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Assessment of the Microbiological Quality and Safety in Takeaway Sushi Meals in Portugal

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Keywords
Sushi · Pathogenic microorganisms · Good practices · Food safety

Abstract
Being a food product that contains perishable ingredients and involves a significant degree of manual handling during preparation, sushi is regarded as a potentially hazardous food, which may lead to foodborne disease outbreaks. In Portugal, consumption of takeaway sushi meals has strongly increased throughout the past few years; however, there is limited information regarding its compliance with food quality standards. Under this context, the present study aimed to evaluate the microbiological quality and safety of take-away ready-to-eat sushi meals in Lisbon, Portugal. Sixty-two samples were collected from different origins (restaurant and hypermarket), and each sample was tested for aerobic mesophilic microorganisms, Enterobacteriaceae, *Escherichia coli*, positive coagulase Staphylococci, presumptive *Bacillus cereus* count, as for detection of pathogenic microorganisms, such as *Salmonella* spp., *Listeria monocytogenes* and *Vibrio parahaemolyticus*, *V. cholerae* and *V. vulnificus*. Results revealed that 48.4% (30/62) were deemed unsatisfactory, 35.5% (22/62) were classified as borderline and only 16.1% (10/62) were considered satisfactory. Even though we did not detect the incidence of potentially pathogenic microorganisms in sushi, the presence of *B. cereus* and coagulase-positive Staphylococci was detected at unsatisfactory levels. Furthermore, significant differences between the place of origin (restaurant vs. hypermarket) and type of fish were also observed. Overall, the high number of samples classified with a level of microbiological quality "unsatisfactory" and "borderline" highlights the need to review good hygiene practices, as well as the quality of the raw materials used, to obtain a final product with a satisfactory quality and safety level.

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Avaliação da qualidade e segurança microbiológica de refeições de sushi prontas a consumir em Portugal

Palavras Chave
Sushi · Microrganismos patogénicos · Boas práticas de fabrico · Segurança alimentar

Resumo
Por ser um género alimentício que contém ingredientes perecíveis e envolve um grau significativo de manipulação manual durante a sua preparação, o sushi é considerado um alimento potencialmente perigoso, que pode causar surtos de doença de origem alimentar. Em Portugal, o consumo de refeições de sushi prontas a consumir tem aumentado ao longo dos últimos anos. No entanto, a informação sobre o cumprimento das normas de qualidade alimentar é limitada. Neste contexto, o presente estudo teve como objetivo avaliar a qualidade e a segurança microbiológica de refeições de sushi prontas para consumo em *take-way*, na região de Lisboa, Portugal. Foram colhidas 62 amostras de diferentes origens (restaurante e hipermercado), e em cada amostra foi efetuada a contagem de microrganismos aeróbios mesófilos, Enterobacteriaceae, *Escherichia coli*, estafilococos coagulase positiva, *Bacillus cereus* presuntivos, e deteção de microrganismos patogénicos, tais como: *Salmonella* spp., *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Vibrio cholerae* e *Vibrio vulnificus*. Os resultados revelaram que 48,4% (30/62) das amostras foram consideradas insatisfatórias, 35,5% (22/62) foram classificadas como "borderline" e apenas 16,1% (10/62) foram consideradas como satisfatórias. Embora não tenham sido detetados microrganismos potencialmente patogénicos nas amostras de sushi, a presença de *B. cereus* e estafilococos coagulase positivos foram detetados em níveis insatisfatórios. Além disso, também foram observadas diferenças significativas entre o local de origem (restaurante *vs.* hipermercado) e tipo de peixe. No geral, o elevado número de amostras classificadas com um nível de qualidade microbiológica insatisfatória e "borderline" evidência a necessidade de revisão das boas práticas de higiene, bem como da qualidade das matérias-primas utilizadas, para obter um produto final com qualidade e segurança satisfatória.

Introduction

Sushi is a well-known traditional Japanese dish made from raw and highly manipulated perishable ingredients [1]. Its microbiological quality depends on several factors, such as the initial microbiological attributes of each ingredient, temperature control during all stages of production, and the maintenance of proper hygiene and food safety practices during handling and preparation [2].

