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DE SÉCURITÉ SANITAIRE
DES ALIMENTS

Maisons-Alfort, 18 february 2009,

OPINION

of the French Food Safety Agency (AFSSA) on the transport of pig carcasses that have not reached the required temperature upon leaving the slaughterhouse

LA DIRECTRICE GÉNÉRALE

1. Review of the request

On 15 September 2008, the Directorate General of Food (DGAl) submitted a request to Afssa to issue its opinion of the modification requested by the French National Pork Trade Union relating to the conditions of transport of pig carcasses that have not reached the required temperature upon leaving the slaughterhouse.

2. Background of the request

2-1. Regulatory background

Annex I of (EC) regulation 853/2004 sets out the modalities for livestock slaughter and the preparation of carcasses. The regulation provides for the possibility to derogate from the 7°C core temperature during the storage, transport and cutting of pig carcasses.

A draft ministry order relating to the health rules applicable to animal products and goods containing such products specifies the provisions of the Community regulation. Afssa has provided its opinion in respect of that order (1). The provisions relating to the exemption are as follows:

" … *the carcasses of domestic ungulates may be transported and cut during cooling, subject to compliance with the following conditions:*

a) *As regards the* ***slaughterhouse:***

   i) ***the transport time is less than two hours****;*

   ii) *the* ***core temperature*** *at the time of loading is* ***equal to or less than 12°C;***

   iii) *a written procedure approved by the Prefect (Departmental Director of Veterinary Services) with a detailed description of the application of these provisions is integrated into the health control plan of the slaughterhouse.*

b) *As regards the* ***consignee establishment****:*

   i) *The* ***core temperature*** *at the time of unloading is* ***equal to or less than 12°C****;…"*

That exemption only applies to establishments that have Community approval.

On the basis of an industry study report, the National Pork Trade Union (NPTU) asked for an easing of the exemption criteria, which are effective since the signing of the ministerial order. Firstly, NPTU would like the maximum permitted core temperature to be raised from 12°C to 15°C as regards pig carcasses when they leave the slaughterhouse and secondly, it is asking for the removal of the limitation on the duration of transport between the slaughterhouse and the cutting plant.

2-2. Carcass cooling

- Purpose of carcass cooling

At the end of the slaughter process, the temperature of the pig carcasses is still approximately 30°C at the surface and 38-40°C within the carcass (56). These temperatures are very conducive to the growth of most bacteria that contaminate carcasses. Cooling is aimed at bringing down the temperature of the carcass as rapidly as possible, in order to protect the microbiological quality of the meat (35, 46, 58). As a result, the slaughter stage is considered

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by some authors (5, 52) to be a critical stage in the control of the microbiological hazards as part of the HACCP approach.

Cooling influences the biochemical reactions (glycolysis, drop of pH, enzyme activity, etc.) and physical changes (loss of water) that occur after the pigs have been slaughtered (35, 58). These reactions and changes have a direct impact on the sensory and technological quality of the meat (61). Rapid refrigeration can improve the ability to retain water and colour, but can also reduce tenderness (46).

- Factors influencing cooling

The air speed, the temperature and the relative humidity (13) and also the space between carcasses (12, 30, 37) are some of the factors that affect the carcass cooling speed.

Cooling is further directly affected by the weight and the thickness of fat in the pig carcasses (5, 13, 52). Its speed is therefore variable depending on the part of the carcass (36).

- Description of the various cooling systems

There are several systems for refrigerating pig carcasses (22, 46):

- conventional (or static) refrigeration: after slaughter, the carcasses are put into refrigeration chambers at temperatures close to 4°C. The air speed inside the chambers is about 1 m/s.
- rapid refrigeration (or in cells): it includes two stages; in the first, the pig carcasses are placed for several hours in pre-chilling cells at temperatures close to -2 to 0°C and air speeds close to 3 m/s and in the second, the carcasses are placed in an equalising room at 4°C.
- ultra-rapid refrigeration (or tunnel refrigeration): it comprises two stages, the first of which consists in placing the pig carcasses at -5 to -8°C for 4-10 hours or at -20°C for one hour with an air speed of 8 m/s, whilst the second stage involves putting the carcasses in an equalising room at 4°C.

During cooling, spraying is sometimes applied (22, 46). It has the benefit of reducing the loss of water by the carcasses.

3. Questions asked

Question 1. Is the study put forward by the petitioner relevant, in terms of the protocol used and the microbiology criteria selected?

Question 2. Could the maximum core temperature limit be raised from 12°C to 15°C within the limit of two hours as regards pig carcasses when they leave the slaughterhouse as part of the warm cutting exemption with no additional risk for consumers?

Question 3. Could the maximum transport time currently set at two hours as part of that exemption be increased with no additional risk for consumers? With a 12°C limit? With a 15°C limit?

4- Expert assessment method

The Microbiology Scientific Panel met on 13 November 2008 and 15 January 2009 and issued the following opinion.

This opinion is organised as follows. First of all, the petitioner's application is presented (part 5). Then, in response to question 1, the relevance of the petition is assessed (part 6). Lastly, questions 2 and 3 are addressed in part 7.

5. Description of the petitioner's application

5-1. Acquisition of cooling kinetics

The petitioner's application is based on a calculation of the theoretical growth potential of four micro-organisms depending on the temperature kinetics measured at the surface of carcasses, on the basis of predictive microbiology models. The growth potential calculated as regards transport in refrigerated lorries was compared with the growth potential of the same micro-organisms during cooling in a refrigerant chamber.

- Cooling kinetics during transport in refrigerant lorries

216 cooling kinetics records were taken from carcasses:

- from 24 different transports. Almost half the transports contained 100% of whole carcasses. In the others, more than 50% of the loads were made up of whole carcasses, and were completed by half carcasses;
- with 3 carcasses per transport;
- and 3 zones per carcass (ham, loin and shoulder).

The ambient temperature was also recorded (minimum, mean and maximum temperature values) in 21 transports out of 24.

