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(71) Applicant(s)
Bernard Matthews Limited

(72) Inventor(s)
Hall, Jeremy

(74) Agent / Attorney
Phillips Ormonde Fitzpatrick, L 16 333 Collins St, Melbourne, VIC, 3000

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(71) Applicant (for all designated States except US): **BERNARD MATTHEWS LIMITED** [GB/GB]; Great Witchingham Hall, Great Witchingham, Norwich, Norfolk NR9 5QD (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HALL, Jeremy** [GB/GB]; Walnut Tree House, The Butts, Kenninghall, Norwich, Norfolk NR16 2EQ (GB). **NORMANTON, John** [GB/GB]; Oxandia Limited, Orchard Lea, Horns Lane, Combe, Witney, Oxfordshire OX29 8NH (GB).

(74) Agent: **BRADLEY, Adrian**; Cleveland, 10 Fetter Lane, London EC4A 1BR (GB).

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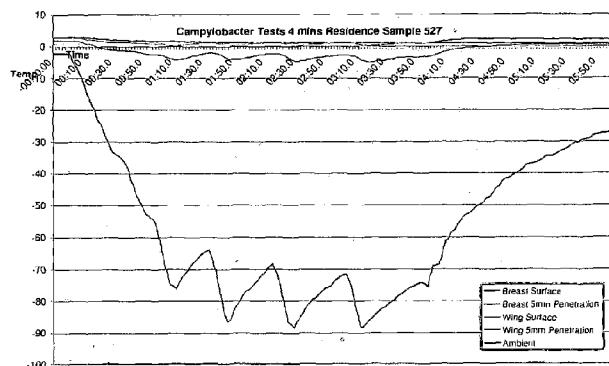
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Figure 1



(57) Abstract: The invention relates to process for reducing the number of viable microorganisms present on the surface of meat, especially poultry. The process comprising the steps of rapidly cooling the surface membrane of a contaminated meat item to a sequence of temperatures sufficient to reduce the number of viable bacteria, whilst at the same time retaining in the muscle meat the organoleptic and nutritional qualities of fresh meat. *Campylobacter spp.* are successfully controlled by the methods disclosed.

WO 2012/168685 A1

Food Hygiene Method and Food Product**Summary**

5 The invention relates to a process for reducing the number of viable microorganisms present on the surface of meat, and to meat items so treated.

Introduction

10 Food-borne pathogens represent a significant and serious threat to human and animal health. Numerous species of microorganism reside naturally on many types of food, some of which are capable of causing diseases in man (and other animals) upon ingestion. Sensible precautions, such as thorough cooking at an appropriate temperature, observation of correct storage protocols for raw and cooked food, and 15 adhering to proper hygiene standards when handling food, can all reduce but not eliminate incidence of such disease.

Amongst the disease-causing pathogens, *Campylobacter* is the most common bacterial cause of food poisoning. It is responsible for around 62,000 reported cases 20 of illness in the UK every year; however, the estimated number of actual incidences is much higher, of the order of 600,000, as it is believed that most individuals affected by the pathogen do not seek medical assistance. The total number of campylobacter cases across the European Union states is calculated at 9 million cases per annum.

25 *Campylobacter* infection is also extremely common in retail poultry. The prevalence of *Campylobacter* in chicken at retail in the UK was 65.2%, based on the results from both methods combined, for the 927 samples tested (Food Survey Information sheet 04/09, *A UK survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale*, UK Food Standards Agency).

30 *Campylobacter* is therefore one of the key organisms that health agencies are tackling in order to reduce levels of food-borne illness. *Campylobacter* can be found on meat, unpasteurised milk, and in untreated water; however there is strong 35 evidence that poultry is the most common cause of illness.

Campylobacter is naturally present in many poultry birds, and is often found in the cecum. Transfer to the skin is thought to occur when the birds are slaughtered and eviscerated.

5 It would be very desirable to kill or remove *Campylobacter* (and other pathogens) from meat, in particular poultry and poultry products, which are intended for human (or animal) consumption.

Brief Description of the Prior Art

10

A known method of disinfecting poultry carcasses involves spraying or immersing the carcasses with water containing antimicrobial agents. However, in Europe, legislation specifies that potable water must be used to wash carcasses.

15 GB 2105570 (A) discloses a method of reducing "weepage" in fresh-pack poultry that includes the steps of first washing the carcasses with jets of unrefrigerated water to pre-cool them and add pick-up moisture, then tumbling the carcasses to equalize the amount of pick-up moisture carried by each carcass, to reduce the general level of pick-up moisture content somewhat, draining the carcasses by gravity to remove
20 surface moisture, and finally subjecting them to a supercold atmosphere, such as may be obtained by directing expanded liquid carbon dioxide in rapidly moving currents over the carcasses for a time to freeze-crust the carcasses.

25 The "freeze-crusting" shrinks the skin to squeeze out pick-up moisture from the fatty fascia layer under the skin to reduce the pickup moisture level to the allowable amount of 8%, and to remove sufficient body heat that when a carcass is then allowed to temper to an even temperature throughout, it will be below the freezing point of water, but above the -3.30 °C freezing point of the flesh, for a very short time. The process is said to reduce seepage from the poultry products so treated.

30

The method is said to inhibit bacterial growth, and thus prolong shelf life. There is no disclosure of the method having any bactericidal effect, however.

35 US 3637405 discloses a process for packaging and preserving meats to give long shelf life and greater tenderness. The portioned or whole chicken is exposed to cold air at a temperature of -40 °C for 1 hour. The crust-frozen chicken is then held at 0 °C for at least 3 hours. The method is said to inhibit bacterial growth, and thus prolong

shelf life. There is no disclosure of the method having any bactericidal effect, however.

WO 2004080189 discloses a method comprising rapidly cooling meat by exposure to

5 a rapid cooling temperature of below about -10 °C for sufficient time to provide a frozen crust on the meat and chilling the resultant crust-frozen meat by exposure of said crust-frozen meat to a chilling temperature greater than the rapid cooling temperature but no higher than about +10 °C to raise the temperature of the surface of the meat and to maintain said surface at a temperature no higher than the freezing

10 temperature of the meat for at least sufficient time to injure lethally and/or kill bacteria, is used to reduce the viability of bacteria on meat. The method is stated to have particular application in the processing of poultry meat to kill bacteria comprising the *Campylobacter* and/or *Salmonella* species.

15 Although the method disclosed in WO 2004080189 apparently achieves the objective of reducing the count of *Campylobacter* (and other bacterial species), the poultry treated according to the process cannot be sold in the EU as "fresh". The sale of poultry either as whole birds or portions is regulated under the EC Poultrymeat Marketing Regulation 1906/90 (which has been incorporated into a combined EC

20 Marketing Regulation 1234/2007). This Regulation categorizes poultry meat and only allows poultry to be marketed as fresh, frozen or quick-frozen. Fresh poultry is defined 'fresh poultry meat' means poultry meat not which has not been stiffened at any time by the cooling process, prior to being kept at a temperature not below -2°C and not higher than +4°C. It is not permitted to market chilled/fresh poultry which has

25 been previously frozen and then thawed.

Studies have been conducted to determine rates of *Campylobacter jejuni* inactivation on poultry exposed to different cooling and freezing temperatures (*Reduction of C. jejuni on the Surface of Poultry by Low Temperature* (J. Food Prot, 66, 4, 2003, 652-655)). A mixture of three strains of *C. jejuni* originally isolated from poultry was inoculated onto chicken wings. Storage of wings at -20 and -30°C for 72 h reduced the population of *C. jejuni* on wings by 1.3 and 1.8 \log_{10} CFU/g, respectively. Protocols were developed to superchill chicken wings with liquid nitrogen at -80, -120, -160, and -196 °C such that the internal portion of each wing quickly reached -3.3 °C but did not freeze. The study concluded that conditions used in the poultry industry to superchill poultry to a nonfrozen-state internal temperature are not likely to substantially reduce *Campylobacter* populations on fresh products.

