

# High microbial loads found in minimally-processed sliced mushrooms from Italian market

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## Abstract

There is an increased consumer interest in minimally processed vegetables that has led to the development of products, such as pre-cut sliced mushrooms. Few data are available on the hygienic condition and the presence of foodborne pathogens in such products. Therefore, the current study aimed to evaluate the safety and hygienic characteristics of both ready-to-eat and ready-to-cook, pre-cut sliced mushrooms obtained from a local Italian market. For the evaluation of the hygienic condition, the aerobic mesophilic bacteria, aerobic psychrotrophic bacteria and *Escherichia coli* enumerations were performed. *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. were considered in the assessment of the foodborne pathogens. High microbial loads were detected, including counts higher than 5 log CFU/g for *E. coli* and 6 log CFU/g for the other bacteria counts considered, but no pathogens were found. Ready-to-eat and ready-to-cook products differed only for aerobic mesophilic counts (7.87 and 8.26 log CFU/g, respectively,  $P=0.003$ ). Strategies to enhance the hygienic level of the mushrooms, particularly the ready-to-eat products, are needed.

## Introduction

The shift to a *fast* lifestyle in several countries has led to an increasing demand for minimally processed foods that are ready-to-eat or easy to prepare. Nowadays, people tend to spend less time preparing meals than in the past, but there is an increasing interest in healthy and high nutritional quality foods (Jeddi *et al.*, 2014). Among these foods, there is an increasing demand for fresh-cut vegetables and fruits (Oliveira *et al.*, 2015), with several products

available in the market, such as minimally processed mushrooms (Venturini *et al.*, 2011) that are sold both for ready-to-eat or ready-to-cook purposes. Ready-to-eat food, as reported by EC Regulation 2073/2005, means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern; ready-to-cook are pre-packaged food prepared for convenience consumption after cooking containing raw vegetables (Chung *et al.*, 2010) and the *need to be cook* condition is highlighted directly in the products label.

Fresh-cut vegetables that undergo minimal processing have physicochemical characteristics that allow the growth of several microorganisms, such as juices rich in nutrients, high humidity and  $a_w$  values (Ayala-Zavala *et al.*, 2008; Mahajan *et al.*, 2008; Ragaert *et al.*, 2007). To date, literature about the hygiene and safety of fresh-cut products has focused mainly on salad vegetables, spinach, carrots and lettuce (Abadias *et al.*, 2008; Brandão *et al.*, 2014; Cardamone *et al.*, 2015; Sagoo *et al.*, 2003a). Although the hygienic and safety condition of fresh, whole (not cut), cultivated mushrooms are reported (McMahon and Wilson, 2001; Venturini *et al.*, 2011; Kim *et al.*, 2016) no data, to our knowledge, are yet available on pre-cut sliced mushrooms sold for ready-to-eat and ready-to-cook purposes. Furthermore, the European Commission (EC) legislation has established process hygienic criteria and safety food criteria for *Salmonella* in pre-cut (ready-to-eat) vegetables (EC Regulation 2073/2005 amended by EC Regulation 1441/2007), but it is not clear if vegetables include mushrooms. According to the legislation, the safety criteria for *Listeria monocytogenes* is applicable to all ready-to-eat foods.

The current study aimed to evaluate the hygienic level of ready-to-eat and ready-to-cook pre-cut sliced mushrooms available in the Italian market and the presence of foodborne pathogens in these products.

## Materials and methods

A total of 50 pre-cut sliced mushrooms (*Agaricus bisporus*, Imbach, 1946), packaged in polystyrene trays covered with stretch-film and stored at 4°C, were randomly sampled from eight point-of-sales in Perugia (central Italy) but produced in various Italian regions. No information were available regarding the gas mix and the permeability of the film used in the packs.

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## Microbiological analysis

The samples, 25 ready-to-eat and 25 ready-to-cook, pre-cut sliced mushrooms, were promptly sent to the laboratory. Each sample was aseptically collected and divided into four aliquots of 25 g of mushrooms: one for the aerobic mesophilic count (AMC) (ISO 4833:2004), aerobic psychrotrophic count (APC) (ISO 17410:2001) and *Escherichia coli* count (AFNOR AES-10/06-10/08); one for *Salmonella* spp. isolation (ISO 6579:2002); one for *L. monocytogenes* isolation and enumeration (EN/ISO 11290-1: 2017a and 11290-2: 2017b) and one for *Campylobacter* spp. isolation (ISO 10272-1:2006). All the analyses were conducted in duplicate, for each product.

