

# Research on mesophilic aerobic microorganisms and *Enterobacteriaceae* in cultivated and commercialized *Agaricus bisporus*

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*Parole chiave:* Carica microbica, funghi freschi, contaminazione, etichettatura, modalità di conservazione

## Abstract

**Background.** Production and consumption of fresh mushrooms reached high levels in recent years but only a few data regarding microbiological quality of these products are available, although their potential microbial load is expected to be high.

EU and Italian legislation have not set a limit on microbial counts in these products and label information is often unclear. This study investigates the microbial quality of samples of fresh cultivated mushrooms sold in Tuscany so that both food business operators and legislators can obtain data about potential microbial risk for consumers and debate about the opportunity of realizing an update on fresh mushrooms labels that should include information to protect consumers' health.

**Study design.** This study reports the microbial load in samples of cultivated and commercialized *Agaricus bisporus*. Samples were obtained from different shops in Florence, chosen among those products whose labels did not indicate how the product should be consumed.

**Methods.** From March through May 2014, 20 couples of samples of *A. bisporus* were acquired in Florence. Microbiological analysis included the quantification of the microbial counts for mesophilic aerobic microorganisms and *Enterobacteriaceae*, as indicators of hygienic practices during cultivation and manufacturing. The analyses were carried out at two subsequent stages: one immediately ( $T_0$ ) and one at the end of the shelf life ( $T_1$ ), i.e. close to the expiry date stated on the label.

**Results.** The high microbial load observed exceeds the reference values set as acceptable for raw foods in Tuscany and is worse than the ones reported in other studies on this subject. The results are particularly alarming in light of the fact that *A. bisporus* is usually consumed raw and there is no mandatory specification on the label that informs consumers that the product must be cooked before being consumed.

**Conclusions.** This research highlights the importance of adequate and complete information on fresh mushrooms labels, that should include information about the need for sanitization before the consumption, the appropriate storage temperature, and the maximum duration of shelf life.

Data obtained could also be useful for food business operators to gather information about the microbial quality of fresh cultivated commercialized mushrooms in order to implement quality controls of the production process and storage conditions.

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## Introduction

In recent years the worldwide consumption of fresh mushrooms has grown considerably and the Food and Agriculture Organization (FAO) of the United Nations has reported that the worldwide production of mushrooms and truffles has increased from 5 million tons in 2003 to 10 million tons in 2013. The biggest producer in the world is China (in 2013, the production in China exceeded seven million tons), followed by Italy, which has increased its harvest in the last few years and produced 792,000 tons of mushrooms and truffles in 2013 (1).

Despite the increasing production and consumption of these products, very limited data about microbiological quality are available.

Usually, mushrooms are nutritionally categorized as vegetables because of their fibre content, low protein load, high percentage of water, and content of vitamins and mineral salts - even though they also provide an important amount of proteins of high biological value (2, 3).

Fresh mushrooms are an ideal medium for microbial growth due to their high water and moisture content and their neutral pH (4). The presence of a high microbial load in cultivated mushrooms can be caused by microbiological contamination during the growth, harvest, processing, packaging, and storage stages.

In the period 2010-2015, the Rapid Alert System for Food and Feed (RASFF) of the European Union reported 67 notifications regarding mushrooms, five of which were regarding sliced mushrooms. Of these notifications, 34.3% were associated with a serious risk decision. In 25.4% of cases, the hazard was related to the presence of pathogenic microorganisms (5). It must be noticed that the notifications regarding sliced mushrooms were probably related to hygienic issues concerning the specific processing and distribution chains.

An alarming trend in notifications has also been observed earlier. In the period 2004-2007, RASFF notified 30 alerts for the presence of potentially pathogenic microorganisms (*B. cereus*, *E. coli*, *Listeria*, and *Salmonella*) in mushrooms (6).

Despite the high microbial load potential of these products, the existing legislation in Italy has not set a limit on microbial counts in freshly cultivated mushrooms. Hence, the product is always considered fit for human consumption, both cooked and raw, in the absence of macroscopic alterations.

