Attached please find a petition submitted by:
North American Natural Sausage Casing Association
Organic Valley-Organic Prairie
Applegate Farms

For Natural Casings.

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Petition for Amending the National List of the USDA’s National Organic Program for inclusion of:

Natural Casings

A Non-organic agricultural substance allowed in processing

Submitted September 1, 2006

by;

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• Jim Pierce, Organic Valley-Organic Prairie / CROPP Cooperative, One Organic Way, La Farge WI 54639 phone (608)625-2602 fax (608)625-3092 email jim.pierce@organicvalley.coop website www.organicvalley.coop.

• Chris Ely, Applegate Farms 750 Route 202 South, 3rd Floor, Bridgewater, NJ 08807 phone (908) 725-5800 fax (908) 725-3383 email chris.ely@applegate.com website www.applegate.com

This petition includes the following attachments;

• Attachment #1; North American Natural Casing Association Membership List
• Attachment #2; Production Narrative and Diagram
• Attachment #3 Excerpts From EU Regulations citing Natural Casings
• Attachment #4 Bulk Labels
• Attachment #5; Antimicrobial properties of salt (NaCl) used for the preservation of natural casings
• Attachment #6; NOSB Evaluation Forms
Petitioners are required to provide the following information as applicable:

Item A, Category for inclusion on the National List:
- §205.606 Nonorganically produced agricultural products allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))"

Item B, Common name:
- Natural Casings, the processed intestines of hogs, cattle and sheep

Item C, Chemical Structure:
- N/A

Item D, Manufacturers name, address and telephone number
- See Attachment #1; North American Natural Casings Association Membership List.

Item E, List of uses, rates and applications for crops and livestock uses, mode of action for handling uses:
- Natural casings are stuffed with sausage. They can be subsequently cooked, smoked or packaged raw. In most instances the casing is consumed as part of the finished product although in the case of some sausage products the natural casing may be removed. The percentage of non-organic natural casing in final formulation will vary depending on the size of the sausage and typically ranges from <0.01% to 0.02%. Examples of sausages using natural casings that are currently available as organic include Bratwurst, Italian, Pepperoni, Breakfast Links, Frankfurters and Kielbasa.

Item F, Sources and detailed description of manufacturing procedures:
- Please refer to Attachment #2 describing manufacture process for natural casings and accompanying machine diagram and "Natural Casing Products - Hog Casings" illustration.

Item G, Summary of any previous reviews by state or private certification agencies:
- There is well established precedent for the use of non-organic natural casings in organic sausage products by both US and foreign certification agencies. In the United States natural casings have been used in products labeled as organic since the National Organic Program became effective on October 21st, 2002. Prior to that time natural casings were used in meat products formulated to organic standards but were prohibited by the USDA from being labeled as organic. Since implementation of the NOP many accredited certification agencies including Oregon Tilth Certified Organic, Quality Assurance International, NOFA New York and Midwest Organic Services Association have approved and are currently allowing natural casings to be used in products labeled as "Organic" or "Made With Organic...". In June 1991 the European Union Council Regulation (EEC) No 2092/91 Organic Regulations listed "casings" as allowed under Section C "Ingredients of agricultural origin which have not been produced organically". The listing originally carried the annotation "until 1 April 2004 only" presumably in anticipation of the advent of organic casings becoming available. In August 2004 the regulation was amended and the annotation was dropped. See Attachment #3; Excerpts From EU Regulations citing Natural Casings. It is not known to the petitioners what the
process and discussion leading up to the 2004 amendment was but it would appear that there was a clear desire not to negatively impact the organic sausage industry that had built and flourished in the thirteen year interim. Indeed organic sausage production and consumption in Europe is a much larger sector than it is in the USA. There are several reasons for this including:

- Meat including sausage has been labeled as organic in Europe since the passage of EU 2092/91 in 1991, eleven years ahead of the USA.
- Sausage consumption in many European nations is significantly higher than in the US so it reasons that the organic market share should also be larger.
- European organic standards also allow the limited use of nitrates to cure meat including sausage making high quality organic products simpler to develop and quicker to bring to market.

**Item H, Regulatory status with EPA, FDA or state authorities:** The Chemfinder website provides links to regulatory status. It will be also helpful to list the international status of the substance according to Canadian regulations, EU regulations and Codex agreements. (see links at NOP website).

- Natural Casings are Regulated by the Food Safety Inspection Service (FSIS) of the USDA under 9 CFR parts 317 and 38. This document is not attached but can be referenced at; http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/94-030F.htm
- Natural casings are regulated internationally according to Chapter 5 of the Harmonized Tariff Schedule of the United States (2006) and can be referenced at http://hotdocs.usitc.gov/docs/tata/hts/bychapter/0612C05.pdf

**Item I, Chemical Abstract Service (CAS) number or other product number, samples of labels:**

- **CAS number** N/A
- **Labels:** See Attachment #4; Bulk Labels

**Item J, Physical properties of the substance and chemical mode of action: including environmental impacts, interactions with other materials, toxicity and persistence, effects on human health, effects of soil organisms, crops or livestock:**

- **N/A**

**Item K, Safety information, including a MSDS (Material Safety Data Sheet) and report from National Institute of Environmental Health Studies (NIEHS):**

- **N/A**

**Item L, Research information, including research reviews and bibliographies:**

- The literature pertaining to the use of Natural Casings in sausage manufacture is well established. Literature specific to the application of natural casings to the manufacture of organic sausage products could not be found. We chose to include one article See Attachment #5; Antimicrobial properties of salt (NaCl) used for the preservation of natural casings because it provides a succinct overview of the history, production, use and relative safety of natural casings.