These recordings were supplemented by measurements at the core of the carcass (ham and loin) during loading.

The petitioner indicates that the records were taken in four slaughter companies representative of the industry.

- Cooling kinetics in refrigeration chambers

Three temperature kinetics at the surface of hams in three different refrigerant chambers are reported. These three kinetics records were used as the bases of comparison to judge the equivalence between the cooling of carcasses during transport and in refrigerant chambers.

5-2. Simulation of bacterial behaviour

- Germs selected

Four germs were used as indicators of microbiological quality: *Pseudomonas*, *Salmonella*, *Listeria monocytogenes* and *Escherichia coli*.

- Models used

Growth simulations were carried out using the Sym'Previus tool. Sym'Previus particularly uses a (secondary) model that makes it possible to predict the growth rate (μ_{max}) of a micro-organism as a function of the temperature, pH, water activity and the interaction between these factors (see Annex 1).

On the basis of the temperature kinetics, the growth rate is estimated for each time interval (eq.1 Annex 1) and the growth of the micro-organisms considered is calculated (eq.2 Annex 1). The overall approach is presented in Figure 1.

- Parameters of the second model

The Sym'Previus simulation tool proposes cardinal values (parameters of the secondary model) for *Salmonella*, *Listeria monocytogenes* and *Escherichia coli*. These values take account of the variability that exists between different strains of the same species.

For *Pseudomonas*, the petitioner has used data from the bibliography to set the parameters of the secondary model (T_{min}, T_{max} ,T_{opt}, pH_{min}, pH_{max}, pH_{opt}, $a_{w\,min}$, $a_{w\,max}$ and $a_{w\,opt}$).

The petitioner used the bibliography as a basis for estimating the optimum growth rate (μ_{opt}) of *Salmonella*, *Pseudomonas* and *Escherichia coli* in pig meat. For *L. monocytogenes*, a value is already proposed by Sym'Previus.

The behaviour of these bacteria was predicted depending on the temperature kinetics and pH and water activity (a_w). The pH was measured on several carcasses on both sides (meat and rind). The value of a_w was set to 0.995. The pH and a_w were considered to be stable over time.

The lag time of all the micro-organisms was set to 0 in all the simulations. The values of N_0 have been selected to be consistent with the self monitoring results.

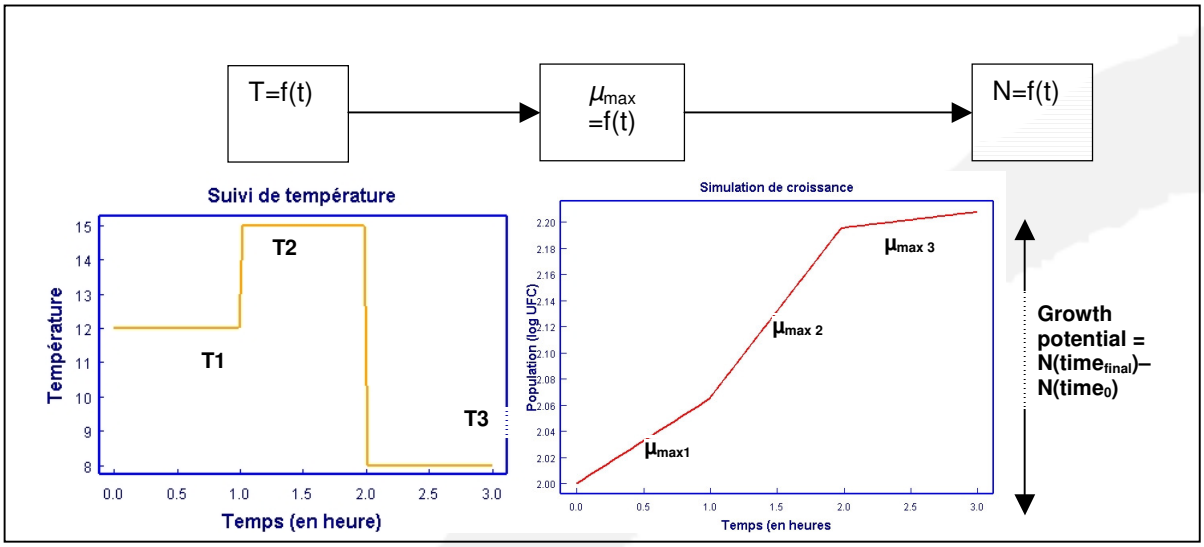

Figure 1. Presentation diagram of the approach used in the petitioner's application

5-3. Presentation of the justification by the petitioner of the request for an exemption change

Out of the 216 carcass temperature kinetics, the petitioner observed that the lowering of the surface temperature was (in almost all the cases) faster as regards loins and shoulders in relation to hams.

The petitioner compared the growth of *Salmonella*, *Pseudomonas* and *E. coli* during the cooling of the hams (where the cooling kinetics are the least favourable for bacterial growth) in refrigerant lorries for each of the 24 transports with the growth of these micro-organisms during the type of refrigeration in refrigerant chambers that is the most conducive to growth (out of the three types tracked).

Out of these 24 growth kinetics in refrigerant lorries, 9 do not lead to growth above that observed during refrigeration in refrigerant chambers for the three micro-organisms listed above.

For these 9 surface temperature cooling kinetics, the petitioner has observed that the core temperature of the hams at the time of loading was below 17°C (in the 15 other cases, the core temperature was above 17°C).

The growth of *L. monocytogenes* at the surface for these 9 kinetics also does not exceed the growth observed during the cooling of the reference refrigerant chamber.

To conclude, the petitioner proposed that the core temperature, when the carcasses are loaded, be changed from 12°C to 15°C (which leaves a safety margin of 2°C in relation to the 17°C temperature found to be satisfactory).

The petitioner did not propose maximum transport time for such transport conditions.

6. Response to question 1: is the study put forward by the petitioner relevant for a change in the exemption, in terms of the protocol used and the micro-organisms selected?

