Is EU regulation on the use of antioxidants in meat preparation and in meat products still cutting edge?

Beniamino T. Cenci-Goga[1,2,*], Musafiri Karama[2], Chrystalleni Hadjichralambous[3],

Paola Sechi [1]& Luca Grispoldi[1]

[1]School of Veterinary Medicine, University of Perugia, Via San Costanzo, 4, 96126, Perugia, Italy

[2]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

[3]Department of Biology, University of Crete, Heraklion, Crete, Greece

*Correspondence to**:** Beniamino T. Cenci-Goga. Email: bengi@mac.com; beniamino.cencigoga@unipg.it

Musafiri Karama. Email: musafiri.karama@up.ac.za
Chrystalleni Hadjichralambous. Email: chad@materials.uoc.gr
Paola Sechi. Email: paola_sechi@outlook.it
Luca Grispoldi. Email: grisluca@outlook.it

Contributions

All authors contributed in equal parts to the manuscript.

Abstract

The use of antioxidants in meat preparation and meat products is highly debated. Regulations define the use of antioxidants mostly in terms of the age-old subdivision between meat preparations and meat products. Best practices are not well represented in regulations. Antioxidants for foodstuffs during processing or before packing protect colour, aroma and nutrient content. As regards food safety regulations, long-term efforts have been made in terms of food standards, food control systems, food legislation and regulatory approaches. These have, however, generated several questions on how to apply the law to diverse food businesses. To answer these questions, a thorough examination of the EU legislator's choices for food additives and definitions is provided and discussed in relation to factors affecting microbial growth. The paper highlights the regulatory aspects along with the correct application and interpretation of the norms

Keywords: Antioxidant; Ascorbic acid; Meat preparation; Meat products; Meat spoilage

Introduction on antioxidants in meat

Among food additives, the use of antioxidants in meat is currently regulated by EC Regulation 1333/2008 [1], amended and supplemented by EU Regulation 1129/2011 [2] and EU Regulation 601/2014 [3].

Antioxidants are described (Annex 1 EC Regulation 1333/2008 [1]) as substances which prolong the shelf life of foods and protect from deterioration that results from oxidation, leading to fat rancidification and colour changes. The antioxidants legally permitted in meat, without using the maximum dosage nevertheless, are: i) ascorbic acid and its salts, ii) citric acid and its salts. These compounds are identified by the E-numbers: E 300 L-ascorbic acid, E 301 sodium L-ascorbate, E 302 calcium L-ascorbate, E 330 citric acid, E 331 sodium citrates, E 332 potassium citrates, E 333 calcium citrates.

Among these, ascorbic acid, also known as vitamin C, is the most commonly used food additive. The food industry uses chemically synthesised, nature-identical L-ascorbic acid [4,5,6]. When used as an antioxidant, however, it cannot be classed as vitamin C. The molecular formula for ascorbic acid is $C_6H_8O_6$ and it appears as a white solid, which dissolves in water (it is one of the water-soluble vitamins) to give a mildly acidic solution. It is an excellent reducing agent, as it tends to oxidise in the presence of oxygen and form dehydroascorbic acid. Its reducing properties prevent not only oxidation responsible for discolouring in meat during storage, but also the formation of metamyoglobin. The addition of ascorbic acid improves meat colour and shelf life [7, 8]. For example, the use of 200–1000 ppm of ascorbic acid delays browning in minced pork [9] and beef [10] and has an inhibitory effect on some pathogens [8, 11, 12].

This paper, by a thorough analysis of EU legislation and criteria behind the legislator's choices, examines the applicability of the law to diverse food businesses.

Chronology of the specific legislation in the EU

The regulations concerning the antioxidants in meat have continually evolved right up to EU Regulation 1333/2008 [1]. Doubts on how they should be interpreted arose as a result of superimposing community regulations on top of national laws and integrating them with other sector-specific legislative provisions, e.g. EC Regulation 853/2004 [13] under the mandatory obligation to apply the regulations to different, national, production circumstances.