Whilst Portugal has been for long a country with high fish consumption, consumers' interest in sushi meals has been increasing exponentially in the past years. Not only the number of establishments offering this type of foodstuff has increased, but given its demand, the markets have expanded to more access points to the consumer. It is already available in hypermarkets and the usual Japanese cuisine restaurants [3].

Being a foodstuff mostly composed of raw fish, without any type of heat treatment that could eliminate or reduce the microbial load present to acceptable levels, the risk of intoxication and infection from bacterial origin increases, hence its monitoring is extremely important, due to the potential health risks for consumers while transmitting various foodborne diseases [4–6].

The scientific literature indicates some outbreaks of foodborne diseases caused by sushi and sashimi. *Salmonella* strains are more frequently involved, but also enterotoxigenic *Escherichia coli* and the virus Norwalk have been found [7–11].

Selling sushi as a takeaway increases the food safety risks, as the product remains uncontrolled during transport until the moment of consumption, which can favour the growth of any existing pathogenic microorganisms [12]. Nonetheless, information about the microbiological ecology of this product has been highly neglected.

European studies have often found that sushi specialities obtained values of total aerobic counts close to, or even above, the level considered satisfactory (10^6 cfu g^{-1}) [13–16], with reports on the presence of pathogenic microorganisms such as *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus* and *Vibrio* spp. in this kind of meal [13, 17]. Reports in Portugal are very scarce, and the few existent reports have evaluated sashimi meals served at different restaurants in Northern Portugal [15, 16], revealing that 63.93% of the analysed samples were deemed "unsatisfactory" due to the high levels of mesophiles, Enterobacteriaceae, *S. aureus*, *B. cereus*, moulds and yeasts [15, 16]. Although sushi meals were not evaluated in these studies, it is well known that the addition of ingredients used in its preparation,

Table 1. Number of samples of each variety of fish analysed and other ingredients that comprised the samples

Commercial origin	Type of fish	Number of samples analysed	Ingredients
Hypermarket	Tuna	11	Sushi rice; tuna; lettuce; cucumber; *nori*; sesame seeds
		2	Sushi rice; tuna; *nori*
	Salmon	1	Sushi rice; salmon; *nori*
		2	Sushi rice; salmon; cucumber; mango; *nori*
		11	Sushi rice; salmon; cucumber; mango; *nori*; sesame seeds
	Shrimp	4	Sushi rice; shrimp; cucumber; lettuce; avocado; *nori*; sesame seeds
Restaurant	Tuna	9	Sushi rice; tuna; arugula; *nori*; sesame seeds
		3	Sushi rice; tuna; *nori*
	Salmon	5	Sushi rice; salmon; mango; fish eggs; *nori*; sesame seeds
		10	Sushi rice; salmon; shrimp; avocado; *nori*
		2	Sushi rice; salmon; *nori*
	Shrimp	2	Sushi rice; shrimp; *nori*

Table 2. Diluents, volumes and weight of each sample used in each assay

Microbiological analysis	Sample weight	Medium used as diluent and respective volume
Salmonella spp. Count of aerobic mesophilic microorganisms, Enterobacteriaceae, *Escherichia coli,* coagulase-positive *Staphylococci* and *Bacillus cereus*	25 g	BPW 225 mL
Listeria monocytogenes	25 g	Half-Fraser 225 mL
Vibrio spp.	25 g	APA 225 mL

BPW, Buffered Peptone Water; APA, Alkaline Peptone Water.

such as fruits, vegetables, and rice, can also be a source of microorganisms, such as *Salmonella* spp., *S. aureus*, *L. monocytogenes* and *B. cereus* [18, 19].

Whilst in Portugal all steps involved in the preparation of sushi must be carried out according to the Hazard Analysis and Critical Control Point (HACCP) principles and comply with the general food hygiene requirements set out in Regulation (EC) No. 852/2004, as well as the specific hygiene rules applicable to food of animal origin established in Regulation (EC) No. 853/2004 [20–22], it was only very recently that the acceptable limits for ready-to-eat raw fish foods were defined [23]. Hence, to our knowledge, there has been no research published concerning compliance of takeaway sushi meals with these limits. Furthermore, no study has been done to understand the role of the commercial origin (restaurants and supermarkets) of the bacterial load of sushi. The influence of different fish species is also a point that should be considered [18] and has been largely neglected. Therefore, the objective of this study was to assess the microbiological quality of different ready-to-eat sushi pieces, acquired in restaurant and hypermarket establishments, through their microbial profile analysis.