**Item M, Petition justification statement - that states why the synthetic substance is necessary, alternatives that could be used, beneficial effects to the environment, etc:**

- Until such time as organic sausage production reaches sufficient size that will entice natural casing manufacturers to cater to this niche by producing natural casings from certified organic livestock it is imperative that they be listed on the National List §205.606 in order to preserve the status quo of this
budding sector of organic food.

Natural casings are the processed intestines of Hogs Cattle and Sheep. Their use in making sausage and preserving meat predates recorded history. Their use in organic sausage production predates the National Organic Program as well as recognized foreign organic certification. Due to their cleanliness and simplicity natural casings have never been prohibited in organic production as an agricultural ingredient not commercially available in organic form. To the contrary natural casings are the preferred choice compared to synthetic alternatives. Their presence in final formulation is low, typically 0.01% - 0.02%, there is no GMO concern nor is there irradiation concerns since irradiation negatively affects the elasticity of the intestine making them unsuitable for use.

There are three alternatives to natural casings; peelable cellulose, edible collagen and no casing whatever. Although each has its benefits and challenges none are an identical equivalent to natural casings.

- **Peelable Cellulose Casings** are synthetic. This material was petitioned to be added to the National List in June 2001, was approved for addition by the NOSB in October 2001 and published to the National List on June 2003. Peelable Cellulose casings serve their purpose well for skinless wieners and other skinless ready to eat sausages and luncheon meats but will not function properly for traditional “skin on” sausages such as Bratwurst, Kielbasa, and Breakfast Links.

- **Eatable Collagen Casings** are synthetic and so would require petition and addition to the National List. Although collagen casings are more widely used in conventional modern sausage manufacture due to their uniformity and adaptability to high tech machinery they represent a step away from the “minimally processed” food paradigm which is at the heart of organic production philosophy. Natural casings are a much better fit for organic sausage production than collagen.

- Some varieties of sausage can and are packaged in bulk without being stuffed into casings. Pork Breakfast Sausage is commonly available as links or patties for example. However many varieties of sausage would either not be possible to manufacture or would be unacceptable to consumers without casings. As stated previously natural casings, organic if available and conventional if not are the best choice for organic sausage production.

While an argument might be made that the organic marketplace should do without sausage labeled as organic or made with organic we feel strongly that such a decision would negatively impact the entire organic meat sector. At the time that this petition is being written the organic sausage business is maturing into a significant sector of the organic food sector. Sausage products are essential to an organic meat program since utilization of the entire organic animal is critical to offset the higher cost of organic production. Without a premium market to use trim meat prices for prime cuts would be forced higher.

The North American Natural Casings Association includes every domestic manufacturer of natural casings. We also have a strong relationship with international casing manufacturers. To the best of our knowledge no casing manufacturer has ever attempted to make natural casings from organic slaughter stock due primarily to the inability to amass enough organic runners for an identity preserved run. While organic natural casings could easily be produced and would certainly be required for use in products labeled as organic the size of the organic sausage market is a long way from being substantial enough for a natural casing manufacturer to find it attractive enough a market. It is reasonable to expect that the organic sector will be catered to with organic casings eventually. Doubtless there will be supply and demand imbalances as organic casings become increasingly available particularly for certain sizes and types of casings. Until such time as the commercial availability of organic casings can be assured natural casings needs to remain listed on section 205.606.
The manufacturer of organic casings would be assured of a market since organic agricultural ingredients are mandated by NOP when available. The producer/marketer of organic sausages using organic casings would in many cases be able to label their products "100% organic since in many formulas now the only non-organic ingredient is the casing. The organic livestock producer and processor would benefit by capturing organic intestines and selling them as organic, hopefully at premium prices. Aside from being taken from organically raised and processed animals there would be no difference in organic natural casings compared to what is now available. The attached processing information reflects the simplicity of this ancient technology. Intestines are stripped, meticulously cleaned and salted. There are no other chemicals or preserving agents used in the manufacture process. That is one of the reasons that natural casings have been so widely accepted by domestic and international organic certifiers.

Since meat products were not permitted to be labeled as organic until the introduction of the NOP in October 2002.