6-1. Micro-organisms selected

L. monocytogenes and *Salmonella* are both main pathogenic agents in pork meat after slaughter (42). *L. monocytogenes* can multiply at temperatures of down to -1°C (4).

E. coli has the advantage of having a minimum growth temperature close to 7°C (55, 59), which is the target temperature to be reached while cooling the carcasses. The growth of *Salmonella* is close to that of *E. coli* (39).

Pseudomonas can also be used to verify the effectiveness of the refrigeration methods (24-26). *Pseudomonas* was considered by the ICMSF to be a bacterium showing one of the fastest multiplication rates in meat (30).

In view of this information, the estimation of the growth potential of these four micro-organisms during the cooling of carcasses seems well justified.

Yersinia enterocolitica, which is a pathogen of interest (19, 38, 48), and *Aeromonas hydrophila* (6, 29, 41), which is potentially present in pork meat, could also have been selected due to their psychrotrophic nature (10, 11).

6-2. Use of time-temperature integration

The general approach of the petition is based on the change in the temperature kinetics in terms of the bacterial growth potential using predictive microbiology models. The approach is also called "time-temperature integration" or "temperature function integration" (43). The approach is fairly widespread and has been described in the literature. The examples below illustrate the use of time-temperature integration in the area of meat cooling.

Gill and colleagues (21-26, 33, 54), Dickson et al. (15), Jericho et al. (32) and also Lovatt et al. (39) used records of the temperatures at the surface or core or carcasses of pigs, sheep and cattle. These temperature records, coupled with growth models developed for *E. coli*, psychrotrophic *Pseudomonas* or *Salmonella* have made it possible to estimate growth during cooling. These studies have made it possible to validate the consequences of specific cooling methods (e.g. spraying during cooling, effect of passing through a refrigeration tunnel, etc.).

In Australia, on the basis of a model predicting the rate of growth of *E. coli* as a function of the temperature, water activity and the lactate concentration (45, 55), the AQIS (Australian Quarantine Inspection Service) established the rules for the cooling of carcasses of animals for export (2). Using recorders placed in the carcasses during cooling, the growth of *E. coli* can be calculated, expressed in the form of a refrigeration index (18). The values calculated for each slaughter site must comply with values that may not be exceeded, to guarantee the validation of the refrigeration applied to the carcasses.

The Meat Industry Research Institute working alongside the New Zealand food safety agency (51) used a process hygiene index (PHI) that estimates the growth of *E. coli* with a model (54) and records over time of the temperature of the carcasses (or cuts of meat). The hygiene index value is used to validate the refrigeration process.

The scientific committee of the Belgian federal agency for food chain safety (Afsca) has also used predictive microbiology models to determine the duration and temperature for the transport of pig carcasses as part of the exemption relating to the warm cutting of carcasses. Using the predictive microbiology tool Pathogen Modeling Program (63), the scientific committee of Afsca calculated the temperature and maximum transport time of carcasses on the basis of the speed of multiplication of *Aeromonas hydrophila*, which is the most psychrotrophic pathogenic agent out of those tested (10, 11).

The purpose of these examples of use of time-temperature integration is not necessarily to estimate the actual growth of micro-organisms. That is because at the surface of meat, particularly during cooling, a loss of water can be observed that reduces water activity (see 4-4 below). Rather, these indicators are used for quantitatively defining good practices in the area of carcass cooling or the equivalence of processes (43).

The use of recorders that log the temperature at the surface of carcasses and time-temperature integration presented in the application of the petitioner is thus appropriate in view of the scientific literature and the existing applications.

6-3. Sym'Previus

The secondary model describing the influence of environmental factors on the growth rate has been published (4). The values of the parameters, used in the application, are given for the parameters that the petitioner had to estimate (for the cardinal temperatures of *Pseudomonas* for example or the μ_{opt} values in meat for *E. coli* and *Salmonella*). On the other hand, for the

parameters already put in place (cardinal temperatures of *E. coli*, *Salmonella*) in the Sym'Previus tool, no value is given (the user of Sym'Previus cannot access them).

To check the relevance of Sym'Previus, the cooling kinetics of the petitioner were used to calculate the growth potential with other models (see Annex 1). The growth potentials calculated with Sym'Previus and given in the report accompanying the referral have been compared with the growth potentials calculated with other models. The different models tested provided potentials close to those obtained with Sym'Previus for *E. coli*, *Listeria* and *Salmonella*. On the other hand, the growth potentials estimated in the report for *Pseudomonas* are not as high as those calculated by other models (see Annex 1). That difference may be put down to the minimum temperature selected, which is 0°C. Several studies indicate that the minimum growth temperature is below -2°C (16, 28, 50). The choice of such a minimum temperature value is liable to bring the growth predictions in the referral documents closer to those of other models.

6-4. Validation of growth potentials calculated

The application submitted with the referral does not contain microbiology data to validate the growth potentials. A bibliography study was carried out to verify the growth values obtained for the four bacteria selected by the petitioner and other bacteria or bacteria groups of interest that are potentially present in pig carcasses.

- *Salmonella*

The petitioner predicted a maximum growth value of 0.2 $\log_{10}$ near the rind and 0.5 $\log_{10}$ near the meat with cooling in refrigerant chambers.

On the basis of a statistical survey of the data from eight studies, Gonzales Barron et al. (27) have shown that the prevalence of pig carcasses that are contaminated before refrigeration was 2.4 times greater than after cooling. That reduction of prevalence was observed (7, 8, 27, 57) regardless of the mode of cooling (in cooling chambers, preceded by time in tunnels or not). That reduction in the number of *Salmonella* seems to be due to the cold shock and the reduction of water activity on the surface (8, 34).

- *E. coli*

The petitioner predicted a maximum growth value of 0.5 $\log_{10}$ near the rind and 0.6 $\log_{10}$ near the meat with cooling in refrigerant chambers.