To compensate for this legislative shortcoming, an appropriate labelling is essential to ensure that consumers receive correct and clear information, such as the maximum durability date and the appropriate storage conditions. However, the use of the specification 'to be consumed after cooking' in labels is mandatory (7) only for mushrooms belonging to a list of species that cannot be eaten raw. This list does not include *A. bisporus* (also called 'champignon'), which is the most commonly cultivated and commercialized species of fresh mushrooms on the Italian market. This fact is particularly worrying, since the Italian 'mycophilic' consumption behaviour (8) implies that cultivated mushrooms are often consumed raw. This fact represents a potential public health issue that could be associated with health costs arising from medical treatments. This threat is often underestimated because of the paucity of studies on this theme.

The aim of this study is to determine the microbial load in a sample of cultivated and commercialized *A. bisporus*, whose label does not indicate specific details about the correct way of consumption, in order to verify the potential microbial risks for consumers and gather useful information for food business operators.

## Materials and methods

### Samples

The *A. bisporus* samples were purchased from shops in mass retail channels in different areas of Florence. From March to May 2014, 40 samples were acquired in five subsequent rounds.

Samples were prepared and transported to the laboratory following the official sampling procedure used by all the local health authorities in Tuscany.

Samples taken to evaluate the hygienic quality of the production process are composed of a single aliquot; the sample units constituting the aliquot must be closed in sterile separate bags and all the bags are closed in one bag bearing a sealing device.

The 40 samples belonged to 20 different lots; two different packs for each lot were taken and the two samples from the same lot were analysed in two subsequent stages: one immediately upon its arrival at the laboratory (T0) and one just before the end of the shelf life (T1), i.e. close to the expiry date stated on the label. Microbiological analyses were carried out at T0 and T1.

Samples for T1 analyses were obtained from different packs within the same lot to avoid secondary contamination from already opened packs. Indeed, during the sampling procedure of packaged foods, unopened packages should be taken to ensure the sterile conditions of the sampling procedure (one unopened package for each aliquot or sample unit).

All the samples purchased included pre-sliced mushrooms. Each of the packages weighted between 250 and 300 g. The packaging was made of PVC and/or food-grade plastic and the mushrooms were stored in an ambient atmosphere.

Samples were taken from the refrigerated sections of shops and maintained at a controlled temperature of 2–4° C during transport and storage. The temperature was

checked at different times to ensure that the cold chain was effective.

A sampling report and a data collection form were filled out.

### Microbiological analysis

Microbiological analysis included the quantification of the microbial counts for mesophilic aerobic microorganisms and *Enterobacteriaceae*, which are indicators of good (or bad) hygienic practices in manufacturing. These indicators do not imply a direct danger to consumer health, but they give information about the degree of 'protection' from microbiological contamination of the mushrooms.

Indicator organisms can be used in the assessment of food product safety because they are usually present in higher numbers than most pathogens and are relatively quick and easy to identify.

Subsamples weighting  $10 \pm 0.1$  g were randomly selected from each package and aseptically removed using a flame-sterilized knife and clamp.

In order to count the number of mesophilic aerobic microorganisms and *Enterobacteriaceae*, UNI EN ISO 4833 - 01.:2013(9) and ISO 21528-2: August 2004 (10) regulations standards were respectively applied.

The total number of mesophilic microorganisms was determined on Plate Count Agar (PCA) (Biolife Italiana Srl) and that of *Enterobacteriaceae* was determined on Violet Red Bile Glucose Agar (VRBGA) (Biolife Italiana Srl). The preparation of the series of decimal dilutions consisted of the following stages: weighing of samples in a plastic envelope; addition of peptone water; the insertion into the stomacher; samples numbering; preparation of the inoculum in Petri dishes (employing a vertical laminar flow safety hood); inoculum in culture medium; incubation of Petri dishes in the thermostats. After that, plate count measurement was conducted and microorganism counts are

Table 1 - Limit values established by resolution DGRT 55/98 for raw food and comparable products, expressed as colony-forming units per gram (cfu x g<sup>-1</sup>)

Microorganisms	Satisfactory	Acceptable	Poor quality
Mesophilic aerobic microorganisms	<10 <sup>6</sup>	≥10 <sup>6</sup> but <10 <sup>7</sup>	>10 <sup>7</sup>
<i>Enterobacteriaceae</i>	<10 <sup>3</sup>	≥10 <sup>3</sup> but <10 <sup>4</sup>	>10 <sup>4</sup>

expressed as number of colony-forming units per gram (CFU x g<sup>-1</sup>).

