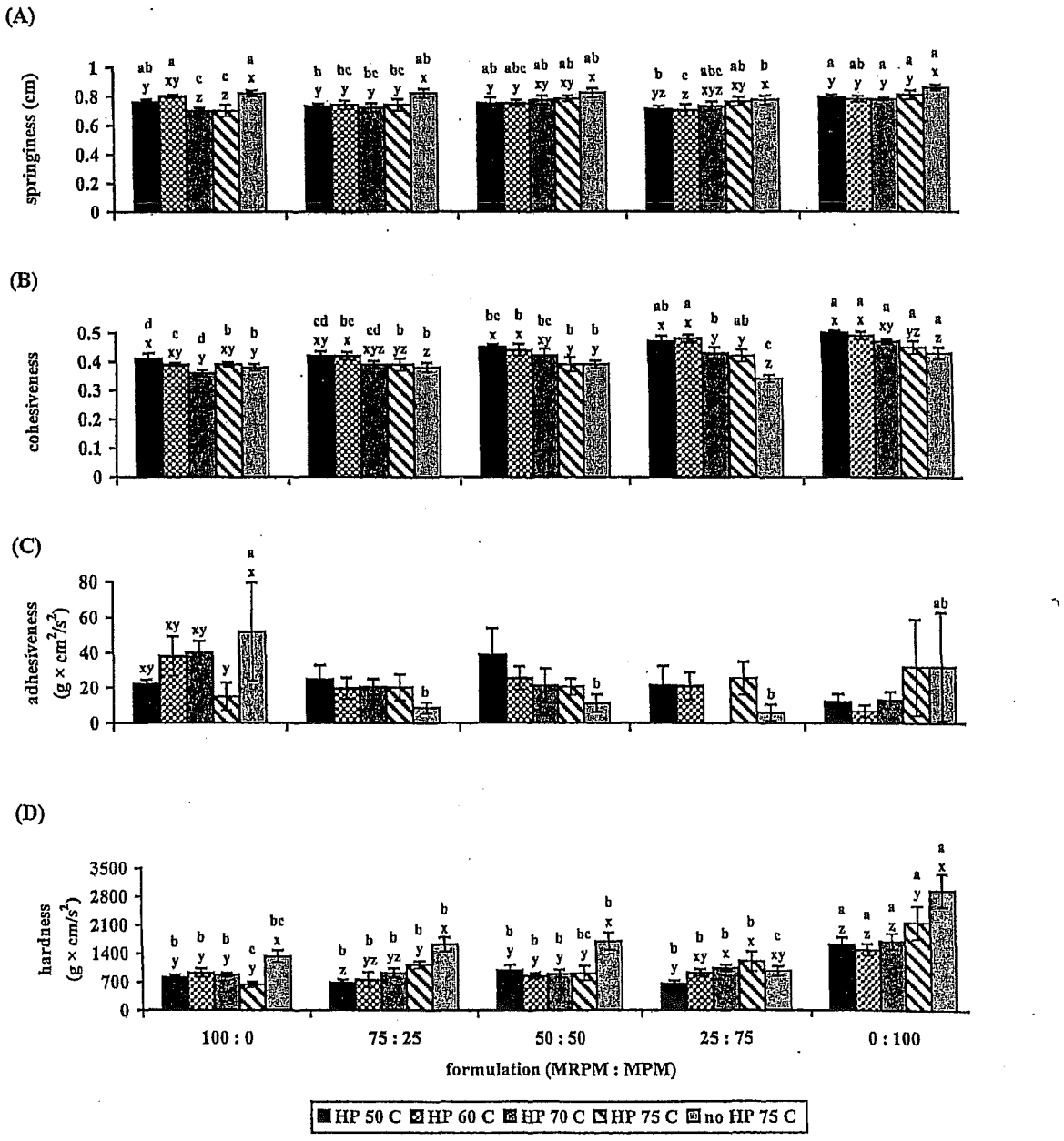

FIGURE 1. Texture profile analysis parameters of sausages containing mechanically recovered poultry meat (MRPM) and minced pork meat (MPM) pressurized (HP) at 500 MPa for 30 min or conventionally cooked (no HP) for 30 min. Results are means from eight replicates. A) springiness; B) cohesiveness; C) adhesiveness; D) hardness. a–d: Means within the same type of bar with no common letter differ significantly ($P < 0.05$). x–z: Means within the same group of bars with no common letter differ significantly ($P < 0.05$).

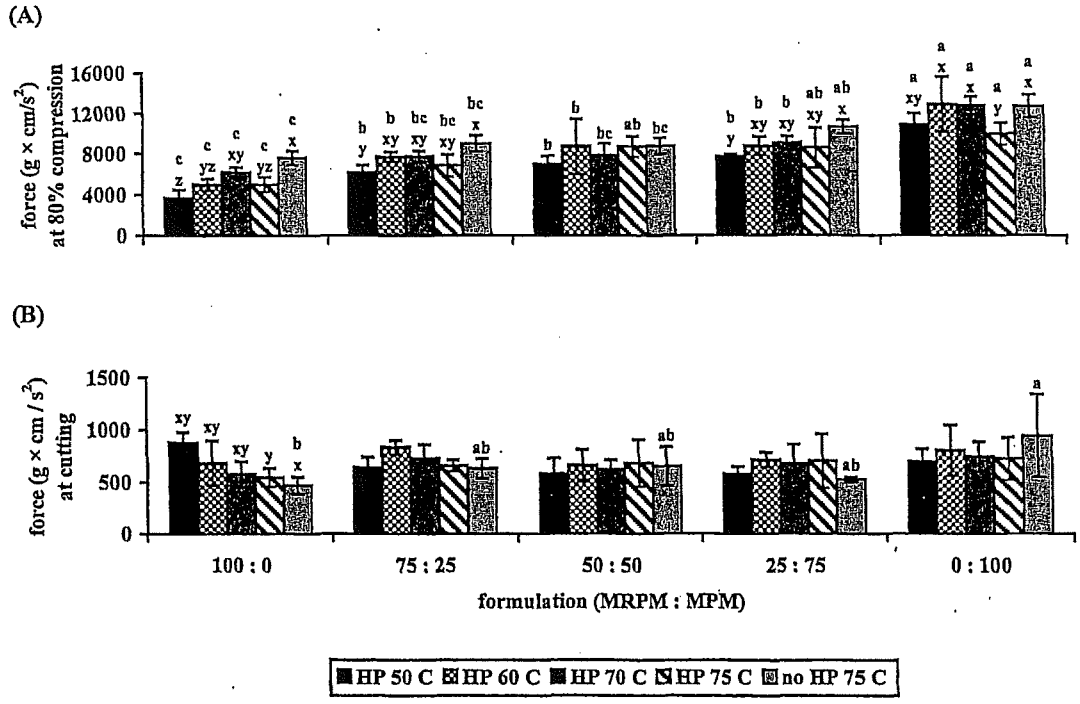

FIGURE 2. Textural parameters of sausages containing mechanically recovered poultry meat (MRPM) and minced pork meat (MPM) pressurized (HP) at 500 MPa for 30 min or conventionally cooked (no HP) for 30 min. Results are means from eight replicates. A) force at 80% compression; B) force at cutting. a–c: Means within the same type of bar with no common letter differ significantly ($P < 0.05$). x–z: Means within the same group of bars with no common letter differ significantly ($P < 0.05$).