For the microbial loads, the results were reported as colony-forming units per gram (CFU/g) and transformed into log<sub>10</sub> values.

## Physicochemical determinations

Additionally, 10 randomly collected samples were analysed for water activity ( $a_w$ ), by using an Aqua Lab water activity meter (Series 3 TB, Decagon Devices Inc., Pullman, WA, USA), and for pH, by inserting a pH probe (Crison Instruments, Barcelona, Spain) into the mushrooms.

## Statistical determination

Descriptive statistics were performed on all data obtained. Comparisons between the average data for ready-to-eat and ready-to-cook mushrooms were evaluated by one-

way analysis of variance (ANOVA), using StatView software (SAS, Cary, NC, USA), followed by Tukey's test with a significance level set at  $P < 0.05$ .

## Results

The average pH value of the mushrooms was 6.88 [standard deviation (SD) = 0.14], ranging from pH 6.62-7.01 and 6.73-7.12 in ready-to-eat and ready-to-cook products, respectively. The  $a_w$  values were always above 0.99, with an average value of 0.997 (SD=0.001).

Table 1 presents the microbial counts obtained for all the samples tested. The average loads were always over 7 log CFU/g for all microbial counts considered. *E. coli* counts in all the samples were over 5 log CFU/g, APC were over 6 log CFU/g and AMC over 7 log CFU/g.

A significant difference was found in the AMC between ready-to-eat mushrooms ( $7.87 \pm 0.37$  log CFU/g) and ready-to-cook mushrooms ( $8.26 \pm 0.49$  log CFU/g) ( $P = 0.003$ ). However, no differences were registered between these two groups of products, regarding the APC and *E. coli* loads (Figure 1).

The products were negative for either *Salmonella* spp., or *Campylobacter* spp. or *L. monocytogenes*. *Listeria* spp. growth was observed on three samples, but the species was identified as *Listeria innocua* by biochemical methods (API Listeria, Oxoid, Basingstoke, UK) (data not reported).

## Discussion

The results highlight high levels of microbial loads for all the bacteria counts considered. Similar data are not available for pre-cut sliced mushrooms at the retail level, but a comparison is attempted with other mushrooms used for decontamination trials or with other minimally processed vegetables, including mushrooms, obtained from the market. In washed and cut mushrooms, used as the control in decontamination trials by pulsed light, Oms-Oliu *et al.*

(2010) found lower values than those observed in the present study. The AMC and APC were above 6 log CFU/g but the mushrooms were cut and immediately processed, which may have influenced the microbial growth rate. The AMC levels reported by Venturini *et al.* (2011) was similar to those found in the present study (average value of 7.7 log CFU/g), even if uncut mushrooms were considered. Moreover, the surface of mushrooms is not favourable for microbial growth, due to the presence of a cuticle, and only after cutting there is an increase in the microbial loads, because of the nutrients released available for the microorganisms (Ragaert *et al.*, 2007). For minimally processed vegetables, there is a broad range of aerobic mesophilic and aerobic psychrotrophic loads in fresh-cut vegetables reported in the literature with values higher (De Oliveira *et al.*, 2011, APC values of 9.4 log CFU/g in arangula, 9.3 log CFU/g in spring onion/parsley mixture and 9.0 log CFU/g in spinach), similar (Soriano *et al.*, 2000, AMC ranging from 3.01-7.81 log CFU/g in 144 ready-to-eat lettuce samples; Valentin-Bon *et al.*, 2008, average AMC from 100 bagged lettuce and spinach mixes was 7 log CFU/g; Santos *et al.*, 2012, APC ranged from 4.65-8.48 log CFU/g in

fresh-cut salads) or lower (Maistro *et al.*, 2012, AMC from 4.00-6.89 log CFU/g in six kinds of minimally processed vegetables with non-modified atmosphere packages; Cerna-Cortes *et al.*, 2015, AMC from 3.0 to 6.6 log CFU/g in ready-to-eat salads; Cardamone *et al.*, 2015, AMC between 5 and 7 log CFU/g in minimally processed leafy vegetables) than those found in pre-cut sliced mushrooms.