Materials and Methods

Sampling and Sample Preparation

A total of 62 sushi samples were purchased in takeaway services; 31 samples were from hypermarkets and 31 samples were from a traditional restaurant. Sampling was carried out for 2

Table 3. Guidelines INSA: hygiene and alteration indicator microorganisms in fish, shellfish (raw, marinated) with or without fully cooked food, raw fruits, vegetables and seaweeds

Hygiene and alteration indicator microorganisms	Satisfactory	Borderline	Unsatisfactory
Microorganisms at 30°C	$<10^6$	10^6 to $\leq 10^7$	$>10^7$
Enterobacteriaceae	$<10^4$	10^4 to $\leq 10^5$	$>10^5$
Escherichia coli	<10 (not detected)	10 to $\leq 10^2$	$>10^2$

Table 4. Guidelines INSA: pathogenic microorganisms in fish, shellfish (raw, marinated) with or without fully cooked food, raw fruits, vegetables and seaweeds

Pathogenic microorganisms	Satisfactory	Unsatisfactory	Unsatisfactory/ potentially high health risk
Staphylococci positive coagulase	$<10^2$	10^2 to $\leq 10^4$	$>10^4$
Bacillus cereus	$<10^3$	10^3 to $\leq 10^5$	$>10^5$
Salmonella spp.	Not detected	Not applicable	Detected
Listeria monocytogenes	Not detected	Detected	$>10^2$
Vibrio spp.	Not detected	Not applicable	Detected

months, between May and June 2019, on Mondays and Wednesdays, between 12:00 and 14:00. The ingredients that made up the samples, as well as the number of samples for each variety of fish, are described in Table 1.

The samples were carried in the original packaging provided by the establishments and transported to the laboratory in refrigeration, using isothermal bags, with a controlled temperature between 1°C and 8°C, for a maximum period of 30 min after collection. The samples were identified by a code number to ensure their traceability throughout the process, and the name of the establishment from which they came, ingredients that made up the sample, time and date of the collection were registered. Subsequently, they were kept in a refrigerator at 3 ± 2°C until the respective microbiological analyses were carried out, which occurred approximately 18–20 h after collection in the Microbiology Laboratory of the Department of Food and Nutrition of Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), accredited by NP EN ISO/IEC 17025 [24].

To each sample, after the necessary weighing, the respective diluents were added, and the bags were properly homogenized in a peristaltic homogenizer (Stomacher 400 Circulator, Seward®) for 60 s at a speed of 230 rpm. For *Salmonella* spp. detection, 1/10 decimal dilution of the samples was carried out with the diluent Buffered Peptone Water, and this suspension was used as a pre-enrichment medium and as the first dilution for counts in the TEMPO® system. For *L. monocytogenes*, the diluent Half-Fraser was added in a 1/10 proportion as a pre-enrichment medium, and for *Vibrio* spp. counts, 1/10 Alkaline Peptone Water was added for dilution and as a first enrichment medium as well. Table 2 describes the diluents, respective volumes and sample weights for each test carried out.

Microbiological Analyses

For enumeration, the TEMPO® method (bioMérieux® SA, Marcy l'Étoile, France) was used for aerobic mesophilic microorganisms, Enterobacteriaceae, *E. coli*, coagulase-positive *Staphylococci* and *B. cereus*, according to the manufacturer's instructions. This is an automated test for the enumeration of food hygiene and quality indicators. It involves a vial of culture medium and a card which are specific for each microorganism. The card is based on the Most Probable Number method and is hermetically sealed so that there is no risk of contamination during subsequent handling. Throughout incubation, the microorganisms present in the card reduce the target metabolite and emit a fluorescent signal, which is detected by the TEMPO® Reader instrument. It was developed to obtain similar results to those of the ISO standards [25]. For *Salmonella* spp. and *L. monocytogenes*, the VIDAS® immunoenzymatic system (bioMérieux® SA) was used according to the manufacturer's instructions. This automatic enzyme immunoassay, like all the method steps, is performed automatically by the equipment, which allows the detection of antigens using the enzyme-linked fluorescent assay method. In the end, the final product (4-methylumbelliferone) emits a fluorescence which is measured at 450 nm. The equipment automatically analyses the results and calculates a test value for each sample, interpreting the result as positive or negative [26].