Force at cutting showed few significant differences, only between nonpressurized sausages containing the maximum percentages of either kind of meat. Samples with 100% MRPM treated at 70 or 75 C required a lower force. This result is more evident when compared with those made with 100% of MPM, which were firmer and more difficult to cut. Force at cutting depends not only on the firmness but also on proper gelling. The different behavior observed in 100% MRPM sausages treated at lower temperatures (50 or 60 C) was due to the lack of the gelled texture of cooked sausages and the soft texture because of the relatively low temperature of treatment and the 100% content of a pasty raw material such as MRPM. In this case, samples were flattened rather than clearly cut, which resulted in a higher resistance (Figure 2B). Although one of the effects of high hydrostatic pressure is gelation of proteins (Cheftel, 1992), pressurization temperatures of 50 or 60 C were not enough to achieve a suitable texture in sausages, regardless of the formulation. Thus, as Schöberl *et al.* (1997) stated, high pressure processing of minced meat as an alternative process of production of "unheated frankfurter-type sausages" does not seem adequate.

Beilken *et al.* (1990) measured Warner-Bratzler shear force on postrigor beef muscles and reported that cooked ones exhibited higher values than pressurized ones (150 MPa) at high temperatures (up to 80 C). Nose *et al.* (1992), working with beef meat, and Pérez Mateos *et al.* (1997) observed, respectively, lower breaking strength and higher breaking deformation in samples treated with high pressure. Carballo *et al.* (1996) found generally higher penetration force and work of penetration values in cooked batters.

Factors that improve binding among meat particles also markedly influenced hardness, force at 80% compression, and force at cutting. Thus, protein denaturation or aggregation or both induced by high pressure processing (Macfarlane *et al.*, 1984; Okamoto *et al.*, 1990; Hayakawa *et al.*, 1992; Ikeuchi *et al.*, 1992b; Yamamoto *et al.*, 1992) and addition of salt, which helps to solubilize myofibrillar proteins, are other important factors to take into account.

Color Analysis

Results are shown in Table 3. The addition of MPM significantly increased lightness of sausages. Although both processes (high pressure or cooking) gave significantly higher L values than those of reference samples, in general, pressurization caused lighter sausages than cooking.

In contrast, pressurized and nonpressurized sausages showed significantly lower a values than reference samples. In general, the higher the MRPM content the greater the a value; this effect is obvious, taking into account the characteristic dark red color of MRPM (Froning, 1976; Jones, 1988) when the fat content is not excessively high. Reference samples containing 100% MRPM showed a much higher a value than the rest; otherwise, the treated samples with 100 and 75% MPM showed the lowest a values. On the other hand, the lowest

b values were observed in nonpressurized sausages. Moreover, those with 100% MPM also presented quite low *b* values in most cases.

Similar changes in color parameters of pressurized minced beef meat, beef patties, and pork meat batters were observed by Carlez *et al.* (1993, 1995), Carballo *et al.* (1997) and Jiménez Colmenero *et al.* (1997), respectively. However, the former reported that the *b* value remained constant. These changes were due to denaturation of the globin moiety of myoglobin molecules and to the partial oxidation of ferrous myoglobin into ferric metmyoglobin caused by pressurization (Carlez *et al.*, 1995). In general, MRPM presents substantial amounts of hemoglobin (Froning, 1981; Field, 1988; Jones, 1988). Thus, it is likely that pressure-induced modifications on this molecule could also generate color changes.

Several authors consider changes in color caused by high pressure processing to be a problem, depending on the product that is treated (Murakami *et al.*, 1992; Carlez *et al.*, 1993; Cheftel and Culioli, 1997). In contrast, in the case of MRPM, pressure-induced discoloration and paleness can be considered beneficial.

Summary and Conclusions

Compared to a standard cooking process, high pressure processing at high temperature yielded less springy and firm but more cohesive sausages, which were also lighter and more yellow.

The addition of MPM increased cohesiveness, hardness, and force at 80% compression. It also caused lighter, less red, and less yellow sausages. In this study, formulation influenced textural parameters more than type of treatment; this effect was very clear, particularly in the case of absence of MRPM.

Significant differences were caused by the three variables (formulation, temperature of treatment, and type of treatment) and also by the interactions among them. Thus, pressurization could be a good choice to achieve desirable characteristics in the case of meat products containing MRPM, because two of the main drawbacks of this meat as an ingredient are its appearance (too dark) and texture (too pasty and soft). Texturization of MRPM would possibly increase the range of products prepared from this raw material (Froning, 1976; Jones, 1988). Moreover, a certain amount of MPM can help to solve the disadvantages and to improve the properties of these products, but this raw material should not be added excessively because it could lead to very firm products. Dhillon and Maurer (1975), Froning (1976), Newman (1981), and Radomyski and Niewiarowicz (1987) stated that combinations of MRPM and hand deboned poultry meat gave desirable sensory and functional properties and economic advantages.

From the results obtained, it can be stated, as reported by Cheftel and Culioli (1997), that pressure treatment with previous, simultaneous, or subsequent cooking is the most

TABLE 3. Color parameters of sausages containing MRPM[1] and MPM[2] pressurized (HP) at 500 MPa for 30 min or conventionally cooked (no HP) for 30 min

	100 MRPM: 0 MPM		75 MRPM: 25 MPM		50 MRPM: 50 MPM		25 MRPM: 75 MPM		0 MRPM: 100 MPM	
Parameter	$\bar{x}$[3]	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
L value										
Reference (untreated)	$43.99^{c,y}$	0.612	$46.78^{b,z}$	0.637	$46.88^{b,z}$	0.916	$52.95^{a,y}$	0.389	$53.07^{a,y}$	0.834
HP 50 C	$48.99^{d,x}$	0.666	$53.34^{c,wx}$	0.544	$54.69^{c,x}$	0.585	$56.46^{b,x}$	0.958	$59.49^{a,vw}$	0.236
HP 60 C	$49.36^{c,x}$	0.502	$53.90^{b,w}$	0.826	$54.22^{b,x}$	0.445	$57.36^{a,x}$	0.690	$58.84^{a,w}$	0.508
HP 70 C	$49.77^{d,x}$	0.225	$53.10^{c,wx}$	0.943	$54.11^{c,x}$	0.428	$56.95^{b,x}$	0.589	$60.59^{a,v}$	1.480
HP 75 C	$49.39^{c,x}$	0.939	$51.87^{b,xy}$	0.834	$54.72^{a,x}$	0.638	$56.06^{a,x}$	0.642	$49.73^{c,z}$	1.959
no HP 75 C	$49.28^{d,x}$	0.577	$50.29^{c,y}$	0.223	$51.87^{c,y}$	0.643	$54.27^{b,y}$	0.427	$56.51^{a,x}$	0.632
a value										
Reference (untreated)	$8.37^{a,w}$	0.298	$5.64^{b,x}$	0.128	$4.68^{c,x}$	0.164	$3.46^{d,x}$	0.202	$4.33^{e,x}$	0.263
HP 50 C	$5.53^{a,x}$	0.129	$3.98^{b,y}$	0.123	$3.40^{c,yz}$	0.048	$2.68^{d,yz}$	0.157	$1.88^{e,z}$	0.040
HP 60 C	$5.28^{a,x}$	0.081	$3.94^{b,y}$	0.195	$3.50^{c,yz}$	0.095	$2.63^{d,z}$	0.102	$2.32^{d,y}$	0.077
HP 70 C	$5.37^{a,x}$	0.101	$4.08^{b,y}$	0.150	$3.62^{c,y}$	0.106	$2.81^{d,yz}$	0.096	$2.30^{e,y}$	0.238
HP 75 C	$4.56^{a,y}$	0.137	$3.94^{b,y}$	0.056	$3.66^{b,y}$	0.072	$2.96^{c,y}$	0.100	$4.56^{a,x}$	1.637
no HP 75 C	$3.89^{a,z}$	0.116	$3.36^{b,z}$	0.101	$3.29^{b,z}$	0.140	$2.60^{c,z}$	0.083	$2.21^{d,y}$	0.103
b value										
Reference (untreated)	$11.17^{ab,wx}$	0.209	$11.67^{a,w}$	0.125	$10.93^{b,x}$	0.395	$10.88^{b,x}$	0.293	$10.01^{c,y}$	0.264
HP 50 C	$10.35^{ab,y}$	0.114	$10.68^{a,xy}$	0.071	$10.43^{ab,x}$	0.127	$9.98^{bc,yz}$	0.369	$9.68^{c,yz}$	0.185
HP 60 C	$10.60^{a,xy}$	0.187	$10.64^{a,y}$	0.107	$10.49^{a,x}$	0.161	$10.43^{ab,xy}$	0.175	$9.85^{b,yz}$	0.240
HP 70 C	$10.59^{a,xy}$	0.163	$10.88^{a,xy}$	0.130	$10.65^{a,x}$	0.142	$10.69^{a,x}$	0.149	$9.53^{b,yz}$	0.782
HP 75 C	$11.27^{a,w}$	0.213	$11.26^{a,wx}$	0.093	$10.81^{ab,x}$	0.172	$10.68^{b,x}$	0.176	$11.27^{a,x}$	1.713
no HP 75 C	9.56^{z}	0.149	9.74^{z}	0.156	9.38^{y}	0.269	9.74^{z}	0.221	9.35^{z}	0.274

