# SAFETY AND CONSUMER HANDLING OF LETTUCE IN THE BELGIAN CONSUMER MARKET

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## ABSTRACT

In the last decades there has been a significant increase in the number of foodborne outbreaks connected to fresh produce as the vehicle for transmission of pathogenic microorganisms. Among these pathogens, the most common are *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. It was the objective of this study to assess risk factors for contamination with the mentioned microorganisms, with a special focus on the role of the consumer.

For this purpose, it was performed a test of prevalence of these pathogens and an enumeration of total coliforms and *Escherichia coli* in samples of fresh lettuce acquired from different retail scenarios to establish a comparison between them. In order to analyze the effect of consumption patterns and handling practices, results of a survey performed in Belgium and Spain were used. Furthermore, an exposure assessment was conducted in order to calculate the probability of consuming an infected portion of lettuce in Belgium.

The prevalence of pathogens determined in this study was very low in the group of gathered samples: 0/150 for *Campylobacter* spp., *Salmonella* spp., and *Escherichia coli* O157:H7 but 3/150 for *Listeria monocytogenes.* Regarding consumer behavior, it was noted a concerning tendency, especially in terms of storage practice, indicating that a considerable percentage of consumers is not complying with the food safety standards. This fact leads to the conclusion that is needed to raise awareness among consumers about the importance of their role in the assurance of food safety in their households.


**Keywords:** food safety; lettuce; consumption patterns; handling practices; consumer behavior; exposure assessment

## INTRODUCTION

In the period of 2007 to 2011, it was registered that 10% of the outbreaks of severe foodborne diseases registered in the area of the European Union (meaning 26% of the cases, 35% of the hospitalizations and 46% of the deaths) were associated with different pathogenic microorganisms in fresh produce, according to the European Food Safety Authority (EFSA, 2013a). It has been noted that the number of cases has dramatically increased in the last decades and therefore fresh produce has been recognized as a potential vehicle for transmission of pathogenic microorganisms known to cause human disease.

Campylobacteriosis has been the most commonly reported foodborne disease in humans in the last years in the EU area, followed by salmonellosis, listeriosis and infections by Vero(cyto)Toxin-Producing *Escherichia coli*, also known as VTEC (EFSA, 2013b; EFSA, 2014). The detection of the pathogens previously mentioned might present some difficulty given to the low prevalence of these in fresh produce, making this activity time consuming with complex methodologies and high consumption of resources due to the high number of samples needed (Cárdenas et al., 2013). The screening of standard representative indicators, such as total coliforms or *E. coli*, is a possible alternative but an accurate relation between the prevalence of indicators and pathogens must be established for the specific case of fresh produce.

Foodborne outbreaks usually present an animal or human origin and affect fresh produce via direct or indirect contamination such as the use of improperly composted manure, contaminated water sources, fecal deposition, among others (Berger et al., 2010). The sources of these infections are difficult to trace due to the complexity of food supply chains that range distinct operations like production, processing, transportation and distribution (FAO/WHO, 2008). Furthermore, new trends in consumption patterns and in production procedures are a challenge to current policies that do not cover these situations.

Finally, consumers play an essential role in preventing the spreading of foodborne diseases through the handling

practices they adopt in their households. Nevertheless, there is a general lack of awareness to this fact and there has been an effort in improving consumers' knowledge about correct procedures to adopt concerning the storage and preparation of food products (Kennedy and Gibney, 2011; Shapiro et al., 2011).

## METHODOLOGY

### Fresh Lettuce Sampling

Fresh lettuce samples were acquired from different supermarkets, independent shops and open markets in the area of Ghent and Bruges, Belgium. In total, 150 samples were tested in this study with 50 samples from each mentioned scenario. Each product was transported to the laboratory after purchase and inspected for its physical appearance before the microbial analysis. Furthermore, information of date and local of collection, cultivar, brand (label), country of origin, packing and price was recorded for the purpose of developing the database used in this study.

Samples of lettuce were halved lengthwise and after discarding one half and the entirety of the stalk, leaves were cut in half and into pieces approximately 3 cm wide. The cut leaves were placed in a bowl and mixed together to homogenize the analyzed samples.

A portion of 10 to 15 g of each sample was aseptically transferred to a stomacher bag. These samples were submitted to a 1:10 dilution in sterile PPS solution of 1 g/L of Neutralized Bacteriological Peptone and 8.5 g/L of Sodium Chloride. The dilutions were homogenized for 1 min using a Colworth 400 Stomacher.

For the enumeration of coliforms, two serial dilutions were made for each homogenized sample in the same PPS solution and inoculated in VRBG by use of a pour plate with a second layer of the same agar solution. Typical colonies present a purple pink color, with or without the presence of a halo, and they were counted after incubation at 37ºC during 24 h.

For the enumeration of *E. coli*, 1 mL from the suspension was added to a pour plate with REC agar. Typical colonies present a violet to pink color and they were counted after incubation at 44ºC during 24 h.

For the enumeration of *Listeria monocytogenes*, 1 mL from the suspension in the stomacher bag mentioned in section 3.1.1 was divided over three spread plates of ALOA®. Typical colonies present a blue color with the presence of a white halo and they were counted after incubation at 37ºC during 48 h.

For the enumeration of *Campylobacter* spp., 1 mL from the suspension in the same stomacher bag was divided over three CFA spread plates. Typical colonies present a burgundy-red to orange-red color and they were counted after microaerophilic incubation using CampyGen at 42ºC during 48 h.