Microorganism counts are expressed as number of colony-forming units per gram (CFU x g<sup>-1</sup>).

In the absence of specific legislation on reference values for the microbiological risk assessment of freshly cultivated mushrooms, the data obtained in this study were compared with the reference values established in the Tuscany Region for microbiological risk assessment of raw food, set out in Resolution DGRT 55/98 (11).

According to the Tuscan law, satisfactory values for mesophilic aerobic microorganisms are below 10<sup>6</sup>CFU x g<sup>-1</sup> and for *Enterobacteriaceae* are below 10<sup>3</sup> CFU x g<sup>-1</sup> (Table 1).

#### Statistical analysis

All the data were analysed with SPSS 22™. For each sample, data on the indicators of microbial contamination were reported in the form of single values, mean, and standard deviation at T0 and T1.

Student t-test for paired data (with a significance level of  $p < 0.05$ ) was performed to determine the statistical significance of differences between the mean values of microbial counts at T0 and T1.

## Results

Table 2 shows the microbial counts for mesophilic aerobes and *Enterobacteriaceae* detected in the 40 samples of freshly cultivated mushrooms that were analysed.

The values of mesophilic aerobes detected at T0 and T1 in all the samples exceed the

‘acceptable’ concentration values defined in DGRT 55/98 for raw food stuffs and similar products (CFU x g<sup>-1</sup> < 10<sup>7</sup>).

*Enterobacteriaceae* were present in 100% of the samples and the detected concentrations exceeded reference values considered as acceptable (CFU x g<sup>-1</sup> < 10<sup>4</sup>) in 90% of the samples analysed at T0 and in 95% at T1.

At T0, mean count of mesophilic aerobic microorganisms was  $8.65 \pm 0.82 \log_{10}$  CFU x g<sup>-1</sup> and that of *Enterobacteriaceae* was  $5.43 \pm 1.68 \log_{10}$  CFU x g<sup>-1</sup>.

The mean microbiological counts of both mesophilic aerobic microorganisms and *Enterobacteriaceae* measured at T0 and T1 significantly differed ( $p < 0.05$ ); values at T1 were higher than the ones reported at T0. Specifically, at T1, the mean count increased by 6.3% for mesophilic aerobes and by 23.4% for *Enterobacteriaceae*.

## Discussion and conclusions

European studies investigating the microbial load of fresh mushroom species reported that the mean counts of mesophilic aerobic microorganisms in *A. bisporus* ranged from 7.0 to 8  $\log_{10}$  CFU x g<sup>-1</sup> (12-15). Regarding mean counts of *Enterobacteriaceae*, the same studies reported a range between 3.2 and 4  $\log_{10}$  CFU x g<sup>-1</sup> (12-15).

For both the microbial indicators, the high microbial load observed in the samples of *A. bisporus* sold in shops in mass retail channels in the entire urban area of Florence exceeds the reference values set by Region Tuscany as acceptable for raw foods and is

Table 2 - Microbial counts for mesophilic aerobes and *Enterobacteriaceae* at T0 and T1, expressed as log<sub>10</sub> (unit of measure CFU x g<sup>-1</sup>)

N° samples	Mesophilic aerobic microorganisms T0 (log <sub>10</sub> ) <sup>*</sup>	Mesophilic aerobic microorganisms T1 (log <sub>10</sub> ) <sup>*</sup>	<i>Enterobacteriaceae</i> (log <sub>10</sub> ) <sup>°</sup> T0	<i>Enterobacteriaceae</i> (log <sub>10</sub> ) <sup>°</sup> T1
1	8.95	9.77	4.77	4.81
2	7.30	7.60	1.48	2.43
3	8.48	8.54	5.78	5.85
4	7.68	8.00	5.08	5.90
5	8.30	8.95	5.85	8.95
6	7.48	7.74	4.30	5.34
7	10.08	10.14	7.95	8.00
8	8.95	9.60	8.95	10.08
9	9.08	9.34	7.08	7.92
10	9.51	9.86	4.69	7.68
11	9.08	9.81	6.08	7.51
12	9.38	10.68	7.99	8.26
13	8.18	8.30	4.90	6.70
14	10.26	10.30	6.26	8.26
15	8.11	9.95	4.70	6.08
16	8.00	10.11	4.60	7.86
17	8.78	8.90	4.91	7.08
18	8.18	8.48	3.78	4.90
19	9.34	9.54	4.08	4.85
20	7.91	8.30	5.40	5.58
Mean	8.65	9.20	5.43	6.70
Standard deviation	0.82	0.92	1.68	1.79