The high microbiological counts registered in both ready-to-eat and ready-to-cook pre-cut sliced mushrooms represent poor hygienic quality, which means that hygienic measures need to be improved during processing. The AMC detected, despite showing a significant difference between ready-to-eat and ready-to-cook mushrooms ( $P < 0.05$ ), highlights that mushrooms could be contaminated before or during the harvest process, or during processing/packaging procedures. During mushroom growing, contamination by soil, irrigation water, manure or sewage sludge use and animals are possible (Ragaert *et al.*, 2007). Then, during the harvesting and post harvesting process, microorganisms can grow, due to physical damage to the plant tissues (Leong *et al.*, 1991; Zagory *et al.*, 1999). Following, all post-harvest mushroom han-

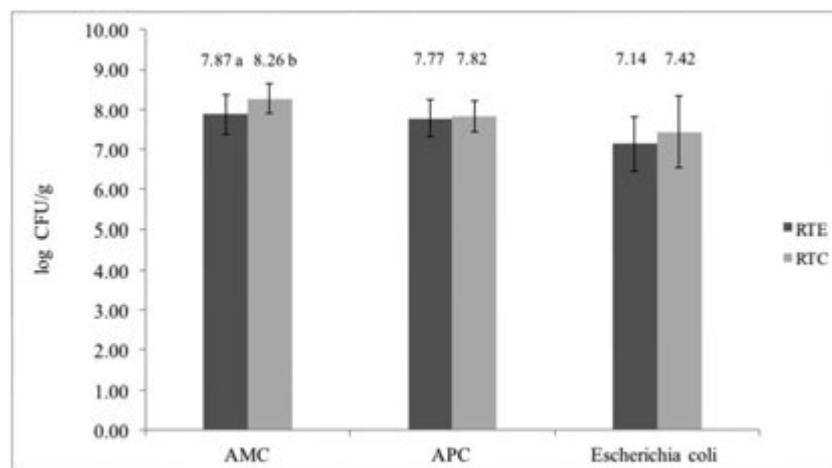


Figure 1. Microbial loads in the two groups of fresh-cut mushrooms.

Table 1. Total microbial loads in fresh-cut mushrooms.

Parameter	Samples (n)	Number of positive samples (%)					Mean log CFU/g	Standard deviation log CFU/g	Range log CFU/g
		$<10^5$ CFU/g	$10^{5-6}$ CFU/g	$10^{6-7}$ CFU/g	$10^{7-8}$ CFU/g	$>10^8$ CFU/g			
Aerobic mesophilic count	50	0	0	0	23 (46)	27 (54)	8.07	0.47	7.20-8.86
Aerobic psychrotrophic count	50	0	0	1 (2)	35 (70)	14 (28)	7.80	0.43	6.48-8.60
<i>Escherichia coli</i>	50	0	3 (6)	19 (38)	19 (38)	9 (18)	7.21	0.81	5.48-9.08

dling stages, including the processes of washing and cutting, could potentially cause hygienic defects. During minimally processing procedures, microbial growth is possible because mushroom slicing creates a large exposed surface area and the lack of a protective barrier (Brennan *et al.*, 1998). Additionally, packaging and storage also allow bacteria to grow (Brennan *et al.*, 2000), due to respiration of fresh mushrooms, with large amounts of water vapour in the package (Singh *et al.*, 2010).

For *E. coli*, Venturini *et al.* (2011) found both *Enterobacteriaceae* (3.0 log CFU/g) and total coliforms (0.3 log CFU/g) with *E. coli* levels below the values found in this study. Most studies documented low *E. coli* counts in ready-to-eat vegetables. Campos *et al.* (2013), find 4% of 50 bagged ready-to-eat salads (split or mixed leaves, carrot, corn) with *E. coli* levels  $\geq 10^2$  CFU/g. Maistro *et al.* (2012) also reported low *E. coli* contamination results, with 10 out of 172 minimally processed green vegetables collected from supermarkets in Campinas (Brazil) testing positive, including two of the 17 whole arugula leaves (3.5 and 3.1 log CFU/g of *E. coli*, respectively). Sagoo *et al.* (2001) analysed 3200 ready-to-eat organic vegetables grown nearby or in contact with soil (including mushrooms), collected from various outlets in the United Kingdom. *E. coli* was detected in 1.5% (48/3200) of the samples and was present at  $10^2$  CFU/g or more in 0.3% (11) samples. Compared to these investigations, both the loads and prevalence of *E. coli* in our study was higher. Regarding food hygiene criteria for pre-cut vegetables, the Regulation states that 2/5 samples are allowed to detect *E. coli*, with the limits from 2-3 log CFU/g during the manufacturing process. However, in the mushrooms sampled at market level, the *E. coli* loads detected (more than 5 log CFU/g) were much higher than referred by the Regulation. These situation, considering the storage temperature used for the mushrooms (4°C), could be due to high level of *E. coli* in the products immediately after packaging.