26 Nov 2013

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

Summary of the Invention

5

In a first aspect, the invention relates to a process for reducing the number of viable microorganisms present on the surface of a meat item, comprising the steps of:

- a) providing an untreated meat item;
- 10 b) exposing the meat item to an atmosphere at a temperature T^1 ;
- c) rapidly reducing the temperature of the atmosphere at a selected cooling rate to a temperature T^2 below -20 °C;
- d) optionally maintaining the atmosphere at temperature T^2 for a selected period of time;
- 15 e) allowing the meat item to warm, optionally at a selected slow warming rate, by adjusting the temperature of the atmosphere to a temperature T^3 ;

characterized in that

20 selected temperatures T^1 , T^2 and T^3 , the selected cooling rate, selected period of time and selected warming rate are selected such that the number of viable microorganisms present on the surface is reduced, whilst the temperature of the meat item does not fall below -2 °C and remains unfrozen.

25 In a second aspect, the invention provides a process for reducing the number of viable microorganisms present on the surface of meat, comprising the steps of:

- a) providing an untreated meat item having a surface membrane and muscle tissue, said untreated meat item having viable microorganisms on the surface membrane;
- 30 b) exposing the surface membrane to cooling at a selected cooling rate until the surface membrane reaches a selected first temperature;
- c) optionally maintaining the surface membrane at the selected first temperature for a selected period of time;
- 35 d) allowing the surface membrane to warm to a selected second temperature higher than the first temperature to give a treated meat item;

characterized in that

the selected rate of cooling, the selected first temperature, selected period of time and selected second temperature are selected such that the number of viable microorganisms present on the surface membrane is reduced, whilst the β -hydroxyacyl-CoA dehydrogenase (HADH) activity of the muscle tissue is not significantly increased in the treated meat item compared with the untreated meat item.

10 According to a third embodiment, there is provided a meat item prepared by the process of the invention.

Summary of the Figures

15 Figure 1 is a graph showing the temperature of the surface, interior and atmosphere of a turkey carcass treated for 4 minutes

Figure 2 is a graph showing the temperature of the surface, interior and atmosphere of a turkey carcass treated for 40 seconds.

Figure 3 is a graph showing the temperature of the surface, interior and atmosphere of a turkey carcass treated for 1.5 minutes.

20 Figure 4 is a graph showing the temperature of the surface, interior and atmosphere of a turkey carcass treated for 2 minutes.

Figure 5 is a graph showing the temperature of the surface, interior and atmosphere of a turkey carcass treated for 1 minute.

Figure 6 is a graph showing a summary of Campylobacter levels on chickens – 25 excluding warm bird data on day K+1.

Figure 7 is a graph showing a summary of Campylobacter levels on chickens – excluding warm bird data on day K+1.

Figure 8 is a graph showing a summary of Campylobacter levels on chickens – excluding warm bird data on day K+1.

30 Figure 9 is a graph showing a summary of Campylobacter levels on chickens – excluding warm bird data on day K+1 with 2 min treatment time.

Figure 10 is a graph showing a summary of Campylobacter levels on chickens – excluding warm bird data on day K+1 with 2.5 min treatment time.

Figure 11 is a graph showing a summary of Campylobacter levels on chickens – 35 excluding warm bird data on day K+1 with 2.5 min treatment time.

Figure 12 is a graph showing the temperature profile of a meat item subject to an embodiment of the inventive process.

Figure 13 is a graph showing the temperature of the surface, interior and atmosphere of a chicken carcass treated for 1 minute.

Detailed Description of the Preferred Embodiments

5

The term "meat" as used herein refers to any form of edible (by human or animal) flesh, and includes, without limitation, pork, lamb, mutton, veal, beef, venison, fish, shellfish, molluscs and poultry. The methods of the invention are, however, particularly appropriate for the treatment of poultry.

10

"Poultry" in this context includes edible birds of any type. Examples of poultry are chicken, turkey, pheasant, quail, duck, goose, guinea fowl and swan. Chicken and turkey are preferred.

15 15 The term "meat item" encompasses whole animal carcasses, both entire and eviscerated, as well as portions of meat (cuts), which comprise at least a proportion of muscle tissue. Examples of meat items are whole, eviscerated poultry carcasses, and poultry breasts, thighs, drumsticks, lower legs and wings.

20 20 The term "untreated meat item" refers to a meat item as hereinbefore defined, which has not been subjected to the inventive process. It may (and preferably will have) undergone a number of preceding steps customary in the preparation of meat, such as stunning, slaughter, evisceration, scalding, removal of head and legs, defeathering, water or air chilling and jointing.

25

25 The term "microorganism" refers to any organism that is capable of causing illness or disease in humans or other animals. Examples of microorganisms include bacteria, fungi, archaea, and protists. Preferred microorganisms which are controlled by the methods of the invention include *Campylobacter spp.*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella spp.*, *Bacillus cereus*, *Shigella spp.*, *Staphylococcus aureus*, *Streptococcus*, *Listeria monocytogenes*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Coxiella burnetii*, *Brucella spp.*, *Corynebacterium ulcerans*, and *Plesiomonas shigelloides*. More preferred microorganisms are *Campylobacter spp.* and *Salmonella spp.* Highly preferred are gram negative bacteria, especially *Campylobacter* species including *Campylobacter jejuni* and *Campylobacter coli*, especially *Campylobacter jejuni*.

The term "viable microorganism" refers to a microorganism as hereinbefore defined, which is capable of causing illness or disease when ingested. It encompasses nonculturable bacteria that are in a state of very low metabolic activity and do not 5 divide, but are alive and have the ability and become culturable once resuscitated, as well as culturable and reproducing microorganisms.

The term "membrane" refers to any biological layer which covers or adheres to the surface of the meat item to be treated, which is not muscle tissue. It includes, for 10 example, skin (dermis and epidermis), as well as layers of fat or cartilage. It also includes the inner membrane of the body cavity of eviscerated poultry fowl.

The term " β -hydroxyacyl-CoA dehydrogenase (HADH) activity" refers to the activity muscle mitochondrial enzyme, β -hydroxyacyl-CoA-dehydrogenase, which is released 15 into the intracellular fluid when the mitochondrial membranes are damaged during freezing and thawing.

β -hydroxyacyl-CoA dehydrogenase (HADH) activity is measured using the protocol described in "The Effect of Superchilling and Rapid Freezing on the HADH Assay for 20 Chicken and Turkey", *J. Assoc. Publ. Analysts*, 2010, **38**, 13-23, a modification of the method described in "Verification of Labelling of Previously Frozen Meat and Poultry by Measurement of HADH Activity" Hargin, K., *J. Assoc. Publ. Analysts*, 1997, **33**, 1-46. These documents are incorporated herein by reference. The protocol is briefly summarised below.

25 A cuboid shaped portion of flesh with base dimensions of approximately 30x30mm and a height of 20mm is used for the HADH test. For the poultry cuboid it is necessary to have six cut surfaces.

30 Preferably, the cuboid is taken from a predetermined depth below the surface of the surface membrane, such as between 1 and 10 mm below the surface.

35 Any excess surface liquid on the sample is wiped off with a tissue. The meat juice is expressed from the sample and diluted with a phosphate buffer. Aliquots of EDTA, phosphate buffer and NADH are added to diluted press juice, followed by acetoacetyl-Coenzyme A solution, in a quartz spectrophotometer cuvette. Using a U.V. spectrophotometer the rate of conversion of NADH to NAD⁺ is measured by the

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rate of decrease in the absorption of the solution. The extinction at 340nm is read. The difference between the two readings ΔE is the decrease in absorption at 340nm.

$$10 \quad \text{HADH activity U/ml} = \frac{V \times \Delta E / \text{min} \times \text{dilution factor of meat juice}}{C \times d \times a}$$

Where V = volume of test mixture = 3ml
 C = extinction co-efficient for NADH at 340nm = 6.3
 d = light path of cell = 1cm
 a = volume of diluted meat juice = 0.1ml
 T = time over which decrease in absorption is measured in minutes

$$15 \quad \Delta E / \text{min} = \underline{\text{Extinction at start of reaction} - \text{extinction at } T \text{ min}}$$

The equation becomes:-

$$20 \quad \text{HADH activity U/ml} = \frac{3 \times \Delta E / \text{min} \times \text{dilution factor of meat juice} *}{6.3 \times 1 \times 0.1}$$

(* e.g. 200 for beef)

25 The juice pressed from meat that has been frozen and thawed will therefore exhibit higher HADH activity than meat which has not been previously frozen. Since some HADH may be released when the meat is cut during sample preparation the method is a comparative one, where the HADH activity is determined on the untreated meat item as received (X_0), and then determined after treatment (X_1). The ratio of the HADH activities (X_1/X_0) is referred to as the R_1 value. If the R_1 value is less than 1.2, preferably less than 1.1, more preferably less than 1.05, then the HADH activity of 30 the meat or poultry is regarded as not significantly increased.