Gill et al. (22, 25) have shown that refrigeration (nine companies with different cooling systems) could lead to a decrease, stagnation or even an increase in the number of *E. coli* on the surface or the percentage of carcasses showing the presence of *E. coli*. The absence of spraying water, and thus the drying of the carcasses during cooling seems to be conducive to the reduction of the number of *E. coli* (22).

Chang et al. (8) and Nesbakken et al. (48) also showed that cooling leads to a significant reduction in the number of *E. coli* by cm^2 at the surface of pig carcasses (not sprayed).

- *L. monocytogenes*

The petitioner predicted a maximum growth value of 0.8 $\log_{10}$ near the rind and 1.2 $\log_{10}$ near the meat with cooling in refrigerant chambers.

In a study carried out in 2000 (9) on the effect of slaughter operations on contamination by *L. monocytogenes* of pig carcasses, it was shown in most cases that the level of contamination reduces in the slaughter line. On the other hand, contamination increases during pre-chilling. In a bibliographic review of *L. monocytogenes* in pig meat and derivative products, Thévenot et al. (62), also reported that the cooling and cutting of carcasses increase contamination by *L. monocytogenes*. That increase is reportedly due to meat re-contamination (49).

In the study by Saide-Albornoz et al. (57), the percentage of positive carcasses remained stable throughout the process of slaughtering and cutting pig carcasses.

Other studies even seem to show that carcass cooling reduces the concentration of *Listeria* in pork (8) and also in beef (47, 53). That decrease appears to be explained by the cold and osmotic shocks that give rise to stress for *Listeria* (17, 47).

- *Pseudomonas*

The petitioner predicted a maximum growth value of 1.5 $\log_{10}$ near the meat (and nil near the rind) with cooling in refrigerant chambers.

No specific study of the behaviour of *Pseudomonas* during the cooling phase of pig carcasses has been identified. However, Gill et al. (25) calculated the potential growth of psychrophilic *Pseudomonas* during the cooling of pig carcasses and compared such calculated growth with that measured with the total flora. In the case of the studied cooling system (tunnel at -20°C for one hour and a passage in the cooling chamber at -2°C with the spraying of water at 5°C for 20 seconds every 10 minutes), an increase of 1 $\log_{10}$ of the total flora was observed. In this study, as in another study of the pre-chilling of cattle carcasses (26), growth was attributed to *Pseudomonas*.

- Other bacteria of interest
  - Coliform bacteria and *Enterobacteriacae*

Yu et al. (64), Chang et al. (8) and Nesbakken et al. (48) showed a significant reduction of the concentration of coliform bacteria at the surface of pig carcasses. On the other hand, Pearce et al. (52) observed a stagnation (loin, ham) or even a rise (neck) in the number of coliform bacteria.

Spescha et al. (60) observed a decrease in the number of carcasses carrying *Enterobacteriacae* with two different cooling systems (tunnel at 8 m/s and -8°C for 45 minutes before transfer to a room with 2 m/s at 2°C and conventional cooling (4 m/s at 2°C).

  - Total flora

As regards the total flora, the results diverge depending on the study. Bolton et al. (5) observed a slight increase during the refrigeration of carcasses. Spescha et al. (60) showed a decrease in the average levels of contamination of pig carcasses with tunnel cooling (8 m/s at -8°C for 45 minutes before a transfer to a room with 2 m/s and 2°C) and conventional cooling (4 m/s at 2°C). These reductions were observed in four areas of the carcass. They were not as marked for the chine, which is the most moist part because the water from the other parts of the carcasses runs on this part (60).

Pearce et al. (52) also observed the influence of the sampling zone on the total mesophilic aerobic flora. The flora was stable during cooling (at 4°C) in hams and loins and increased in necks.

In a study reproducing the conventional or tunnel conditions experimentally, Chang et al. (8) observed a reduction in the aerobic flora between 1 and 3 $\log_{10}$. These results are confirmed by Yu et al. (64), who observed a reduction of the aerobic flora from 0.6 to 1.3 $\log_{10}$ depending on the method used for sampling carcasses.

In two studies covering nine pig slaughterhouses (possibly with a passage in a tunnel and/or spraying of carcasses during refrigeration), Gill et al. (22, 25) observed stagnation of the aerobic flora during cooling in six slaughterhouses, a significant increase in two others and a significant decrease in the last one. The use of tunnels or spraying during cooling in chambers can not explain these differences.

  - *Staphylococcus aureus*

Spescha et al. (60) showed that the cooling of carcasses makes it possible to reduce the percentage presence of coagulase-positive staphylococcus. In another study, Saide-Albornoz et al. (57) observed an opposite effect where a larger number of carcasses tested positive for the presence of *Staphyloccus aureus* after 24 hours of refrigeration at 4°C. The authors attributed this increased percentage to recontamination by the personnel. Besides, the ability of this bacterium to multiply in the cold is poor, with minimum growth temperatures ranging from 5 to 10°C depending on the strain (14).

  - *Yersinia enterocolitica*

Tunnel refrigeration (-21°C for 70 minutes, then 5°C for one hour) does not affect the occurrence of *Yersinia* at the surface of carcasses (48).

- *Campylobacter*

Campylobacter is sensitive to the cooling applied to pig carcasses (6, 8, 48) and particularly to the drying of the surface of carcasses (30). For example, tunnel (-21°C for 70 minutes then 5°C for one hour) at the surface of hams decreases the percentage of over 50% hams with *Campylobacter* to less than 2% (48).

- *Aeromonas hydrophila*

No data specific to the effect of pre-chilling have been identified with *A. hydrophila*. However, it appears that *A. hydrophila* are frequently found in refrigerated raw pork meat (6, 29, 41).

An analysis of all these results makes it possible to conclude that actual bacterial growth is certainly not as high as predicted bacterial growth. The microbiological behviour observed from various studies show high variability of results. The drying of meat at its surface and the speed at which the temperature drops are some of the most influent factors in the behaviour of bacterial population. Depending on the conditions, pre-chilling may lead to growth, stagnation or even a decrease in bacterial population. It must also be noted that these bibliographical sources report the data observed during cooling in chambers.