Item N, Commercial Confidential Information Statement - describing information that is considered to be confidential business or commercial information:

This petition for the addition of Natural Casings to the National List section 205.606 is being submitted by the North American Natural Casings Association, Organic Valley/Organic Prairie and Applegate Farms for the benefit of everybody presently manufacturing organic sausages. There is nothing confidential or exclusive in this document. It is our sincere hope that the information presented here is sufficient to approve the addition of natural casings to section 205.606 of the National List prior to June 2007 in order to seamlessly allow production of organic sausages to continue. To that end the services of the signatories of this petition are available to the National Organic Program and the National Organic Standards Board regarding any questions, concerns or additional information necessary to expedite this process.
Additional information;
National Organic Standards Board (NOSB)
Policy Development and Handling Committee
Final Recommendation for the Establishment Commercial Availability Criteria
March 30, 2006

Excerpts:
The NOSB recommends using the procedures currently in place for petitioning materials onto 205.606. The document entitled “Information to be Included in a Petition”, shown on the NOP website, should be amended to include a description of the information needed for the determination of commercial availability of non-organically produced agricultural products. The following additions to this document are recommended:

1. Add the following bullet to Item A:
   - Agricultural (nonorganic) substance allowed in or on processed product labeled as "organic."
     ➢ This information is included in Item A.

2. Add the following bullets to Item B #12
   - When petitioning for the inclusion on the National List of nonorganically produced agricultural products the petition must state why the product should be permitted in the production or handling of an organic product. Specifically, the petition must include current industry information regarding availability of and history of non-availability of an organic form of the product, and all factors that may present a challenge to a consistent organic supply.
     ➢ This information is included in Item M.
Attachment #1; North American Natural Casing Association  
Membership List

REGULAR/BROKER MEMBERS

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Email: mike@canadacomponent.com

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Fax: (718) 617-6894  
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San Antonio, TX 78219-6112  
(210) 661-6161  
Fax: (210) 662-6112  
Web: www.dewed.com

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Fax: (905) 475-0038  
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Fax: (973) 777-5338  
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NATURAL CASING COMPANY  
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Fax: (978) 562-3933  
Email: nccom@nari.net

THE STANDARD CASING CO., INC.  
165 Chubb Ave  
Lyndhurst, NJ 07071  
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Email: casings@standardcasing.com

WORLD CASING CORPORATION  
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Maspeth, NY 11378  
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CONTINENTAL CASING CORPORATION  
145 Broadway  
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DAKOTA NATURAL CASING  
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Fax: (212) 695-7153  
Email: info@lvlcorp.com

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Subsidiary in Canada: (800) 615-4474  
Email: mmayo@overseacasing.com  
Web: www.overseacasing.com

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June 26, 2006

Jim Pierce
Organic Valley/CROPP Cooperative
1 Organic Way
LaFarge WI 54639

Dear Jim,

The source of Pork, Beef and Lamb Casings is either the small and/or the large intestine. Essentially all of the intestines are processed in the same manner.

After gutting the animal, workers strip the gut from the connective fat and deliver it to the machines. Please note the only ingredient used in the cleaning procedure is water.

The machines 1 and 2 remove the manure and the machines 3 and 4 remove the mucosa lining of the intestine. This lining is the raw material for the wonder drug, Heparin. Heparin is the most widely prescribed drug in hospitals. It prevents blood clots, and this is recovered only from the Hog small intestine in this Nation.

The machine number 5 is the Finisher which removes the outer shell or ribbon and that leaves the RUNNER (this is an unselected casing). It is then put into a tank with cold water and sometimes salt is added to increase and assist the removal of blood from the gut.......nothing else is added. The next step is to salt, cure and pack. After curing the runners are put into 50 gallon drums in order to assist in shipping for further processing. Again let us state that during the entire process there is absolutely nothing added to the product other than water and salt.

Selecting: The Runners (unselected intestines) are sent to plants where the product is desalted, flushed with water to grade and remove any damaged strands with holes or tears and put into proper sizes, usually with 2 meter restrictions, and then into 100 yard units we call HANKS. These are re-salted and packed in drums, and then are ready to be shipped to the Casing Company plants for distribution to their Sausage Manufacturing Customers.

Very Truly Yours,

[Signature]

Allan Ross
Hog Casings are used for Cooked Sausage, Country Style Sausage, Fresh Pork Sausage, Pepperoni, Italian Sausage, large Frankfurters, Kishka, Kielbasa and Bratwurst...to name just some of the best-selling items.

Hog Casings are sold in "bundles" or "hanks." This unit of measure equals approximately 91 meters.

Hog Casings - unstuffed, shown in hanks, tubed, and in tube; also shown are examples of stuffed hog casings.

Hog Casings are also sold in bundles called "shorts." Shorts are 1 to 2 meter lengths and usually classified as 35mm and up or 35mm and down. NOTE: "Green Weights" refers to the weight of a stuffed casing, prior to cooking or smoking, per 91 meter lengths.

**HOG CASINGS by Bundle or Hank**

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<td>30 mm/down</td>
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<td>Frankfurters, Italian Sausage</td>
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<tr>
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<td>Smoked Sausage, Pepperoni, Bratwurst, Italian Sausage</td>
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<td>38-42 mm</td>
<td>57-61 kg</td>
<td>Smoked Sausage, Kielbasa, Pepperoni, Rope Sausage</td>
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<td>42-44 mm</td>
<td>59-64 kg</td>
<td>Smoked Sausage, Kielbasa, Pepperoni</td>
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<tr>
<td>44 mm/up</td>
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<td>Specialty Items</td>
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</table>
Attachment #3 Excerpts From EU Regulations citing Natural Casings

**SECTION C — INGREDIENTS OF AGRICULTURAL ORIGIN WHICH HAVE NOT BEEN PRODUCED ORGANICALLY, REFERRED TO IN ARTICLE 5(4) OF REGULATION (EEC) No 2092/91**