A portion of 25 g of each fresh lettuce sample was aseptically transferred to a stomacher bag. It was added 225 mL of Bolton Broth to the stomacher bag. The homogenization was performed during 1 min with a Colworth 400 Stomacher. The samples were placed in a jar and incubated in a microaerophilic environment using CampyGen during 4 h at 37ºC and then 40–48 h at 42ºC.

The obtained enrichments were used to inoculate CampyFood Agar plates by streaking. Typical colonies present a burgundy-red to orange-red color and they were counted after microaerophilic incubation using CampyGen at 42ºC during 48 h.

A portion of 25 g of each sample was aseptically transferred to a filter stomacher bag. It was added 225 mL of BPW solution. The homogenization was performed during 1 min with a Colworth 400 Stomacher. Samples were incubated during 18 – 24 h at 37ºC.

From the enriched samples, 50 µL of suspension were added to Extraction Pack Food 1 Lysis Tubes. The lysis was performed using a Multi-Blok Heater at 100ºC during 10 min. Samples were centrifuged by use of a MicroCL 17 Microcentrifuge during 2 min at 10 000 g. Additionally, three Eppendorf tubes were filled with 1.8 mL of enriched samples stabilized in glycerol to be kept frozen at a temperature of -75ºC in case of need of further confirmation of positive results in this analysis. This detection is performed through a simultaneous detection of genes encoding Shiga toxins 1 and 2 (stx1 and stx2), intimin (eae), the *E. coli* O157:H7 antigen (rfbE), and *Salmonella* spp.-specific genes. Results are considered automatically positive in the GeneDic® Cycler for amplifications corresponding to a cycle threshold inferior to 40 (Ct < 40).

Centrifuged lysed samples were submitted to a 1:2 dilution in Dilution Buffer (Pall Corporation, Bruz, France). 36 µL of the supernatant of these dilutions were added to the compartments of a GeneDisc® Plate STEC & Salmonella spp. 06 (Pall Corporation, Bruz, France) together with 36 µL of Mastermix 21013SR003 (Pall Corporation, Bruz France). After the execution of a vacuum step, 4 drops of mineral oil were added to each compartment and the vacuum step was repeated. To proceed with the analysis, the plates were placed in the GeneDisc® Cycler, which performs a qPCR assay in order to detect the presence of the mentioned microorganisms and records the results.

Samples which presented a positive result in the GeneDisc® analysis were submitted to a confirmation procedure through a classic detection method. Resuscitation of the cells after storage in the freezer was

executed through an enrichment in the same BPW solution mentioned before. After an incubation period of 18 h, samples were submitted to a selective enrichment by inoculation in screw-capped tubes with 10 mL of two different media (RVS Broth and MKTTn Broth) inoculated with 0.1 mL and 1 mL of the BPW enrichment respectively. The first enrichment was incubated at a temperature of 41.5ºC while the latter at 37ºC, both during 24 h.

The selective enrichments were used to inoculate XLD agar plates in order to isolate colonies of *Salmonella* spp. by regrowing them in a nutrient agar. Typical *Salmonella* colonies in XLD agar present a black color and they were identified after incubation at 37ºC during 24 h.

Confirmation of positive samples for *Salmonella* spp. was achieved by use of the BBL Crystal™ Enteric/Nonfermenter Identification System. Test inoculum was prepared with the inoculum fluid and used to fill the reactions wells. The incubation was performed at a temperature of 37ºC during 24 h and after the kits were observed for identification of color changes. Results were used to compare with the BBL Crystal E/NF ID database and thus identify the bacteria present in the analyzed samples.

The results obtained from laboratorial work were analyzed using the software IBM SPSS Statistics 22. To establish comparisons between different groups it was used a GLM Univariate Analysis procedure with the Bonferroni post hoc range test and multiple comparisons and the test of normality was executed using the Kolmogorov-Smirnov testing. All the analysis in this work were performed considering the traditional significance level of 0.05.

## Analysis of the Survey Data

The original database contained 1 967 respondents in Belgium and 830 in Spain originated through a survey that was conducted from November 2010 until October 2011. It was selected the age group of 18 to 65, leading to a total number of 1 883 respondents in Belgium and 786 in Spain. For the Analysis of Food Consumption, 278 individuals in Belgium and 203 in Spain were not considered because they did not answer any question of this section of the survey, leading to a total number of 1 605 respondents in Belgium and 583 in Spain who answered at least one question of the referred section. For the same reason, 234 individuals in Belgium and 167 in Spain were not considered for the Analysis of Food Preservation Time Practices with a total of respondents of 1 649 in Belgium and 619 in Spain. In the Analysis of Food Preservation Method, Peeling and Washing Practices, 258 respondents in Belgium and 175 in Spain were not considered leading to total number of respondents of 1 625 in Belgium and 610 in Spain.

To perform the analysis of the acquired data it was used the application *Excel 2013* available in the productivity suite *Microsoft Office Professional Plus 2013*. For each question of the survey, the respondents were divided in consumers, non-consumers and blank answers. Relative frequencies of different answers were calculated using the total number of consumer respondents for each item.

## Exposure Assessment

The exposure assessment presented in this work was performed by use of the work of Delbeke, Jacxsens and Uyttendaele (2014). This models contains the prevalence of *Salmonella* spp. and VTEC at retail level and the influence of consumer behavior (storing and washing) together with consumption data. The model does not contemplate transport, for being of short duration, or the effect of drying after washing lettuce due to its low significance in the reduction of the presence of pathogens. The prevalence of pathogens was acquired during the execution of this work and the final data, after confirmation through culture methods, was inserted in the model using @Risk through a β-distribution:

$$Prevalence = RiskBeta\big((p+1);(n-p-1)\big) \qquad (1)$$

with n representing the total number of samples and p representing the number of positive samples for the presence of *Salmonella* spp. or VTEC, respectively.