The most debated question regards the possibility of admixing antioxidants to minced meat preparations, made in retail shop preparation areas. The Italian legislation expressly (explicitly?) prohibited butchers from using antioxidants in minced meat under the Royal Decree Law No. 3298 of 20 December 1928 [14], which prohibited the sale of pre-prepared minced meat for consumer protection. EC Regulation 853/2004 [13] gave specific details for the production of food of animal origin. Specifically, in Annex I it defined minced meat as "boned meat that has been minced into fragments and contains less than 1% salt" and meat preparations as "fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat". EU Regulation 1133/2008 [1] and EU Regulation 1129/2011 [2], which amended and completed the previous legislation, established the European Union

list of food additives and provided a new interpretation of legislation on food additives. More recently, the EU Regulation 601/2014 [3] introduced the most significant amendments regarding meat preparations, which will be further discussed below.

The main difference in the framework of the most recent regulations (2008, 2011 and 2014) compared to preceding laws is basically a formality. The different categorisation of foods makes it easier to understand. The new laws actually divide the foods into categories and specific sub-categories, identified by ascending numbers, whereby the authorised additives and the relevant conditions of their use are stated. Nevertheless, the framework remains based on the principle of the positive list. Additional principles, as stated on the Commission website, EUR-LEX (https://eur-lex.europa.eu), are as follows: (i) to be included in the approved EU list, a food additive must not be a health risk and must not mislead consumers; (ii) it must be used for a reasonable requirement, which cannot be fulfilled in any other way; (iii) the additive must provide some benefit to consumers, including the preservation of the nutritional quality of the food, aid the manufacture, processing, preparation, treatment, packing, transport or storage of food; (iv) satisfy special dietary needs; (v) the minimum quantity of additives required should be used to achieve the desired effect, and that quantity must take into account not only the acceptable daily intake, but also the needs of special consumer groups; (vi) lastly, additives should not usually be used in unprocessed food, or for infants and babies, unless envisaged for a specific use.

Classification of additives and food categories under EU Regulation 1333/2008 with emphasis on meat and antioxidants

The annexes of EU Regulation 1333/2008 [1] provide a list of food categories and classify additives as follows: (i) (Part A) foods in which the presence of an additive may not be permitted by virtue of the carry-over principle set out in article 18, paragraph 1, letter a; (ii) all the additives (Part B, list of all additives: 1. colours, 2. sweeteners, 3. additives other than colours and sweeteners); (iii) (Part C) the definitions of the groups of additives (group I, group II—food colours authorised quantum satis, group III—food colours with combined maximum limit, group IV—polyols and other additives that may be regulated combined); (iv) (Part D) the food categories and (v) (Part E) the authorised food additives and conditions of use in food categories.

Meat is included in category 08, Part A and is further divided into sub-categories. For the antioxidants ascorbic acid (E 300), sodium ascorbate (E 301) and calcium ascorbate (E 302) for meat preparations, the EC Regulation 853/2004 [13] indicates the maximum level as quantum satis and for the restrictions/exceptions it considers that their use is allowed for: “only gehakt, prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added”.

The initial draft of EC Regulation 1333/2008 [1] envisaged the possible addition of antioxidants only for "prepacked preparations of freshly minced meat". As mentioned above, subsequent amendments were made, the most significant of which were those introduced by EU Regulation 601/2014 [3]. More specifically, by rewording Annex II of EC Regulation 1333, it permitted the use of antioxidants for: “only gehakt, prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added”. Furthermore, it corrected Article 18 and established that the carry-over principle could be applied to meat preparations. As a result, the presence of an unauthorised food

additive is considered legal, when the additive is authorised for one of the ingredients used in it.

Therefore, according to the provisions of EC Regulation 1333/2008 [1], as amended by EU Regulation 601/2014 [3], antioxidants can be admixed with “only prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added”.