35 Cooling may be achieved by any one of a number of methods. Meat items may be placed in a chamber containing air (or other refrigerant gas) held at an appropriate temperature to achieve the selected cooling rate. One or more gas circulation means (such as fans, blowers etc.) may be provided to increase the flow of cold air over the meat item, to increase the rate of cooling of the surface membrane.

Alternatively, and preferably, the surface membrane of the meat item may be cooled with a cryogen. Suitable cryogens include liquefied gasses, such as liquid nitrogen, liquid air, liquid carbon dioxide and liquid argon, and solids, such as solid carbon dioxide ("dry ice", preferably in finely divided form), and combinations of these.

5 Liquid nitrogen is a preferred cryogen.

Cryogen may be applied to the surface membrane as a jet, spray or bath. It is preferred that cryogen is applied as a spray. Various types of spray nozzle, such as roses or spraybars may be utilised, depending on the size and shape of the meat 10 item to be treated.

Preferably, the cryogen is applied in combination with an impinging gas. Impinging gas serves to convey the cryogen to the surface membrane of the meat item and thus increases the rate of cooling. Suitable impinging gasses include nitrogen, air, 15 and carbon dioxide, and mixtures of these. The pressure of the impinging gas, and the amount relative to the cryogen, are adjusted so as to achieve the selected cooling rate.

One embodiment of the process utilizes impingement type gas flow of cryogen, such 20 as carbon dioxide or nitrogen gas, in a straight pass-through configuration. The meat item is loaded into one end of the apparatus, and is removed with the surface membrane at a selected first temperature at the opposite end. Conveyors or driven shackle lines may be used to convey the meat item through the cooling apparatus and process.

25 In certain embodiments, the meat item is conveyed for surface cooling along a passage formed between a pair of impingement plates through which a cooling flow of a cryogen, such as carbon dioxide or nitrogen gas, is circulated to cool the surface membrane. In an alternative embodiment, the impingement plates may be disposed 30 to one side, as opposed to being beneath the blower which circulates the cryogen. In embodiments where the meat item is a poultry carcass, the conveyer in these embodiments is a shackle conveyor.

In an alternative embodiment, a cryogenic food cabinet freezer is used. A suitable 35 device is the CRYOLINE® CF, supplied by LINDE AG, Linde Gases Division, Seitnerstrasse 70 82049 Pullach, Germany. This embodiment is more appropriate for batch freezing and chilling processes where an in-line system is not appropriate.

In one embodiment, cryogen is supplied to the interior of a meat item by means of a spray nozzle connected to a cannula or probe. This embodiment is convenient for the internal sterilisation of the body cavity of eviscerated poultry carcasses, for 5 example.

As used herein, the term "temperature of the meat item" refers to the average temperature throughout the item. In some embodiments, the temperature of the meat item does not fall below -2 °C at any point during the procedure. Preferably, no 10 part of the meat item falls below -2 °C at any point during the procedure. Preferably, no part of the meat item is frozen at any point during the inventive process.

As used herein, "surface temperature" refers to the average temperature of the whole or part of the surface of a meat item, as measured by a surface temperature 15 thermocouple probe.

As used herein, "interior temperature" refers to the average temperature at a predetermined depth below the surface of the meat item measured by a temperature probe. Preferably, the interior temperature is measured at a depth of at least 3mm 20 below the surface, more preferably at least 5mm. Preferably, the temperature of the interior does not fall below -2 °C at any point during the inventive process.

The temperature of the meat item may be (in the case of freshly slaughtered whole poultry carcasses, for instance), be around or just below body heat, such as between 25 25 and 37 °C. Alternatively, the interior temperature may be around ambient temperature, such as between 15 and 25 °C.

However, it has been found preferable that the temperature of the meat item is below ambient temperature, such as below 20 °C. More preferably, the interior temperature 30 is below 15 °C. Still more preferably, interior temperature is below 10 °C. Still more preferably, the interior temperature is below 5 °C. When employing such initial temperatures, good control of bacteria and consistency of results is achieved.

Preferably, the temperature of the meat item is above 0 °C. More preferably, the 35 interior temperature is above 1 °C. Still more preferably, the interior temperature is above 2 °C.

The untreated meat item is exposed to an atmosphere at an initial temperature T^1 . The atmosphere may be air, nitrogen or any other suitable gas. Preferably, T^1 is between -5 and 20 °C. More preferably, T^1 is between -3 and 10 °C.

5 As used herein, the term "selected cooling rate" refers to the average rate of cooling from initial temperature T^1 to maintenance temperature T^2 . This is illustrated schematically in Figure 12, and is calculated as the difference between T^1 and T^2 (referred to as ΔT^1) divided by the time taken to achieve this reduction (referred to as Δt^1), and is expressed in terms of °Cs⁻¹. The skilled person will appreciate that it is
10 not always practicable or desirable to provide a linear decrease in temperature.

The selected cooling rate is preferably between 0.1 and 10 °Cs⁻¹, more preferably between 0.3 and 1.5 °Cs⁻¹.

15 Temperature T^2 is preferably between -20 and -120 °C. Temperature T^2 is more preferably between -20 and -100 °C. More preferably, temperature T^2 is between -25 and -95 °C. More preferably, is between -40 and -90 °C. Still more preferably, T^2 is between -50 and -80 °C.

20 In preferred embodiments, the atmosphere is maintained at the selected temperature T^2 . Some variation of surface temperature may occur during this maintenance phase, as when cooling is periodically applied. Preferably, such deviations from T^2 are no more than ± 20 °C, more preferably ± 15 °C, still more preferably ± 10 °C.

25 In preferred embodiments, the atmosphere is maintained at the selected temperature T^2 for a selected period of time. The period of time is selected in order to optimise bacterial control in the overall process, whilst avoiding freezing of the of any part of the meat item. It has been found in particular that the duration of the maintenance phase (i.e. the selected period of time at which the atmosphere is maintained at T^2) is
30 a factor in determining, in certain embodiments, the warming rate in the subsequent process step.

35 Preferably, the atmosphere is maintained at the selected temperature T^2 for a period of between 10 seconds and 10 minutes, more preferably between 30 seconds and 5 minutes, still more preferably between 1 minute and 4 minutes.

In the third phase of some embodiments, the atmosphere is warmed at a selected warming rate to a temperature T^3 . Temperature T^3 is above temperature T^2 , such as above -20 °C, more preferably above -10 °C, more preferably above 0 °C. Preferably, T^3 is between -1 and 4 °C.

5

Warming may be achieved by any conventional means, and is most conveniently achieved by simply ceasing the application of cooling. In this case, the latent heat stored in the interior of the meat item will permeate through to the surface of the meat. Alternatively, the meat item can be transferred to a warmer environment, or warm air 10 or other warm gas may be applied to the surface of the meat.

In an alternative embodiment, the ambient temperature is gradually raised by passage of the meat item through an area having a temperature gradient, such as an in-line processing tunnel having a temperature which varies along its length.

15

As used herein, the term "selected warming rate" refers to the average rate of warming from maintenance temperature T^2 to final temperature T^3 . This is illustrated schematically in Figure 12, and is calculated as the difference between T^2 and T^3 (referred to as ΔT^2) divided by the time taken to achieve this reduction (referred to as 20 Δt^2), and is expressed in terms of °Cs⁻¹. The skilled person will appreciate that it is not always practicable or desirable to provide a linear increase in temperature.

Preferably, the selected warming rate is less than 20 °Cs⁻¹, more preferably less than 10 °Cs⁻¹, more preferably less than 5 °Cs⁻¹, more preferably less than 2 °Cs⁻¹, more 25 preferably less than 1 °Cs⁻¹, still more preferably less than 0.5 °Cs⁻¹, most preferably less than 0.1 °Cs⁻¹.

Surprisingly, it has been found that warming the meat item at a slower warming rate from maintenance temperature T^2 to final temperature T^3 leads to enhanced 30 reduction of the number of viable microorganisms present on the surface of a meat item. This was contrary to the expectations of the inventors, and the reasons for this phenomenon are not properly understood.