For the building up of the model, an initial concentration at retail level was considered. This value was calculated through a Pert distribution in @Risk considering a minimum value of 1 CFU/10 g, a maximum of 100 CFU/g and a most likely value of 5 CFU/g. The mentioned values were determined by expert discussion.

The survival of these pathogens in butterhead lettuce was determined by the authors of the model through lab experiments for the temperatures of 7ºC, 15ºC and 22ºC corresponding to the situations of storage in the fridge, in the basement or at room temperature, respectively. The storage procedures from consumers were inserted as a probability in the model while storage time was described using a cumulative distribution.

The reduction by washing was retrieved from literature to be inserted in the model through a Triang distribution created in @Risk by the authors, featuring the different scenarios of washing by rinsing, immersion or a combination of both. The washing procedures from consumers were inserted as a probability function in the model, based on the data obtained of the consumer behavior survey, as explained before.

The consumption data was retrieved from the survey described in this work, considering only the age group of 18 to 65 years old. The results of this analysis were inserted in the model using discrete functions to portray

the distribution of number of portions consumed in a year and of the portion size.

In the scope of this work, only Belgian data was considered for the exposure assessment although the model also included Spanish data. With this data, the model was simulated with 100 000 iterations in @Risk in order to achieve the results presented in this work.

## RESULTS

### Fresh Lettuce Sampling

The collection of samples was limited to the availability of lettuce in establishments of the three different scenarios in the scope of this work. During the initial phase of this work (from February to April), due to the climacteric conditions of Belgium and the outdoors location of farmers' markets, samples were only acquired from supermarkets and independent stores since farmers' markets were scarce and with low availability of lettuce. For this reason, the majority of lettuce samples from farmers' markets were retrieved in the month of May of 2014.

An overview of the results obtained in the enumeration of microbial hygiene indicators is presented in Table 1. Total Coliforms (TC) were found in the majority of samples, with only 5 samples (approximately 3% of the total number of gathered samples) that presented a concentration below the limit of detection (LOD = 10 CFU/g). Regarding the case of enumeration of *E. coli*, these could only be counted in 10 samples retrieved during the month of May and 9 of these were collected in farmers' markets. For this reason, while coliforms were submitted to further analysis, the number of positive samples for *E. coli* were not enough to establish relations between different groups of characteristics and therefore this exercise was not considered in this work.

Table 1 – Determination of the prevalence of indicators of hygiene in the lettuce samples gathered.

| | | Supermarkets | Independent Stores | Farmers' Markets |
|---|---|---|---|---|
| Total Coliforms | < 10 CFU/g | 4 (8%) | 0 (0%) | 1 (2%) |
| | ≥ 10 CFU/g | 46 (92%) | 50 (100%) | 49 (98%) |
| | | Concentration (CFU/g) | | |
| | Minimum | 10 | 20 | 10 |
| | Maximum | 72 000 | 76 000 | 580 000 |
| | Median | 830 | 865 | 5 500 |
| *Escherichia coli* | < 10 CFU/g | 50 (100%) | 49 (98%) | 41 (82%) |
| | ≥ 10 CFU/g | 0 (0%) | 1 (2%) | 9 (18%) |
| | | Concentration (CFU/g) | | |
| | Minimum | - | 30 | 10 |
| | Maximum | - | 30 | 430 |
| | Median | - | 30 | 20 |

The results for the enumeration of coliforms in each sample of the different scenarios together with indication of the date of purchase are represented in Figure 1. This representation suggests the existence of an increase of the number of coliforms along the sampling period.

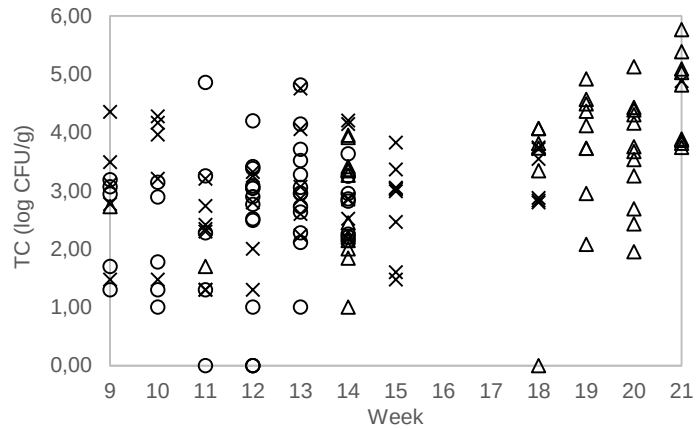


Figure 1 – Concentration of total coliforms, in log CFU/g, in the gathered samples according to date of purchase expressed in weeks of the year 2014. Samples gathered from supermarkets are represented by circles, from independent stores by crosses and from farmers' markets by triangles.

In a comparison between the different scenarios of place of purchase, a significant difference could be found between these groups (p-value < 0.001). Supermarkets and independent stores can be grouped together but concentrations of TC in farmers' markets display a distinctness towards the other scenarios. An examination through SPSS demonstrates that samples retrieved from farmers' markets present generally a higher concentration of coliforms.