\* paired t-tests, t=-4.192 (19 df), p=0.001

° paired t-tests, t=-5.624 (19 df), p<0.001

worse than the ones reported in the studies cited above (12-15).

It should be remembered that sampling procedures carried out to avoid the interruption of the cold chain tend to prevent microbial growth better than at the consumer's house. Therefore, citizens' risk could be even worse.

Storage temperature is one of the most important factors for maintaining the quality of fresh mushrooms (14). However, modes of transport and storage conditions used by an average consumer can easily cause an interruption of the cold chain, thus leading

to higher microbial counts than the ones observed in this analysis.

Also, the duration of storage, even at controlled temperature, proved to be important for microbial growth; microbiological counts increased significantly from T0 to T1. This increase could be indicative of the presence of psychrotrophic species among *Enterobacteriaceae* or mesophilic aerobic microorganism; among *Enterobacteriaceae*, the prevalence of *Ewingella americana* in fresh cultivated mushrooms has been previously evaluated (13) and it was the predominant species between *Enterobacteriaceae spp.*

in three different species of cultivated mushrooms. As psychrotrophic mesophilic aerobic microorganisms, *Pseudomonas* could cause the microbial growth observed in T1 because it is proved to be the predominant bacterial population in different species of fresh cultivated mushrooms (included *A. bisporus*) (13); these bacterial species should be controlled regarding their capability to develop under refrigeration.

The collected samples were sliced mushrooms, which partly explains the high microbial counts observed; slicing causes an increase in the respiration rate and creates a larger surface area for microbial growth (16).

Information on the microbial quality of freshly cultivated commercialized mushrooms is very limited, but the high values of microbiological indicators observed in this study highlight the need of implementing quality controls along the production process and regarding storage conditions.

Our microbiological examination considers only two microbial species that are indicators of the hygienic quality of the product and also indicative of the potential contamination by pathogenic microbial agents associated with the risk of clinical manifestations.

The enlargement of the microbial species to be investigated in freshly cultivated mushrooms might better define the magnitude of risk to consumers' health. For example, microorganisms of the *Enterobacteriaceae* family like *Escherichia*, spread through faecal contamination, indicate the possible presence of enteric pathogenic bacteria more efficiently than *Enterobacteriaceae*.

However, since previous research (15) has demonstrated the absence of any of the enteric pathogenic microorganisms in freshly cultivated mushrooms contaminated by *Enterobacteriaceae* and mesophilic aerobic microorganisms, those microorganisms are quality indicators.

The high level of both indicators (mesophilic aerobic microorganisms and *Enterobacteriaceae*), in some samples could raise concerns about the consumption of those food items in the raw form, as is usually done for the species *A. bisporus*. Since these microorganisms are killed by heat, adequate cooking can protect consumers.

It should be recalled that all the mushroom samples selected for the study had incomplete labels, particularly with regard to the pattern of consumption. Specific features of product labelling are defined in Legislative Decree No. 109/1992 (17) (and its subsequent amendments) and in EU Regulation No. 1169/2011 (18).

In light of the existing law and the high microbial counts detected in commercialized mushrooms in Tuscany, it is essential to raise awareness among food business operators about the opportunity of implementing transparency and risk management measures in line with EU Regulation 852/04 EU (19). Food business operators should ensure hygienic production by means of the hazard analyses and critical control points (HACCP) system at all the production stages. For this purpose, it would be prudent not to sell sliced fresh mushrooms, because slicing is associated with a potential increase of microbial growth (16), and to provide complete information to the consumers on the label.