A number of factors affect the warming rate. These include the maintenance 35 temperature T^2 , the selected period of time at which the meat item is held at this temperature, the initial temperature T^1 , the mass of the meat item and its surface

area to mass ratio. In particular, a slow warming rate which favours bacterial control is favoured by a low temperature T^1 .

5 In some embodiments, the surface membrane of the meat is exposed to cooling conditions until the surface membrane reaches the selected first temperature. The selected first temperature is chosen such that at least a proportion of the viable microorganisms present on the surface become non-viable when allowed to warm to the selected second temperature.

10 The prior art indicates that in order to achieve successful control of microorganisms present on the surface of meat, rapid cooling to a temperature of less than -10 °C for sufficient time to provide a frozen crust on the meat, followed by subsequent warming, is necessary. Surprisingly, the inventors have found that an extremely effective antimicrobial effect can be achieved by rapid surface chilling to temperatures well 15 above -10 °C. In addition, the prior art indicates that a frozen crust is necessary to achieve the sterilisation effect. Surprisingly, the inventors have found that providing a frozen crust is not a requirement, and indeed superior results are obtained in certain instances when the surface membrane remains unfrozen.

20 Preferably, the selected first temperature is below 2 °C. More preferably, the selected first temperature is below 1 °C. More preferably, the selected first temperature is below 0.5 °C. Still more preferably, the selected first temperature is below 0 °C. Still more preferably, the selected first temperature is below -0.5 °C. Preferably, the selected first temperature is above -5 °C. More preferably, the selected first 25 temperature is above -4 °C. Still more preferably, the selected first temperature is above -3.5 °C. Still more preferably, the selected first temperature is above -2.5 °C. Most preferably, the selected first temperature is above -2 °C. Utilising a temperature above -2 °C has the advantage that meat treated according to the 30 process fulfils the requirement that 'fresh poultry meat' is to be kept at a not below -2 °C at any time

35 Preferably, the selected first temperature being in the range of between -5 °C and 2 °C. More preferably, the selected first temperature is between -3 °C and 1 °C. Still more preferably, the selected first temperature is between -2 °C and 0.5 °C

The selected first temperature and selected second temperature refer to temperature of the surface as measured by infrared thermometer or a probe inserted within or just below the membrane.

5 Prior to exposure of the surface membrane to the cooling step of the invention, the meat item may be at ambient temperature (for example, between 20 °C and 29 °C), or it may be (and preferably is) pre-chilled to below ambient temperature. In one embodiment, the meat item is pre-chilled to a temperature below ambient temperature, such that the temperature of the meat item is substantially uniform
10 throughout the meat item. Preferably, the meat item is pre-chilled to below 15 °C, more preferably to below 10 °C, still more preferably to below 5 °C, still more preferably to below 2 °C.

15 It may be more convenient, however, to carry out the process of the invention on meat items which arrive from the preceding steps in the processing plant without a preliminary step of refrigeration.

20 The cooling rate is selected such that the desired temperature reduction of the surface membrane occurs sufficiently rapidly that no substantial change in the temperature of the underlying muscle occurs. The skilled person will be able to determine a suitable cooling rate for each particular type of meat item.

25 In a particularly preferred embodiment, the meat item is cooled by reducing the ambient temperature (i.e. the temperature of the atmosphere surrounding the meat item) at a rate of between 0.1 °Cs⁻¹ and 5 °Cs⁻¹, more preferably at a rate of between 0.2 °Cs⁻¹ and 3 °Cs⁻¹, most preferably at a rate of between 0.5 °Cs⁻¹ and 3 °Cs⁻¹.

30 Preferably, the ambient temperature of the atmosphere surrounding the meat is cooled to a temperature of between -10 °C and -150 °C, more preferably between -20 °C and -100 °C, still more preferably between -30 °C and -90 °C.

35 Preferably, the meat item is exposed to the surface cooling conditions for as short a period of time as feasible. This ensures that the temperature gradient through the meat item is high, and a large differential between the temperature of the surface membrane and the temperature of the underlying muscle is maintained.

Optionally, and preferably, the surface membrane of the meat item is maintained at the preselected temperature for a period of time. This period of time is selected such that insufficient heat is transferred from the muscle meat to cause a significant increase in the activity of HADH.

5

In one preferred embodiment, the cooling is achieved by use of a refrigerated tunnel having a predetermined temperature profile. The rate of passage of the meat items through the tunnel is adjusted so as to obtain the correct rate of cooling. This embodiment is particularly convenient when the process of the invention is part of the 10 production line for the preparation of meat products.

Preferably, the surface membrane of the meat item is maintained at the selected first temperature for between 30 seconds and 20 minutes, preferably between 1 minute and 10 minutes, more preferably between 1.5 minutes and 7 minutes, most 15 preferably between 2 and 6 minutes.

Alternatively, the meat item need not be maintained at the selected first temperature, but on reaching the selected first temperature may instead be warmed or allowed to warm to the selected second temperature.

20

The selected second temperature is higher than the selected first temperature. The differential between the selected first and selected second temperature is selected such that the number of viable microorganisms is reduced, and also to fit efficiently with subsequent steps in the processing of the meat item. Preferably, the selected 25 second temperature is above -1 °C. Preferably, the selected second temperature is below 20 °C, more preferably below 15 °C, still more preferably below 10 °C, still more preferably below 5 °C, most preferably below 4 °C. Very preferably, the selected second temperature is between -1 °C and 4 °C. Maintaining the temperature not higher than +4 °C at any time fulfils the requirement for fresh poultry 30 meat under European legislation.

Quantitative assessment of the number of viable microorganisms present on surface membrane is carried out in accordance with appropriate protocols. For example, *Campylobacter* is quantified according to ISO/TS 10272-2:2006(E) "Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 2: Colony-count technique". Other microorganisms may be quantified using different techniques. The term "number of viable

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microorganisms" will be understood by the skilled person as referring to the number of such microorganisms present per unit surface area of surface tissue.

The rate of cooling, the selected first temperature and the selected second temperature are chosen such that the reduction in the number of viable microorganisms present on the surface of the meat observed is at least statistically significant within the limits of the quantification method used. Preferably, at least a log (ten-fold) reduction in the number of microorganisms present is achieved. More preferably, at least a 2-log (hundred-fold) reduction in the number of microorganisms present is achieved.

Preferably, the R_i of the HADH activity in the treated item is less than 2, more preferably less than 1.5, more preferably less than 1.2, more preferably less than 1.1.

Because the muscle tissue remains largely unaffected by the treatment of the skin (or other surface membrane) according to the present invention, the meat item retains the organoleptic and nutritional qualities of fresh meat.

Subsequent to treatment with the inventive process, the treated meat items may be subjected to any further processes customary for the treatment of fresh meat, such as jointing, chilling (air and water chilling are contemplated), and packing or wrapping. Preferably, the inventive process is one of the last steps on the production line prior to packing to prevent the possibility of recontamination of the treated meat item.

In some embodiments, the present food hygiene methods may be combined with additional food-hygiene treatments. Suitable additional treatments include chemical treatments (such as chlorine, chloramine, chlorite, chlorine dioxide, ozone, organic acids (such as citric and lactic acid), hydrogen peroxide and potassium permanganate. Other treatments include gamma wave irradiation, treatment with steam, treatment with electrified water, and crust-freezing.

The process may also include further process steps such as curing, smoking, salting, pickling, or cooking.

35 **Examples**

The process involved using a CRYOLINE® CF unit which has a temperature control system with an ability to inject cryogenic gases into the unit for cooling purposes.

5 The cabinet has an inner chamber into which slaughtered poultry carcasses were hung by their legs, which allowed the neck skin to hang clear of the bird, and the body cavity entrance to be open.

10 The unit was set to a temperature that allowed rapid cooling of the skin of the bird and other outer or exposed membranes such that a temperature of below -2 °C was achieved, with no parts going below -3.8 °C. This was achieved via the use of a cryogenic gas spray bar which introduced liquid nitrogen into the cabinet, and a circulation fan able to deliver impingement, or rapid temperature exchange, on the exposed membranes.

15 The flesh of the bird was not frozen, but the skin and membranes underwent rapid cooling. This was followed by removal from the unit, and the carcass was then allowed to rise back in temperature to the normal chilled holding range of -1 °C to +4 °C.

