

## Microbiological Quality of Sandwiches from Local Cafés and Food Vending Machines in Turin, Italy

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### Abstract

The microbiological quality of ready-to-eat sandwiches (60 samples in total, 30 from food vending machines and 30 from local cafés) was analyzed for the presence and amount of *E. coli*, coagulase-positive *staphylococci*, sulfite-reducing anaerobes, aerobic colony count (ACC), and *Enterobacteriaceae* count. Microbiological quality was unsatisfactory in about 5% of the samples. None of the samples tested positive for sulfite-reducing anaerobes or coagulase-positive *staphylococci*. *E. coli* was detected only in the samples from the cafes, and the ACC and the total *Enterobacteriaceae* count were also higher in these samples, indicating that the industrially processed sandwiches more consistently met the criteria for standard microbiological quality than the manually prepared sandwiches.

**Keywords:** Ready-To-Eat Sandwiches; Microbiological Quality; Cafe; Food Vending Machines; Modified Atmosphere Packaging

### Abbreviations

S: Satisfactory; B: Borderline; U: Unsatisfactory; N/A: Not Applicable

### Introduction

The rapid rise in points of sale of ready-to-eat foods has radically changed the food landscape in industrialized countries [1]. In response to the greater demand for time-saving convenience and consumer choice, local grocery stores, sandwich bars, and food vending machines provide for round the clock availability of quick takeout meals and sandwiches [2]. Sandwiches, pre-packaged or not, owe their appeal to their fresh taste and appearance, low cost, and nutrient value. Sandwich breads stuffed with a mix of various different ingredients (vegetables, cheeses, meats, etc.) and seasoned with condiments have become a diet staple.

Sandwiches are multi-ingredient foods. As such, they may harbor pathogens that cause foodborne illnesses. Indeed, they are also the most frequently reported sources of foodborne illness: multi-ingredient foods were associated with 34.3% of total illnesses in Canada from 1976 to 2005 [3] and 14.0% of illnesses in the United States from 1999 to 2008 [4]. In Europe, ready-to-eat foods pose a direct health risk to consumers mainly owing to Salmonella contamination [5].

Other factors besides the initial microbiological load on ingredients that influence the growth rate of microorganisms at the point of sale include food handling, processing, storage, and display. This is especially critical for ready-to-eat foods sold via self-service food vending machines, where products are left unattended until purchase. To overcome these issues, the food packaging industry utilizes modified atmosphere packaging (MAP) to preserve product quality and extend its shelf life. MAP involves the elimination of O<sub>2</sub> from the packaging

and the introduction of different concentrations of CO<sub>2</sub> and N<sub>2</sub>. Under adequate refrigeration, the growth of aerobic microorganisms, proteolytic bacteria, yeasts, and fungi is inhibited and the product's organoleptic characteristics are maintained [6].

Here, we evaluated the microbiological quality of ready-to-eat sandwiches obtained from local cafés and vending machines for the presence and amount of *Escherichia coli*, coagulase-positive staphylococci, sulfite-reducing anaerobes, aerobic colony count and total *Enterobacteriaceae* count. The microbiological quality of sandwiches from cafés and vending machines was compared against the criteria outlined in the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [9].

## Materials and Methods

### Sample collection

A total of 60 food samples were randomly collected from 30 different local cafes and 30 vending machines between October and November 2016. Twenty-nine of the 30 industrially produced sandwiches from the vending machines were packaged under modified atmosphere and 1 in ambient atmosphere. The 30 sandwiches from the cafés were handmade and displayed at ambient temperature. Sandwich stuffing consisted of various different ingredients (processed meats, chicken, vegetables, cheeses, tuna, tomatoes, mushrooms, eggs). The samples were collected into sterile containers under aseptic conditions and brought refrigerated to the Food Control Laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Turin, for analysis within 24h of sampling.

### Preparation of Samples

Ten grams of each sample were weighed under aseptic conditions and homogenized with 90 mL of buffered peptone water (BPW). Serial dilutions were prepared and the pour plate method was used for counting the number of colony-forming units (CFUs) on selective media.