6-5. Justification of the application for exemption

The hypotheses selected by the petitioner for calculating the growth potentials during the cooling of the carcasses in refrigerant transport lorries are safe in that the lag time before growth is considered to be nil and the possible drying of the meat the surface is not taken into account.

However, the application for exemption that is submitted relies on the equivalence in terms of bacterial growth potential between one particular kind of cooling kinetics in refrigerant chambers (the most favourable to growth out of the three presented in the report) and the different refrigerant lorry transports.

That comparison of the cooling of carcasses in lorries with the situation in refrigerant chambers that is the most favourable to bacterial growth is not justified. That is because no information is given about the representativeness of those cooling kinetics in refrigerant chambers. Also, while there are records of the temperatures at the surface of carcasses during transport in lorries, no information is given about the time spent by the carcasses in refrigerant chambers (and the temperature) before they are loaded and unloaded. That is why it is not possible to compare cooling in refrigerant chambers and that in refrigerant lorries because the temperature recording periods are not identical.

In the current state of knowledge, if no limit is set on the transport time of carcasses transported when warm (15°C core temperature as proposed by the petitioner), it is impossible to make sure that the safety of such meat is identical to that of meat cooled to the required core temperature of 7°C before transport or that of meat transported under the exemption for warm cutting.

It should be pointed out again that in the absence of data about technical characteristics (air speed, relative humidity, distance between carcasses, etc.) and microbiology characteristics, Afssa is not in a position to provide an opinion of the relevance of the use of refrigerant transport as a means to cool carcasses.

7. Answers to questions 2 and 3:

Could the maximum core temperature limit be raised from 12°C to 15°C within the limit of two hours as regards pig carcasses when they leave the slaughterhouse as part of the warm cutting exemption with no additional risk for consumers?

Could the maximum transport time currently set at two hours as part of that exemption be increased with no additional risk for consumers? With a 12°C limit? With a 15°C limit?

As part of warm cutting, an exemption relating to the transport of carcasses having a core temperature of 12°C is provided, within a two-hour limit.

An increase in the core temperature of carcasses from 12°C to 15°C over a same transport time of two hours or the extension of transport times above two hours for carcasses with a core temperature of 12°C would lead to greater growth of micro-organisms. As a result, the safety of meat transported in such conditions would be not as high as that of meat transported for two hours at 12°C.

However, it is possible to calculate the temperature conditions and transport time that will lead to microbial growth equivalent to growth at the temperature and for the time defined in the exemption.

Three scenarios are proposed. They are based on the potential growth of *A. hydrophila* and *Salmonella* at the core of the meat and the potential growth of *L. monocytogenes* on the surface. A. *hydrophila* has been selected by reference to the opinion of the scientific committee of Afsca relating to the warm carcass transportation exemption (10, 11). *Salmonella* and *L. monocytogenes* have been selected for their major health implications.

7-1. Equivalence based on the growth of *A. hydrophila*

The approach presented here is similar to that used by the scientific committee of Afsca as part of the exemption relating to the transport of warm carcasses (10, 11).

A. hydrophila, which is potentially present in pig carcasses (6, 29, 41), shows one of the fastest growth rates out of psychrotrophic bacteria according to the models published (31, 63). The growth potential of *A. hydrophila*, calculated on the basis of the cooling kinetics provided in the application submitted with the referral, shows that the growth of that bacterium is greater than *Y. enterocolitica*, *L. monocytogenes* and *Pseudomonas* (data not presented).

If the lag time of *A. hydrophila* is considered to be nil, growth in anaerobiosis (at the core of the meat) at pH 6.2 for two hours at 12°C is 0.19 $\log_{10}$ CFU according to the Pathogen Modeling Program, which represents less than a doubling of the population. With the same program, it is possible to calculate the maximum transport time depending on the core temperature of the carcasses at the time of loading that will not lead to a growth of *A. hydrophila* greater than 0.19 $\log_{10}$ CFU. Those maximum transport times are given in Figure 2.

7-2. Equivalence based on the core growth of *Salmonella*

The presence of bacteria at the core of meat is a possible phenomenon (3, 20, 40), particularly with *Salmonella* (3).

If the lag time of *Salmonella* is considered to be nil, the core growth at pH 6.2 for two hours at 12°C is 0.11 $\log_{10}$ CFU according to the growth model proposed by Combase Predictor[1]. Such growth represents less than a doubling of the population. With the same model, it is possible to calculate the maximum transport time depending on the core temperature of the carcasses at the time of loading that will not lead to growth of *Salmonella* greater than 0.11 log10 CFU. Those maximum transport times are given in Figure 2.

7-3. Equivalence based on the surface growth of *L. monocytogenes*

This pathogenic bacterium is regularly detected on the surface of pig carcasses (9, 62). The consumption of pork meat and products made from these carcasses can give rise to cases of listeriosis (62).

According to the data supplied in the application submitted with the referral, a relationship has been established between the temperatures measured at the core and the surface (see Annex 2). That relationship makes it possible to determine the temperature at the surface from the core temperature (and vice versa).

If the lag time of *L. monocytogenes* is considered to be nil, growth in aerobiosis at pH 6.2 for two hours at 10°C at the surface (12°C core) is 0.13 $\log_{10}$ CFU according to the Pathogen Modeling Program, which represents less than a doubling of the population. With the same program, it is possible to calculate the maximum transport time depending on the core temperature of the carcasses at the time of loading that will not lead to growth of *L.*

[1] Aerobic growth model.

monocytogenes greater than 0.13 $\log_{10}$ CFU at the surface. Those maximum transport times are given in Figure 2.