In parallel with the enumeration of these indicators, it was analyzed the prevalence of Campylobacter spp., Listeria monocytogenes, Salmonella spp., and E. coli O157:H7 (VTEC). The results of this study can be found in

Table 2 Table 2.

The performed tests for detection of Campylobacter, Salmonella and VTEC revealed that the prevalence of these pathogens was below the limit of detection in the entirety of the samples retrieved. Nevertheless, Listeria monocytogenes was found in three samples in a concentration of 10 CFU/g with one sample retrieved from a supermarket and two from independent shops.

An analysis of the characteristics of samples that retrieved positive results for the presence of *L. monocytogenes* does not support the establishment of a pattern between these, mostly due to the reduced size of the population. Appearance was generally clean in these samples with the exception of one that presented bruises on the leaves and none of them was kept in cold storage. However, although these samples were acquired in two different places (samples 80 and 84 were acquired in the same

independent shop), the date of purchase is relatively close (7 days apart).

Table 2 – Determination of prevalence of pathogens in the lettuce samples from the three different scenarios of place of purchase.

| | Supermarkets | Independent Shops | Farmers' Markets |
|---|---|---|---|
| *Campylobacter* spp. | 0 (0%) | 0 (0%) | 0 (0%) |
| *L. monocytogenes* | 1 (2%) | 2 (4%) | 0 (0%) |
| *Salmonella* spp. | 0 (0%) | 0 (0%) | 0 (0%) |
| *E. coli* O157:H7 | 0 (0%) | 0 (0%) | 0 (0%) |

## Analysis of the Survey Data

In a first approach, the results obtained through the survey were targeted for the age group of 18 to 65 years old. This procedure created a population of 1 722 and 714 respondents in Belgium and Spain, respectively. A characterization of these populations revealed that in both cases there was a higher number of female respondents. Regarding occupation, the majority of the respondents were workers, followed by students and people who currently are unemployed or stay-at-home parents (categorized by the expression "at home").

An examination of the results presented in Table 4 leads to the conclusion that consumption patterns of fresh lettuce in Belgium and Spain present several differences. Spanish respondents manifest a higher number of consumers with 95% of the respondents claiming that they consume this vegetable while in Belgium only 3 in 4 people do it. Furthermore, Belgian consumers revealed a stronger tendency of buying lettuce in a seasonal way (45% of the respondents against 8% in Spain). Regarding the frequency of consumption, Spanish consumers eat more regularly lettuce with the majority reporting the consumption of lettuce several times a week or daily. On the other hand, consumption in Belgium is more commonly done in a weekly basis, followed by a frequency of several times a week and once in a month. Finally, portion consumption is more similar comparing both countries with smaller portions being more commonly consumed.

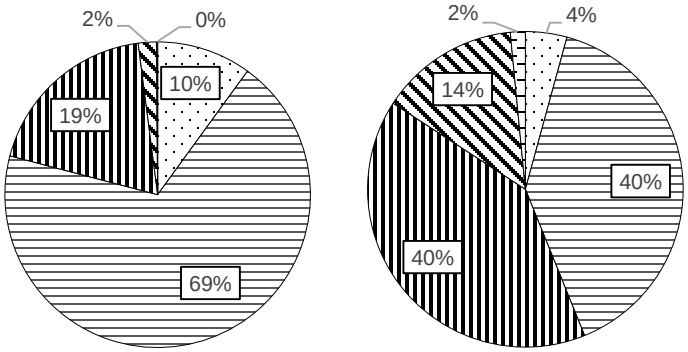


Figure 2 – Duration of storage after purchase of fresh lettuce by the respondents of the survey in Belgium (left, n = 1 649) and Spain (right, n = 619). The dotted area represents respondents who consume fresh lettuce the same day they buy it, horizontal stripes represent storage for 1 to 3 days, vertical stripes represent 4 to 7 days, diagonal stripes represent for longer than a week, and the dashed area represents a period longer than the expiration date.

Storage procedures were also evaluated in this work. In Figure 2, the data relative to the duration of storage of fresh lettuce in the household, after being bought and before being consumed, is represented. It is noticeable that these results present a significant difference between the two countries in scope. The majority of respondents in Belgium consume lettuce after storing it for 1 to 3 days whereas in Spain this practice is equally divided with consumption after 4 to 7 days of storage. In general, in comparison with Belgian respondents, Spanish respondents store fresh lettuce in longer periods of time before consuming it.

Table 3 – Composition of men and women from the age group of 18 to 65 years old in function of occupation responding the survey in Belgium and Spain before data cleaning.

| Country | Occupation | Men | | Women | | Total | |
|---|---|---|---|---|---|---|---|
| | | Number | Percentage | Number | Percentage | Number | Percentage |
| Belgium | Student | 123 | 7,2% | 373 | 21,6% | 496 | 28,8% |
| | Worker | 288 | 16,7% | 708 | 41,1% | 996 | 57,8% |
| | At Home | 34 | 2,0% | 101 | 5,9% | 135 | 7,9% |
| | Other | 24 | 1,4% | 71 | 4,1% | 95 | 5,5% |
| | Total | 469 | 27,3% | 1 253 | 72,7% | 1 722 | 100,0% |
| Spain | Student | 39 | 5,5% | 36 | 5,0% | 75 | 10,5% |
| | Worker | 209 | 29,3% | 357 | 50,0% | 566 | 79,3% |
| | At Home | 20 | 2,8% | 53 | 7,4% | 73 | 10,2% |
| | Total | 268 | 37,5% | 446 | 62,5% | 714 | 100,0% |