$^{a-e}$Means within a row with no common superscript differ significantly ($P < 0.05$).

$^{v-z}$Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]MRPM = mechanically recovered poultry meat.

[2]MPM = minced pork meat.

[3]n = 6.

suitable way of processing fresh whole or minced meat, taking into account the modifications induced by pressurization. Final cooked meat products would be obtained directly from this process.

Cooked sausages containing MRPM with better appearance and texture than the traditional ones can be obtained by means of high pressure processing. Moreover, the ability of pressurization to inactivate microorganisms and, therefore, to enhance the safety and to extend the shelf-life of some food products must be emphasized (Hoover *et al.*, 1989; Hayashi, 1991; Ludwig *et al.*, 1992; Yuste *et al.*, 1998). Thus, high pressure processing is a technique with a promising future in the processing of meat and meat products and, in general, in food technology.

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Núm. 5:

Pressure- vs. heat-induced bacterial stress in cooked poultry sausages: a preliminary study (1999)

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Pressure- vs. heat-induced bacterial stress in cooked poultry sausages: a preliminary study

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J. YUSTE, M. MOR-MUR, M. CAPELLAS AND R. PLA. 1999. Vacuum-packaged poultry cooked sausages were pressure-treated at 500 MPa by combinations of time (5–45 min) and temperature (2–80 °C) and later stored at 6–8 °C for 12 we. Mesophile and psychrotrophe counts were determined 1 d, 3, 6, 9 and 12 we after treatment and compared with those of cooked sausages pasteurized at 80–85 °C for 40 min. Both pressure and heat treatments offer great possibilities for preservation. Sausages pressurized at 65 °C for 15 min showed mesophile numbers below 2 log cfu g^{-1} throughout the chill storage. Pressurization, unlike heat treatment, causes a reversible bacterial stress. Thus, injured cells recovered during storage and, at 6 and 12 we, after a temperature abuse (room temperature for approx. 24 h), counts increased up to 6.5–7.5 log units. Psychrotrophes were more sensitive to both treatments; no growth was detected the day after (a lethality of more than 4 log units).

INTRODUCTION

High hydrostatic pressure is an increasingly investigated method in food processing. It causes microbial inactivation and therefore extends the shelf-life and enhances the safety of food products. Pressurization does not modify the wholesomeness of foods and is evenly and instantaneously transmitted throughout the sample, obtaining, therefore, products with great homogeneity (Shigehisa *et al.* 1991; Cheftel 1992; Mertens 1993).

Bacteria are more or less sensitive to pressure depending on several factors such as the strain or the phase and state of cells. Thus, vegetative cells in the logarithmic growth phase are very sensitive. Gram-positive organisms are usually more resistant than Gram-negative ones (Cheftel 1992). The cell membrane is believed to be an important site of injury (i.e. modifications in permeability and ion exchange). In addition, pressurization causes changes in morphology and biochemical reactions, protein denaturation and inhibition of genetic mechanisms (Hoover *et al.* 1989; Cheftel 1992).

In the present study, high pressure processing was applied to cooked poultry sausages. The objectives of this work were, first, to study the bactericidal effect of pressure and heat treatment and to follow the microbiological quality of treated sausages through chill storage (6–8 °C) and, second, to evaluate the stress caused by both treatments and the microbial recovery under real storage conditions. The experiment was designed to obtain results applicable to industry. Thus, food samples, instead of buffered solutions, were used and storage at 6–8 °C with some temperature abuse was tested.

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MATERIALS AND METHODS

Sample

Pasteurized (80–85 °C for 40 min after packaging) and non-pasteurized cooked sausages were provided by an industrial company. Sausage formulation contained 47% of poultry and 16% of lean pork meat, 2% of salt and 125 p.p.m. of sodium nitrite. These cooked sausages were vacuum-packaged, six by six, by the company. Packages were kept at 2 °C until pressure or heat treatment.

High pressure treatment, chill storage, incubation and temperature abuse

For high pressure processing, the equipment used was a discontinuous isostatic press (ACB, Nantes, France). The

time needed to achieve the treatment pressure was about 120 s and the decompression time was approx. 30 s. The pressure chamber and the liquid inside were cooled or heated to the treatment temperature with a constant flow of an ethanol-water mixture (3:1) or water, respectively. When necessary, refrigerated sausages were allowed to reach the temperature of treatment in this chamber before pressurization. Nonpasteurized sausages were pressure-treated at 500 MPa by combining different temperatures (2, 20, 50, 60, 65 and 80 °C) and times, continuous pressurization (5, 15, 30 and 45 min) and cycle oscillatory pressurization (3 × 5, 2 × 15 and 3 × 15 min). After processing, packages pressurized at high temperature were cooled in running tap water for 30 s. The experience was repeated twice.

Sausages were stored at 6–8 °C and analysed 1 d, 3, 6, 9 and 12 we after pressure or heat treatment. Samples analysed the day after were distributed as follows: two replicates of each treatment were directly analysed, two were incubated at 37 °C for 24 h and two at 37 °C for 24 h in brain heart infusion (BHI; Oxoid, Basingstoke, UK). After 1, 6 and 12 we of storage, some samples were submitted to an abuse of temperature (room temperature for approx. 24 h) and then also analysed.

Microbiological analysis and characterization

Twenty-five grams of sausage were homogenized in 225 mL of peptone water (Oxoid) for 1.5 min in an electromechanical blender and decimal dilutions were also prepared with peptone water (Pascual 1992). For samples previously incubated in BHI, this medium was the first diluent and incubation was carried out before preparing the rest of decimal dilutions. Plate count agar (PCA; Oxoid) was used to enumerate aerobic mesophilic and psychrotrophic bacteria; duplicate plates were incubated at 30 °C for 72 h (Pascual 1992) and at 7 °C for 10 d (Cousin *et al.* 1992), respectively. The *most probable number* (MPN) procedure was also used to determine bacterial counts when negligible growth in PCA plates was expected. For this purpose, tryptone soya broth (Oxoid) was used; tubes were incubated at 30 °C for 48 h for mesophiles and at 7 °C for 10 d for psychrotrophes. To confirm microbial growth, a loopful of this medium was transferred to tryptone soya agar (Oxoid) plates (Peeler *et al.* 1992), which were incubated at 30 °C for 48 h for both populations.

From PCA plates of samples pressurized at high temperature, several colonies were randomly selected, isolated and characterized according to the standard methods.

Statistical analysis

Numbers of mesophiles and psychrotrophes ($n = 4$) were subjected to analysis of variance of the General Linear Models procedure of SAS® software (the SAS® System for Windows™, release 6.12, SAS Institute, Inc., Cary, NC) to determine if there were significant differences ($P < 0.05$) among treatments or incubations or storage times.