No *Salmonella* was detected in pre-cut sliced mushrooms in accordance to Venturini *et al.* (2011), that could not isolate *Salmonella* spp. from 402 mushroom samples, representing 22 species of wild and cultivated mushrooms sold in retail markets and supermarkets in Zaragoza (Spain). *Salmonella* spp. was not detected also in fresh-cut vegetables (Jeddi *et al.*, 2014; Santos *et al.*, 2012; Seow *et al.*, 2012), while a few studies found low percentages of this pathogen (Sant'Ana *et al.*, 2011,

4/512 packages of minimally processed vegetables were found positive for *Salmonella* spp.; Gómez-Govea *et al.*, 2012, *Salmonella Typhi* E1 was detected at 0.66% total frequency and at <3 most probable number/g in six fresh produce vegetables).

Regarding *L. monocytogenes* previous studies found positive samples in mushrooms obtained from retail market. Venturini *et al.* (2011) found 6.5% (26/402) and Abadias *et al.*, (2008) found 0.6% (1/156) positive samples of mushroom collected from several retail establishments in Spain. On the contrary, Leong *et al.* (2015) found no *L. monocytogenes* in pre-packaged, whole, fresh, refrigerated mushrooms (*A. bisporus*) acquired from a supplier in Ireland. In other minimally processed vegetables, low levels of contamination by *Listeria* microorganism have been highlighted (Giusti *et al.*, 2010; Johannessen *et al.*, 2002). De Oliveira *et al.* (2011) detected *L. monocytogenes* in 1.2% (2/162) and *L. innocua* in 2.4% (4/162) samples of minimally processed leafy vegetables commercialised in Brazil. Our study confirmed no *L. monocytogenes* in 50 pre-cut sliced mushroom samples, but *L. innocua* was detected in three ready-to-eat samples (6%). The results is in agreement with Guerra *et al.* (2001) that found *L. innocua* in 4% (1/23) ready-to-eat vegetable samples but no *L. monocytogenes*. Nonetheless, according to the EU regulation 2073/2005 pre-cut sliced ready-to-eat mushroom species can support the growth of *L. monocytogenes* as pH and  $a_w$  are favourable for this pathogen. Moreover, a challenge test showed that *A. bisporus* did not support the growth of *L. monocytogenes* (Leong *et al.*, 2015).

No *Campylobacter* spp. was found in our research, in agreement with McMahon *et al.* (2001) and Sagoo *et al.* (2003a, 2003b).

Although we compared our results with the European Union regulation for the hygienic criteria, that refers to the loads during manufacturing process instead of the selling period, the high microbial load detected need a correct analyses of the level before the products are available in the market and the rate of growth during storage/distribution. Furthermore, good agricultural practices, good manufacturing practice and hazard analysis and critical control points throughout mushroom processing are crucial, and decontamination strategies, like ultraviolet light (Brennan *et al.*, 2000), acidic electrolysed water (Ding *et al.*, 2011) and bacteriocins (Randazzo *et al.*, 2009), could be used to reduce microor-

ganisms in fresh-cut mushrooms and minimize risk.

## Conclusions

This study provides a general overview of the microbiological quality of pre-cut sliced mushrooms in Italy. The poor level of hygiene is not associated with the presence of pathogens even if the high level of *E. coli* detected warrants further investigation and the need to define the presence of enterohemorrhagic *E. coli*. Moreover, a general recommendation to consumers could be to wash pre-cut vegetables and mushrooms before consumption.

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