

Review

# EU regulation on the use of antioxidants in meat preparation and in meat products

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**Abstract:** Antioxidants for foodstuffs during processing or before packing protects 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 the diverse food businesses. To answer these questions, a thorough examination of the EU legislator's choices for food preservation and definitions are provided and discussed with factors affecting microbial growth.

**Keywords:** ascorbic acid; meat preparation; meat products, meat spoilage

## 1. Introduction

Antioxidants are food additives and their use 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 food shelf life and protect them from deteriorating as a result of oxidation, such as rancid fat 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. Of these, ascorbic acid, also known as Vitamin C, is the one most used as a food additive. The food industry uses chemically synthesised, nature-identical L-ascorbic acid [4-6]. 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 with discolouring in meat during storage, but also the formation of metamyoglobin. The addition of ascorbic acid improves meat colour and shelf

life [7,8]. 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].

This review, by a thorough review of EU legislation and criteria behind the legislator's choices, answers the question on how to apply the law to the diverse food businesses.

## 2. Chronology of the specific legislation in the EU

The regulations on use have continually evolved right up to EU Regulation 1333/2008 [1]. Doubts on how it should be interpreted arose as a result of superimposing community regulations on top of national laws and integrating it with other sector-specific legislative provisions, e.g. EC Regulation 853/2004 [12] under the mandatory obligation to apply them to different, national, productive circumstances.

The most debated question regards the possibility of admixing antioxidants to minced meat preparations, made in retail shop preparation areas. Italian legislation expressly prohibited butchers from using antioxidants in minced meat under Royal Decree Law No. 3298 of 20 December 1928 [13], which prohibited pre-prepared minced meat for consumer protection. EC Regulation 853/2004 [12] gave specific details for the production of food of animal origin. In Annex I defines: *minced meat*: "boned meat that has been minced into fragments and contains less than 1 % salt" and *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". EU Regulation 1133/2008 [1] and EU Regulation 1129/2011 [2], which amended and completed the legislation, established the European Union list of food additives and provide a new interpretation of legislation on food additives. In this way EU Regulation 601/2014 [3] introduced the most significant amendment as regards meat preparations.

The main difference in the framework of the most recent regulations 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, which states the authorised additives and the relevant conditions of use. Nevertheless, the framework remains based on the principle of the positive list. Additional principles, as stated on the Commission website, EUR-LEX (<http://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.

## 3. Classification of additives and food categories under EU Regulation 1333/2008

The annexes of EU Regulation 1333/2008 [1] list: 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 which is further divided into sub-categories. The items ascorbic acid (E 300), sodium ascorbate (E 301) and calcium ascorbate (E 302) for meat preparations, as defined in EC Regulation 853/2004 [12], indicate the maximum level as *quantum satis* and for the

restrictions/exceptions considerations that the 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 "pre-packed preparations of freshly minced meat". As said before, 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 [14] 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 was replaced with the similar pre-packed, but without changing the meaning.

The new 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 and salt have been added at store level (Tables 1, 2, 3).

#### 4. Meat preparations and meat products

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

The numerous circulars and ministerial notes (cf. note 10194 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 on 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, redox potential and temperature)[15-17].

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

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

In order 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 to produce two sister cells: a further division will give 4 cells, the next 8 and so on. 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 5 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 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 we will see at a later stage). Lastly, changes in the menstuum induced by exponential development interrupt this phase, as the key nutrients are depleted and growth inhibiting metabolites accumulate: the culture moves into the stationary phase.