In the meantime, EU Regulation 1169/2011 [15] regarding the provision of information on food to consumers came into force, giving the following definition in Article 2, paragraph 2, letter e): «prepacked food» “means any single item for presentation as such to the final consumer and to mass caterers, consisting of a food and the packaging into which it was put before being offered for sale, whether such packaging encloses the food completely or only partially, but in any event in such a way that the contents cannot be altered without opening or changing the packaging; «prepacked food» does not cover foods packed on the sales premises at the consumer’s request or prepacked for direct sale”. Thus, the previous adjective pre-packaged, used in EC Regulation was replaced with the similar prepacked, but without changing the meaning.

The draft of EC Regulation 1333/2008 [1] raised numerous uncertainties in its interpretation. A reading of both regulations and some national rules shows that freshly minced meat preparations made in retail stores cannot be admixed. The use of antioxidants is, on the other hand, permitted for meat, to which ingredients other than additives or salt have been added at store level (Tables 1, 2, 3).

Table 1 Definitions of minced meat, meat preparation and meat products

Source	Product	Definition
Regulation (EC) No 853/2004 of the European parliament and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin	Minced meat	Boned meat that has been minced into fragments and contains less than 1% salt
	Meat preparations	Fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat
	Meat products	Processed products resulting from the processing of meat or from further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat
Commission regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Definitions as amended by EU Regulation 601/2014	Meat preparation —category 08.2	As defined by EC Regulation 853/2004
	Meat products —category 08.3	As defined by EC Regulation 853/2004

Table 2 Antioxidants allowed in meat

Matrix	Reference for definition	Reference for use of E300, E301 ed E302	Use
Minced meat	Reg. 853/2004	Reg. 1333/2008	No
Meat preparation (in EU regulation, there is no explicit difference between prepacked and non-prepacked)	Reg. 853/2004	Art. 18 reg. 1333/2008 carry-over principle (modified by 601/2014 (without technological function)	Yes
		Reg. 1129/2011	No
		Reg. 601/2014	Yes (only in meat preparation)
Prepacked preparations of fresh minced meat	Reg. 1333/2008, annex II	Reg. 1333/2008, annex II	Yes
Prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added	Reg. 1333/2008 (as amended by Reg. 601/2014)	Reg. 601/2014	Yes
Preparations in which ingredients have been injected	Reg. 601/2014	Reg. 601/2014	Other additives of group I
Meat preparations composed of meat parts that have been handled differently: minced, sliced or processed and that are combined together	Reg. 601/2014	Reg. 601/2014	Other additives of group I
Fresh minced meat (< 1% di salt) (in EU regulation there is no explicit difference between prepacked and non-prepacked)	Reg. 853/2004 Reg. 601/2014	Reg. 853/2004 Reg. 601/2014	No
Prepacked preparations of fresh minced meat (> 1% salt)	Reg. 853/2004 Reg. 601/2014	Reg. 853/2004 Reg. 601/2014	Yes

Table 3 Authorization for use of E300, E301 and E302

Matrix	Reg. 1333/2008	Reg. 1129/2011	Reg. 601/2014
Minced meat	No	No	No
Meat preparation	Yes	No	Yes
Preparations of fresh minced meat	No	No	Yes
Prepacked preparations of fresh minced meat	Yes	Yes	Yes
Meat preparations to which other ingredients than additives or salt have been added (in EU regulation there is no explicit difference between prepacked and non-prepacked)	No	No	Yes
Preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added	Yes	Yes	Yes
Fresh minced meat (< 1% di salt)	No	No	No
Prepacked preparations of fresh minced meat (> 1% salt)	Yes	Yes	Yes

Meat preparations and meat products

Some difficulties in interpretation of the EU regulations arise from the age-old subdivision between meat preparations and meat products.

The numerous circulars and ministerial notes (cf. note 10,194 of 18 March 2014 "EC Regulation 1333/2008 on food additives and application of the «carry over principle» to meat preparations; note 1308 of 20 January 2014 "Food additives—publication on the European Commission's website of the «Guidance document describing the food categories in Part E of Annex II to Regulation (EC) No 1333/2008 an Food Additive» and note 44979-P-03 of 12 December 2014 "EU Regulation No. 601/14—Query regarding the correct application of the instructions in Annex II Part E during control and assessment activities) show the legislator's desire to highlight the need to identify foods, in which the majority of micro-organisms have a reduced ability to multiply and to produce toxins (Tables 2, 3 and 4) as a result of their intrinsic characteristics (including their pH, free water content, redox potential and temperature) [16,17,18].