20 This process was designed to rapidly cool the surface membrane and *Campylobacter* bacteria present upon the carcass surfaces, and to render them into a cold tempered state, similar to that seen just before the product passes through the latent heat phase, thus avoiding full freezing of the poultry carcasses. The rapid cold shock administered to the bacteria on the bird renders them damaged such that during the 25 temperature rise back to normal chill temperatures the *Campylobacter* bacteria are severely damaged or killed, rendering them unable to create infection.

30 The birds were rapidly chilled in the cabinet and this was completed satisfactorily without the freezing of the muscle units of the birds, in times from around 30 seconds up to a dwell time of five minutes, depending upon carcass size and ingoing temperature.

Example 1

35 The cabinet was set to a temperature of -80 °C (other temperatures delivered the same result but took more or less time to deliver the cold tempered state).

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In some cases the campylobacter bacteria were fully destroyed, and in all cases in excess of a two log reduction was delivered. This damage and destruction of Campylobacter is delivered by faster and simpler means than any previous process.

5 Table 1 indicates the *Campylobacter* count achieved after treatment of naturally infected chicken or turkey carcasses according to this protocol.

Figures 1 to 5 indicate the temperature profile of the surface of turkey carcasses exposed to rapid chilling conditions at i) the surface and ii) 5mm depth penetration of
10 the wing and breast.

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Table 1

Sample No	Temperature/ Time	SURFACE TEMP °C IN	SURFACE TEMP °C OUT	Campylobacter count cfu/g
1	-80°C 2 mins	1-1.5°C	0.5°C	0.00E+00
2	-80°C 2 mins	1-1.5°C	0.5°C	0.00E+00
3	-80°C 4 mins	1-1.5°C	-1.5°C	0.00E+00
4	-80°C 4 mins	1-1.5°C	-1.5°C	0.00E+00
5	-80°C 6 mins	1-1.5°C	0.3 to -0.3°C	0.00E+00
6	-80°C 6 mins	1-1.5°C	0.3 to -0.3°C	0.00E+00
7	-80°C 5 mins	1-1.5°C	-0.2°C	0.00E+00
8	-80°C 5 mins	1-1.5°C	-0.2°C	0.00E+00
9	-80°C 5 mins	1-1.5°C	-1.1 to -1.9°C	0.00E+00
10	-80°C 5 mins	1-1.5°C	-1.1 to -1.9°C	0.00E+00
11	CONTROL	~	~	1.00E+02
12	CONTROL	~	~	5.00E+01
13	CONTROL	~	~	5.00E+01
14	CONTROL	~	~	3.00E+02
15	CONTROL	~	~	2.00E+01
16	CONTROL	~	~	1.00E+01
17	CONTROL	~	~	2.00E+02
18	CONTROL	~	~	1.00E+01
19	CONTROL	~	~	1.00E+01

5

Example 2

All birds were pre chilled to 4 °C as testing was completed externally. They were treated for between 30 seconds and for 2 minutes under the conditions used for

10 Example 1. The reduction in campylobacter count is shown in Table 2.

Table 2

	Sample Description	Day 1			Day 7		
		Aerobic Plate Count per g	Count of presumptive Campylobacter per g	Count of confirmed Campylobacter per g	Aerobic Plate Count per g	Count of presumptive Campylobacter per g	Count of confirmed Campylobacter per g
1	Chicken Control	710,000	500	300	12,000,000	<10	<10
2	Chicken Control	3,200,000	370	370	25,000,000	<10	<10
3	Chicken Control	23,000,000	460	460	12,000,000	90	
4	Chicken Control	1,900,000	210	210	16,000,000	<10	<10
5	Chicken Control	140,000	60	60	13,000,000	<10	<10
6	Chicken Control	380,000	490	490	14,000,000	<10	<10
7	Chicken Control	350,000	830	830	16,000,000	340	
8	Chicken Control	1,100,000	80	<10	21,000,000	<10	<10
11	Chicken Treated	130,000	<10	<10	12,000,000	<10	<10
12	Chicken Treated	84,000	<10	<10	14,000,000	<10	<10
13	Chicken Treated	660,000	<10	<10	16,000,000	<10	<10
14	Chicken Treated	140,000	<10	<10	14,000,000	<10	<10
15	Chicken Treated	140,000	<10	<10	12,000,000	<10	<10
16	Chicken Treated	740,000	110	<10	12,000,000	<10	<10
17	Chicken Treated	450,000	<10	<10	14,000,000	<10	<10
18	Chicken Treated	80,000	<10	<10	13,000,000	<10	<10
19	Chicken Treated	75,000	<10	<10	13,000,000	<10	<10
20	Chicken Treated	120,000	<10	<10	14,000,000	<10	<10
21	Turkey Control	26,000	50	<10	13,000,000	<10	<10
22	Turkey Control	58,000	300	60	16,000,000	50	
23	Turkey Control	26,000	140	84	12,000,000	<10	<10
24	Turkey Control	51,000	300	240	19,000,000	60	
27	Turkey Treated	17,000	10	10	21,000,000	10	
28	Turkey Treated	17,000	<10	<10	23,000,000	<10	<10
29	Turkey Treated	15,000	20	20	16,000,000	<10	<10
30	Turkey Treated	35,000	90	90	18,000,000	<10	<10
31	Turkey Treated	9,900	50	30	19,000,000	20	
32	Turkey Treated	52,000	90	18	23,000,000	20	

This example used only chickens. All birds were pre chilled to 4 °C. They were treated for between 1 minute and 2.5 minutes under the conditions used for Example 1. The reduction in campylobacter count is shown in Table 3.

5

Table 3

Initial Bird Condition	Treatment Time	Day	Control Campylobacter Count (log)	Treated Campylobacter Count (log)	Difference
Cold	1 min	1	2.48	1.9	0.58
Cold	2 min	1	2.48	1.98	0.50
Cold	1.5 min	1	3.75	3.69	0.06
Cold	2.5 min	1	3.75	2.62	1.13
Cold	2.5 min	1	2.44	1.61	0.83
Cold	2.5 min	1	2.80	2.47	0.33
Cold	2.5 min	7	2.38	1.65	0.73
Cold	2 min	1	1.88	1.61	0.27
Cold	2 min	7	1.40	1.04	0.36

Example 4

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Chilled turkey carcasses (10 in total) at 4 °C are treated under the conditions described in example 1 and held in the cryogenic cabinet for 2 minutes to an average surface temperature of -2 °C. All turkeys are allowed to warm to 4 °C. Five turkeys are allowed to warm over a period of 1 minute. The remaining turkeys are allowed to 15 warm over a period of 10 minutes. Warming rates are varied by allowing ingress of ambient temperature air at differing rates. The carcasses allowed to warm more slowly exhibited lower counts of campylobacter compared with those allowed to warm over 1 minute.

20 **Example 5**

Chilled chicken carcasses (10 in total) at 4 °C were treated under the conditions described in example 1 and held in the cryogenic cabinet for 1 minute or 45 seconds with the atmospheric temperature at -80 °C. Some birds were removed from the

cabinet immediately after treatment, and allowed to warm to 4 °C. A further set of birds were allowed to remain in the cabinet after the cessation of refrigeration, for a period of between 45 seconds and 2 minutes (referred to as "with dwell" birds). The reduction in campylobacter count is shown in Table 4.

5

	GEOMETRIC AVERAGE	LOG ₁₀ VALUES	LOG DIFFERENCE (compared to control)
CONTROL	1,525	3.18	
45 seconds (all data)	1,324	3.12	-0.06
45 seconds (no dwell)	1,511	3.18	0.00
45 seconds (with dwell)	1,199	3.08	-0.10
1 minute	361	2.56	-0.63

Conclusions:

- 45 second treatment with no dwell in the cabinet produced almost exactly the same value as control.
- 10 • 45 second treatment plus dwell in the cabinet produced a small 0.1 log difference.
- 1 minute treatment looks even more effective than shorter residence times.