C.1. Unprocessed vegetable products as well as products derived therefrom by processes referred to under definition 2(iii) of the introduction of this Annex:

C.1.1. Edible fruits, nuts and seeds: acorns Quercus spp. cola nuts Cola acuminata gooseberries Ribes uva-crispa maracujas (passion fruit) Passiflora edulis raspberries (dried) Rubus idaeus red currants (dried) Ribes rubrum

C.1.2. Edible spices and herbs: nutmeg Myristica fragrans, until 31.12.2000 only pepper green Piper nigrum, until 30.4.2001 only pepper (Peruvian) Schinus molle L horseradish seeds Armoracia rusticana lesser galanga Alpinia officinarum safflower flowers Carthamus tinctorius watercress herb Nasturtium officinale

C.1.3. Miscellaneous: algae, including seaweed, permitted in conventional foodstuffs preparation

C.2. Vegetable products, processed by processes as referred to under definition 2(ii) of the introduction of this Annex

C.2.1. Fats and oils whether or not refined, but not chemically modified, derived from plants other than: cocoa Theobroma cacao coconut Cocos nucifera olive Olea europaea sunflower Helianthus annuus palm Elaeis guineensis rape Brassica napus, rapa safflower Carthamus tinctorius sesame Sesamum indicum soya Glycine max

C.2.2. The following sugars, starches and other products from cereals and tubers: beet sugar, until 1.4.2003 only fructose rice paper unleavened bread paper starch from rice and waxy maize, not chemically modified

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C.2.3. Miscellaneous: coriander, smoked Coriandrum sativum until 31.12.2000 only pea protein Pisum spp. rum, only obtained from cane sugar Juice kirsch prepared on the basis of fruits and flavourings as referred to in section A.2 of this Annex mixtures of crops permitted in conventional foodstuffs preparation, and giving colouring and tasting qualities to confectionary, only for preparation of 'Gummi Bärchen', until 30.9.2000 only mixtures of the following peppers: Piper nigrum, Schinus molle and Schinus terebinthifolium, until 31.12.2000 only

C.3. Animal products:

C.3.1. Aquatic organisms, not originating from aquaculture, and permitted in conventional foodstuffs preparation:

C.3.2. Animal products:

buttermilk powder until 31.8.2001 only gelatin honey until 28.2.2001 only lactose until 31.8.2001 only whey powder 'herasuola' casings until 1 April 2004 only

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,
Having regard to Council Regulation (EEC) No 2092/91, of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs(1), and in particular the second and third indents of Article 13 thereof,

Whereas:
(1) Following the accession of new Member States to the European Union, the typography defined for the Community logo by the graphic manual laid down in Annex V, part B.4, to Regulation (EEC) No 2092/91 no longer has all the necessary characters and accents of all the official languages. Therefore, a further typography has to be allowed in the graphic manual.
(2) Annex VI, Section C, to Regulation (EEC) No 2092/91 lists the ingredients of agricultural origin which have not been produced organically but which may be used in the preparation of foodstuffs in accordance with the conditions laid down in Article 5 of that Regulation, provided that it has been shown that such ingredients obtained by the organic production method are not available in sufficient quantities within the Community.
(3) After it had been established that organically produced casings were not available in sufficient quantities within the Community, Annex VI, Section C, to Regulation (EEC) No 2092/91 was amended by Commission Regulation (EC) No 473/2002(2), in order to include casings in the list of agricultural ingredients, for a transitional period expiring on 1 April 2004.
(4) It appears, however, that the availability in organically produced casings still remains very limited and that it is unlikely that sufficient quantities will be available in the future. It is therefore necessary to allow the use of casings not produced according to the organic farming method without any limit in time.
(5) Regulation (EEC) No 2092/91 should therefore be amended accordingly.
(6) The measures provided for in this Regulation are in accordance with the opinion of the Committee set up in accordance with Article 14 of Regulation (EEC) No 2092/91,

HAS ADOPTED THIS REGULATION:

Article 1
Regulation (EEC) No 2092/91 is amended as follows:
1. In Annex V, part B.4, Section 2.4 (Typography) is replaced by the following:
«Use Frutiger or Myriad bold condensed in capitals for the wording. The letter size of the wording shall be reduced according to the norms set down in Section 2.6.»
2. In Annex VI, Section C, point C.3, in the entry for «casings», «until 1 April 2004 only» is deleted.

Article 2
This Regulation shall enter into force on the seventh day following that of its publication in the Official Journal of the European Union.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 19 August 2004.

For the Commission
Franz Fischler
Member of the Commission

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<th>Lb</th>
<th>Net WT</th>
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SIOUX FALLS, SD 57103 USA
Antimicrobial properties of salt (NaCl) used for the preservation of natural casings

J.J. Wijnker, G. Koop, L.J.A. Lipman

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Received 10 August 2005; accepted 20 November 2005

Abstract

The antimicrobial properties of salt (NaCl) used for the preservation of natural casings were studied by investigating the survival of six bacterial species in natural casings at different water activity (aw) levels. Individual sheep casings were inoculated with ca. 10^5 colony-forming units (cfu) g\(^{-1}\) of Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens and 10^2 cfu g\(^{-1}\) of E. coli O157:H7.