Table 4 - Frequency and portion consumption by seasonal and non-seasonal consumers of fresh lettuce in Belgium and Spain.

| Consumption of Fresh Lettuce | | Belgium (n = 1605) | | Spain (n = 583) | |
|---|---|---|---|---|---|
| | | Seasonal 543 (45%) | Non Seasonal 663 (55%) | Seasonal 42 (8%) | Non Seasonal 510 (92%) |
| Few times a year (6/365) | Handful of lettuce (43,10 g) | 24 (4%) | 34 (5%) | 2 (5%) | 7 (2%) |
| | Half of a lettuce (195,85 g) | 3 (1%) | 5 (1%) | 0 (0%) | 1 (0%) |
| | ¾ of a lettuce (293,80 g) | 0 (0%) | 1 (0%) | 0 (0%) | 0 (0%) |
| | Whole lettuce (391,70 g) | 0 (0%) | 0 (0%) | 1 (2%) | 0 (0%) |
| | Total | 27 (5%) | 40 (6%) | 3 (7%) | 8 (2%) |
| Monthly (12/365) | Handful of lettuce (43,10 g) | 53 (10%) | 137 (21%) | 2 (5%) | 13 (3%) |
| | Half of a lettuce (195,85 g) | 10 (2%) | 31 (5%) | 0 (0%) | 1 (0%) |
| | ¾ of a lettuce (293,80 g) | 0 (0%) | 5 (1%) | 0 (0%) | 2 (0%) |
| | Whole lettuce (391,70 g) | 0 (0%) | 2 (0%) | 0 (0%) | 1 (0%) |
| | Total | 63 (12%) | 175 (27%) | 2 (5%) | 17 (3%) |
| Weekly (52/365) | Handful of lettuce (43,10 g) | 232 (43%) | 211 (32%) | 7 (17%) | 42 (8%) |
| | Half of a lettuce (195,85 g) | 51 (9%) | 65 (10%) | 1 (2%) | 8 (2%) |
| | ¾ of a lettuce (293,80 g) | 8 (1%) | 10 (1%) | 0 (0%) | 4 (0%) |
| | Whole lettuce (391,70 g) | 4 (1%) | 1 (0%) | 2 (5%) | 7 (2%) |
| | Total | 295 (54%) | 287 (43%) | 10 (24%) | 61 (12%) |
| Several times a week (3/7) | Handful of lettuce (43,10 g) | 90 (16%) | 88 (13%) | 10 (24%) | 157 (31%) |
| | Half of a lettuce (195,85 g) | 26 (5%) | 37 (6%) | 4 (10%) | 69 (13%) |
| | ¾ of a lettuce (293,80 g) | 8 (1%) | 7 (1%) | 3 (7%) | 21 (4%) |
| | Whole lettuce (391,70 g) | 3 (1%) | 1 (0%) | 1 (2%) | 15 (3%) |
| | Total | 127 (23%) | 133 (20%) | 18 (43%) | 262 (51%) |
| Daily (365/365) | Handful of lettuce (43,10 g) | 24 (5%) | 16 (3%) | 2 (5%) | 81 (16%) |
| | Half of a lettuce (195,85 g) | 7 (1%) | 8 (1%) | 4 (10%) | 41 (8%) |
| | ¾ of a lettuce (293,80 g) | 0 (0%) | 1 (0%) | 1 (2%) | 9 (2%) |
| | Whole lettuce (391,70 g) | 0 (0%) | 1 (0%) | 1 (2%) | 12 (2%) |
| | Total | 31 (6%) | 26 (4%) | 8 (19%) | 143 (28%) |
| Several times a day (1095/365) | Handful of lettuce (43,10 g) | 0 (0%) | 1 (0%) | 0 (0%) | 11 (2%) |
| | Half of a lettuce (195,85 g) | 0 (0%) | 1 (0%) | 1 (2%) | 6 (2%) |
| | ¾ of a lettuce (293,80 g) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0%) |
| | Whole lettuce (391,70 g) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0%) |
| | Total | 0 (0%) | 2 (0%) | 1 (2%) | 19 (4%) |
| Consumers | | 1206 (75%) | | 552 (95%) | |
| Non consumers | | 43 (3%) | | 5 (1%) | |
| Not answered | | 356 (22%) | | 26 (4%) | |

Regarding place of storage, the results are illustrated in Figure 3. Respondents in Belgium and Spain revealed similar patterns with a vast majority using the refrigerator as the place to store fresh lettuce before consumption. Nevertheless, it was registered a higher frequency in Belgium of consumers that store this vegetable in the basement or at room temperature.

The temperature of the refrigerator was also assessed in the survey and the results are represented in Figure 4. Respondents in both countries present once again a similar distribution of behaviors with a substantial majority reporting a temperature between 4 to 7ºC. However, there is a slightly higher percentage in Belgium of people that do not control the temperature of the refrigerator used in their households.

Finally, it was performed an analysis of washing procedures in the households. Respondents were inquired about their practices and the results of this activity are portrayed in Figure 5. This study revealed that only a small percentage of the enquired people stated that they do not wash lettuce before consuming it (2% in Belgium and 1% in Spain). Most of Belgian consumers also perform a step

of drying after the washing, however in Spain this practice is not so common.