As for *Vibrio* spp., the ISO 21872-1: 2017 standard was used. The entire process was carried out respecting the rules described in the standard ISO 7218: 2007/Amd 1: 2013 [27, 28].

Criteria Used for Microbiological Evaluation

To evaluate the microbiological quality of the samples, the INSA guide values were used [23], as described in Tables 3 and 4 for indicator microorganisms and pathogenic microorganisms, respectively.

Statistical Analysis

Data analysis was performed using descriptive and inferential statistics using the SPSS-24.0 software. To carry out the inferential analysis and considering the fulfilment of the necessary criteria for the performance of parametric tests, and after carrying out the Kolmogorov-Smirnov normality test, and given that the p value result was <0.05 for the variables under study, we assumed that the

Table 5. Microbiological counts expressed in log cfu g^{-1} and respective standard deviation, and minimum and maximum values obtained, per microbiological parameter analysed in the total sample in each type of commercial surface

	Total					Hypermarket					Restaurant				
	n	ave	SD	min.	max.	*n*	ave	SD	min.	max.	*n*	ave	SD	min.	max.
log AC	62	6.67	1.06	4.57	8.69	31	6.62	1.02	4.83	8.08	31	6.73	1.12	4.57	8.69
log EB	62	4.16	1.22	1.00	6.83	31	4.50	1.09	2.72	6.83	31	3.81	1.26	1.00	6.23
log STA	15	1.44	0.44	1.00	2.18	2	1.59	0.83	1.00	2.18	13	1.42	0.41	1.00	2.11
log BC	26	1.74	0.72	1.00	3.43	12	1.91	0.79	1.00	3.43	14	1.60	0.66	1.00	2.72

The total number of samples for all tested parameters was 62; 31 being from supermarkets and 31 from restaurants. *Staphylococcus* coagulase and *Bacillus cereus* were not detected in all samples and *Escherichia coli* was only detected in one sample; therefore, they are not represented in the Table. AC, mesophilic aerobic microorganisms; EB, Enterobacteriaceae; STA, coagulase-positive *Staphylococci*; BC, *Bacillus cereus*; *n*, number of samples in which each parameter was found positive; ave, average; SD, standard deviation; min., minimum; max., maximum.

sample did not follow a normal distribution. In this sense, non-parametric tests were used. To compare the variables under study as a function of the surface, the Mann-Whitney test was applied, which is the appropriate non-parametric test to compare the distribution functions of at least an ordinal variable measured in two independent samples [29].

To correlate the logs of microorganisms, and given that the sample, in this case, presented a normal distribution, according to the Kolmogorov-Smirnov normality test whose *p* value was >0.05, Pearson's correlation coefficient was used, which measures the intensity and direction of the linear-type association between two quantitative variables [29]. For comparing the microorganisms' counts and the surface, the χ^2 test was applied.

Results

Microbial Counts and Respective Bacteriological Quality in the Analysed Sushi Samples

Table 5 shows the average number and the minimum and maximum values obtained for each microbiological parameter analysed in the totality of hypermarket and restaurant samples. Results show that the average number for aerobic mesophilic microorganisms was 6.67 log cfu g^{-1} varying between 4.57 and 8.69 log cfu g^{-1}. Regarding their distribution per type of commercial area, we obtained 6.62 log cfu g^{-1} (4.83–8.08) for the sushi from hypermarket origin and 6.73 log cfu g^{-1} (4.57–8.69) for restaurant samples.

As for the global mean of Enterobacteriaceae, the average number obtained was 4.16 log cfu g^{-1} and the values varied between 1.00 and 6.83 log cfu g^{-1}, yielding 4.50 log cfu g^{-1} (2.72–6.83) and 3.81 log cfu g^{-1} (1.00–6.23) for hypermarket and restaurant commercial establishments, respectively (Table 5).

In coagulase-positive *Staphylococci* counts, the global mean was 1.44 log cfu g^{-1} and the values varied between 1.00 and 2.18, with 1.59 log cfu g^{-1} (1.00–2.18) and 1.42 log cfu g^{-1} (1.00–2.11) in hypermarkets and restaurants, respectively (Table 5).