RESULTS AND DISCUSSION

Counts the day after treatment, incubation and bacterial characterization

The effect of heat and pressure treatment on mesophilic bacteria is shown in Table 1. Pressurization at 65 °C for

Table 1 Aerobic mesophile counts (log cfu g^{-1}) of heat-treated (80–85 °C for 40 min) or pressurized (500 MPa) poultry cooked sausages

			Incubated*	Inc. in BHI†
Untreated		$4{\cdot}95^{b,v}$	$8{\cdot}44^{a,x}$	$9{\cdot}95^{a}$
Heat-treated		$2{\cdot}81^{b,wx}$	$4{\cdot}50^{b,y}$	$8{\cdot}90^{a}$
Pressurized				
°C	min			
2	5	$3{\cdot}66^{b,vw}$	$8{\cdot}70^{a,x}$	$10{\cdot}07^{a}$
	15	$3{\cdot}71^{b,vw}$	$8{\cdot}57^{a,x}$	$10{\cdot}12^{a}$
20	15	$2{\cdot}70^{b,wxy}$	$9{\cdot}47^{a,x}$	$10{\cdot}06^{a}$
	3 × 5	$1{\cdot}95^{b,wxyz}$	$8{\cdot}72^{a,x}$	$10{\cdot}20^{a}$
	30	$2{\cdot}70^{b,wxy}$	$8{\cdot}76^{a,x}$	$9{\cdot}57^{a}$
	2 × 15	$2{\cdot}68^{b,wxy}$	$8{\cdot}69^{a,x}$	$9{\cdot}95^{a}$
	45	$2{\cdot}98^{b,wx}$	$8{\cdot}34^{a,x}$	$10{\cdot}01^{a}$
	3 × 15	$2{\cdot}37^{b,wxyz}$	$8{\cdot}18^{a,x}$	$9{\cdot}45^{a}$
50	5	$2{\cdot}61^{b,wxy}$	$5{\cdot}98^{a,y}$	n.a.‡
	15	$3{\cdot}04^{b,wx}$	$4{\cdot}91^{a,y}$	n.a.
60	5	$2{\cdot}70^{b,wxy}$	$4{\cdot}76^{a,y}$	n.a.
	15	$1{\cdot}36^{xyz}$	$0{\cdot}70^{z}$	n.a.
65	5	$1{\cdot}60^{b,xyz}$	$2{\cdot}13^{b,z}$	$9{\cdot}56^{a}$
	15	$0{\cdot}95^{b,yz}$	$0{\cdot}70^{b,z}$	$10{\cdot}26^{a}$
80	5	$0{\cdot}60^{b,z}$	$0{\cdot}61^{b,z}$	$10{\cdot}17^{a}$
	15	$0{\cdot}60^{b,z}$	$0{\cdot}54^{b,z}$	$10{\cdot}10^{a}$

$n = 4$.

[a–b] Means within a row lacking a common superscript differ significantly ($P < 0{\cdot}05$).

[v–z] Means within a column lacking a common superscript differ significantly ($P < 0{\cdot}05$).

* Incubation at 37 °C for 24 h before microbiological analysis.

† Incubation at 37 °C for 24 h in brain heart infusion before microbiological analysis.

‡ n.a. not analysed.

15 min and at 80 °C caused significantly greater inactivation than heat treatment: counts were below 1 log cfu g^{-1} (a lethality of more than 4 log units). Simultaneous application of high pressure and mild heating to poultry meat and UHT milk is found more lethal for *Escherichia coli* O157:H7 and *Staphylococcus aureus* than heat treatment alone by Patterson and Kilpatrick (1998) too. Ludwig *et al.* (1992) also observe the greatest *E. coli* inactivation in different aqueous solutions combining pressure with heat. Treatments at 2 °C were the least effective.

After incubation at 37 °C for 24 h, sausages treated at 2 and 20 °C presented numbers higher than 8 log units, similar to those of untreated sausages. All pressurized samples incubated in BHI reached 9 and, even, 10 log units.

These results prove that some cells were just injured, but not inactivated. Pressurization at elevated temperature and heat treatment occasioned stronger sublethal injury and probably higher percentage of stressed cells than the rest of pressure treatments. Increases in counts observed after incubations were due both to the recovery of injured cells and to the multiplication of survivors. After incubation at 37 °C for 24 h, sausages pressurized at 60 °C for 15 min, 65 and 80 °C showed the significantly lowest counts, which were between 2 and 4 log units smaller than that of heat-treated sausages. In these pressurized samples, injured bacteria did not recover. So, pressure causes different bacterial inactivation rate and stress depending on the treatment temperature. In contrast, a highly enriched medium, such as BHI, allowed populations from all treated samples to recover from injury. Likewise, the sausage seemed to prevent recovery of damaged cells, especially when a heat treatment was administered. Patterson and Kilpatrick (1998) also observe bacterial stress caused by combined pressure-heat treatments, with the greatest injury occurring at extremes of pressure and temperature.

In samples pressurized at high temperature, surviving bacteria were preponderantly Gram-positive cocci. This is in agreement with Shigehisa *et al.* (1991), Patterson *et al.* (1995) and Ludwig and Schreck (1997), who find cocci very pressure-resistant. The latter, moreover, conclude that there seems to be no correlation with the Gram type. In the present study, some Gram-positive sporeforming rods were also isolated. Thus, besides recovery of stressed bacteria, incubation probably also induced spore germination.

Chill storage and temperature abuse

Table 2 shows mesophile counts during 12 week storage at 6–8 °C of heat- and pressure-treated sausages. This not very severe and oscillatory refrigeration temperature was assayed to follow the bacterial evolution under real conditions usually applied to chilled foods during transport and distribution. Both treatments offer great possibilities for preservation. Counts remained very low and stable all through the storage. Samples pressurized for 15 min presented numbers below 2 log cfu g^{-1} during all the study. O'Brien and Marshall (1996) pressurize raw ground chicken (10 min at room temperature) and, throughout 3 months of storage at 4 °C, at 616 MPa find similar results to those from the present work whereas at 408 MPa observe a shorter shelf-life.

The bacterial response to the temperature abuse (room temperature for approx. 24 h) was similar for all treated samples a week after treatment (Table 2). Counts increased up to 5 log units approx. In contrast, temperature abuse at 6 and 12 we caused very different effects depending on the treatment. In heat-treated sausages counts remained below 2 log units, whereas in pressure-treated sausages numbers substantially increased, up to 6.5–7.5 log units. All these abuses of temperature were carried out to determine the microbiological changes caused by unexpected temperature increase that may occur at any step after manufacturing.

Pressure-induced stress is reversible. When cells had enough time, 1 and, especially, 6 and 12 we, they recovered from injury and were able to develop in sausages if the temperature was raised. Hoover *et al.* (1989) report that some pressure effects on microorganisms, such as morphological changes, enzyme denaturation or inhibition of genetic processes, are, in some cases, reversible. The day after treatment, in samples pressurized at 60 °C for 15 min, 65 and 80 °C, not even the incubation at 37 °C originated significant differences in bacterial numbers (Table 1). So, storage time is a very weighty factor to allow stressed cells to recover and, consequently, to grow and multiply again.

Heat causes other types of modifications, which are more permanent. Damage was still not repaired after 12 we of storage and cells were not able to multiply in sausages after the temperature abuse. On the other hand, the treated sausages subjected to the temperature abuse a week after treatment, were kept under chilling conditions; 12 weeks later, counts in heat-treated samples changed very little, from 4.39 to 5.03 log cfu g^{-1} (data not included in Table 2). Initially, some injured bacteria managed to grow, but afterwards were unable to survive refrigeration; Capellas *et al.* (1996), Ponce *et al.* (1998) and Yuste *et al.* (1998) observe the same on studying the behaviour of several microorganisms surviving high pressure during storage of various food products.

