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Annex A – Protocol for the Scientific opinion on the microbiological safety of aged meat

A.1.   Introduction
A.1.1.   Introduction and scope of this document

This annex includes the protocol for the scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) on the microbiological safety of aged meat.

The protocol was developed as per the framework for protocol development for EFSA's scientific assessments (EFSA, 2020) and tailored to accommodate the characteristics of the mandate. Thus, the planning presented below is as comprehensive as required to develop an outline approach to addressing the ToRs of the mandate, taking limitations such as lack of information and/or data into account. For instance, the extent of planning can vary from being very comprehensive to generic outlines of the approach for performing the assessment and its rationale, limitations and related sources of uncertainty. The reasons for limited planning can be multiple: e.g. limited resources, limited time, or no need.

After establishing the objectives of the assessment, including clarification and interpretation of the Terms of Reference (TOR) received (section 2), both steps in the protocol development are described, starting with the formulation of the problem (section 3) followed by the planning of the methods for conducting the assessment (section 4).

A.1.2. Terms of Reference (TOR) as provided by the requestor

EFSA is asked to deliver a scientific opinion on the microbiological safety of aged meat.

More specifically, EFSA is asked:

TOR 1. To provide an overview of the current practices used by food business operators for dry-ageing and wet-ageing of meat (e.g. time, temperature, relative humidity, air flow, type of packaging)

TOR 2. To identify public health-relevant microbiological hazards and spoilage bacteria occurring in the process of prolonged dry-ageing and wet-ageing of meat, also considering its possible use for the production of minced meat and mechanically separated meat

TOR 3. To assess the impact that dry-ageing and wet-ageing of meat, produced according to selected current practices, could have on the load of public health-relevant microbiological hazards and spoilage bacteria, when compared to standard fresh meat

TOR 4. To provide those conditions during the production of dry-aged and wet-aged meat and possible further storage that would result in a similar or lower load of the relevant microbiological hazards and spoilage bacteria as compared to standard fresh meat before consumption (i.e. at the end of the shelf-life)

TOR 5. To recommend additional good hygiene practices specific to the production and storage of dry-aged and wet-aged meat, as compared to those relevant for the production and storage of standard fresh meat.

A.2 Problem formulation

A.2.1. Clarification of the ToRs

- The dry-aging will cover beef only while the wet-aging applies to beef, pork and lamb.

- Meat aging done in meat plants and restaurants is covered in the mandate, but meat aging performed at home in a domestic setting is excluded.

- Aged meat is not currently allowed to be used for the production of minced meat or MSM, due to the time-restrictions indicated in the law (Regulation (EC) No 853/2004). but will be included in the assessment as there is interest in knowing the impact of the use of aged meat, for this type of production.

- The end point of the assessment is the end of shelf-life. This will be covered in the answer to ToR1.

- There is no standard definition for 'standard fresh meat' or no agreed 'standard' methods for meat maturation. This may, for example, require hanging of the carcasses for a set period of time or carcass chilling for 48 hours followed by cutting into primals, vacuum packaging and storage at 2°C for 4 to 6 weeks.

- The term 'prolonged' in ToR 2 will be considered to be the longest period typically used for dry- or wet-aging. Dry aging usually takes longer than wet aging and is usually up to 45 days. However, some customers may request a longer period of 60 to 90 days but this is rare as the beef develops a strong 'blue cheese odour'.

- The assessment will include microbial hazards capable of growth during the aging process.

- For ToR3, the comparison in the counts of pathogenic and spoilage bacteria on dry- and wet-aged meat versus 'standard fresh meat' will be expressed as the log difference rather than a prediction of the absolute bacterial concentrations on each type (dry- and wet-aged vs standard) of meat.

- Inactivation (i.e. reductions in bacterial numbers) will not be considered in the modelling exercise (ToR 3), although this may lead to an overestimation of the log increase. Thus the models will predict the 'worst case scenario'. This approach will be justified in the 'Methods section' and the overall impact included in the uncertainty analysis.

A.2.1.1. Definitions

Fresh meat refers to meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.

Mechanically separated meat or **MSM** means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure.

Current practices refers to practices and procedures occurring in the present time (timeframe 2000-2021)

Domestic ungulates means domestic bovine (including Bubalus and Bison species), porcine, ovine and caprine animals, and domestic solipeds.