### Microbiological Analysis

Tryptone Bile X-glucuronide (TBX) agar was used for the detection and enumeration of *E. coli* (dilution from 10<sup>-1</sup> to 10<sup>-5</sup> in peptone water followed by incubation at 44°C for 18-24h) [10]. Plate count agar (PCA) was used for aerobic colony count (ACC) (dilution from 10<sup>-1</sup> to 10<sup>-5</sup> in peptone water followed by incubation at 30°C for 48 - 72h) [11]. Violet Red Bile Glucose (VRBG) agar was used for the detection and enumeration of *Enterobacteriaceae* (dilution from 10<sup>-1</sup> to 10<sup>-5</sup> in peptone water followed by incubation at 37°C for 24h). The oxidase test was used for confirmation [12]. Baird-Parker Agar (BPA) with rabbit plasma fibrinogen (RPF) supplement was used for the detection and enumeration of coagulase-positive *staphylococci* (CPS) (dilution from 10<sup>-1</sup> to 10<sup>-3</sup> in peptone water followed by incubation at 37°C for 48h) [13]. Perfringens agar base (PAB) agar was used for the detection of sulfite-reducing anaerobes (dilution from 10<sup>-1</sup> to 10<sup>-2</sup> in peptone water followed by incubation at 44°C for 18 - 24h in anaerobic jars) [14]. The laboratory and all test procedures are accredited by the Italian accreditation body ACCREDIA according to ISO 17025:2005 [15].

## Results and Discussion

Indicator bacteria, because associated with the presence of pathogens, are useful for the assessment of food safety [9]. The presence of indicator bacteria in ready-to-eat food, although not inherently a health hazard, can be indicative of poor quality of raw materials and food components, undercooking, cross-contamination, poor cleaning, poor temperature and time control. The range of CFU/g for target microorganism found in the samples is presented in Tables 1 and 2. No sulfite-reducing anaerobes or CPS were detected in any of the samples. *E. coli* was detected only in 2 samples of sandwiches from cafés (250 and 10 CFU/g, respectively). *Enterobacteriaceae* was detected in 6 (20%) food vending machines samples (10 to 210 CFU/g) and in 20 (66.7%) samples from the cafés (50 to 15.000.000 CFU/g). Similarly, the ACC was, on average, higher in the samples from the cafés than from the vending machines (7000 to > 30.000.000 CFU/g vs. 50 to > 30.000.000 CFU/g).

		10 <sup>-</sup>	10 <sup>2-</sup>	10 <sup>3-</sup>	10 <sup>4-</sup>	10 <sup>5-</sup>	10 <sup>6-</sup>	
		<10	<10 <sup>2</sup>	<10 <sup>3</sup>	<10 <sup>4</sup>	<10 <sup>5</sup>	<10 <sup>6</sup>	
Sulfite-reducing anaerobes	30	-	-	-	-	-	-	-
Coagulase-positive staphylococci	30	-	-	-	-	-	-	-
<i>E. coli</i>	30	-	-	-	-	-	-	-
Enterobacteriaceae	24	5	1	-	-	-	-	-
Aerobic Colony Count	-	1	1	1	3	9	5	10

**Table 1:** Microbiological test results of 30 sandwich samples collected from vending machines.  
Range expressed as CFU/g.

		10 <sup>-</sup>	10 <sup>2-</sup>	10 <sup>3-</sup>	10 <sup>4-</sup>	10 <sup>5-</sup>	10 <sup>6-</sup>	
		<10	<10 <sup>2</sup>	<10 <sup>3</sup>	<10 <sup>4</sup>	<10 <sup>5</sup>	<10 <sup>6</sup>	
Sulfite-reducing anaerobes	30	-	-	-	-	-	-	-
Coagulase-positive staphylococci	30	-	-	-	-	-	-	-
<i>E. coli</i>	28	1	1	-	-	-	-	-
Enterobacteriaceae	10	1	8	4	2	1	3	1
Aerobic Colony Count	-	-	-	1	2	2	11	14

**Table 2:** Microbiological test results of 30 sandwich samples collected from cafes.  
Range expressed as CFU/g.

For the purposes of this study we compared the microbial test results against the criteria outlined in the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [9]. The Guidelines identify three categories of microbiological quality (satisfactory, borderline, unsatisfactory) for each target microorganism. The microbiological quality parameters for ACC distinguish between food products that have undergone MAP or not. As can be seen in Tables 3 and 4, there were notable differences in microbiological quality between the sandwiches collected from the food vending machines and those from the local cafes. *E. coli* was detected in only 2 samples, both collected from the cafes, in one of which the *E. coli* count was 250 CFU/g and therefore classified as unsatisfactory (>10<sup>2</sup> CFU/g) [9]. *E. coli* is widely used as an indicator of food contamination; its detection suggests inadequate handling and storage conditions and general lack of good hygienic practices