Food labelling should provide complete information that must be true and demonstrable, easily recognizable, legible, and understandable to the average consumer. Its purpose is not to mislead the consumer about product characteristics but to protect consumer's health. Label information on prepacked food should specify the appropriate storage conditions and instructions for use, if the omission thereof may preclude proper use of the product. Regarding fresh mushrooms, the existing legislation does not stipulate any mandatory labelling with regard to the fact that all species must be

cooked prior to consumption. The use of the specification 'to be consumed after cooking' in labels is mandatory (7) only for mushrooms belonging to a list of species that cannot be eaten raw. Our analysis reveals that even fresh mushrooms not included in this list (*A. bisporus*) should be consumed after cooking, in light of the high microbial load observed.

In conclusion, adequate and complete information on fresh mushrooms labels should include information about the need for sanitization before the consumption, the appropriate storage temperature, and the date of minimum durability.

If fresh mushrooms are considered 'ready-to-eat' products (20), it should be necessary to carry out specific stages of processing: selection, sorting, possible weeding and cutting, washing, drying, and packaging in sealed envelopes, possibly with the use of protective gas atmosphere packaging. This kind of food has a very short shelf life (about seven days) and it should be stored at refrigerated temperatures of 0–4 °C. Currently, the production system of fresh mushrooms requires sanitization before consumption.

## Riassunto

### Ricerca di microrganismi mesofili aerobi e di *Enterobacteriaceae* in *Agaricus bisporus* coltivati e commercializzati

**Introduzione.** Negli ultimi anni, la produzione ed il consumo di funghi freschi hanno raggiunto alti livelli ma, nonostante la potenziale elevata carica microbica di questi prodotti, sono disponibili solo pochi dati relativi alla loro qualità microbiologica.

La legislazione italiana non ha indicato un limite per la carica microbica nei funghi freschi coltivati e le informazioni riportate sulle etichette alimentari di questi prodotti risultano spesso poco chiare.

Questo studio valuta la qualità microbiologica di campioni di funghi freschi coltivati commercializzati in negozi della Toscana, in modo da informare sia gli operatori del settore alimentare che i legislatori sul

loro potenziale rischio microbiologico e sulla necessità di aggiornare le etichette alimentari di questi prodotti affinché siano inserite indicazioni a tutela della salute dei consumatori.

**Materiali e metodi.** La ricerca ha lo scopo di determinare la carica microbica in *Agaricus bisporus* coltivati e commercializzati. I campioni sono stati acquistati in differenti negozi di Firenze, scegliendo tra i prodotti le cui etichette alimentari non fornivano indicazioni sulle modalità di consumo.

Nel periodo compreso tra Marzo e Maggio 2014, 40 campioni di *A. bisporus*, appartenenti a 20 lotti diversi (2 per lotto) sono stati acquistati ed analizzati quantificando la carica di microrganismi mesofili aerobi e di *Enterobacteriaceae*, in quanto indicatori di igiene del processo di produzione. Le analisi sono state effettuate in due differenti fasi: immediatamente dopo l'acquisto del campione (T0), e alla fine della vita commerciale del prodotto (T1).

**Risultati.** I valori di carica microbica riscontrati superano i limiti considerati come accettabili per i cibi crudi dalla normativa toscana e sono più elevati rispetto a quelli riportati in precedenti studi. Tale risultato è particolarmente allarmante in considerazione del fatto che i funghi *A. bisporus* sono generalmente consumati crudi e non vi sono disposizioni specifiche per informare il consumatore riguardo la necessità di assumere il prodotto previa cottura.

**Conclusioni.** Questo studio pone l'attenzione sull'importanza di porre informazioni complete ed utili sulle etichette alimentari di funghi freschi. Dovrebbe essere indicata la necessità di sanificare il prodotto prima di consumarlo, l'appropriata temperatura di conservazione ed il termine massimo di conservazione. I dati ottenuti potrebbero essere utili agli operatori del settore alimentare per conoscere la qualità microbiologica dei funghi coltivati commercializzati allo scopo di implementare i controlli di qualità del processo produttivo e le condizioni di stoccaggio.