Wild ungulates, that are hunted for human consumption and are considered to be wild game under the applicable law in the Member State concerned, including mammals living in enclosed territory under conditions of freedom similar to those of wild game.

Pathogen is an organism (e.g. bacterium, virus and parasite) that can cause disease.

Spoilage bacteria are bacteria which limit the shelf life of foods by producing objectionable odours, colour or slime.

Meat maturation refers to the endogenic enzymatic activity, or proteolysis, which causes muscle tissue to mature, become tender and develop a typical taste.

Meat ageing is the process during which microbes and enzymes act upon the meat to help breakdown the connective tissue to tenderise the meat. There are two ways ageing can be accomplished; **Wet ageing** by storing meat under vacuum conditions in sealed bags and **Dry ageing** by storing meat in a temperature and humidity-controlled environment.

A.2.2. Assessment questions based on the interpretation of the mandate

Step 1: Formulation of the problem (what)

Step 1 consists of the translation of the mandate into assessment question(s) (AQs) (step 1.1) and the definition of the sub-questions (SQs) (step 1.2) of each assessment question and their relationship (conceptual model).

Table A1 provides, for each of the ToR, the translation of the mandate into AQs as included in the second column (step 1.1), while the SQs are included in the third column (step 1.2). The approach for each SQ, i.e. whether to apply a quantitative, qualitative or semi-quantitative approach, has been specified in the fourth column (step 1.3). There was no need to prioritise SQs over others.

A.3. Methods for conducting the assessment

Step 2: Planning of the methods for conducting the assessment (how)

This step includes the overall approach (step 1.3) as well as the evidence needs and the methods (step 2.1) for answering each assessment question (AQ) and associated sub-question (SQ) arising from the ToRs. The methods to be used for obtaining the required information and data (e.g. a literature search), data extraction and evidence appraisal are summarised. Sources of uncertainty and the methods for prioritising and analysing them are also briefly described.

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The methods that will be used for evidence integration across SQs and for accounting for the remaining uncertainty is provided in Table A2.

Table A1. Assessment questions and sub-questions for TORs for the scientific opinion on the microbiological safety of aged meat

ToR	Step 1.1. AQ	Step 1.2. SQ	Step 1.3. Approach	Step 2.1. Evidence needs	Step 2.1. Description of method to be used
ToR1: To provide an overview of the current practices used by food business operators for dry-ageing and wet-ageing of meat (e.g. time, temperature, relative humidity, air flow, type of packaging)	**AQ1:** What are the practices and processes used by meat FBOs and restaurants in the EU for the dry aging of beef and the wet aging of beef, pork and lamb? Specifically, what are the processing conditions (e.g. time, temperature and relative humidity) for each of these processes	**SQ1:** What practices and processes are used by meat FBOs and restaurants in the EU to **dry** age beef? **SQ2**: What practices and processes are used by meat FBOs and restaurants in the EU to **wet** age beef, pork and lamb? **SQ3**: What is the shelf-life of dry aged beef and wet aged beef, pork and lamb? SQ4: what are the resultant characteristics of the meat (surface temperature, pH, aw and concentrations of antimicrobials such as lactic acid)?	Qualitative approach All questions are equally important	Literature review Primary data collection (questionnaire)	**a. Eligibility criteria:** The aim is to retrieve information on the practices and processes used during the dry aging of beef and the wet aging of beef, pork and lamb in the EU. This will be derived through a literature review and a questionnaire that will be sent to food safety competent authorities, meat processor associations and/or representative of the restaurant sector at European, national and regional level. The eligibility criteria for the literature review related to the study characteristics include: – Population: dry aging of beef and wet aging of beef, pork or lamb in the EU (and outside the EU in case of limited information inside the EU) – Outcome: information about the meat aging practices, processes and meat characteristics (here you would have to cover the info that you would extract from the records) – Setting: mainly industrial but including studies undertaken in pilot or laboratory settings. Those related to report characteristics are: – Language of the full text: English – Time: 2000 onwards (as the mandate covers current rather than historical practices)

| | | | | | – Publication type: peer reviewed paper, industry report, review or book (chapter)

b. Definition of the search strategy: The search string for Web of Science (WOS, BCI, CABI, FSTA, MEDLINE) is provided in Table A3 (String 1). Grey literature resources will be also considered

c. Methods for selecting studies for inclusion/exclusion: The screening process will be undertaken in three steps: screening of (1) titles, (2) abstracts and then (3) full-text documents to further identify records to be included/excluded based on the relevance of the information and data provided.
All data gathered through the questionnaire will be considered when addressing ToR 1.