These factors are divided into intrinsic, which includes nutrients, pH, redox potential, antimicrobial obstacles and water activity and extrinsic, i. e. those that can be traced to the conditions of conservation: 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 and it is at a competitive disadvantage compared to bacteria, which are able to use it. For example, the ability to synthesise amylolytic enzymes (to denaturise starch) facilitates the growth of micro-organisms on cereals. «pH and buffer capacity» regulates bacterial growth but certain organic acids act as a bacteriostatic regardless of the solution pH. This is very useful if used to raise food conservation times: pH values that are too low 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 and fish usually suffers bacterial changes faster than meat at refrigeration temperatures: the pH of mammal muscles after rigor mortis is approximately 5.6, whereas that of fish is 6.2. High positive «redox potential» values ( $E_h$ ) indicate that the oxidised species is a strong oxidising agent and the reduced species is a weak reducing agent. High negative values indicate the opposite. If there is a larger quantity of oxidising species compared to that of the reducing agent, the redox potential increases and with it the medium's oxidising ability also increases. Where there is air, the redox potential is high and the majority of the other pairs are oxidised. The «redox potential» intrinsic factor is clearly linked to the «gaseous atmosphere» extrinsic factor, i.e. to the food conservation atmosphere. For example, mincing simultaneously increases the air and the redox potential, whereas the opposite phenomenon can be observed in vacuum packs. The same 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. The redox potential has a major effect on bacterial development in food.

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 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. Cytoplasm is an aqueous solution and must have a lower  $a_w$  than that of pure water: this allows water molecules to pass into the cytoplasm. If the germ does not tolerate this passage, it increases in volume until it bursts. Bacteria, fungi and algae have a rigid wall capable of resisting the osmotic pressure of the cytoplasm: up to 30 atm in Gram-positive bacteria, lower than 5 atm in Gram-negative bacteria. If  $a_w$  in the area surrounding the germ 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 in order to develop.

## 6. Comments on the legislator's choice

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

For instance, in Italy the definitions in Presidential Decree 309/1998 [18] and in Legislative Decree 537/1992 [19] (Table 1) introduced an easily interpreted parameter (the appearance of the cut surface), leaving discretion and 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" (Photographs 1 and 2). This approach stems from a precise desire of the European legislator who, with Directive 92/5/EC [20] (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 [21] - 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.

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 assign 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 that "*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 hygiene 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, which english-speaking authors call "hurdle technology", in which food health and safety is guaranteed by the contribution of several factors (Table 4).

Although technology has taken gigantic steps forward, in order 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) [22], 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. This depends on the temperature at which the analyses were conducted and the time required for the measurement instrument to reach a balance in terms of relative humidity (water vapour) between the sample and head space. Minimum temperature variations, leading to even centesimal differences in instrument readings would make the result completely unusable, 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 [23]. Lastly, as regards the classic definition of meat preparation, we should also bear in mind (Photograph 3) that, although many meat-based products have free water values below 0.97, they maintain the appearance of fresh meat.

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Figure and Tables

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 the 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

Table 4. Behaviour of microbial groups with reference to factors which influence microbial growth [15,16,24].

Microorganism or group	18-20°C	a <sup>w</sup> 0,96	pH = 5,3	anaerobiosis	nitrites
<i>Escherichia coli</i>	+	-	+/-	+	+/-
<i>Serratia</i>	+	+	+/-	+	+/-
other Enterobacteriaceae	+	-	+/-	+	+/-
<i>Vibrio</i>	+	+	+/-	+	+/-
<i>Campylobacter</i> spp.	-	-	-	-	-
<i>Pseudomonas</i> spp.	+	-	+/-	-	+/-
<i>Micrococcus</i> spp.	+	+	+	-	+
<i>Staphylococcus</i> spp.	+	+	+	+	+
Lactobacilli & lactococci	+/-	+	+	+	+
Enterococci	+	+	+	+	+
<i>Pediococcus</i> spp.	+/-	+	+	+	+
<i>Leuconostoc</i> spp.	+	+	+	+	+
<i>Bacillus cereus</i>	-	-	+	-	+/-
<i>Cl. botulinum</i> & <i>Cl. perfringens</i>	+	+	+	+	-
<i>Brochothris thermosphacta</i>	+	+	+	+	-
<i>Listeria monocytogenes</i>	+	+	+	+	+/-

Photograph 1. Hamburger. Meat preparation where the treatment is *insufficient to modify the internal cellular structure of the meat*.  $a_w > 0,97$



Photograph 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.  $a_w < 0.97$





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