The casings were stored at 20 ± 1.5 °C in different brines and dry salt, giving aw-levels of 0.90aw, 0.87aw, 0.85aw, 0.83aw and 0.75aw. Samples were taken at day 1, 3, 6, 8, 13, 20, 27 and 30 after inoculation and the number of bacteria present was determined. Based on survival curves, death rates (day\(^{-1}\)) were calculated to quantify the reduction in log\(_{10}\) cfu g\(^{-1}\) per day.

The influence of aw on death rates was higher for Gram-negative bacteria than for Gram-positive bacteria. The death rates were overall higher for Gram-negatives than for Gram-positives. No clear reduction in the survival of C. perfringens in relation to any aw level was observed in this study.

These results indicate that the antimicrobial properties of salt used for the preservation of natural casings are sufficient to reduce the bacterial contamination (except for Clostridium spores) well below acceptable levels at a water activity level of 0.85 or lower during a 30-day storage period.

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Keywords: Natural casings; Water activity; Salt; Preservation; Salmonella

1. Introduction

As sausage is known to be the oldest and longest established form of processed meat, the intestines of sheep, hogs and cattle have been used for thousands of years as an edible container for this well-regarded product. The basic technique for the cleaning of casings has also remained unchanged over all this time (Ockerman and Hansen, 2000) and a comparative study (Koolmees et al., 2004) revealed no significant difference in the cleaning efficacy of manual or mechanical cleaned sheep casings. In general, the remaining submucosa layer is known as the natural casing (Bakker et al., 1999) and, based on the animal it originates from, is used for a wide variety of sausages (Houben, 2005).

Casings are usually preserved by salting, curing and/or drying (Fischer and Schweißlinghaus, 1988). By using salt, the water activity (aw) of the product is decreased and together with temperature and pH, is one of the major parameters influencing bacterial growth and survival.

The lethal effect of a low aw (Gutierrez et al., 1995) is linked to the fact that the turgor pressure in a cell is established as a result of the intracellular aw and the aw in the surrounding medium. A process known as plasmolysis (Clonka, 1989), describes how hypertonic shock causes an instantaneous efflux of water, accompanied by a decrease in the cytoplasmatic volume of the cell. A universal response to the temporary loss of turgor is the cytoplasmatic accumulation of so-called 'compatible
load of sheep and hog casings at different steps of the cleaning process (Utrecht University, 1995; Ockerman and Hansen, 2000), with $4.31 \times 10^5 \text{cfu g}^{-1}$ for *Staphylococcus aureus* and $4.26 \times 10^4 \text{cfu g}^{-1}$ for *Enterobacteriaceae* after the final cleaning step. Since sheep casings were found to be more contaminated than hog casings, these were used for this study. The microbial survival of food pathogens was based on readily available information on the Internet (www.food-info.net, www.cfsan.fda.gov).

2.1. Bacteria

The following bacterial species were chosen: *E. coli* (ATCC 10536), *E. coli O157:H7* (ATCC 43895), *Salmonella typhimurium* (ATCC 14028), *Listeria monocytogenes* (ATCC 7644), *S. aureus* (ATCC 29213) and *Clostridium perfringens* (ATCC 13124). Each bacterial species was grown in 10 ml TSB (Tryptone Soy Broth, Oxoid CM 129), for 24 h at 37°C, diluted and counted to create final inoculum (ca. $10^5 \text{cfu g}^{-1}$). An exception was made for *E. coli O157:H7* where a lower final inoculum was made of $10^4 \text{cfu ml}^{-1}$ due to the high pathogenicity of this species. Specific media for culture and enrichment are listed in Table 2 for each bacterial species.

2.2. Sample preparation

Dry salted Australian sheep casings (AA 22-24) were made available by the Van Hessen Company, The Netherlands. These casings were taken from stock and were previously cleaned, scraped, and processed according to Company Standard Operating Procedures. The casings were desalinated in water, checked for holes and divided into pieces of approximately 1 m in length. The casings were tied up at one end, re-salted and stored under ambient conditions according to the company’s specifications.

2.3. Preservation media

To preserve the natural casings under controlled conditions, 4 different brines with specific salt concentrations (molarity: 2.8, 4.0, 5.2 and 6.2 M) and dry salt were prepared for storage of the inoculated casing samples (AW LAB Set H, Novasina, Switzerland). Temperature and pH measurements were made during the entire period of 30 days at regular intervals.