Regarding *B. cereus*, the global average was 1.74, ranging from 1.00 to 3.43, with 1.91 log cfu g^{-1} (1.00–3.43) in hypermarket samples and 1.60 log cfu g^{-1} (1.00–2.72) in restaurant samples (Table 5).

Table 6 shows the relations between the microorganisms studied and the surface, in the total sample and in each of the sushi varieties, with the obtained Pearson's correlation coefficients and *p* values for each of the tests. Regarding the number of aerobic mesophilic microorganisms, 40.3% (25/62) of the samples were classified as presenting unsatisfactory microbiological levels, of which 22.6% (14/62) came from hypermarkets and 17.7% (11/62) from restaurants. In the Enterobacteriaceae count, 17.7% (11/62) of the samples were also classified as having an unsatisfactory microbiological level, of which 9.7% (6/62) were hypermarket samples and 8% (5/62) were restaurant samples. Moreover, while *E. coli* was detected in only one restaurant sample, and was classified at a borderline microbiological level with a value of 1.00 log cfu g^{-1}, coagulase-positive *Staphylococci* were detected at unsatisfactory levels, although not posing a potentially high health risk, in 4/62 samples, of which 1.6% (1/62) were from hypermarket and 4.8% (3/62) from restaurant commercial surfaces. As for *B. cereus* count, only one hypermarket sample revealed unsatisfactory levels (again, although not posing a potentially high health risk), with 3.43 log cfu g^{-1}. Finally, the detection of *Salmonella* spp., *L. monocytogenes* and *Vibrio* spp. was per-

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Table 6. Relation between the microorganisms and the surface, in the total sample and in each sushi variety, with the obtained Pearson's correlation coefficients and *p* values for each test

Classification			Total sample				Tuna				Salmon				Shrimp			
			hyper.	rest.	total	*p*	hyper.	rest.	total	*p*	hyper.	rest.	total	*p*	hyper.	rest.	total	*p*
log AC	Unsatisfactory	*n*	14	11	25		8	7	15		4	3	7		2	1	3	
		%	56	44	100		53	47	100		57	43	100		67	33	100	
	Borderline	*n*	7	13	20		3	3	6		2	10	12		2	0	2	
		%	35	65	100		50	50	100		17	83	100		100	0	100	
	Satisfactory	*n*	10	7	17		2	2	4		8	4	12		0	1		
		%	59	41	100		50	50	100		67	33	100		0	100	100	
	Total	*n*	31	31	62		13	12	25		14	17	31		4	2	6	
		%	59	41	100	0.261	52	48	100	0.987	45	55	100	0.037	67	33	100	0.223
log EB	Unsatisfactory	*n*	6	5	11		2	3	5		2	2	4		2	0	2	
		%	55	45	100		40	60	100		50	50	100		100	0	100	
	Borderline	*n*	17	9	26		6	5	11		9	4	13		2	0	2	
		%	65	35	100		55	45	100		69	31	100		100	0	100	
	Satisfactory	n	8	17	25		5	4	9		3	11	14		0	2	2	
		%	32	68	100		56	44	100		21	79	100		0	100	100	
	Total	*n*	31	31	62		13	12	25		14	17	31		4	2	6	
		%	50	50	100	0.055	52	48	100	0.834	45	55	100	0.044	67	33	100	0.055
log BC	Unsatisfactory	*n*	1	0	1		1	0	1		0	0	0		0	0	0	
		%	100	0	100		100	0	100		0	0	0		0	0	0	
	Borderline	*n*	1	1	2		0	0	0		0	0	0		0	0	0	
		%	50	50	100		0	0	0		0	0	0		0	0	0	
	Satisfactory	*n*	29	30	59		12	12	24		14	17	31		4	2	6	
		%	49	51	100		50	50	100		46	54	100		67	33	100	
	Total	*n*	31	31	62		13	12	25		14	17	31		4	2	6	
		%	50	50	100	0.537	52	48	100	1.000	46	54	100	0.444	67	33	100	1.000
log STA	Unsatisfactory	*n*	1	3	4		0	2	2		1	0	1		0	1	1	
		%	25	75	100		0	100	100		100	0	100		0	100	100	
	Borderline	*n*	0	0	0		0	0	0		0	0	0		0	0	0	
		%	0	0	0		0	0	0		0	0	0		0	0	0	
	Satisfactory	*n*	30	28	58		13	10	23		13	17	30		4	1	5	
		%	52	48	100		57	43	100		43	57	100		80	20	100	
	Total	*n*	31	31	62		13	12	25		14	17	31		4	2	6	
		%	50	50	100	0.278	50	50	100	0.889	46	54	100	0.467	67	33	100	0.347