d. Methods for extracting data from included studies. Selected full-text documents will be screened with one reviewer (member of the WG) extracting the information required to answer the SQs. Data obtained in the questionnaires will be included.

e. Methods for appraising evidence. The information gathered will be initially appraised by the reviewer extracting the data and later by the WG members. This process with include reading, reviewing, summarising the information extracted |

					in the draft Opinion and discussion/review by the entire WG. **f. Sources of uncertainty and definition of the methods for prioritising them**. There are multiple sources of uncertainty including the variable impact of practices on extrinsic and intrinsic parameters of the meat. These will be captured in the uncertainty table including 'impact of the uncertainties on the conclusions' as the Opinion is developed. These will not be prioritised. **g. Methods for synthesising evidence**. The methods used for synthesizing evidence will be qualitative. **h. Methods for analysing uncertainties**. The sources of uncertainty will be identified and listed in the 'Uncertainty table'. The methods used for uncertainty analysis will be qualitative.
ToR2: To identify public health-relevant microbiological hazards and spoilage bacteria occurring in the process of prolonged dry-ageing and wet-ageing of meat, also considering its possible use	**AQ2**: What are the relevant microbiological hazards and spoilage bacteria that occur and which of these can grow and/or produce toxins during the dry aging of beef and the wet aging of beef, pork and lamb and on the subsequently stored product, including in minced meat or MSM	**SQ4**: What pathogenic and spoilage bacteria occur and which of these can grow on dry aged beef and **wet** aged beef, pork and lamb? **SQ5**: Which moulds are found on dry aged beef and do these represent a hazard for human health (ie. do they produce mycotoxins under the conditions used)?	Qualitative approach No sub-question is prioritized over any other	Literature review Primary data collection (questionnaire)	**a. Eligibility criteria:** The aim is to retrieve information on the microbiological hazards and spoilage bacteria that occur in dry aged beef and wet aged beef, pork and lamb. This will be undertaken using a review of the scientific and grey literature as well as using a questionnaire. The eligibility criteria for the literature review are: – Population: any publication in the peer reviewed or grey literature that

for the production of minced meat and mechanically separated meat	prepared from the aged meat.	**SQ6:** What pathogenic and spoilage bacteria are relevant considering the use of dry aged beef and wet aged beef, pork and lamb for the production of minced meat and mechanically separated meat?			identifies and/or quantifies the pathogenic bacteria, spoilage bacteria or mycotoxin formation on beef during the dry aging process or on beef, pork or lamb during the wet aging process (from chilled carcasses to the end-of shelf-life) including minced meat or MSM prepared from the aged meats. – <u>Outcome</u>: The outcome of this review will be data extracted on pathogenic and spoilage bacteria and mycotoxin development including, prevalence and concentration of relevant bacteria and/or mycotoxins and how these change at the various stages during the dry and wet aging processes. -<u>Setting</u>: mainly industrial (for literature review and questionnaire) but will include pilot and laboratory-based studies. Those related to report characteristics are: – <u>Language of the full text</u>: English – <u>Time</u>: 2000 onwards – <u>Publication type</u>: peer reviewed paper, industry report, review or book (chapter) **b**. **Definition of the search strategy**: The search string for Web of Science (WOS, BCI, CABI, FSTA, MEDLINE) is provided in Table A4 (string 2). **c. Methods for selecting studies for inclusion/exclusion**: As for ToR 1.

					d. Methods for extracting data from included studies. As for ToR 1. **e. Methods for appraising evidence**. As for ToR 1. **f. Sources of uncertainty and definition of the methods for prioritising them**. There are several sources of uncertainty including the design of the studies used to enumerate/quantify hazards (bacterial pathogens and mycotoxins) and spoilage bacteria, the sampling and detection methods used, the confirmation methods used, etc. If necessary, these will be prioritised based on expert discussion within the WG. **g. Methods for synthesising evidence**. As for ToR 1. **h. Methods for analysing uncertainties**. The methods used for uncertainty analysis will be qualitative. Uncertainty sources will be identified and analysed individually and where appropriate as combinations, by expert discussion within the WG.