Table 4 Behaviour of microbial groups with reference to factors which influence microbial growth [16, 17, 25]

Microorganism or group	18–20 °C	a_w 0.96	pH = 5.3	Anaerobiosis	Nitrites
Escherichia coli	+	–	±	+	±
Serratia	+	+	±	+	±
Other Enterobacteriaceae	+	–	±	+	±
Vibrio	+	+	±	+	±
Campylobacter spp.	–	–	–	–	–
Pseudomonas spp.	+	–	±	–	±
Micrococcus spp.	+	+	+	–	+
Staphylococcus spp.	+	+	+	+	+
Lactobacilli and lactococci	±	+	+	+	+
Enterococci	+	+	+	+	+
Pediococcus spp.	±	+	+	+	+
Leuconostoc spp.	+	+	+	+	+
Bacillus cereus	–	–	+	–	±
Cl. botulinum and *Cl. perfringens*	+	+	+	+	–
Brochothris thermosphacta	+	+	+	+	–
Listeria monocytogenes	+	+	+	+	±

For instance, when the legislator shows interest in the official sampling to verify the safety criteria for *Listeria monocytogenes* (such as in EC Regulation 2073/2005), foods with $pH \leq 4.4$ or $a_w \leq 0.92$ and those with $pH \leq 5$ and $a_w \leq 0.94$ are considered as «foods that do not constitute a favourable medium for the growth of *L. monocytogenes*».

Factors which influence the growth and survival of micro-organisms in food

To understand why the legislator identified the parameters of pH and free water as those to be taken into account, some preliminary remarks are needed on microbial growth and on the factors which influence it. Microbial growth is a self-catalytic process: there is no growth unless at least one living cell is present, and the growth rate increases according to the increase in the living "biomass" present. Bacterial cells divide by binary fission thus the growth rate and total number of cells doubles after each division. However, exponential growth actually occurs for a limited period of time (exponential growth phase). There are four phases: delay (or lag), acceleration, exponential growth (or log), stationary and decline. In the initial phase, there is no apparent growth, as the inoculated bacteria has to adjust to the new environment. The necessary enzymes are synthesised and the damage caused by previous noxa (e.g. freezing, dehydration, heating) is repaired. The log phase (or exponential growth) features an increase in cell numbers, which follows the simple, general formula (2, 4, 8, 16, 32, 64, etc.). The typical curve slope for the micro-organism under examination depends on numerous factors (intrinsic and extrinsic, as discussed below). Lastly, changes in the cell cycle induced by exponential development interrupt this phase, as the key nutrients are depleted and growth inhibiting metabolites accumulation: the culture moves into the stationary phase.

The factors that affect microbial growth are divided into intrinsic, which include nutrients, pH, redox potential, antimicrobial obstacles and water activity (free water) and extrinsic, i.e. those that can be traced to the environmental conditions: relative humidity, temperature and gaseous atmosphere. Among intrinsic factors, with reference to the «presence of nutrients», a micro-organism's inability to use a particular food component restricts its development, posing a competitive disadvantage compared to bacteria, which are able to use it. For