2.4. Inoculation procedure

The prepared casings were rinsed in brines with similar molarities as their final preservation brine to remove any salt particles. The cleaned casings were weighed and corresponding amounts of inocula in a 1:1 ratio with TSB were brought into the casings using a blunt-tipped needle resulting in a primary contamination of...
Dilutions were made to create countable dilutions. Specific department protocols were based on Houben (2003) for tetthuis (2003) for textbook peptone (Colworth Stomacher casings were weighed into a 19-fold volume of buffered water (BPW), according to the protocol by Houben (2005). This dilution was mixed using a Stomacher Lab System Model 400 bags, Seward, London, UK) for 120s. Decimal dilutions were made to create countable dilutions. Specific department protocols were based on ISO methods and the textbook Microbiology of Foodstuffs by Dijk and Groothuis (2005) for further bacterial isolation and confirmation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plating medium</th>
<th>Pre-enrichment</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>MacConkey Agar</td>
<td>Buffered peptone water (BPW)</td>
<td>(24h, 37°C)</td>
</tr>
<tr>
<td>O157:H7</td>
<td>CT SMAC</td>
<td></td>
<td>nTSB + norethioptic</td>
</tr>
<tr>
<td>S.salivarius</td>
<td>Brilliant Green Agar (BGA)</td>
<td>Buffered peptone water (BPW)</td>
<td>(24-48h, 42°C)</td>
</tr>
<tr>
<td>S.aureus</td>
<td>XLD Agar</td>
<td></td>
<td>Selenite broth</td>
</tr>
<tr>
<td>L.monocytogenes</td>
<td>Compass medium</td>
<td>Half-Frider broth</td>
<td>Frazier broth</td>
</tr>
<tr>
<td>S.aureus</td>
<td>Baird-Parker medium (BP)</td>
<td></td>
<td>Gioldini Cantoni broth</td>
</tr>
<tr>
<td>C.perfringens</td>
<td>Iron Sulfate agar (ISA)</td>
<td></td>
<td>Rapid perfringens medium (RPM)</td>
</tr>
<tr>
<td>L.monocytogenes</td>
<td>ID 12190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.aureus</td>
<td>ID 6988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.perfringens</td>
<td>ID 7937</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

468 log_{10} CFU g^-1 for E.coli, 2.30 log_{10} CFU g^-1 for E.coli O157:H7, 4.78 log_{10} CFU g^-1 for S.typhimurium, 5.29 log_{10} CFU g^-1 for L.monocytogenes, 4.51 log_{10} CFU g^-1 for S.aureus and 4.18 log_{10} CFU g^-1 for C.perfringens. The open end of each casing was tied up and the inocula were distributed through each casing. Subsequent batches of 8 pieces (corresponding to inoculum and preservation medium) were weighed into an equal volume of its specific preservation medium and shaken (120 oscillations min^-1) for 1h, after which they were returned to their original brine. All inoculations were done in duplicate and representative samples of the casings were checked prior to inoculation on the presence of contaminating bacteria in order to create a negative control; no contamination was present. The procedures used for these checks prior to inoculation were similar to the procedures used for the final microbiological analysis (Table 2).

2.5. Microbiological analysis

At 1, 3, 6, 8, 13, 20, 27 and 30 days after inoculation, a single piece of casing for each aw was cut into 3 cm pieces. To avoid any negative interference from the salt, the casings were weighed into a 19-fold volume of buffered peptone water (BPW), according to the protocol by Houben (2005). This dilution was mixed using a Stomacher (Colworth Stomacher 400, Stomacher Lab System Model 400 bags, Seward, London, UK) for 120s. Decimal dilutions were made to create countable dilutions. Specific department protocols were based on ISO methods and the textbook Microbiology of Foodstuffs by Dijk and Groothuis (2003) for further bacterial isolation and confirmation. Since monocolonies were used for the inoculations, less selective media could be used to provide better yields and more accurate cell counts. The selected ISO methods and media used for plating and enrichment are given in Table 2.

The first step in the analysis of C. perfringens spores was the heat treatment of the tubes containing the first dilution (in BPW) to eliminate vegetative cells. A thermostatically controlled water bath was used at 75±0.1°C for exactly 15 min after the temperature in a control tube reached 75°C. According to Hooben (2005) the Iron Sulphite Agar (ISA) was a suitable medium for Clostridium spores in natural casings and therefore incorporated into our protocols.

2.6. Statistical analysis

According to Lenovitch (1987), the graphic illustration of microbial survival can be obtained by plotting the logarithm of survivors against time. Since these plots are generally linear, survival curves can be described by linear correlations. The slopes of the survival curves were calculated using regression analysis. The absolute values of these slopes were defined as death rates (day^-1). The statistical analyses were carried out using SPSS Version 12.0.1 software (SPSS, IL, USA).

3. Results and discussion

The brine concentrations and dry salt samples were measured repeatedly for their water activity, giving aw levels of 0.90aw, 0.87aw, 0.85aw, 0.83aw, and 0.75aw, respectively. The survival characteristics of the non-spore forming bacteria at these aw levels are presented in Table 3. Overall it can be stated that Gram-positive bacteria are less sensitive to lowered aw than Gram-negative bacteria. This
is consistent with the fact that Gram-positive bacteria are better equipped to cope with osmotic stress than Gram-negatives (Mellefont et al., 2003). Gram-positive bacteria have constitutive transport systems for the uptake of compatible solutes, whereas Gram-negatives need to implement transport systems (Mellefont et al., 2003). Until this implementation has taken place, accumulation of potassium and its counterion glutamate is used to maintain turgor, giving a more severe plasmolysis during osmotic stress (Gutierrez et al., 1995). This impairment of enzymes together with the more severe plasmolysis gives an explanation for the higher death rates observed for Gram-negative bacteria at lower \( a_w \).