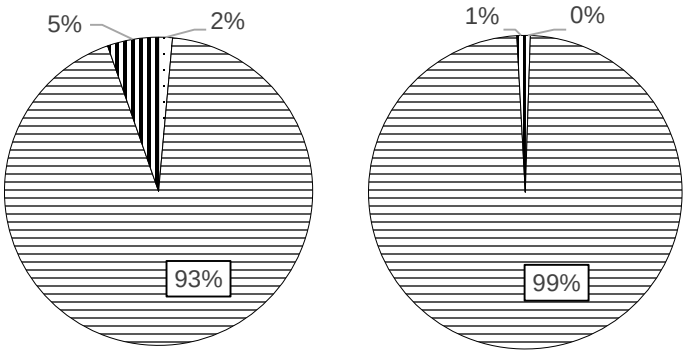


Figure 3 – Place of storage after purchase of fresh lettuce by the respondents of the survey in Belgium (n = 1 625), on the left, and Spain (n = 610), on the right. The dotted area represents respondents who store fresh lettuce at room temperature, horizontal stripes represent storage in the refrigerator, and vertical stripes represent storage in the basement.

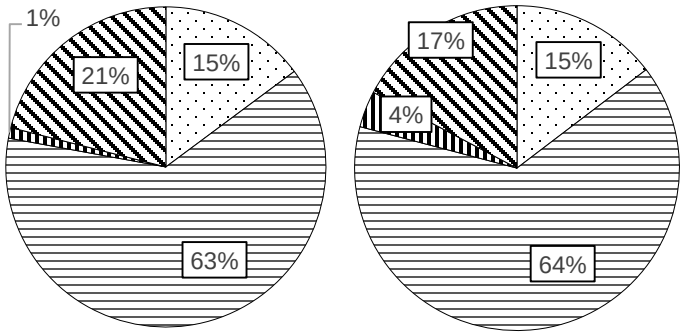


Figure 4 – Temperature of refrigerator reported by the respondents of the survey in Belgium (n = 1619), on the left, and Spain (n = 608), on the right. The dotted area represents respondents who keep the temperature of the refrigerator below 4ºC, horizontal stripes represent a temperature between 4 to 7ºC, vertical stripes represent a temperature above 7ºC, and the diagonal stripes represent respondents who do not know the temperature of their refrigerator.

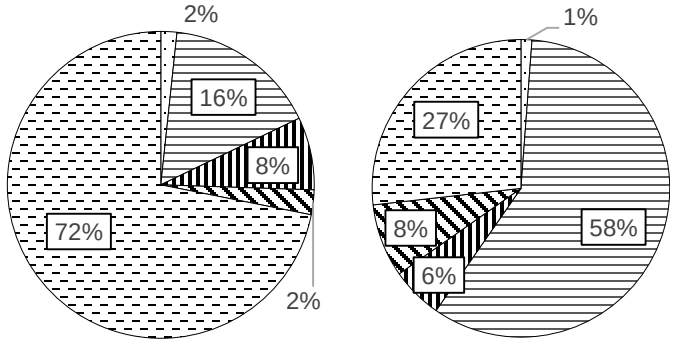


Figure 5 – Washing procedures of fresh lettuce adopted by the respondents of the survey in Belgium (n = 1422), on the left, and Spain (n = 590), on the right. The dotted area represents respondents who do not wash lettuce before consumption, horizontal stripes represent washing without drying, vertical stripes represent washing and drying, diagonal stripes represent washing by scrubbing and drying, and the dashed area represents washing and drying with a salad spinner.

## Exposure Assessment

The results from the exposure assessment are presented for the situations of Belgian consumer's exposure to *Salmonella* spp. and VTEC. These include the effect of consumer's practice (storage and washing) on the concentration of these pathogens in the moment of consumption, together with a quantification of the number of ingested contaminated portions. The results presented were obtained through the use of the model developed by Debelke, Jacxsens and Uyttendaele (2014).

The distribution of concentration of the pathogens was used, together with the distribution of consumption of fresh lettuce in Belgium, to determine the exposure of Belgian consumers to *Salmonella* spp. and VTEC, after storage and washing, as it is represented in Figure 6 and in Figure 7. Due to the similarity of the results, it was chosen to represent only the situation of washing by rinsing.

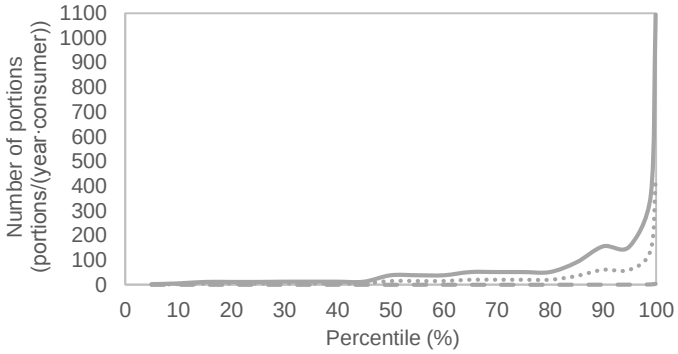


Figure 6 – Distribution of the number of consumed lettuce portions contaminated with *Salmonella* spp. after storage and washing (rinsing) by a Belgian consumer in one year. The solid line represents consumption of lettuce, the dotted line represents consumption of lettuce contaminated with *Salmonella* without taking prevalence into account and the dashed line represents consumption of lettuce contaminated with *Salmonella* taking prevalence into account.