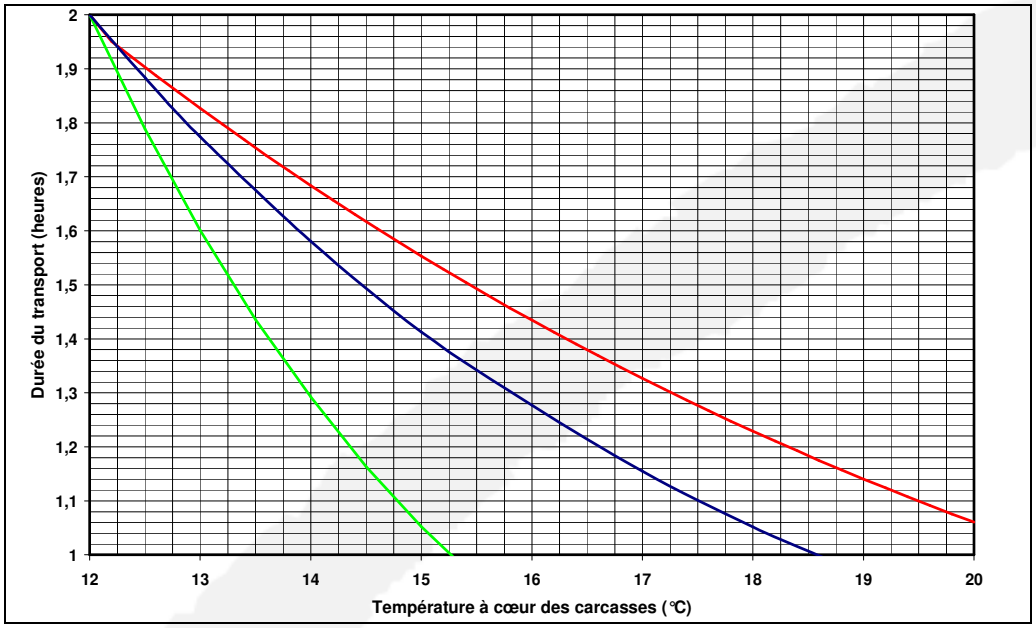

Figure 2. Maximum transport time depending on the core temperature of carcasses leading to a theoretical growth of *A. hydrophila* (—), *L. monocytogenes* (—) *Salmonella* (—) identical to that with transport with a core temperature of 12°C for two hours.

It must be stressed that the different growth simulations are safe simulations. That is because the temperature of the meat is considered to be stable during transport. The lag times are considered to be nil, even when it is likely that the bacteria contaminating the carcasses are in a physiological condition that requires adaptation before exponential growth (17, 47). The models used have been established in liquid culture media and not in meat. The growth model used for *Salmonella* is an aerobic model, even though what is considered is core growth. Lastly, water activity is considered to be optimal even at the surface, when partial drying is possible (35, 58).

8- Conclusion

As part of the warm cutting exemption, the transport of carcasses at core temperatures of 15°C with no time limit, does not guarantee that the safety standards of theses carcasses will be equivalent to those of carcasses transported under the current exemption.

The transport of carcasses at core temperatures at the time of loading ranging from 12 to 15°C implies transport times below two hours. The results presented in paragraph 7-3 are based on bacterial growth estimated with safe hypotheses and make it possible to establish those transport conditions (core temperature and transport time).

It should be pointed out again that in the absence of data about technical characteristics (air speed, relative humidity, distance between carcasses, etc.) and microbiology characteristics, Afssa is not in a position to provide an opinion on the relevance of the use of refrigerant transport as a means to cool the carcasses. Afssa recommends the performance of a study of these technical and microbiology characteristics.

9. Bibliography

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conditions sanitaires auxquelles doivent satisfaire les établissements d'abattage de volailles et l'arrêté du 4 novembre 1963 relatif aux conditions de collecte et de commercialisation des œufs, le projet d'arrêté abrogeant des arrêtés relatifs aux règles sanitaires applicables à certains produits d'origine animale et aux denrées en contenant, pris en application du " paquet hygiène ". Afssa.

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10. Keywords

carcass; cooling; hygiene package; exemption; predictive microbiology

Director General of the French Food Safety Agency
(AFSSA)

Annex 1: Growth models – description and comparison

Models presented in the application of the petitioner

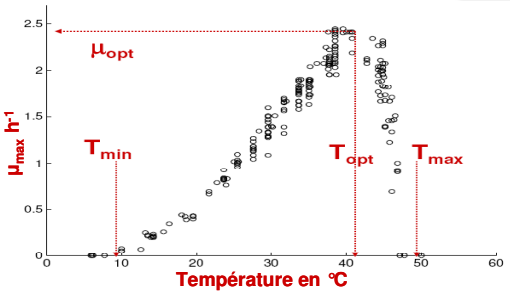
The general form of the model is as follows (4):

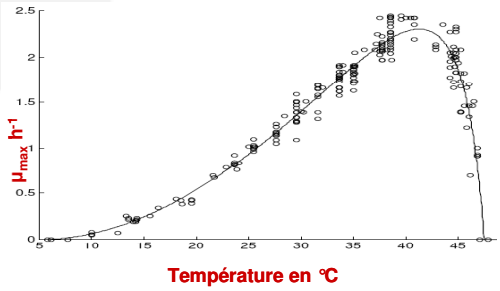
$$\mu_{\max} = \mu_{opt} \cdot \gamma(T) \cdot \gamma(aw) \cdot \gamma(pH) \cdot \gamma(\text{interactions}) \quad \text{(eq. 1)}$$

The effect of each factor (T, pH, and a_w) is described by a mathematical function with parameters having a biological significance (cardinal values).

For example, the relationship between the growth rate and the temperature is as follows:

where $\gamma(T) = \dfrac{(T - T_{\max}) \cdot (T - T_{\min})^2}{(T - T_{\min}) \cdot [(T_{opt} - T_{\min}) \cdot (T - T_{opt}) - (T_{opt} - T_{\max}) \cdot (T_{opt} + T_{\min} - 2T)]}$





The values of T_{min}, T_{max} and T_{opt} are characteristic of a micro-organism and independent of the foodstuff. That reasoning may be extended to a_w and pH. On the other hand, the value of the optimal growth rate (μ_{opt}) depends on the foodstuff in question.