As illustrated in the introduction, there are very few studies done on the survival of pathogens in natural casings preserved in salt. Only the study by Gribis and Siliker (1974) on the survival of *Salmonella* spp. showed a decrease of approximately one log-cycle in 3 days in saturated brine (0.75aw). A death rate of 0.33 day\(^{-1}\) can be calculated, which corresponds to our findings. In recent years, the survival of *E. coli* O157:H7 in many other foodstuffs has been the subject of several studies. Rousk et al. (1996) showed a decrease of approximately 0.14 log cfu g\(^{-1}\) day\(^{-1}\) at 20\(^\circ\)C and 0.90aw in salami and Park and Beachot (2000) showed a decrease of approximately 4.40 log cfu g\(^{-1}\) week\(^{-1}\) in starch. These values are higher than the death rates found in this study, which could be explained by the relatively low inoculation levels used for *E. coli* O157:H7 and the differences in composition of the foods used in the different models (Park and Beachot, 2000).

A study on the survival of *L. monocytogenes*, (Nolan et al., 1992) recorded a reduction of five log-cycles in 7 days at 0.91aw in TSB-YE and added sodium chloride. This is a markedly higher death rate than was observed in our study, but the data from the Nolan study were obtained at \( a_w \) levels in the bacterial growth range whereas lower \( a_w \) ranges as reported by Uzelac and Stille (1977), will increase survival. These findings correspond to our findings where *L. monocytogenes* survived for up to 30 days at an \( a_w \)-level of 0.85.

Table 1 summarized survival of bacterial species in natural casings at different \( a_w \)-levels

<table>
<thead>
<tr>
<th>Species</th>
<th>0.90aw</th>
<th>0.85aw</th>
<th>0.83aw</th>
<th>0.81aw</th>
<th>0.75aw</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.11 [0.07-0.13]</td>
<td>0.66 [0.05-0.09]</td>
<td>0.71 [0.06-0.11]</td>
<td>0.21 [0.04-0.09]</td>
<td>0.79 [0.00-0.09]</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>0.10 [0.08-0.16]</td>
<td>0.66 [0.05-0.11]</td>
<td>0.67 [0.08-0.09]</td>
<td>0.31 [0.06-0.09]</td>
<td>0.90 [0.07-0.13]</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>0.17 [0.13-0.20]</td>
<td>0.88 [0.05-0.11]</td>
<td>0.80 [0.06-0.12]</td>
<td>0.32 [0.08-0.12]</td>
<td>0.88 [0.06-0.14]</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>0.07 [0.05-0.09]</td>
<td>0.84 [0.05-0.12]</td>
<td>0.89 [0.08-0.09]</td>
<td>0.10 [0.02-0.08]</td>
<td>0.90 [0.05-0.08]</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.11 [0.08-0.14]</td>
<td>0.79 [0.09-0.14]</td>
<td>0.82 [0.09-0.14]</td>
<td>0.11 [0.06-0.14]</td>
<td>0.81 [0.08-0.13]</td>
</tr>
</tbody>
</table>

\(a_w\)-values as low as 0.86 (Abee and Wouters, 1999; Gutierrez et al., 1995). This is caused by the highly effective transport systems of *S. aureus* for compatible solutes. Nevertheless, a severe decrease in viable cells was observed over the 30-day period. The reason for this subsequent cell-death could be found in a shortage of compatible solutes or substrate for compatible solutes, since *S. aureus* needs exogenous supply of these substances (Gutierrez et al., 1995). Shortage of energy can be another factor leading to death of the bacteria, since many mechanisms required to maintain turgor are energy-dependent (Abee and Wouters, 1999; Verheul et al., 1997).

No clear reduction in the survival of *C. perfringens* in the relation to any \( a_w \)-level. *S. aureus* is known to be the most halotolerant non-halophilic bacterium, and can grow at \( a_w \)-values as low as 0.86 (Abee and Wouters, 1999). This left only a few cells to sporulate, which were re-grown using the enrichment procedures as described in Table 2. Due to the fact that *C. perfringens* is a natural inhabitant of the intestinal tract of many animals (Brynestad and Osvan, 2000), the *Clostridium* spores were not only found during the casing processing (Ockerman and Hansen, 2000), but their presence was also confirmed in salted natural casings, sometimes at high concentrations (Horben, 2003). The recurring re-growth of bacterial spores as found in our study (results not shown) clearly demonstrates the resistance to low \( a_w \)-levels of *Clostridium* spores.

In this study, cultures were diluted in TSB, prior to insertion of the inoculum into the casing samples. Since one of the ingredients of TSB is soy-peptone, a known substrate for several compatible solutes (Sletor et al., 2003), this may have had a protective effect on the bacteria. The presence of soy-peptone may have therefore artificially increased the survivability of the bacteria. The temperature at which the casings were stored (20±1.5\(^\circ\)C) was within the range which provides the highest tolerance against osmotic stress (Mellefont et al., 2003). The measured pH was close to neutral during the storage of the casings and no detrimental influence was to be
expected. The results found in this study may therefore represent the maximal survival of specific pathogens in salt-preserved natural casings. With the exception for L. monocytogenes, all non-spore forming bacteria could not be positively identified after the mandatory 30-day preservation period for natural casings at a water activity level of 0.85 or lower (Fig. 1). L. monocytogenes could not be positively identified at a water activity level of 0.75 after 30 days. In general the casing industry uses either dry salt (aw-level 0.75) or saturated brine (aw-level between 0.75 and 0.80) for preservation and the storage period will exceed the minimum required 30-day period. This way a clear safety margin exists for all non-spore forming bacteria to be well below any microbiological critical limit currently in use for preserved natural casings (Table 1).