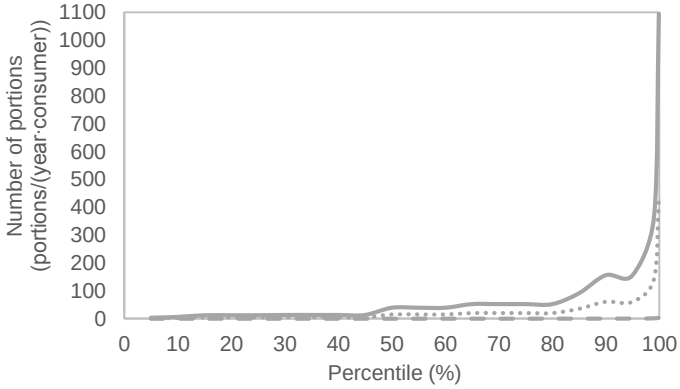


Figure 7 – Distribution of the number of consumed lettuce portions contaminated with VTEC after storage and washing (rinsing) by a Belgian consumer in one year. The solid line represents consumption of lettuce, the dotted line represents consumption of lettuce contaminated with VTEC without taking prevalence into account and the dashed line represents consumption of lettuce contaminated with VTEC taking prevalence into account.

A strategy of washing by immersion is the scenario that leads to a higher final concentration of bacterial pathogens while a combination of this strategy with rinsing leads to the best results. Considering the prevalence determined in this work of 0/150 for *Salmonella* spp., it was determined that half of the consumers are not exposed to contaminations of this pathogen through consumption of fresh lettuce if they proceed to at least a step of washing by rinsing. Nevertheless, 1 in 100 consumers (P99) are exposed to 1 to 3 contaminated portions and 1 in 1 000 (P99.9) are exposed to a number of 1 to 7, according to the adopted washing strategy. Regarding the situation of VTEC and taking into consideration the prevalence recorded in this work for this pathogen of 0/150, it was determined that, once more, half of the consumers are not exposed to contaminations of *E. coli* H157:O7 through consumption of fresh lettuce if at least a step of washing by rinsing is applied. Additionally, 1 in 100 consumers (P99) are exposed to 1 to 3 contaminated portions and 1 in 1 000 (P99.9) are exposed to a number of 1 to 7, according to the adopted washing strategy.

## DISCUSSION

### Fresh Lettuce Sampling

Regarding total coliforms, the determination of concentration of these in the gathered samples revealed an overall increasing tendency along with the date of purchase. Samples retrieved in the month of May 2014 (weeks 18 to 21) present a generally higher concentration of coliforms than samples collected in March or April (weeks 9 to 15). Due to the characteristics of farmers' markets, it was extremely difficult to retrieve lettuce samples from these outdoors establishments in the beginning of the sampling period due to the rainy weather conditions. For this reason, sample collection had to be adjourned until the month of May, when it was possible to retrieve all the samples necessary for this work from farmers' markets.

As a result of this situation, it is not possible to confirm if the existence of an increase of the number of coliforms along the sampling period is related to the different scenario of place of purchase (higher counts of TC in farmers' markets) or due to the change of climacteric conditions in this period. Nevertheless, it could be established that supermarkets and independent shops do not present a significant difference in the concentration of this hygiene indicator. This fact can be explained by the similarity that smaller establishments are nowadays able to present, like supermarkets, appropriately equipped facilities in order to ensure the safety of their food products.

The gathered data concerning the impact of source (place of purchase) in the concentration of total coliforms in fresh lettuce led to a result, expressed in terms of median concentrations, of 830 CFU/g in supermarkets, 865 CFU/g in independent shops and 5 500 CFU/g in farmers' markets. Comparing with previous literature published on this topic (Viswanathan and Rhandir, 2001; Aycicec et al., 2006; Mdluli et al., 2013; Brandão et al., 2014; Moneim et al, 2014), the results in this work are consistent and relatively low comparing with the situations described.

Literature on this matter was scarce and the retrieved studies lack resemblance with this work in what concerns location. Furthermore, as it has been stated previously in this work, different authors have declared that total coliforms might not constitute a reliable indicator of fecal contamination in fresh produce due to the ability of these microorganisms to grow in different environments (Won et al., 2013).

Regarding the study of *E. coli* as a hygiene indicator for fresh vegetables, it was not possible to perform this study since there was not enough positive results for the presence of this microorganism. The entirety of these samples was collected in the month of May 2014 and, in total, 10 samples gathered in this work had a positive result for the presence of *E. coli*, corresponding to approximately 7% of the overall of samples. Once more, the higher frequency of *E. coli* in samples from farmers' markets cannot be separated from the date of purchase leading to the impossibility of indicating if there is actually a difference between different kinds of establishments in terms of concentration of *E. coli* and, thus, safety of food products sold in them.

The analysis of prevalence of pathogens was performed in order to investigate the existence of cases of fresh lettuce available to the customer that were infected with the four most common foodborne diseases: *Campylobacter* spp., *Escherichia coli* O157:H7 (VTEC), *Listeria monocytogenes, and Salmonella* spp. For this purpose, different testing procedures were performed leading to the result that the entirety of the samples were below the limit of detection for *Campylobacter*, VTEC and *Salmonella.* However, 3 samples in total (2% of the total sample size) retrieved a positive result for the presence of *Listeria monocytogenes.*

*L. monocytogenes* was the only pathogen in the scope of this work that was able to be detected in the gathered samples of lettuce. The prevalence of this microorganism corresponds to 2% of the total sample size. Comparing with other similar works, Abadias et al. (2008) retrieved a prevalence of 0.7% in lettuce samples and Wijnands et al. (2014) stated a prevalence ranging up to 0.11%. Thus, the prevalence of *Listeria monocytogenes* determined in this

work appears as relatively high, although the concentrations presented low values in all the positive samples (10 CFU/g). No correlation could be found relating this data to their reported characteristics leading to the conclusion that these correspond to individual cases of infection. Nevertheless, cases like these can still lead to serious consequences due to the high mortality rate of this microorganism associated with its high resistance to grow in environments with a low temperature, such as the refrigerator where lettuce is most commonly stored.