TOR3. To assess the impact that dry-ageing and wet-ageing of meat, produced according to selected current practices, could have on the load of public health-relevant microbiological hazards and spoilage bacteria, when compared to standard fresh meat	**AQ** what is the increase in the relevant microorganisms (from AQ2) during the dry-ageing of beef and the wet aging of beef, pork and lamb (from AQ1) and during subsequent storage, as compared to 'standard fresh meat'?	**SQ7**: which are the scenarios that represent current practices of dry-ageing of beef, wet aging of beef, pork and lamb and standard fresh meat maturation (time/temperature, pH, aw/relative humidity, with/out bag, with/out competition, etc.) including minced meat and MSM preparation from the aged meat? **SQ8**: which are the relevant pathogenic bacteria, spoilage bacteria and/or mycotoxigenic moulds to evaluate (able to grow and/or produce toxin)? **SQ9**: What is the log change for each scenario and each microorganism and/or toxin produced by moulds?	Quantitative approach when the appropriate models and the required data are available, otherwise semi-quantitative. Semi-quantitative or qualitative (if no quantitative tools, or insufficient data, are available for a given relevant microorganism)	Primary data collection (questionnaire and information from hearing expert(s) Literature review (outputs from ToR1 and ToR2) and data in databases covering the behaviour of microorganisms (ComBase Browser) Predictive microbiology models (secondary models and/or open access tools) Pending the outcome of Tor 1 and 2 it may be required to do an EKE to estimate some of the evidence and its uncertainties	**Sources of information:** mainly outputs from ToR1 and ToR2, including the literature review, questionnaire and information provided by hearing expert(s). In case the available open access tools for predictive microbiology simulation do not cover the relevant microorganisms to be assessed, and/or the literature search done for ToR2 does not provide quantitative information on the behaviour of relevant microorganisms, an additional specific literature search will be considered. **Sources of uncertainty and definition of the methods for prioritising them**. The usual limitations related to data, definition of scenarios, and modelling (the application of predictive models not necessarily developed in and for, or validated for meat aging), the use of challenge test results, and the need to make assumptions, etc. will be associated with a number of uncertainties. If necessary, these will be prioritised based on sensitivity analyses (for relevant scenarios) when possible or expert discussion within the WG. **Methods for synthesising evidence**. The methods used for synthesizing evidence will be quantitative assuming

					that quantitative tools will be available for the relevant microorganisms to be evaluated. Semi-quantitative or qualitative methods will need to be applied in cases where no quantitative tools are available for a given relevant microorganism **Methods for analysing uncertainties**. The sources of uncertainty will be identified and listed in the 'Uncertainty table'. The methods used for uncertainty analysis will be qualitative and/or quantitative (e.g. application of predictive models to assess the impact of assumptions).
TOR4. To provide those conditions during the production of dry-aged and wet-aged meat and possible further storage that would result in a similar or lower load of the relevant microbiological hazards and spoilage bacteria as compared to standard fresh meat before consumption (i.e. at the end of the shelf-life)	**AQ4** What are the conditions for producing, handling (including trimming, cutting, packaging) and storing dry-aged beef and wet aged beef pork and lamb to ensure similar or lower prevalence and counts/load/concentrations of pathogenic microorganisms, spoilage bacteria and, if relevant, mycotoxins at the end of shelf-life as compared to standard fresh meat?	**SQ10:** What are the conditions for producing, handling (including trimming, cutting, packaging) and storing dry-aged beef pork and lamb to ensure similar or lower prevalence and counts/load/concentrations of pathogenic microorganisms, spoilage bacteria and, if relevant, mycotoxins at the end of shelf-life as compared to standard fresh meat? **SQ11:** What are the conditions for producing, handling (including trimming, cutting, packaging) and storing wet aged beef pork and lamb to ensure similar or lower prevalence and	As for ToR 3.	As for ToR 3. If predictive models are available and there is sufficient data to describe scenarios to indicate conditions resulting in similar loads.	As for ToR 3.