Pressurization at 65 °C seemingly generated similar injury and rate of stressed cells to heat treatment, but the stress caused by the latter likely became irreversible. Patterson *et al.* (1995) and Cheftel and Culioli (1997) emphasize the negative implications of microbial stress: injured cells are not generally determined, which makes pressure inactivation be often overevaluated, but they can grow during the storage of pressure-processed foods.

Psychrotrophe counts during 12 we of chill storage are shown in Table 3. Psychrotrophic bacteria were more sen-

Table 2 Aerobic mesophile counts (log cfu g^{-1}) during 12 week chill storage (6–8 °C) of heat-treated (80–85 °C for 40 min) or pressurized (500 MPa at 65 °C) poultry cooked sausages

		Week 1		Week 6			Week 12	
	Day 1	(t.a.*)	Week 3	No t.a.	t.a.	Week 9	No t.a.	t.a.
Untreated	$4{\cdot}48^{b}$	$8{\cdot}93^{a,x}$	$8{\cdot}41^{a,x}$	$8{\cdot}56^{a,x}$	$8{\cdot}66^{a,x}$	$8{\cdot}61^{a,x}$	$8{\cdot}86^{a,x}$	$8{\cdot}80^{a,x}$
Heat-treated	2·24	$4{\cdot}39^{y}$	$2{\cdot}20^{y}$	$2{\cdot}50^{y}$	$1{\cdot}68^{y}$	$2{\cdot}22^{y}$	$1{\cdot}66^{y}$	$1{\cdot}95^{y}$
Pressurized for 5 min	$2{\cdot}18^{b}$	$4{\cdot}72^{ab,y}$	$2{\cdot}24^{b,y}$	$1{\cdot}83^{b,y}$	$7{\cdot}22^{a,x}$	$2{\cdot}04^{b,y}$	$2{\cdot}30^{b,y}$	$6{\cdot}89^{a,x}$
Pressurized for 15 min	$1{\cdot}83^{c}$	$5{\cdot}20^{b,y}$	$1{\cdot}73^{c,y}$	$1{\cdot}60^{c,y}$	$7{\cdot}52^{a,x}$	$1{\cdot}83^{c,y}$	$1{\cdot}95^{c,y}$	$6{\cdot}54^{ab,x}$

$n = 4$.
[a–c] Means within a row lacking a common superscript differ significantly ($P < 0{\cdot}05$).
[x–y] Means within a column lacking a common superscript differ significantly ($P < 0{\cdot}05$).
* t.a. temperature abuse (room temperature for approx. 24 h) before microbiological analysis.

Table 3 Aerobic psychrotrophe counts (log cfu g^{-1}) during 12 week chill storage (6–8 °C) of heat-treated (80–85 °C for 40 min) or pressurized (500 MPa for 15 min at 65 °C) poultry cooked sausages

		Week 6		Week 12	
	Day 1	No t.a.*	t.a.	No t.a.	t.a.
Untreated	$4{\cdot}23^{b,x}$	$8{\cdot}61^{a,x}$	$8{\cdot}72^{a,x}$	$8{\cdot}86^{a,x}$	$8{\cdot}85^{a,x}$
Heat-treated	†n.d.^{y}	n.d.^{y}	$0{\cdot}60^{c}$	n.d.^{y}	n.d.^{z}
Pressurized	n.d.^{b,y}	$0{\cdot}30^{b,y}$	$7{\cdot}01^{a,y}$	$0{\cdot}48^{b,y}$	$6{\cdot}53^{a,y}$

$n = 4$.
[a–b] Means within a row lacking a common superscript differ significantly ($P < 0{\cdot}05$).
[x–z] Means within a column lacking a common superscript differ significantly ($P < 0{\cdot}05$).
* t.a. temperature abuse (room temperature for approx. 24 h) before microbiological analysis.
† n.d. nondetected growth.

sitive to both heat and pressure treatments than mesophilic ones. In heat-treated samples, no growth was detected throughout the chill storage. In pressurized samples, counts were minimal. This sensitivity of psychrotrophes is very beneficial as they are the most common spoilage organisms in refrigerated poultry products. In a previous work, the authors treat mechanically recovered poultry meat mixed with nisin and also find psychrotrophes highly pressure-sensitive (Yuste *et al.* 1998). However, at 6 and 12 we, after the temperature abuse, the same behaviour as in mesophiles was observed: there was a great bacterial recovery in pressurized sausages, whereas counts did not increase in heat-treated sausages.

In conclusion, high pressure processing at mild temperature gives encouraging results in order to improve the microbiological quality of poultry sausages if they are stored under controlled refrigeration conditions. This confirms the ability of some combined treatments to preserve minimally processed food products (Crawford *et al.* 1996; Patterson and Kilpatrick 1998; Yuste *et al.* 1998). Thus, by means of pressurization, the same preserving effect as with standard heat treatment is obtained using lower temperature and shorter time. Bacterial sublethal injury and recovery mechanisms are being in-depth investigated to know more about the effects of high hydrostatic pressure on microorganisms.

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Núm. 6:

High hydrostatic pressure applied to cooked sausages: pathogenic and spoilage populations during chill storage

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High Hydrostatic Pressure Applied to Cooked Sausages: Pathogenic and Spoilage Populations during Chill Storage

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Key words: high hydrostatic pressure treatment, pathogenic bacteria, spoilage bacteria, chill storage, cooked sausages

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ABSTRACT

Vacuum-packaged cooked sausages were pressurized at 500 MPa for 5 or 15 min at 65°C and later stored at 2 and 8°C for 18 weeks. Counts of aerobic mesophiles and psychrotrophes, lactic acid bacteria, enterobacteria, Baird-Parker microbiota and *Listeria* spp. were determined 1 day, 3, 6, 9, 12, 15 and 18 weeks after treatment and compared with those of cooked sausages treated at 80-85°C for 40 min. Pressure treatment occasioned substantial reductions, of about 4 log CFU/g the day after, in psychrotrophes and lactic acid bacteria. Enterobacteria and *Listeria* proved the most pressure-sensitive; insignificant or no growth was detected all through the study. Heat treatment generated inactivation rates of psychrotrophes and enterobacteria very similar to pressurization. *Listeria monocytogenes* and enterotoxigenic *Staphylococcus aureus* were not found in treated samples. In general, there are not significant differences in any populations either among treatments or between storage temperatures. High pressure processing at mild temperature is an effective preservation method that can replace heat pasteurization applied after packaging to some cooked meat products.

In recent years, high hydrostatic pressure is being thoroughly investigated as a new food preservation process. Microbiological studies have focused mostly on strains suspended in buffers rather than indigenous microbiota of food products. Unlike heat treatment, pressurization does not change the wholesomeness of foods *(11)* and is evenly and instantaneously transmitted throughout the sample, which allows products with greater homogeneity to be obtained *(5, 24)*. High pressure is especially suitable for foods with attributes and properties that are extensively modified by heat.

Regarding pressure-induced microbial inactivation, the primary site of pressure damage is the cell membrane (i.e., modifications in permeability and ion exchange). In addition, pressurization causes changes in morphology and biochemical reactions, protein denaturation and inhibition of genetic mechanisms. Eucaryotic microorganisms are generally more sensitive to pressure than procaryotic ones *(13)*. Vegetative bacterial cells, mainly those in the logarithmic growth phase, are highly sensitive: they are inactivated by pressures in the region of 400 to 600 MPa, whereas spores of some species can even survive pressures above 1000 MPa, at least at low or at room temperature. Gram-positive are said to be more resistant than Gram-negative *(5, 10)*. However, Ludwig and Schreck *(15)* state that there seems to be no correlation with the Gram type but with the cell morphology. Thus, they found the most sensitive bacteria are rod-shaped, the most resistant are spheres, and medium sensitive bacteria are a mixed assortment of forms between short rods and cocci.