example, the ability to synthesise amylolytic enzymes (to hydrolyze starch) facilitates the growth of certain micro-organisms on cereals. «pH and buffer capacity» control bacterial growth but certain organic acids can act as bacteriostatic agents regardless of the solution pH. This is very useful if used to increase food conservation times without lowering the pH, as a low pH will alter the organoleptic characteristics of the food. Bacteria develop in environments with a pH between 4.5 and 9, with an optimum of between 6.5 and 7.5. For example, fish usually suffer bacterial changes faster than meat at refrigeration temperatures since the pH of mammal muscles after rigor mortis is approximately 5.6, whereas that of fish is 6.2. The redox potential (E_h) in a food matrix is related to the amount of all the oxidizing and reducing species found in the medium. When the concentration of oxidizing species (dissolved oxygen, free radicals, hydrogen peroxide, some oxidized metal ions) increases, the redox potential increases as well as the medium's oxidizing ability. The redox potential is clearly linked to the «gaseous atmosphere» extrinsic factor, i.e. the food environment. For example, the mincing process increases the amount of air in the food matrix and therefore the redox potential E_h increases too, whereas the opposite phenomenon can be observed in vacuum packed products. In the contrary, microbial growth in a food reduces the E_h value, due to the use of oxygen and the production of reducing compounds, such as hydrogen. Also, the redox potential has a major effect on bacterial development in food and every micro-organism possesses a favorable value of redox for growth.

Overall, all food of animal origin contains sufficient water for all micro-organisms to develop. The water content, however, gives no indication of its availability (i.e. so-called «water activity»). From a microbiological viewpoint, the physical state of the water is more important than its concentration. In fact, water that has not bonded with other molecules, i.e. free water, is available for bacterial metabolism. A useful parameter to understand water movement from the environment to the cytoplasm and vice versa is water activity, a_w. Substrate water activity is defined as the ratio between vapour pressure of the solution and the vapour pressure of pure water at the same environmental pressure and temperature. A parameter linked to water activity is osmotic pressure, which can be described as the force per surface unit required to interrupt the flow of water molecules from one high a_w area to a low a_w area. The cytoplasm is an aqueous solution and must maintain a lower a_w than that of pure water: this drives water molecules to pass from the extracellular space into the cytoplasm. If the germ does not tolerate this flow, it increases in volume until it bursts. Bacteria, fungi and algae have a rigid wall capable of resisting the osmotic pressure: up to 30 atm in Gram-positive bacteria, lower than 5 atm in Gram-negative bacteria. If a_w in the area surrounding the micro-organism decreases or the osmotic pressure increases, a_w in the cytoplasm has to be lower and the osmotic pressure higher. For this purpose, bacteria produce solutes, which do not interfere with the cytoplasm functions. As a_w decreases, the number of bacteria capable of multiplying is reduced. Each microbial species has minimum a_w values to develop.

Comments on the legislator's choice

Initially, the legislator intended to use easily applied definitions to clearly and unmistakeably distinguish the shelf life and food safety of different meats.

For instance, in Italy, the definitions in Presidential Decree 309/1998 [19] and in Legislative Decree 537/1992 [20] (Table 1) introduced an easily interpreted parameter (the appearance of the cut surface), leaving the responsibility for subsequent decisions, especially regarding food health and safety, to the preparation, training and experience of the competent authority.

Thus, the legislator intended to distinguish between "meat preparations … to which food products, condiments or additives had been added or which had been subjected to processing which would not have modified the central cell structure of the meat and removed the characteristics of fresh meat" and “products obtained from meat or with meat processed to the extent that the central cut surface shows a lack of the characteristics of fresh meat" (Figs. 1 and 2). This approach stems from a precise desire of the European legislator who, with Directive 92/5/EC [21] (containing the definition of meat-based products–meat products: products prepared from or with meat which has undergone treatment such that the cut surface shows that the product no longer has the characteristics of fresh meat), and with Directive 94/65/EC [22]—containing the definition of meat preparations—meat preparations: meat within the meaning of Article 2 of Directives 64/433/EEC, 71/118/EEC and 92/45/EEC (1), and meat satisfying the requirements of Articles 3, 6 and 8 of Directive 91/495/EEC (2) which has had foodstuffs, seasonings or additives added to it or which has undergone a treatment insufficient to modify the internal cellular structure of the meat and thus to cause the characteristics of the fresh meat to disappear) introduced an innovation, which the competent authority and veterinary services did not fully appreciate. Where the legislator identified a cultural and discretionary ability among professionals and, therefore, in Italy, among the National Health Service veterinarians above all, the Minister of Health at the time found a series of dubious interpretations.