		counts/load/concentrations of pathogenic microorganisms, spoilage bacteria and, if relevant, mycotoxins at the end of shelf-life as compared to standard fresh meat?			
TOR5. To recommend additional good hygiene practices specific to the production and storage of dry-aged and wet-aged meat, as compared to those relevant for the production and storage of standard fresh meat.	**AQ5:** What additional GHPs could be employed to minimise the prevalence and/or concentration of pathogenic and spoilage bacteria and mycotoxin formation (if relevant) on dry and wet aged meat?	**SQ11**: What GHPs are currently used in meat plants and restaurants that produce dry aged beef and wet aged beef, pork and lamb and for the production and storage of 'standard fresh meat'? **SQ12**: Based on the outcomes from ToR3, are additional GHP required for dry and wet aged meat to assure its food safety? **SQ13**: Based on the outcomes from ToR4 and a literature review what changes in current practices formulated as GHP(s) could be developed to achieve a similar or lower load of relevant microbiological hazards and spoilage bacteria on dry and wet aged meat as	Qualitative approach No sub-question is prioritized over any other	Literature review with a particular focus on industry guidelines including those that can be accessed through national regulatory authorities. Review of the conditions that must be achieved to reduce the microbial hazards and spoilage bacteria on dry and wet aged meat as provided by the answer to ToR4.	**a. Eligibility criteria**: The aim is to retrieve information on the current and possible additional GHPs to be used during the dry aging of beef and the wet aging of beef, pork and lamb in the EU. This will be derived through a literature review. The eligibility criteria for the literature review are: -Population: any publication in the peer reviewed or grey literature that describes the GHP used in the dry and wet aging of meat in the EU -Outcome: the identification of current and possibly additional GHPs -Setting: industry, pilot and where necessary laboratory-based studies. Those related to report characteristics are: -Language of the full text: English -Time: 2000 onwards -Publication type: peer reviewed paper, industry report, review or book (chapter) **b. Definition of the search strategy:** The search would be as for ToR 1 with the addition of 'GHP' OR 'prerequisites' **c. Methods for selecting studies for inclusion/exclusion:** As for ToR 1.

		compared to standard fresh meat?			**d. Methods for extracting data from included studies.** As for ToR 1. **e. Methods for appraising evidence.** As for ToR 1. **f. Sources of uncertainty and definition of the methods for prioritising them.** As for ToR 1. **g. Methods for synthesising evidence.** As for ToR 1. **h. Methods for analysing uncertainties.** As for ToR 1.

Table A2. Integration of evidence across sub-questions and remaining overall uncertainty

Step 2.2		
ToRs as clarified	**Integration of evidence between sub-questions**	**Addressing overall uncertainty**
ToR1	The direct evidence from the literature survey and the questionnaire will be used to answer the SQs and these will be integrated qualitatively to answer ToR1 . The information obtained from the literature review and the questionnaire will be extracted, summarized and used to build the description of the processes used in dry aging of beef and wet aging of beef, pork and lamb.	There may be uncertainty about the specific processes and associated parameters such as temperature, pH and aw due to a lack of reliable information. This uncertainty will be qualitatively analysed by identifying and highlighting these information gaps.
ToR2	As for ToR1, direct evidence from relevant studies published in the scientific literature will used to address the SQs and these will be integrated qualitatively to addressToR2. The information and specific data (prevalence and/or concentrations of pathogenic and spoilage bacteria and/or mycotoxins) will be extracted and summarized using both text and, where relevant, tables.	There may be uncertainty about the prevalence and/or concentration of pathogenic and spoilage bacteria and/or mycotoxins produced by moulds (dry aging only) due to a lack of information/data or due to the study designs, detection, confirmation and characterization methods used. This uncertainty will be qualitatively analysed by identifying and highlighting these information gaps.
ToR3	The outputs of ToR1 (reasonably foreseeable practices) will be used to define the scenarios to be assessed, including worst case, mean/most frequent case, best case scenarios (SQ6). etc. The outputs of ToR2 will be used to qualitatively answer SQs 7, and 8 on the basis of agreed criteria (e.g. growth capability under the aging conditions) and the availability of predictive tools and/or published experiments on the behavior of the target microorganisms.	The usual limitations related to the definition of scenarios and the uncertainties identified and addressed in ToR1 and ToR2, the application of predictive models (expected to be not developed in and for / nor expensively validated for meat aging), the use of challenge test results, the need to make assumptions, etc. will be associated with a number of uncertainties. The methods used for uncertainty analysis will be qualitative and, whenever possible, quantitative approaches through the application of predictive models to assess the impact of assumptions in e.g. defining the scenarios. Data gaps will be identified in order to suggest recommendations for future research and actions.
ToR4	From the outputs from previous ToRs, the combination of conditions during processing, handling and storage of age meat ensuring similar or lower prevalence and counts/load of pathogenic microorganisms at the end of shelf-life as compared to standard fresh meat will be identified.	The uncertainties associated with ToR3 will also be uncertainties for ToR4. The methods used for uncertainty analysis will be qualitative and, whenever possible, quantitative approaches through the application of predictive models to assess the impact of assumptions in e.g. defining the scenarios. Data gaps will be identified in order to suggest recommendations for future research and actions.