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The claims defining the invention are as follows:

1. A process of reducing the number of viable microorganisms present on the surface of meat, comprising the steps of:
 - a) providing an untreated meat item having a surface membrane and muscle tissue, said untreated meat item having viable microorganisms on the surface membrane;
 - b) exposing the surface membrane to a spray of liquid nitrogen for an exposure time of between 30 seconds and 2 minutes until the surface membrane reaches a first temperature as measured by a probe inserted within or just below the membrane of between -5 °C and 2 °C;
 - c) allowing the surface membrane to warm to a second temperature higher than the first temperature to give a treated meat item;
- wherein
the number of viable microorganisms present on the surface membrane is reduced, whilst the β -hydroxyacyl-CoA dehydrogenase (HADH) activity of the muscle tissue is not increased by a factor of more than 2 in the treated meat item compared with the untreated meat item.
2. A process according to claim 1, wherein the surface membrane is maintained at the first temperature for a period of time.
3. A process according to claim 1 or claim 2 wherein the meat item is selected from a whole poultry carcass, optionally eviscerated.
4. A process according to claim 1 wherein the meat item is a poultry portion.
5. A process according to any one of the preceding claims wherein the cryogen is applied in combination with an impinging gas.
6. A process according to any one of the preceding claims wherein the selected second temperature is below 4 °C.
7. A process according to any one of the preceding claims wherein the microorganisms comprise at least *Campylobacter* species.

8. A process according to any one of the preceding claims comprising at least one further step of curing, smoking, salting, pickling, or cooking the treated meat item.
9. A treated meat item obtained by the process according to any one of claims 1 to 8.

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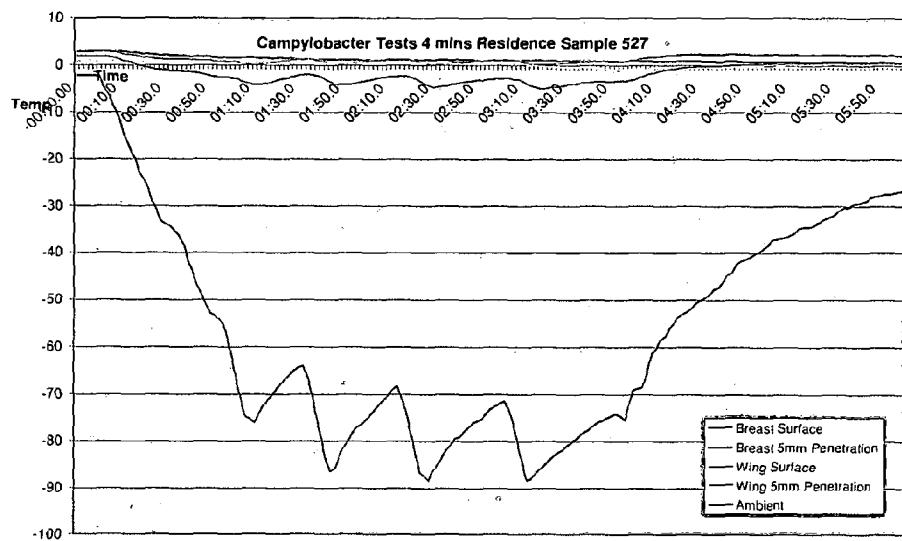


Figure 1

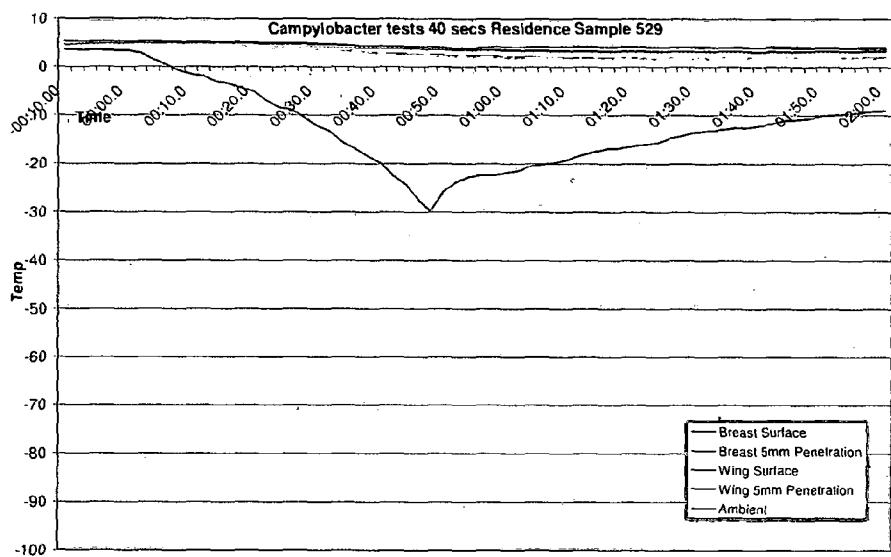


Figure 2

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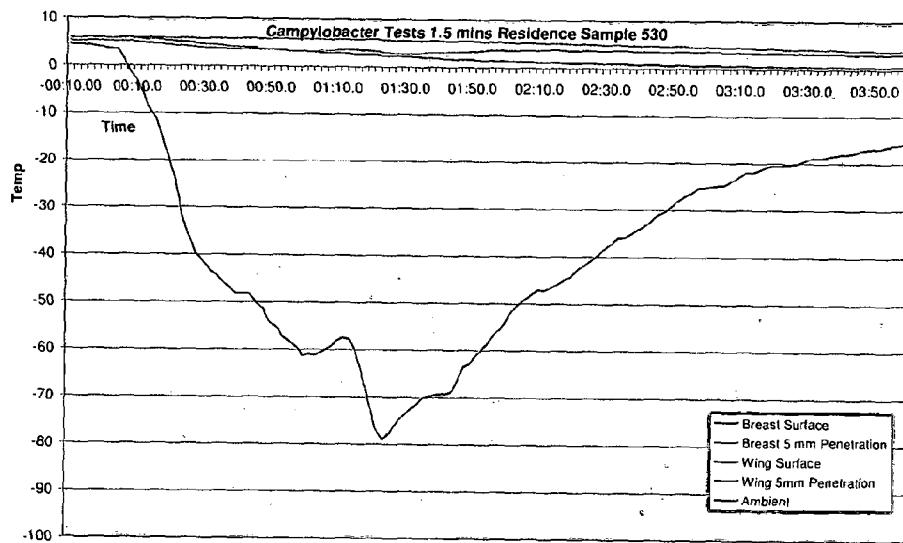


Figure 3

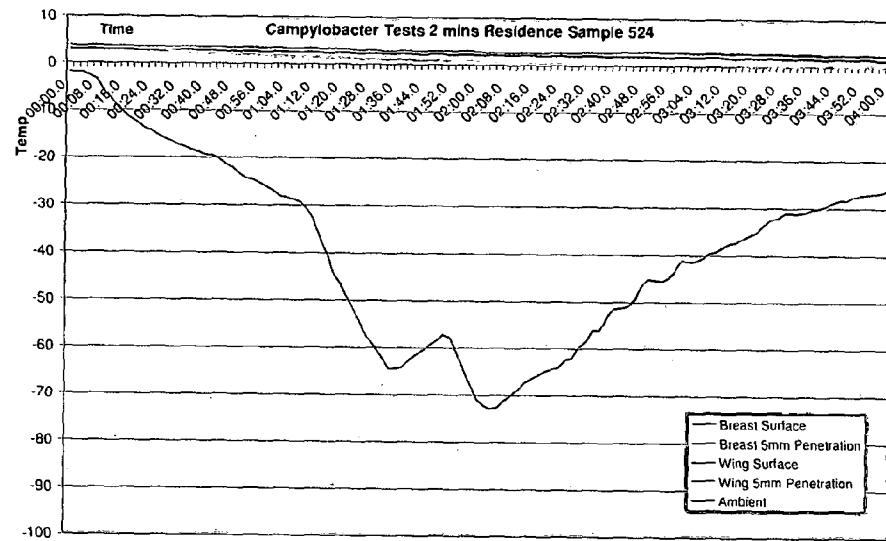


Figure 4

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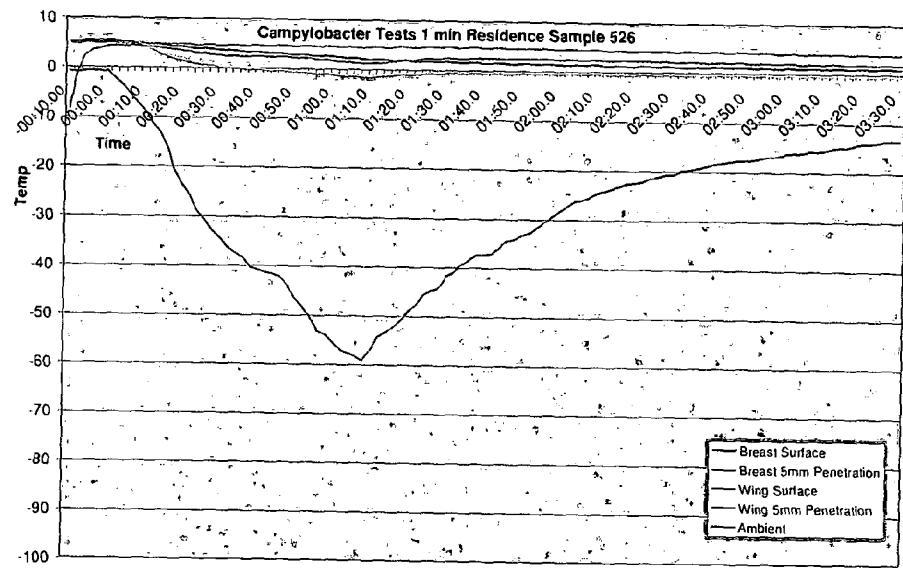