Fig. 1. Hamburger. Meat preparation where the treatment is insufficient to modify the internal cellular structure of the meat. $a_w > 0.97$

Fig. 2. Dry salami. Product which has undergone treatment such that the cut surface shows that the product no longer has the characteristics of fresh meat. $a_w < 0.97$

Objective parameters were required instead of the European legislator's subjective definitions and the choice fell on the parameter of "water activity *(a_w)*". The choice of the value of a_w below 0.97 was mainly the result of the need to be able to correctly define the product "sausage" which, as seen before, includes cold meat products which differ in their technological production specifications. As the scientific community agreed that fresh meat should have levels of a_w no lower than 0.98, the value of 0.97 was chosen exclusively as a threshold value, with which to supplement the definition of meat preparation as meat which has had foodstuffs, seasonings or additives added to it or which has undergone a treatment insufficient to modify the internal cellular structure of the meat and thus to cause the characteristics of the fresh meat to disappear".

It is actually the "lack of the characteristics of fresh meat" which directed the attention of the experts. The value of a_w over 0.97 is actually a characteristic of fresh meat and not a health safety parameter. In fact, the identification of the parameters, which enable a product to be classified as perishable or not, should be sought in other sources of law, such as those quoted above. More specifically, to identify the microbial stability of a product, we should resort to an association of factors, called "hurdle technology", in which food safety is guaranteed by the contribution of several factors (Table 4).

Although technology has taken gigantic steps forward, to measure a_w, laboratory technicians are always faced with an analysis method with restricted sensitivity and specificity. The reference method is described in the standard ISO 21807:2004 (Microbiology of food and animal feeding stuffs—determination of water activity) [23], which puts us on guard against the loss of homogeneity of certain foods, such as minced meat and sausages, and against the repeatability of measurements. Water activity values depend on the temperature at which the analyses are conducted and the time required for the instrument to reach an equilibrium in terms of relative humidity (water vapour) between the sample and head space. Slight temperature variations, leading to even centesimal differences in instrument readings may render the measurement unreliable, bearing in mind the fact that the difference between a meat preparation ($a_w < 0.97$) and a meat-based product ($a_w > 0.97$) actually depends on hundredths [24]. Lastly, regarding the classic definition of meat preparation, we should also bear in mind (Fig. 3) that, although many meat-based products have free water values below 0.97, they maintain the appearance of fresh meat.

Fig. 3. Dry salami. Product which still shows the characteristics of fresh meat regardless of $a_w < 0.97$

Acknowledgements

The authors wish to express sincere appreciation to members of Polyglot, Perugia for careful reading and comments on the manuscript. This paper was produced during the EFSA EU-FORA action Grant—agreement number—GP/EFSA/AFSCO/2017/08—GA16—title: Risk Assessment for Listeria Monocytogenes in Ready to Eat Food

References

1.Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (2008). L 354 Official Journal of the European Union. Brusels

2.Commission regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives (2011). L 295 Official Journal of the European Union. Brussels

3.Commission regulation (EU) No 601/2014 of 4 June 2014 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food categories of meat and the use of certain food additives in meat preparations (2014). L 166 Official Journal of the European Union. Brussels

4.Lee JS, Chang Y, Lee ES, Song HG, Chang PS, Han J (2018) Ascorbic acid-based oxygen scavenger in active food packaging system for raw meatloaf. J Food Sci 83(3):682–688. https://doi.org/10.1111/1750-3841.14061

5.Kanatt SR, Siddiqui A, Chawla SP (2018) Antioxidant/antimicrobial potential of *Emblica officinalis* Gaertn and its application as a natural additive for shelf life extension of minced chicken meat. Biointerface Research in Applied Chemistry 8(4):3344–3350

6.Johnson DR, Inchingolo R, Decker EA (2018) The ability of oxygen scavenging packaging to inhibit vitamin degradation and lipid oxidation in fish oil-in-water emulsions. Innov Food Sci Emerg Technol 47:467–475. https://doi.org/10.1016/j.ifset.2018.04.021