| ToR5 | Direct evidence of the GHPs currently used will be obtained from the relevant scientific and grey literature (eg. prerequisite programme guidance documents), which will also serve as source of potential additional GHPs that could be applied to improve hygiene in the dry and wet meat aging processes. This information will be extracted, summarized in the answers to the 3 SQs and integrated qualitatively to address ToR5, using both text, and where relevant, tables. | There may be uncertainty about both currently used and potentially applicable GHPs. Moreover, it is unlikely there will be direct evidence that the latter are effective in practice. These uncertainties will be highlighted and analysed qualitatively. |

Table A3. String 1 for literature review to address ToR 1

Set	Query	Notes	Hits as 21.07.2021
#16	#15 and English (Languages)	+ Language limit (English)	**194**
#15	#14	+ Time limit (2000-2021)	204
#14	#13 and Books or Review Articles (Document Types)	+ Document type Limit (Books/Reviews only)	301
#13	#12 OR #11	Combination	8,227
#12	TI=((Beef OR Meat OR Steak OR Lamb OR Pork OR Sheep OR Loin OR Striploin OR Primal* OR Subprimal*) AND ("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "Ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness"))	Meat and Ageing in Title	5,269
#11	#10 AND #9	Meat, all types of ageing and process concept	4,191
#10	TS=("Process" OR "processes" OR Procedure* OR Protocol* OR Practice* OR "Proceeding" OR Operation* OR "Method" OR "Methods" OR Step* OR Technique* OR System* OR "Modus Operandi" OR "Environment Condition*" OR Temperature OR RH OR "Relative humidity" OR Aw OR "Water activity" OR Ventilation OR Day* OR Week* OR "Airflow")	Process concept	59,669,281
#9	#8 OR #6 OR #3	Meat and all types of ageing	4,779
#8	#7 AND #1	Meat and Extra info	2,059
#7	TS=(("Smart" OR "Postmortem" OR "Post-mortem" OR "Prolonged" OR "Extended" OR "Bag") NEAR/7 ("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR	Extra info (ageing synonyms less specific)	10,138

	"Ripened" OR "ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness"))		
#6	#5 AND #4	Dry Ageing Meat II	1,639
#5	TS=(("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness") NEAR/7 ("Dry" OR "Drying" OR "Dried" OR "Dehydrating" OR "Dehydrated" OR "Dehydrate" OR "Dehydration" OR "Desiccation" OR "Desiccating" OR "Desiccate" OR "Desiccated"))	Dry Ageing	13,126
#4	TS=(Beef* OR Meat* OR Steak* OR Carcass* OR Loin* OR Striploin* OR Primal* OR Subprimal*)	Meat II	729,571
#3	#2 AND #1	Wet Ageing Meat I	1,597
#2	TS=((Wet OR Moist OR Moisture OR Humid* OR Vacuum OR Vacuity OR "Vacuum-pack*" OR VP OR Void OR "Empty-pack*" OR "Empty-spac*" OR Sousvid* OR "Sous-vid*") NEAR/7 ("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "Ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness"))	Wet Ageing (or Vacuum-pack)	30,375
#1	TS=(Beef OR Meat OR Steak OR Lamb OR Pork OR Sheep OR Primal* OR Subprimal*)	Meat I	1,137,289

The search has been restricted to 'books' and 'reviews'.