Some cooked meat products, for example on portioning, slicing, or removing the casing of emulsion-type sausages, have to be handled after cooking and, in consequence, are usually recontaminated before packaging. Bacteria of human origin, such as staphylococci, are likely the main group of microorganisms carried to these products *(9, 12, 14)*. So, subsequent pasteurization is essential to guarantee a satisfactory preservation. Otherwise, the shelf-life would drastically decrease. Early spoilage would imply a lot of waste and, therefore, substantial economic losses for manufacturers. Standard heat pasteurization applied after vacuum packaging involves some nutrient losses in meat products; moreover, it requires use of expensive heat-resistant packages. This heat treatment could be replaced with high pressure processing at less severe temperature and time conditions.

In the present study, high pressure at mild temperature was assayed in cooked sausages. The experiment was designed to obtain results applicable to industry. Thus, food samples, instead of buffered suspensions, were used and 18 week storage both at 2 and at

8°C was tested. The objectives were to compare the bactericidal effect of pressurization with that of heat treatment on several populations and to follow the microbiological quality of treated sausages through chill storage, and, thus, to determine whether high pressure processing is a valid alternative method to heat pasteurization of cooked sausages. Conclusions can be applied to all those cooked meat and poultry products which are handled after cooking.

MATERIALS AND METHODS

Experimental procedure. Figure 1 shows schematically sausage preparation and processing. Treatments assayed after vacuum-packaging are the basis of the present study.

Sample, physicochemical analysis, and chill storage. Frankfurter-type sausages of 1.6 cm diameter, manufactured with 47% of poultry and 16% of pork meat, were provided by an industrial company in six-unit vacuum packages and kept at 2°C until treatment. The AOAC official methods of analysis were applied to determine the composition of these sausages *(16)*. Packages were divided into four groups and processed as follows: one group remained untreated after cooking, one was heat-treated (80-85°C for 40 min) and the other two were pressure-treated (500 MPa at 65°C) for different exposure times (5 and 15 min). From each group, one batch was stored at 2°C and another at 8°C, both for 18 weeks. So, eight different batches were analysed. The complete experience was performed twice.

High pressure treatment. The equipment used was a discontinuous isostatic press (ALSTOM, Nantes, France). The time needed to achieve the treatment pressure was about 120 s and the decompression time was approximately 30 s. The pressure chamber and the liquid inside were held at the appropriate temperature by circulating hot water. Sausage packages were allowed to reach the treatment temperature in this chamber before pressurization. After processing, they were cooled in running tap water for 30 s.

Microbiological analysis and identification. First, aerobic mesophiles and psychrotrophes were enumerated immediately after industrial cooking. Second, counts of mesophiles, psychrotrophes, lactic acid bacteria, enterobacteria, Baird-Parker microbiota and *Listeria* spp. were determined 1 day, 3, 6, 9, 12, 15, and 18 weeks after heat or

pressure treatment. Twenty-five grams of sausage were homogenized in 225 ml of peptone water (Oxoid, Basingstoke, U.K.) for 1.5 min in an electromechanical blender and decimal dilutions were also prepared with peptone water *(18)*. The media and incubation conditions used are described in Table 1. The MPN procedure, with a detection limit of 3 CFU/g, was applied to determine counts when negligible growth in plates was expected. For this purpose, liquid media were first used and, to confirm microbial growth, a loopful of these media was transferred to the corresponding solid media *(21)*. From Baird-Parker agar plates, several colonies were randomly selected, isolated, and identified. Gram staining and various tests (KOH 3%, catalase, oxidase, Voges Proskauer, fermentation/oxidation, nitrate reduction, and growth anaerobically) were performed. In addition, the coagulase (Difco, Detroit, Mich.) and DNase tests were carried out to detect enterotoxigenic *Staphylococcus aureus*. The β-haemolysis and API Listeria (bioMérieux, Marcy l'Etoile, France) tests were used to detect *Listeria monocytogenes* from PALCAM plates.

Statistical analysis. Microbiological counts ($n = 4$) were subjected to analysis of variance of the General Linear Models procedure of SAS® software (the SAS® System for Windows™, release 6.12, SAS Institute, Cary, N.C.). Level of significance was set for $P < 0.05$.

RESULTS

Proximate composition and microbiological counts after cooking. Proximate composition of sausages was: total solids, 35.3% (± 0.13); fat, 15.5% (± 0.14); total nitrogen, 2.3% (± 0.04); ash, 3.3% (± 0.01). Samples analysed immediately after industrial cooking presented very low mesophile and psychrotrophe counts, 0.35 and 0.30 log CFU/g, respectively.

Aerobic mesophiles (Fig. 2). There are not significant differences in numbers between storage temperatures (2 and 8°C). Untreated sausages already showed high counts, about 8 log CFU/g, at 3 weeks of storage. In contrast, in treated samples, mesophile numbers did not reach 3 log units during the whole period tested. Pressurization for 15 min occasioned very low counts, below 2 log units throughout the 2°C storage, except at 15 weeks (2.2 log units).

Aerobic psychrotrophes (Fig. 3). The two chill temperatures do not cause significantly different numbers either. Counts in untreated samples were very similar to those of mesophiles. However, treated sausages showed much lower bacterial numbers; no growth (a reduction about 4.5 log CFU/g) was detected the day after treatment; in general, counts were minimal all through the study: in heat-treated and 15-min-pressurized sausages, they were less than 1 log unit.

Lactic acid bacteria (Fig. 4). In untreated sausages, almost 8 log CFU/g were found after 3 and 6 weeks of storage at 8 and 2°C, respectively. So, bacterial numbers appear to considerably rise later in samples kept at the lowest temperature. Pressure causes high inactivation rate; an initial decrease above 3.5 log units was observed; in any pressurized sausages stored at 2°C and in 15-min-treated ones stored at 8°C, counts did not reach 1 log unit throughout the study.

Enterobacteria (Fig. 5). Comparing between chill temperatures, evolution of counts in untreated samples is significantly different. Thus, at 2°C, numbers were still below 5 log CFU/g at 3 weeks and about 6 log units (the highest point) at 9 weeks. In contrast, after 3 weeks of storage at 8°C, counts were already more than 6 log units; at this temperature, they reached the highest point earlier and then gradually decreased in a more marked way than at 2°C. Heat treatment and pressurization prove very effective to inactivate enterobacteria. Insignificant or no growth was detected regardless of the treatment and the storage temperature and time.

Baird-Parker microbiota (Fig. 6). There are not significant differences in numbers between storage temperatures. High pressure is effective to decrease counts. In samples treated for 15 min, with an initial reduction of 3.5 log CFU/g, numbers did not exceed 1.4 log units all through the study. With regard to microbiological identification, Baird-Parker microbiota was preponderantly composed of *Bacillus* (52.2%), *Staphylococcus* (17.4%), and *Micrococcus* (8.7%). Enterotoxigenic *S. aureus* was found in some untreated sausages but never in heat-treated and pressurized samples.

***Listeria* spp.** (Fig. 7). As in lactic acid bacteria, chill temperature seems to influence bacterial counts slightly. At 8°C, numbers in untreated samples were already 4.6 log CFU/g after 3 weeks and more than 5 log units after 6 weeks. In contrast, at 2°C, the

maximum count (4.6 log units) was not observed until 18 weeks. Heat and, especially, pressure treatment show a remarkable lethal effect. No growth was detected in any pressurized sausages irrespective of the storage temperature and time. *Listeria monocytogenes* was found in some untreated sausages but not in heat-treated samples.

DISCUSSION

The low counts found in samples analysed immediately after industrial cooking indicate that this process would be enough to preserve sausages. However, considerable contamination is subsequently introduced through extensive handling prior to packaging. The substantial increases observed in mesophile and psychrotrophe counts, above 4 log CFU/g, confirm this point. So, an additional treatment to inactivate pathogenic and spoilage microorganisms is essential after sausages are packaged. Without this treatment, rapid and marked increases in all the populations studied are observed. This fact, besides meaning an economic disadvantge, obviously implies sanitary risk.