7.Bauernfeind JC, Pinkert DM (1970) Food processing with added ascorbic acid. Adv Food Res. https://doi.org/10.1016/S0065-2628(08)60371-7

8.Mahmoud MH, Abu-Salem AM (2015) Synergistic effect of ascorbic acid and soybean bioactive compounds in extending shelf life of refrigerated burger. Res J Pharm Biol Chem Sci 6(6):17–26

9.Watts B, Lehmann B (1952) Ascorbic acid and meat color. Food Technol 6:194

10.Shivas SD, Kropf DH, Hunt MC, Kastner CL, Kendall JLA, Dayton AD (1984) Effects of ascorbic acid on display life of ground beef. J Food Prot 47(1):11–19

11.King AM, Glass KA, Milkowski AL, Seman DL, Sindelar JJ (2016) Modeling the impact of ingoing sodium nitrite, sodium ascorbate, and residual nitrite concentrations on growth

parameters of Listeria monocytogenes in cooked, cured pork sausage. J Food Prot 79(2):184–193. https://doi.org/10.4315/0362-028X.JFP-15-322

12.Rossitto PV, Cullor JS, Crook J, Parko J, Sechi P, Cenci-Goga BT (2012) Effects of uv irradiation in a continuous turbulent flow uv reactor on microbiological and sensory characteristics of cow's milk. J Food Prot 75(12):2197–2207. https://doi.org/10.4315/0362-028X.JFP-12-036

13.Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin (2004). L 139 Official Journal of the European Union Brussels

14.R.D. 20 dicembre 1928, n. 3298, Approvazione del regolamento per la vigilanza sanitaria delle carni (1928). GU 36, 12-02-1929.

15.Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 (2011). L 304 Official Journal of the European Union. Brussels

16.Cenci Goga B (2012) Fattori che regolano la crescita e la sopravvivenza dei microrganismi negli alimenti. In: Colavita G (ed) Igiene e tecnologie degli alimenti di origine animale. Point Vétérinaire Italie, Milano, pp 19–25

17.ICMSF (1996) Characteristics of microbial pathogens. [ICMSF] International Commission on Microbiological Specification for Foods, vol 5. Blackie Academic Professional, London

18.FDA (2015) Evaluation and Definition of Potentially Hazardous Foods - Chapter 3. Factors that Influence Microbial Growth. U. S. Food and drug administration. https://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094145.htm-top. Accessed January 2016

19.Decreto presidente della Repubblica 3-8-1998 n. 309 - Regolamento recante norme di attuazione della direttiva 94/65/CE, relativa ai requisiti applicabili all'immissione sul mercato di carni macinate e di preparazione di carni. (1998). GU Serie Generale 199, 27-08-1998. Rome

20.Decreto legislativo 30-12-1992, n. 537—Attuazione della direttiva 92/5/CEE relativa a problemi sanitari in materia di scambi intracomunitari di prodotti a base di carne (1992). GU Serie Generale 7, 11-01-1993.

21.Council Directive 92/5/EEC of 10 February 1992 amending and updating Directive 77/99/EEC on health problems affecting intra-Community trade in meat products and amending Directive 64/433/EEC (1992). L 57 Official Journal of the European Union. Bruseels

22.Council Directive 94/65/EC of 14 December 1994 laying down the requirements for the production and placing on the market of minced meat and meat preparations (1994). L 368 Official Journal of the European Communities. Brusels

23.ISO (2017) Foodstuffs—determination of water activity. International Organization for Standardization, Geneva

24.Rödel W (2001) Water activity and its measurement in food. In: Kress-Rogers E, Brimelow CJB (eds) Instrumentation and Sensors for the Food Industry, 2nd edn. Woodhead, Cambridge, pp 453–483

25.Zambonelli C, Papa F, Romano P, Suzzi G, Grazia L (1992) Microbiologia dei salumi. Edagricole, Bologna