## References

1. Food and Agriculture Organization (FAO). Food and Agriculture Commodities Productions 2013. Available from: <http://faostat.fao.org/> [Last accessed 2017, May 22].
2. Tavaglini O, Tavaglini R. Valore alimentare dei funghi. In: Atlante dei funghi commestibili della Basilicata. 2.ed. Consiglio Regionale della Basilicata, 2003: 34-7.
3. Gruppo Micologico Bresadola Trento. Parliamo di funghi. II Tossicologia, commercializzazione, legislazione. Manuale per i corsi di formazione

- per il rilascio dell'attestato di micologo. Provincia autonoma di Trento: Giunta, 2007.
4. Martinez-Carrera D, Sobal M, Aguilar A, Navarro M, Bonilla M, Larqué-Saavdera A. Canning technology as an alternative for management and conservation of wild edible mushrooms in Mexico. *Micol Neotrop Apl* 1998; (11): 35-51.
  5. RASFF Portal. Available from: <https://webgate.ec.europa.eu/rasff-window/portal/> [Last accessed: 2017, May 22].
  6. RASFF. Annual Reports 2004-2007. Available from: [http://ec.europa.eu/food/safety/rasff/index\\_en.htm](http://ec.europa.eu/food/safety/rasff/index_en.htm) [Last accessed: 2017, May 22].
  7. Legislative Decree No. 376, July 14, 1995. Regolamento concernente la disciplina della raccolta e della commercializzazione dei funghi epigei freschi e conservati. Official Gazette of the Italian Republic September 11, 1995, No. 212.
  8. Peintner U, Schwarz S, Mešić A, Moreau P-A, Moreno G, Saviuc P. Mycophilic or mycophobic? Legislation and guidelines on wild mushroom commerce reveal different consumption behaviour in European countries. *PLoS One* 2013; **8**(5): e63926. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3660358&tool=pmcentrez&rendertype=abstract> [Last accessed: 2017, May 22].
  9. UNI EN ISO 4833- 01. ISO 4833-1:2013 Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique. 2011.
  10. UNI EN ISO 21528-2. ISO 21528-2:2004 Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method. 2004.
  11. Legislative Decree No. 55, March 17, 1998. Toscana. Piano regionale di programmazione e coordinamento degli interventi in materia di vigilanza degli alimenti e delle bevande. Bollettino Ufficiale della Regione Toscana n.25, 24 giugno 1998.
  12. Doores S, Kramer M, Beelman RB. Evaluation and bacterial populations associated with fresh mushrooms (*Agaricus bisporus*). In: Wuest PJ, Royse DJ, Beelman RB, eds. *Cultivating Edible Fungi*. Amsterdam: Elsevier, 1986: 283-94.
  13. Reyes JE, Venturini ME, Oria R, Blanco D. Prevalence of *Ewingella americana* in retail fresh cultivated mushrooms (*Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus*) in Zaragoza (Spain). *FEMS Microbiol Ecol* 2004; **47**(3): 291-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19712317> [Last accessed: 2017, May 22].
  14. González-Fandos E. Quality and shelf life of packaged fresh sliced mushrooms stored at two different temperatures. *Agric Food Sci* 2006; **15**(January): 414-22.
  15. Venturini ME, Reyes JE, Rivera CS, Oria R, Blanco D. Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. *Food Microbiol* 2011; **28**(8): 1492-8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21925034> [Last accessed: 2017, May 22].
  16. Brennan MH, Gormley TR. Extending the Shelf Life of Fresh Sliced Mushrooms. Research report no 2. Natl Food Centre, Dunsinea, Castleknock, Dublin, 1998; 30.
  17. Legislative Decree No. 109, January 27, 1992 Attuazione delle direttive (CEE) n. 395/89 e (CEE) n. 396/89, concernenti l'etichettatura, la presentazione e la pubblicità dei prodotti alimentari. Official Gazette of the Italian Republic February 17, 1992, No. 39(Suppl.).
  18. 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. Official Journal of the European Union 22.11.2011.
  19. REGULATION (EC) No 852/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union 30.04.2004.
  20. Legislative Decree No. 77, May 13, 2011. Disposizioni concernenti la preparazione, il confezionamento e la distribuzione dei prodotti ortofrutticoli di quarta gamma. Official Gazette of the Italian Republic June 1, 2011, No. 126.