Table A4. String 2 for literature review to address ToR 2

Set	Query	Notes	Hits as 21.07.2021
#15	#14	Meat, all types of ageing and microbiological hazard (time limit 2000-2021)	**949**
#14	#13 AND #9	Meat, all types of ageing and microbiological hazard	1,445
#13	#12 OR #11 OR #10	Microbiological Hazard (safety, bacteria and moulds)	19,728,456
#12	TS=("Food borne disease*" OR "Food borne illness*" OR "Foodborne disease*" OR "Foodborne illness*" OR "Safe" OR "Safety" OR "Security" OR "Hazard" OR "Hazards" OR "Prevent" OR "Preventing" OR "Prevention" OR "Risk" OR "Risks" OR "Danger*" OR Contaminat* OR Microbial* OR "Microbiological")	Safety	11,702,635
#11	TS=(Aflatoxin OR Aspergillus OR Aureobasidium OR Candida OR Cladosporium OR Debaryomyces OR Deoxynivalenol OR Fumonisin OR Fung* OR Fusarium OR Mold OR Mould* OR Mucor OR Mycotoxin* OR Ochratoxin OR Patulin OR Penicillium OR "Pilaria anomala" OR Rhizopus OR Rhodotorula OR Thamnidium OR Yeast OR Zearalenone)	Moulds	3,617,726
#10	TS=(Achromobacter OR Acinetobacter OR "Aerobic plate count" OR Aeromonas OR Alcaligenes OR Alteromonas OR Anaerob* OR AnPC OR Arcobacter OR Ardenticatenales OR Arhtrobacter OR APC OR Bacillus OR Bacter* OR Bacteroidales OR Brochotrix OR Campylobacter* OR Carnobacterium OR Citrobacter OR Clostridium OR Coliform* OR Corynebacterium OR E.coli OR Enterobacter* OR Enterococcus OR "Escherichia coli" OR Flavobacterium OR Firmicutes OR "Gram-negative" OR Gram- OR Gram+ OR "Gram-positive" OR Hafnia OR Klebsiella OR Kluyvella OR Kocuria OR Kurthia OR "L. monocytogenes" OR LAB OR "Lactic acid bacteria" OR Lactobacill* OR Lactococc* OR Leuconostoc* OR Lister* OR Microbacter* OR Micrococcus OR Microorganism* OR Moraxella OR Morganella OR Paenibacillus OR Pantoea OR Pathogen* OR Plesiomonas OR Proprionibacterium OR Proteus OR Providencia OR Pseudomona* OR Psychrophil* OR Psychrotroph* OR Psychrotrophs OR Rahnella OR Rhizobiales OR "S. Aureus" OR Salmonell* OR Serratia OR Shewanella OR Shigell* OR Sphingobacterium OR Spoilage OR	Bacteria	10,365,879

	Spore OR SSO OR Staphylococc* OR Stenotrophomonas OR STEC OR Streptococc* OR "Total bacterial count" OR "Total viable count" OR "Total aerobic count" OR "Total plate count" OR TBC OR TPC OR TVC OR Vibrio OR VTEC OR Weissella OR "Y. Entercolitica" OR Yersini*)		
#9	#8 OR #6 OR #3	Meat and all types of ageing	4,779
#8	#7 AND #1	Meat and Extra info	2,059
#7	TS=(("Smart" OR "Postmortem" OR "Post-mortem" OR "Prolonged" OR "Extended" OR "Bag") NEAR/7 ("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness"))	Extra info (ageing synonyms less specific)	10,138
#6	#5 AND #4	Dry Ageing Meat II	1,639
#5	TS=(("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness") NEAR/7 ("Dry" OR "Drying" OR "Dried" OR "Dehydrating" OR "Dehydrated" OR "Dehydrate" OR "Dehydration" OR "Desiccation" OR "Desiccating" OR "Desiccate" OR "Desiccated"))	Dry Ageing	13,126
#4	TS=(Beef* OR Meat* OR Steak* OR Carcass* OR Loin* OR Striploin* OR Primal* OR Subprimal*)	Meat II	729,571
#3	#2 AND #1	Wet Ageing Meat I	1,597
#2	TS=((Wet OR Moist OR Moisture OR Humid* OR Vacuum OR Vacuity OR "Vacuum-pack*" OR VP OR Void OR "Empty-pack*" OR "Empty-spac*" OR Sousvid* OR "Sous-vid*") NEAR/7 ("Ageing" OR "Aging" OR "Aged" OR "Conditioned" OR "Conditioning" OR "Matured" OR "Maturation" OR "Maturating" OR "Ripe" OR "Ripened" OR "Ripening" OR "Tenderize" OR "Tenderized" OR "Tenderise" OR "Tenderised" OR "Tenderness"))	Wet Ageing (or Vacuum-pack)	30,375
#1	TS=(Beef OR Meat OR Steak OR Lamb OR Pork OR Sheep OR Primal* OR Subprimal*)	Meat I	1,137,289