Figure 5

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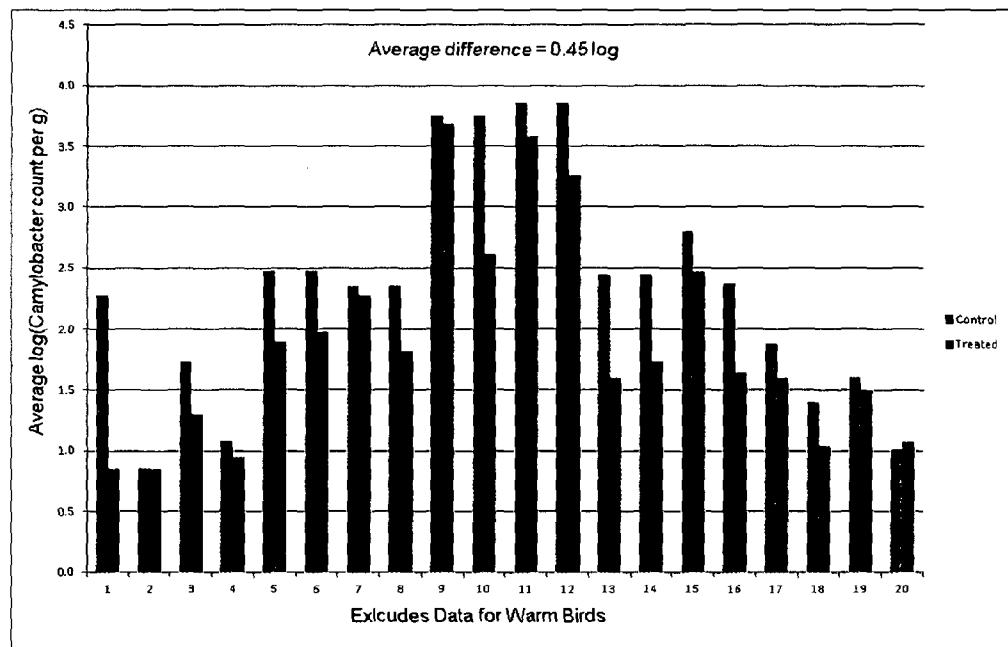