4. Conclusions

The results found in this study can be directly applicable to the natural casing industry and may support the known antimicrobial properties of salt used in the traditional methods for the preservation of natural casings. The inoculation levels used reflect the actual bacterial counts to the natural casing industry and may support the known hurdles for the preservation of natural casings. By measuring the water activity level at which no bacteria could be found or where the hurdles represent the maximal survival of specific pathogens in salt-preserved natural casings.

Acknowledgements

The enthusiastic and skilled assistance of Ali Eggenskamp, Angelie Timars and Ibra Awil, Department of Public Health and Food Safety, is gratefully acknowledged.

References

Roccella, M., Clavero, S., Beuchat, L.R., 1996. Survival of E. coli О157:H7 in broth and processed ultrasound as influenced by pH, water activity,

Web references
http://www.food-info.net/uk/intj/intro.htm
http://www.cfsan.fda.gov/~mow/intro.htm
## Category 1. Adverse impacts on humans or the environment?

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>N/A¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Are there adverse effects on environment from manufacture, use, or disposal? [§205.600 b.2]</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2. Is there environmental contamination during manufacture, use, misuse, or disposal? [§6518 m.3]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Is the substance harmful to the environment? [§6517c(1)(A)(i), §6517c(2)(A)(i)]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Does the substance contain List 1, 2, or 3 inert? [§6517 c(1)(B)(ii); §205.601(m2)]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Is there potential for detrimental chemical interaction with other materials used? [§6518 m.1]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Are there adverse biological and chemical interactions in agro-ecosystem? [§6518 m.5]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Are there detrimental physiological effects on soil organisms, crops, or livestock? [§6518 m.5]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Is there a toxic or other adverse action of the material or its breakdown products? [§6518 m.2]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Is there undesirable persistence or concentration of the material or breakdown products in environment? [§6518 m.2]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Is there any harmful effect on human health? [§6517 c(1)(A)(i); §6517 c(2)(A)(i); §6518 m.4]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Is there an adverse effect on human health as defined by applicable Federal regulations? [§205.600 b.3]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Is the substance GRAS when used according to FDA’s good manufacturing practices? [§205.600 b.5]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? [§205.600 b.5]</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.
# Category 2. Is the Substance Essential for Organic Production?

## Substance: Natural Casings

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
<th>Documentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is the substance formulated or manufactured by a chemical process?</td>
<td></td>
<td>X</td>
<td></td>
<td>[6502 (21)]</td>
</tr>
<tr>
<td>2. Is the substance formulated or manufactured by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral, sources?</td>
<td></td>
<td>X</td>
<td></td>
<td>[6502 (21)]</td>
</tr>
<tr>
<td>3. Is the substance created by naturally occurring biological processes?</td>
<td>X</td>
<td></td>
<td></td>
<td>[6502 (21)]</td>
</tr>
<tr>
<td>4. Is there a natural source of the substance? [§205.600 b.1]</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Is there an organic substitute? [§205.600 b.1]</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Is the substance essential for handling of organically produced agricultural products? [§205.600 b.6]</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Is there a wholly natural substitute product? [§6517 c (1)(A)(ii)]</td>
<td>X</td>
<td></td>
<td></td>
<td>Casings are wholly natural. And agricultural.</td>
</tr>
<tr>
<td>8. Is the substance used in handling, not synthetic, but not organically produced? [§6517 c (1)(B)(iii)]</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Is there any alternative substances? [§6518 m.6]</td>
<td>X</td>
<td></td>
<td></td>
<td>Peelable Cellulose (synthetic), Eatable Collagen (synthetic)</td>
</tr>
<tr>
<td>10. Is there another practice that would make the substance unnecessary?</td>
<td></td>
<td>X</td>
<td></td>
<td>If an uninterrupted supply of organic natural casings were commercially available non-organic natural casings would not be necessary</td>
</tr>
</tbody>
</table>

1If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.
Category 3. Is the substance compatible with organic production practices?

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
<th>Documentation (TAP; petition; regulatory agency; other)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is the substance compatible with organic handling? ([205.600 b.2])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Is the substance consistent with organic farming and handling? ([6517 c (1)(A)(iii); 6517 c (2)(A)(ii)])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Is the substance compatible with a system of sustainable agriculture? ([6518 m.7])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Is the nutritional quality of the food maintained with the substance? ([205.600 b.3])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Is the primary use as a preservative? ([205.600 b.4])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Is the primary use to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law, e.g., vitamin D in milk)? ([205.600 b.4])</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories: a. copper and sulfur compounds; b. toxins derived from bacteria; c. pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; d. livestock parasiticides and medicines?</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If the substance under review is for crops or livestock production, all of the questions from 205.600 (b) are N/A—not applicable.