## Analysis of the Survey Data

The survey in analysis in the present work was conducted in Belgium and Spain, as two distinct examples of consumers in Europe. This study was carried in order to study consumption habits regarding selected fruits and vegetable. In the scope of this work, only the data relative to lettuce consumption was taken into account.

A previous study (Jacxsens et al., 2012) stated that Spain and Belgium present cultural differences that are reflected in the food consumption habits of their populations. Mediterranean countries, such as Spain, present a higher consumption of fruits and vegetables. In contrast, eating habits in Belgium correspond to the usual diet adopted in the Western countries. This diet generally includes a high intake of saturated fatty acids and a low consumption of products such as fiber, fruits or vegetables.

An obvious remark in this analysis is the fact that lettuce is clearly a vegetable that is consumed by a vast majority of population: only 3% of the respondents in Belgium and 1% in Spain stated that they do not consume this vegetable. Regarding frequency of consumption, Spain presents a higher percentage of consumers eating lettuce several times a week and even in a daily basis in comparison to Belgium that presents a lower consumption of fresh lettuce. These results coincide with the statement that consumption of vegetables is more frequent in Mediterranean countries, such as Spain, than in the Western countries, such as Belgium.

It is recommended that lettuce should not be stored in a period longer than one to three days. Moreover, this vegetable should be stored in the refrigerator in a temperature between 4-7ºC (Jacxsens et al., 2012). In an analysis of the duration of storage practiced, it is noticeable a clear distinction between Belgian and Spanish consumers. In Belgium, the majority of the consumers are in compliance with the correct practices of storage of lettuce while Spanish consumers present a majority that keeps lettuce in storage for a period longer than the advised. This situation should be the target of awareness campaigns in order to improve handling practices of the consumer.

Storage procedures were the most critical aspect detected during this work concerning consumer behavior. Consumers often believe that foodborne diseases are originated outside their homes and they think correct procedures will be a superfluous consumption of money and time during the preparation of their meals. It is portrayed through the results of this study that consumers need to be educated about correct storage practices but, at the same time, made aware of the importance of following these practices.

Regarding washing procedures, it is recommended that fresh lettuce is washed by placing it under running tap water while scrubbing its leaves with the hands and, after washing, drying it with a paper towel or a lettuce centrifuge (Jacxsens et al., 2012). The collected results indicate that only a small percentage of consumers in Belgium and Spain eat lettuce without performing a step of washing before. Relatively to the step of drying, answers differed between these two countries since a vast majority of the respondents in Belgium performs a step of drying while in Spain this situation does not arise. Most of the Spanish consumers enquired stated they perform no step of drying lettuce after washing it. These results appear to be optimistic although more awareness could be raised about the effect of applying a drying step in the maintenance of food safety during the preparation of lettuce for consumption.

## Exposure Assessment

The exposure assessment presented in this work was conducted in order to determine the probability of consuming a portion of lettuce that was infected with a given pathogen and the respective concentration of the pathogen in these portion. To accomplish an exposure assessment it is necessary to model the growth, survival and inactivation of pathogens along the entirety of the supply chain until the moment of consumption. The model used in this study was based on the work of Delbeke, Jacxsens and Uyttendaele (2014) and it was only available for *Salmonella* spp. and VTEC. For this reason, *Campylobacter* spp. and *Listeria monocytogenes* were not considered in this exercise and focus was made on the Belgian consumers, because samples for prevalence estimation in lettuce were taken in Belgium.

The data of frequency consumption and portion size from the survey was used in order to create a distribution of consumption of lettuce in Belgium. With this information, the distribution of number of infected portions consumed in a year could be determined taking into consideration the value determined in this work. The fact that it was established the same value for the prevalence of both pathogens is a reason why the distributions of the number

of infected portions present a strong similarity between the situations for the two pathogens. Additionally, this fact can also be explained by the fact that these microorganisms present similar death rates in these conditions (Debelke et al., 2014).

It was concluded with the results of this exposure assessment that consumption of lettuce is, in fact, highly frequent in the population of Belgium. This detail leads to a situation where consumers are exposed to infection through the consumption of this vegetable even though the entities participating in the supply chain are in compliance with food safety standards in order to guarantee a low prevalence of pathogens in these food products.

Other studies have conducted risk assessments focusing on the consumption of lettuce (Franz et al., 2010; Danyluk and Schaffner, 2011; Ottoson et al., 2011; Pielaat et al., 2014; Sant'Ana et al., 2014). Comparing with the mentioned articles, the model used in this work presents the novelty of considering prevalence of the pathogens at retail level, in the moment of purchase, together with the subsequent effect of consumer handling, including storage practices (method and length) and different scenarios of washing procedures.

## CONCLUSION

The data collected in this study regarding consumption patterns and consumers' handling practices can be used for further research and specifically for the development of scenario-analysis for the development of a microbial risk assessment. This exercise can be performed in order to analyze the influence of the behavior of the consumer regarding consumption patterns, storage practices and washing procedures in the maintenance of food safety concerning fresh fruits and vegetables.

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