The rate of growth is then used in a primary model to simulate the growth of each micro-organism depending on the time (t):

$$\ln(x(t)) = \ln x_o \quad ,t \le lag$$
$$\ln(x(t)) = \frac{\ln x_{\max}}{\ln\left(1 + \left(\frac{x_{\max}}{x_o} - 1\right)\exp(-\mu_{\max} \cdot (t - lag))\right)} \quad ,t \ge lag \quad \text{(eq. 2)}$$

The number of bacteria at a time *t*, ln(*x*(*t*)), depends on the initial number of cells (x_0), the growth rate (μ_{max}), the lag time (*lag*) and the number of cells reached in stationary phase (x_{max}).

The growth potential can be predicted

Other models used for comparison

- Secondary models for *E. coli*

The first model tested (23, 54) can make it possible to estimate the growth of *E. coli* (μ, expressed generations per hour) depending on the temperature (*T*) in aerobic conditions:

$$\mu = \begin{cases} [(0.0513 \cdot T) - 0.17]^2 & 7°C < T \le 30°C \\ [(0.027 \cdot T) + 0.55]^2 & 30°C < T \le 40°C \\ 2.66 & 40°C < T \le 47°C \\ 0 & T \le 7°C \quad T > 47°C \end{cases} \quad \text{(eq. 3a)}$$

That rate of growth is then integrated in a Process Hygiene Index - PHI. The PHI is calculated as follows:

$$PHI = \sum_{1}^{n} \mu_i \cdot (t_{i+1} - t_i) \quad \text{(eq. 3b)}$$

The PHI is also expressed in the total growth of *E. coli* (39) $N_{E.\ coli:}$

$$N_{E.coli} = 2^{PHI}$$ (eq. 3c)

The second model tested is that developed by Ross et al. (55) and validated by Mellefont et al. (45). This model predicts the growth (*r*, expressed in generation per hour) as a function of temperature (T), pH, lactic acid concentration ([LAC]) and water activity (a_w).

$$\sqrt{r} = 0.2790 \times ((T - 4.14) \times (1 - \exp(0.2636 \times (T - 49.55))) \times \sqrt{(a_w - 0.9508)} \times \sqrt{(1 - 10^{(3.909 - pH)})} \times \sqrt{(1 - 10^{(pH - 8.860)})} \times \sqrt{(1 - [LAC]/10.433)} \times (1 + 10^{(pH - 3.86)}) \times \sqrt{(1 - ([LAC]/(995.509 \times (1 + 10^{(3.86 - pH)}))))}) \pm 0.0054$$ (eq. 4a)

The model is now provided to professionals in the form of a downloadable software programme (18). It makes it possible to calculate the refrigeration index (*RI*) that corresponds with the growth of *E. coli* in the cooling period:

$$RI = \log_{10}\left(\frac{N_n}{N_0}\right) = \sum_1^n r_i \cdot (t_{i+1} - t_i) \cdot 0.303$$ (eq. 4b)

- Secondary models for *Pseudomonas*

One of the tested models (the second being taken from Combase Modelling Toolbox – see below) for *Pseudomonas* has been published by Gill & Jones (24). It makes it possible to estimate the growth of *Pseudomonas* (μ, expressed in generations per hour) as a function of temperature (*T*) in aerobic conditions:

$$\mu = \begin{cases} [(0.033 \cdot T) + 0.27]^2 & -2°C < T \le 25°C \\ 1 & 25°C < T \le 35°C \\ 0 \quad T \le -2°C & T > 35°C \end{cases}$$ (eq. 5a)

The growth of *Pseudomonas* is calculated as follows:

$$N_{Pseudomonas} = 2^{\sum_1^n \mu_i \cdot (t_{i+1} - t_i)}$$ (eq. 5b)

- Combase Modelling Toolbox (31)

The IFR (Institute of Food Research) proposes a modelling tool, named Combase Modelling Toolbox, which may be used online. The tool makes it possible to predict the microbial response of environmental factors. The temperature, pH and water activity (or rate of NaCl) are the factors taken into account for the growth of 15 micro-organisms. For some micro-organisms, a fourth factor (nitrite concentration, CO_2 concentration, etc.) is also implemented.

The models used are polynomial models, the parameters of which are not accessible to the user. The data used for building the models are culture media data. The authors conclude that the growth predictions are safe.

Like Sym'Previus, the tool makes it possible to address dynamic temperature profiles.

The secondary models for *Pseudomonas, Salmonella*, and *Listeria* from this tool were used.

- Pathogen Modeling Program (63)

The tool Pathogen Modeling Program (PMP) is accessible on line. This tool for the prediction of the behaviour of micro-organisms is one of most commonly used. The current version includes over 35 models for 11 pathogens. The environmental factors taken into account are temperature, pH, NaCl salt rate (or water activity) and other factors for some micro-organisms (organic acids, composition of the atmosphere, etc.) The predictions may be exported and the bibliography references from which the models are derived are given.

The drawback of this model is that only fixed temperature conditions can be implemented (44).