	Range CFU/g	Category*	Sample No. (%)
Enterobacteriaceae	<10 <sup>2</sup>	S	14 (46.7)
	10 <sup>2</sup> - ≤10 <sup>4</sup>	B	1 (3.3)
	>10 <sup>4</sup>	U	-
		N/A	15
Aerobic Colony Count (for MAP products)	<10 <sup>6</sup>	S	14 (46.7)
	10 <sup>6</sup> - <10 <sup>8</sup>	B	9 (30)
	>10 <sup>8</sup>	U	-
		N/A	6

Aerobic Colony Count	<10 <sup>5</sup>	S	1 (3.3)
	10 <sup>5</sup> - <10 <sup>7</sup>	B	-
	>10 <sup>7</sup>	U	-
		N/A	-

**Table 3:** Microbiological quality of vending machine sandwich samples evaluated according to the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [9] for Enterobacteriaceae and ACC.

\*S: Satisfactory; B: Borderline; U: Unsatisfactory; N/A: Not Applicable.

Range expressed as CFU/g.

	Range CFU/g	Category*	Sample No. (%)
Enterobacteriaceae	<10 <sup>2</sup>	S	9 (30)
	10 <sup>2</sup> - ≤10 <sup>4</sup>	B	8 (26.7)
	>10 <sup>4</sup>	U	-
		N/A	13
Aerobic Colony Count	<10 <sup>5</sup>	S	1 (3.3)
	10 <sup>5</sup> - <10 <sup>7</sup>	B	10 (33.3)
	>10 <sup>7</sup>	U	2 (6.7)
		N/A	17
<i>E. coli</i>	<20	S	29 (96.7)
	20 - ≤10 <sup>2</sup>	B	-
	>10 <sup>2</sup>	U	1 (3.3)
		N/A	-

**Table 4:** Microbiological quality of café sandwich samples evaluated according to the Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [9] for Enterobacteriaceae, ACC, and *E. coli*.

\*S, satisfactory; B, borderline; U, unsatisfactory; N/A not applicable.

Range expressed as CFU/g.

The detection of *Enterobacteriaceae*, a large family of Gram-negative bacteria comprising *E. coli* and other pathogenic and non-pathogenic bacteria, indicates post-processing contamination or inadequate cooking. *Enterobacteriaceae* was detected in both types of samples (from 10 to 210 CFU/g in vending machine samples and from 50 to 15,000,000 CFU/g in samples from cafés). Seven of the sandwiches from the cafés exceeded the limit of >10<sup>4</sup> CFU/g. However, this criterion cannot be applied in this case because the sandwich stuffing contained vegetable ingredients which can have high levels of *Enterobacteriaceae* as part of their normal micro-flora [9].

Aerobic colony count (ACC) is used as a microbiological hygiene indicator of general quality assessment, including that of extended shelf-life foods. High counts may suggest quality issues and possible inadequate temperature control [9]. In the present study, the ACC was higher in the samples from the cafés: 2 of the 30 samples were classified as unsatisfactory according to this parameter. The other unsatisfactory samples contained a matrix (cheeses, vegetables, salamis, olives) with naturally occurring high levels of microorganisms [9]. Of the total of 60 sandwiches, 5% were classified as unsatisfactory. Except for sulfite-reducing anaerobes and CPS, which were not detected in any of the samples, the bacterial count for all other target microorganisms was higher in the samples from the cafés.

We speculate that this difference most likely resulted from the better control of bacterial growth afforded by MAP technology (29 of 30 vending machines samples), which can prolong potential shelf-life by 50 to 400% [17], and provided by storage in a temperature-controlled environment. The sandwiches from the cafés were kept at ambient temperature. Perishable foods such as sandwiches and other

ready-to-eat products should be displayed refrigerated to reduce the growth of mesophilic microorganisms. Furthermore, the higher *Enterobacteriaceae* count in the samples from the cafés could have been due to poor hygiene practices during food preparation and storage. Finally, the overall better microbiological hygiene of the samples from the vending machine may reflect compliance with food safety standards by food manufacturers.

### Conclusion

Our findings indicate that the microbiological quality of industrially processed sandwiches for sale via automatic vending machines is higher than that of manually prepared sandwiches sold in the cafés that we investigated. Since the lower microbiological hygiene of hand-made sandwiches can constitute a potential risk to consumer health, not only the quality of raw materials but also the preparation and presentation of perishable food are crucial factors for ensuring food product safety. Compliance with good food manufacturing practices may help to improve the microbiological quality of ready-to-eat food products.

### Conflict of Interest

There is not any financial interest or any conflict of interests.

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