Pressurization at 65°C was selected because it proved effective to decrease plate counts of mesophilic and psychrotrophic bacteria and to keep them low *(29)*. Results from other studies point up the suitability of using mild temperature in pressure treatment. Patterson and Kilpatrick *(19)* combined high pressure (up to 700 MPa) with mild heating (up to 60°C) to treat poultry and UHT milk and found it more lethal for *Escherichia coli* O157:H7 and *S. aureus* than either treatment alone. On the other hand, Carlez et al. *(4)* and O'Brien and Marshall *(17)* pressurized at room temperature and then stored (3-4°C) minced beef meat and chicken, respectively. At pressures about that applied in the present study, they found very different mesophile counts: more than 9 log CFU/g after 22 days, the former, and 3.5 log units after 14 weeks, the latter. A longer shelf-life under refrigeration is achieved in the present study, even at 8°C. This is the temperature that really matters to manufacturers, rather than severe chilling, because it is usually applied to chilled foods during transport and distribution.

Both heat and pressure treatment preserve sausages for a considerable period of time. Mesophile numbers remained low and quite stable throughout the study. High pressure substantially reduces psychrotrophe and lactic acid bacterial counts. Heat treatment generates an inactivation rate of psychrotrophes similar to pressurization. Psychrotrophes are usually more sensitive to heat and pressure processing than mesophiles. This is because, after treatment, they lose the ability to grow at refrigeration temperature.

Thus, very low counts were found during the whole experiment, which is remarkable in view of the important role of these bacteria in spoilage of chilled meat, poultry, and derived products. The marked sensitivity of psychrotrophes to pressurization has already been reported by the authors *(27, 29)*.

Holzapfel *(12)* and Johnston and Tompkin *(14)* state that lactic acid bacteria often make up the typical spoilage association of vacuum-packaged emulsion-type meat products kept under refrigeration. Following the evolution of the different populations studied, it is clear that bacterial load of cooked sausages indeed consisted mostly of these bacteria, which exerted a competitive inhibition, especially at 8°C, that is reflected by gradual decreases in enterobacteria counts of untreated sausages. The low lactic acid bacterial numbers observed in pressurized sausages during all the study, confirm the ability of high pressure to markedly delay spoilage. Carlez et al. *(4)* found *Lactobacillus* spp. highly pressure-sensitive: these bacteria were not detected during 22 days at 3°C.

Presence of Baird-Parker microbiota in sausages was also considerable. The genera found in higher rates, *Bacillus*, *Staphylococcus*, and *Micrococcus*, are those described as the most common growing on Baird-Parker agar. As observed by Ludwig and Schreck *(15)*, Patterson et al. *(20)*, Shigehisa et al. *(24)*, and Timson and Short *(26)*, these genera are the most pressure-resistant. However, enterotoxigenic *S. aureus* was not found in any treated samples. According to Bennett et al. *(1)*, because 10^6 *S. aureus* cells/g are required for detectable levels of enterotoxin to be produced, it is important to prevent the contamination and subsequent outgrowth of *S. aureus*.

High pressure is an excellent method to inactivate enterobacteria and *Listeria*, which proved the most pressure-sensitive. In a previous work, the authors did not detect growth of noninoculated *Listeria* in pressurized mechanically recovered poultry meat stored at 2°C for 2 months either *(28)*. Considerable reductions of inoculated *L. monocytogenes* and *Listeria innocua*, a nonpathogenic indicator microorganism for *L. monocytogenes (7)*, counts have also been reported by several authors on pressurizing various foods *(3, 8, 20, 22, 25, 28)*. Regarding heat-treated samples, the inactivation rate of enterobacteria is very similar to that achieved with pressurization. It is of concern for food safety and public health the absence in treated samples of outstanding pathogens, such as *L. monocytogenes* and the aforementioned enterotoxigenic *S. aureus*.

In general, there are not significant differences in any populations either among treatments or between temperatures of storage. In those cases that, at certain storage times,

bacterial numbers significantly differ between 2 and 8°C, counts were eventually equal. So, this difference in storage temperature is not enough to modify the shelf-life of sausages.

Both heat and pressure treatments can also sublethally injure bacterial cells, which is probably the reason for the slight oscillations in counts observed in some occasions during storage. These irregular counts are likely due both to the inability of some stressed cells to survive refrigeration and to the recovery of some other damaged cells at some point of chill storage. Capellas et al. *(2)*, Ponce et al. *(23)*, and Yuste et al. *(28)* come to similar conclusions. Stress and recovery of microorganisms and subsequent ability to develop and perform their usual metabolic activity must be well known to avoid the negative implications that they have for the storage of processed foods.

In conclusion, high pressure processing at mild temperature substantially extends the shelf-life and enhances the safety of refrigerated cooked sausages. The same preserving effect as with standard heat pasteurization is achieved using lower temperature and shorter time. Thus, products with better nutritional quality are obtained and, on the other hand, heat-resistant packages are not necessary, which reduces manufacturing costs and so the price of final products. Likewise, color is not modified because the nitrosyl-haemochromogen molecule is fairly resistant to high pressure *(6)*. All these practical benefits for manufacturers make pressurization an interesting alternative method to the second heat treatment applied to some cooked meat and poultry products.

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TABLE 1. *Media and incubation conditions used to enumerate bacterial populations of cooked sausages*

	Plate count media	Incubation	MPN			
			Liquid media	Incubation	Solid media	Incubation
Mesophiles	PCA[a,h]	30°C / 72 h	TSB[b,h]	30°C / 48 h	TSA[c,h]	30°C / 48 h
Psychrotrophes	PCA	7°C / 10 days	TSB	7°C / 10 days	TSA	30°C / 48 h
Lactic acid bacteria	MRS agar[i]	30°C / 72 h	MRS broth[i]	30°C / 72 h	MRS agar	30°C / 72 h
Enterobacteria	VRBGA[d,i]	37°C / 24 h	EE broth[e,h]	37°C / 24 h	VRBGA	37°C / 24 h
Baird-Parker microbiota	Baird-Parker agar[h]	37°C / 24-48 h	Giolitti-Cantoni broth[h]	37°C / 24-48 h	Baird-Parker agar	37°C / 24-48 h
***Listeria* spp.**	PALCAM[f,i]	37°C / 48 h	UVM broth[g,i]	37°C / 48 h	PALCAM	37°C / 48 h

[a] PCA: plate count agar.
[b] TSB: tryptone soya broth.
[c] TSA: tryptone soya agar.
[d] VRBGA: violet red bile glucose agar.
[e] Glucose-buffer-brilliant green-bile.
[f] PALCAM agar base with added PALCAM Listeria selective supplement.
[g] Listeria enrichment broth base (UVM formulation) with added Listeria primary selective enrichment supplement.
[h] Oxoid, Basingstoke, U.K.
[i] Biokar Diagnostics, Beauvais, France.