Figure 6

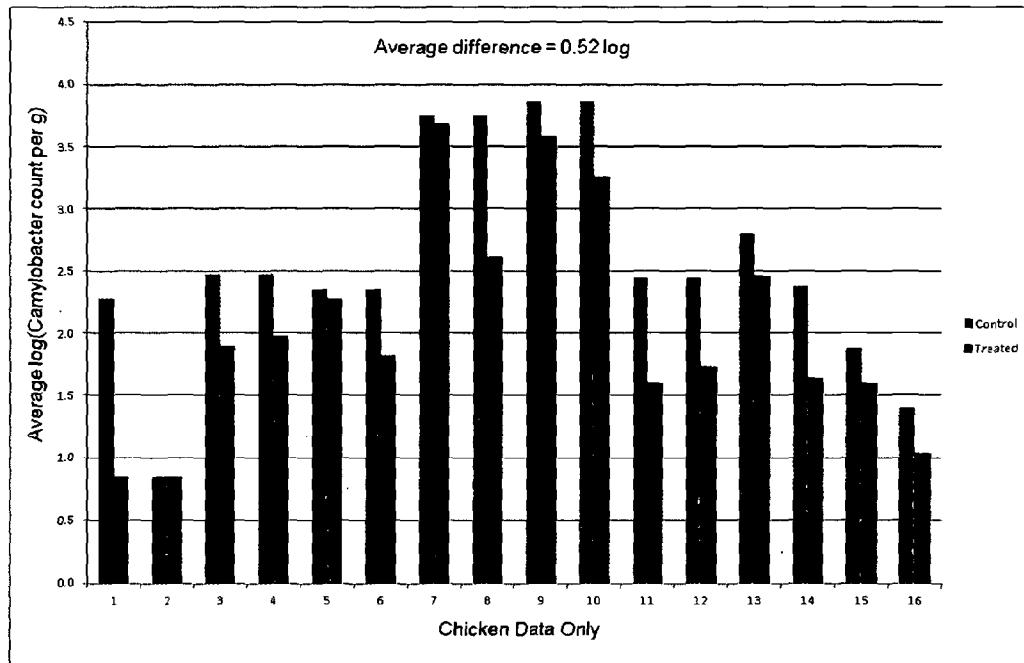


Figure 7

- 5/7 -

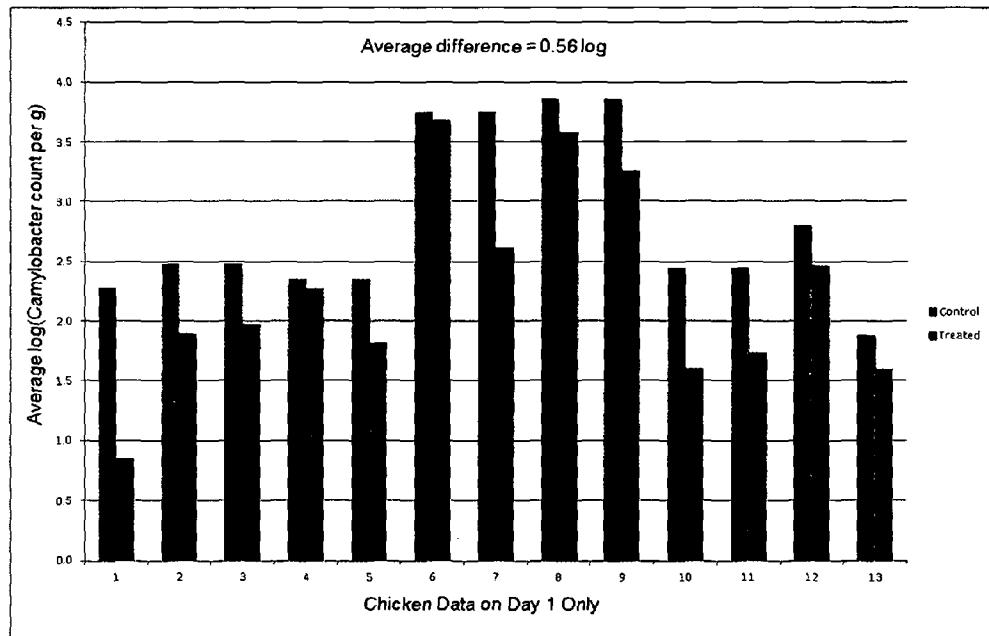


Figure 8

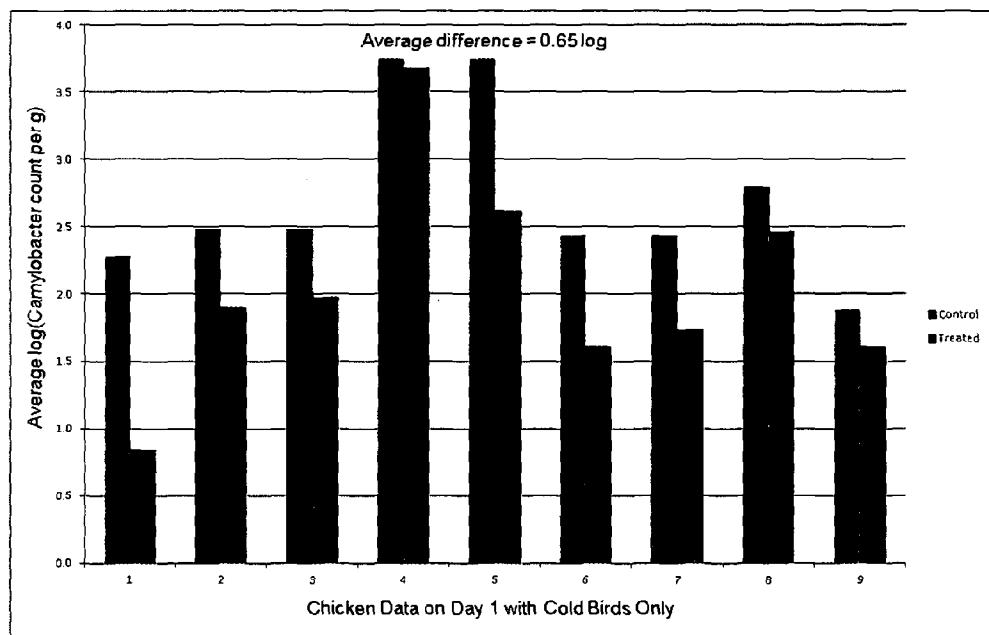


Figure 9

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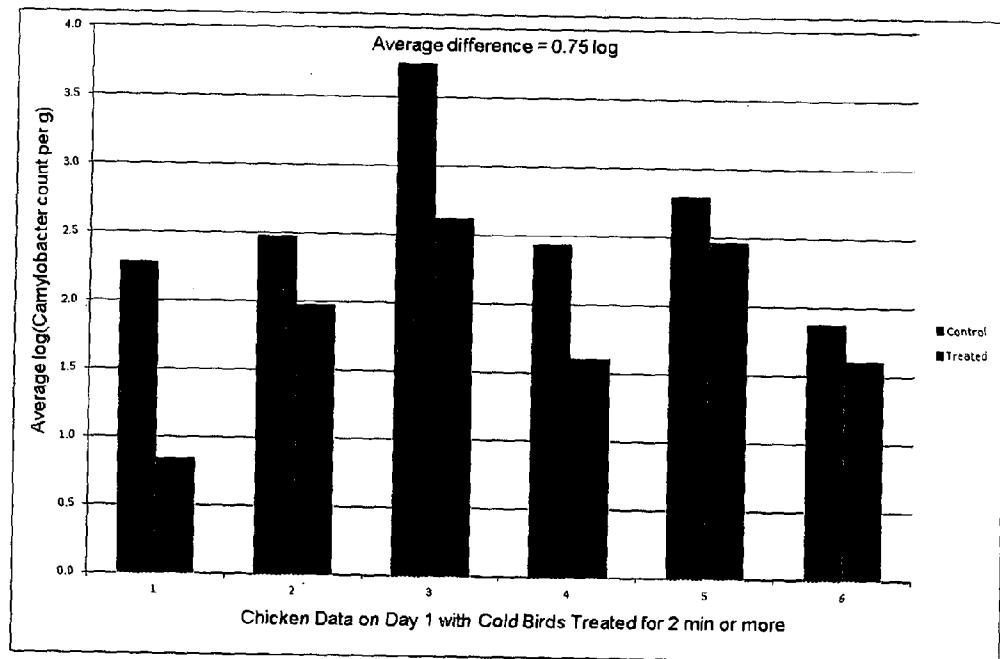


Figure 10

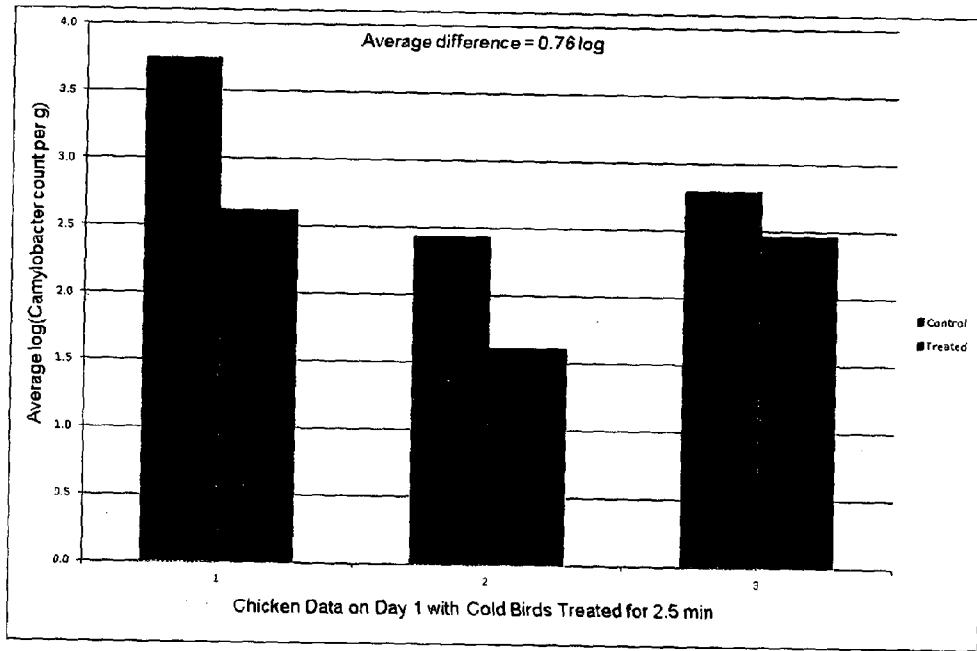


Figure 11

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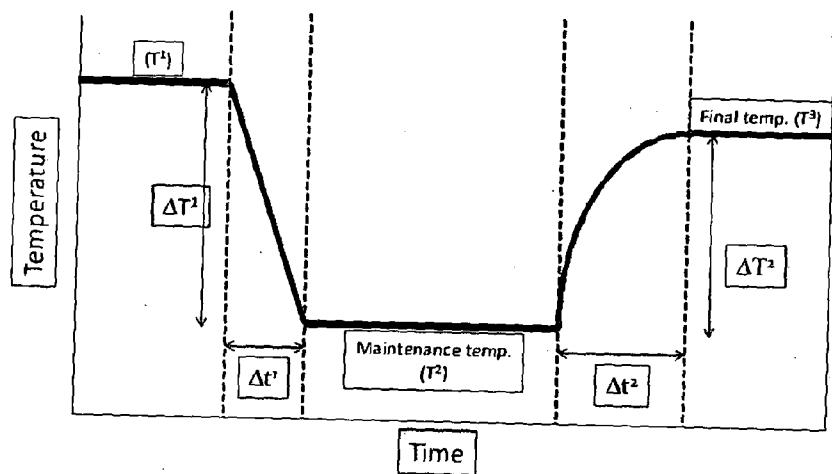


Figure 12

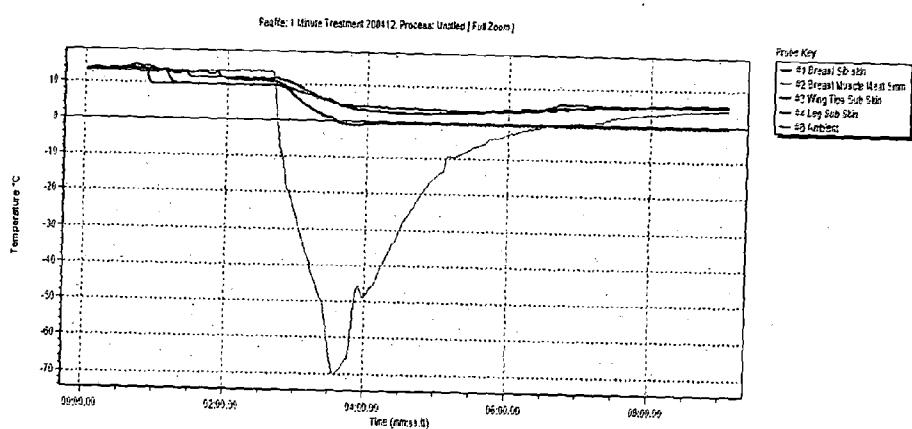


Figure 13