Comparison of estimated growth potentials in the petitioner's application and the potential estimated with other models

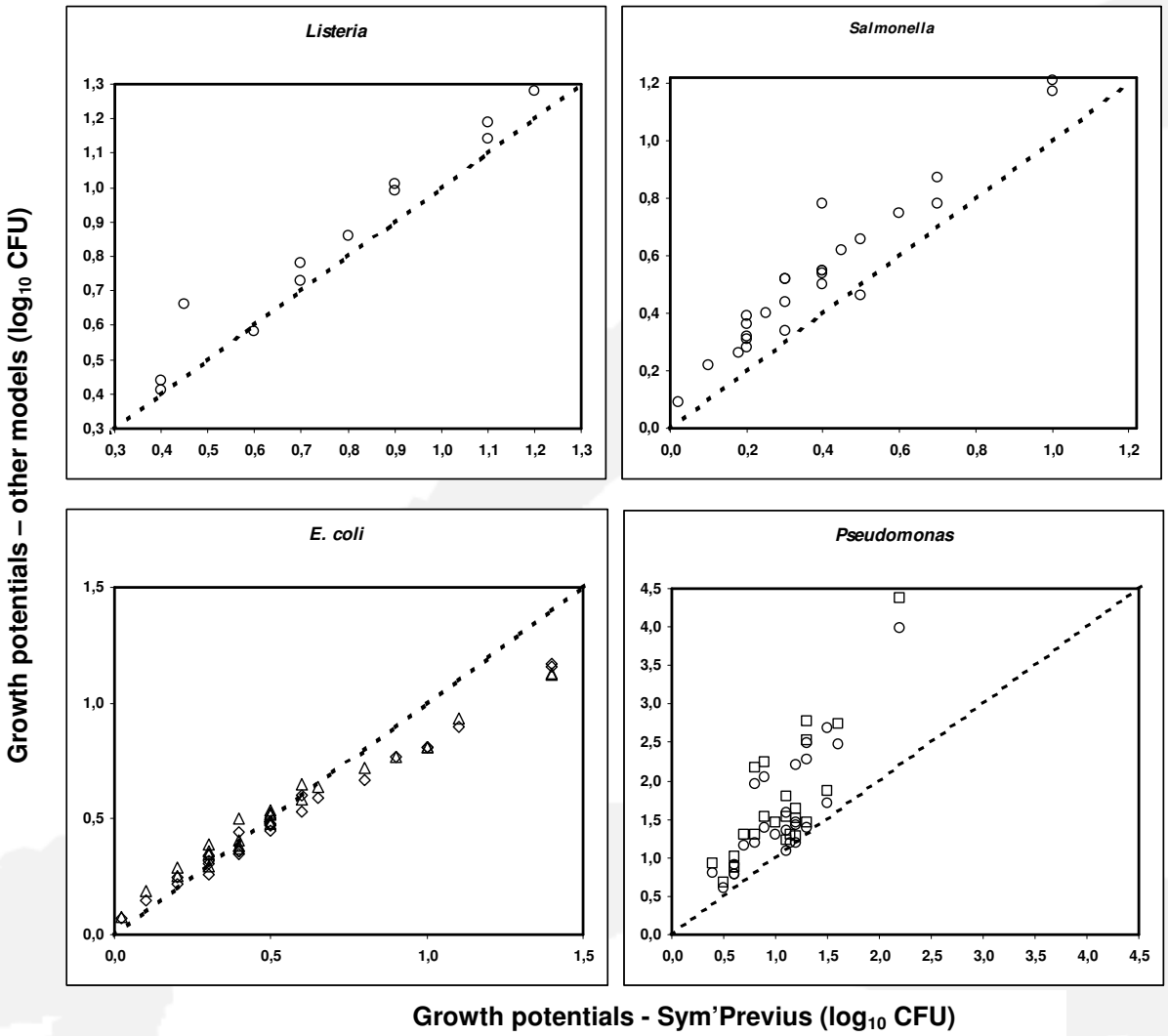


Figure A1. Comparison of the growth potential estimated by Sym'Previus for the different types of cooling in transport and in refrigerant chambers with the potentials estimated using the following models: (○) Combase Modelling Toolbox (31) ; (◇) Gill et al. (23); (△) Ross et al. (55); (□) Gill & Jones (24).

Annex 2: Relationship between surface temperature and temperature at the core of carcasses

The petitioner has measured the core temperature of hams at the time of loading. The temperatures measured at the surface of the same hams at the time of loading are directly correlated with those core temperatures (Figure A2).

Those two temperature measurements are both liable to random fluctuations, so orthogonal regression has been used to determine the relationship between the two temperatures. Instead of minimising the squared deviation along the horizontal or vertical axis, the straight line of orthogonal regression (or major axis) implies the two variables.

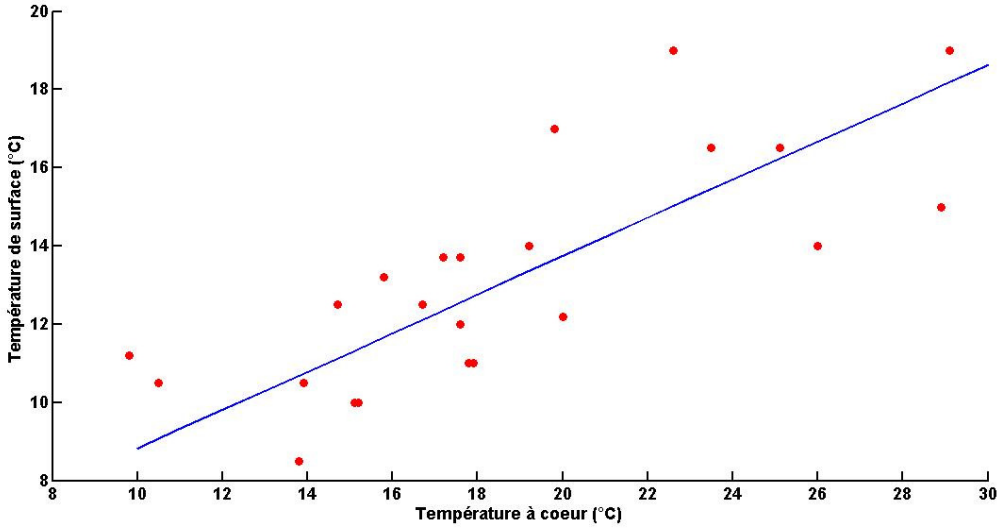


Figure A2. Surface temperatures (T_S) versus core temperatures (T_C) of hams at the time of loading (•). Major axis (—) : T_S=0.4866 T_C+3.99.