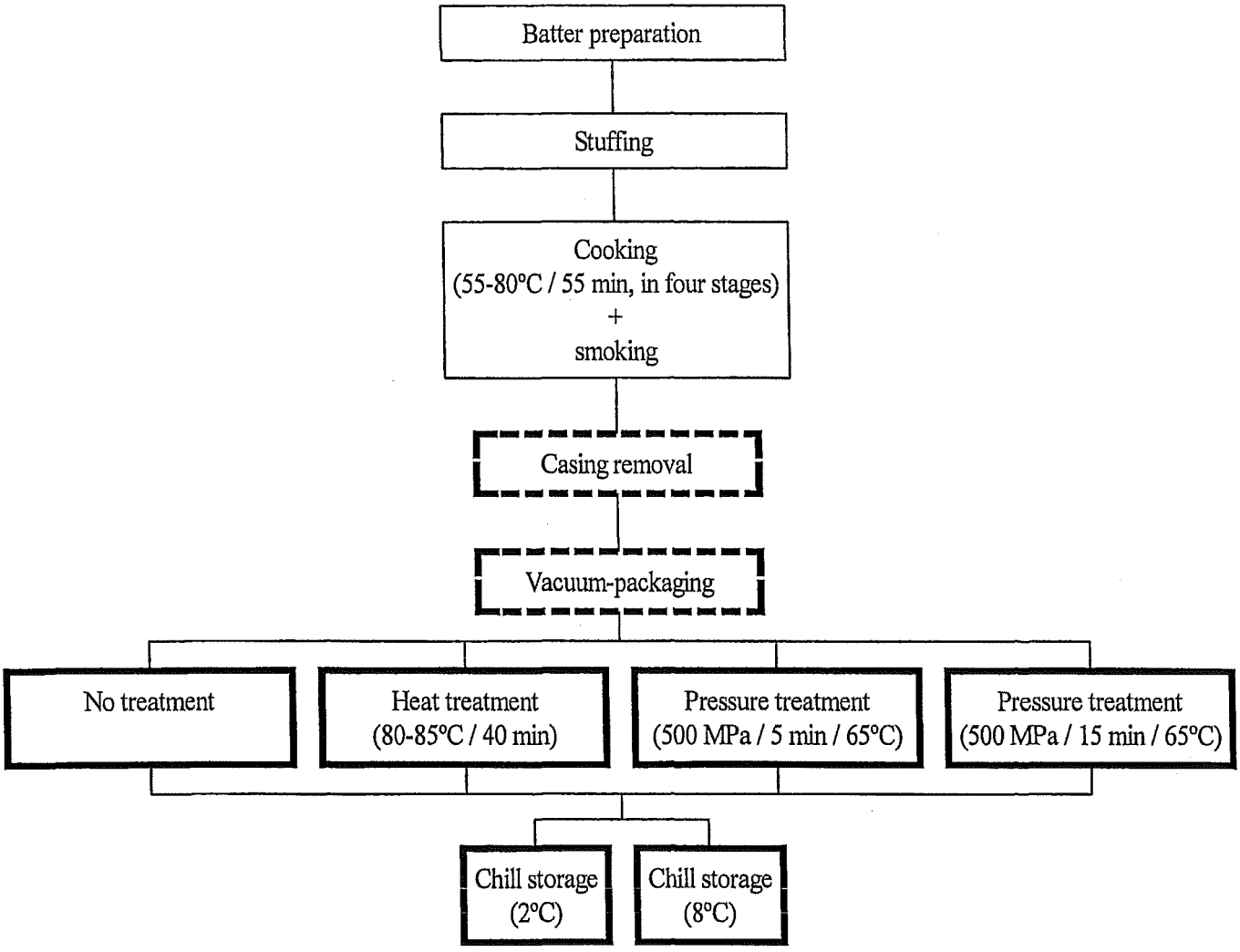
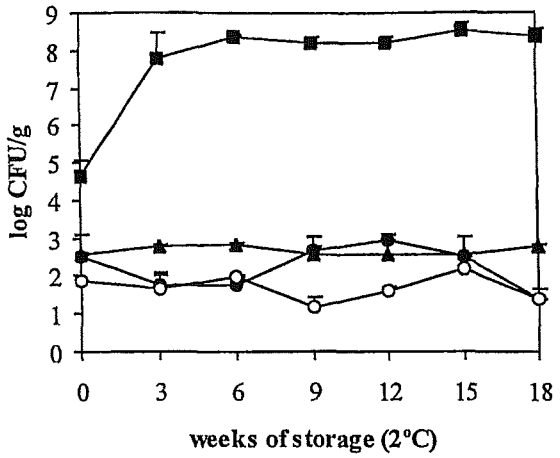


FIGURE 1. *Flow diagram of cooked sausage manufacture, processing and storage. Stages involving considerable risk of microbiological contamination after cooking are put in broken thick frames. The different treatments and storage temperatures tested in the present study are put in continuous thick frames.*

(a)



(b)

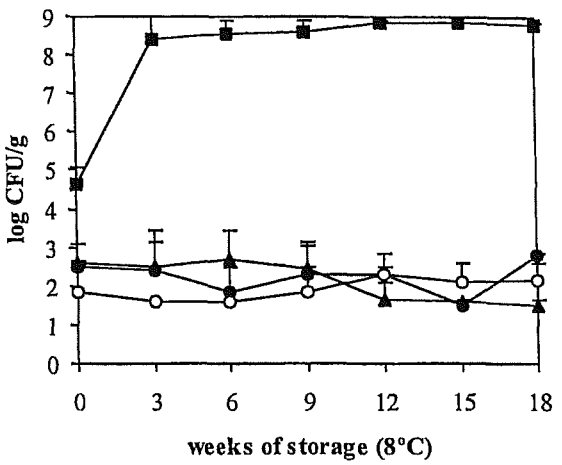
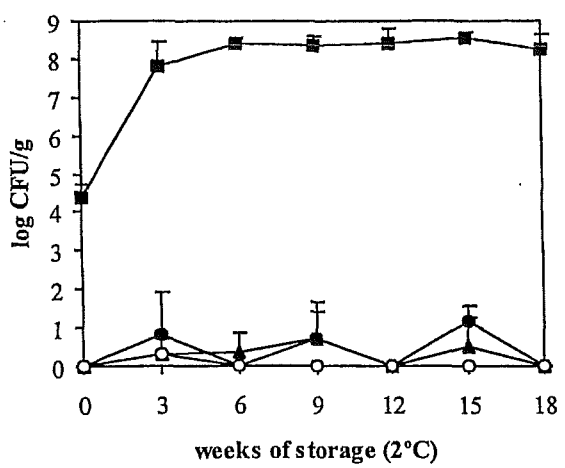


FIGURE 2. *Aerobic mesophile counts of heat-treated or pressurized cooked sausages.* ***(a)*** *Storage at 2°C. Least significant difference = 1.327.* ***(b)*** *Storage at 8°C. Least significant difference = 1.869. (■), untreated; (▲), 80-85°C / 40 min; (●), 500 MPa / 5 min / 65°C; (○), 500 MPa / 15 min / 65°C. Zero in the X-axis corresponds to the day after treatment.*

(a)



(b)

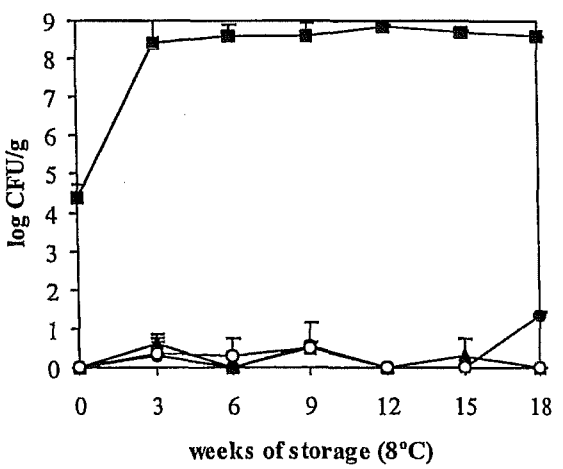


FIGURE 3. *Aerobic psychrotrophe counts of heat-treated or pressurized cooked sausages.* ***(a)*** *Storage at 2°C. Least significant difference = 1.921.* ***(b)*** *Storage at 8°C. Least significant difference = 1.269. (■), untreated; (▲), 80-85°C / 40 min; (●), 500 MPa / 5 min / 65°C; (○), 500 MPa / 15 min / 65°C. Zero in the X-axis corresponds to the day after treatment.*