Table 6 (continued)

Classification			Total sample				Tuna				Salmon				Shrimp			
			hyper.	rest.	total	*p*	hyper.	rest.	total	*p*	hyper.	rest.	total	*p*	hyper.	rest.	total	*p*
log EC	Unsatisfactory	*n*	0	0	0		0	0	0		0	0	0		0	0	0	
		%	0	0	0		0	0	0		0	0	0		0	0	0	
	Borderline	*n*	0	1	0		0	1	1		0	0	0		0	0	0	
		%	0	100	0		0	100	100		0	0	0		0	0	0	
	Satisfactory	*n*	31	30	62		13	11	24		14	17	31		4	2	6	
		%	51	49	100		54	46	100		46	54	100		67	33	100	
	Total	*n*	31	31	62		13	12	25		14	17	31		4	2	6	
		%	50	50	100	0.440	50	50	100	1.000	46	54	100	0.325	67	33	100	0.089

The total number of samples for all tested parameters was 62; 31 being from supermarkets and 31 from restaurants. Classification of unsatisfactory, borderline and satisfactory was obtained according to Tables 3 and 4. hyper, hypermarket; rest, restaurant; AC, mesophilic aerobic microorganisms; EB, Enterobacteriaceae; STA, coagulase-positive *Staphylococci*; BC, *Bacillus cereus*; *n*, number of samples in which each parameter was found positive in the total sample in each type of commercial surface.

formed in 25 g of each of the 62 samples under study, and none of these pathogens was detected in any of the samples analysed.

The Quality of Sushi Samples Is Influenced by the Type of Establishment and the Type of Fish Used

Overall, of the 62 samples analysed, significant differences were observed between the two types of commercial establishments ($p<0.05$). Of the total samples analysed, 48.4% (30/62) were classified as having an unsatisfactory microbiological level, corresponding to 54.8% (17/31) of the samples from hypermarkets and 41.9% (13/31) of restaurant samples. As for the remaining samples, 35.5% (22/62) were classified with borderline level (corresponding to 32.3% [10/31] and 38.7% [12/31] in hypermarkets and restaurants, respectively) and only 16.1% (10/62) were classified as having a satisfactory microbiological level (corresponding to 12.9% [4/31] and 19.4% [6/31] for hypermarket and restaurant samples, respectively) (Fig. 1).

Figure 2 shows the bacterial quality of the sushi samples, by fish variety. Results show that there were significant differences ($p<0.05$) in the microbiological levels obtained by type of fish. Overall, of the 62 sushi samples analysed, 40.3% (25/62) were made with tuna (variety A; Fig. 2a), 50% (31/62) were made with salmon (variety B; Fig. 2b), and 9.7% (6/62) were made with shrimp (variety C; Fig. 2c). Concerning the 25 samples of variety A, made with tuna, 64% (16/25) were classified as unsatisfactory, 24% (6/25) were classified as borderline, and only 12% (3/25) of the samples were classified as having a satisfactory microbiological level (Fig. 2).

The microbiological evaluation of the 31 samples of variety B, made with salmon, revealed 29% (9/31) with unsatisfactory microbiological level, 48.4% (15/31) with borderline level and 22.6% (7/31) with a satisfactory microbiological level (Fig. 2). As for variety C, made with shrimp, 83.4% (5/6) were classified with an unsatisfactory microbiological level and 16.6% (1/6) with a borderline microbiological level (Fig. 2), and although the number of samples is reduced in this variety, none of the 6 samples analysed was considered to have a satisfactory microbiological level.

Discussion/Conclusion

In this study, it was our goal to evaluate the microbiological quality and safety of takeaway ready-to-eat sushi meals in the region of Lisbon, Portugal. All samples were

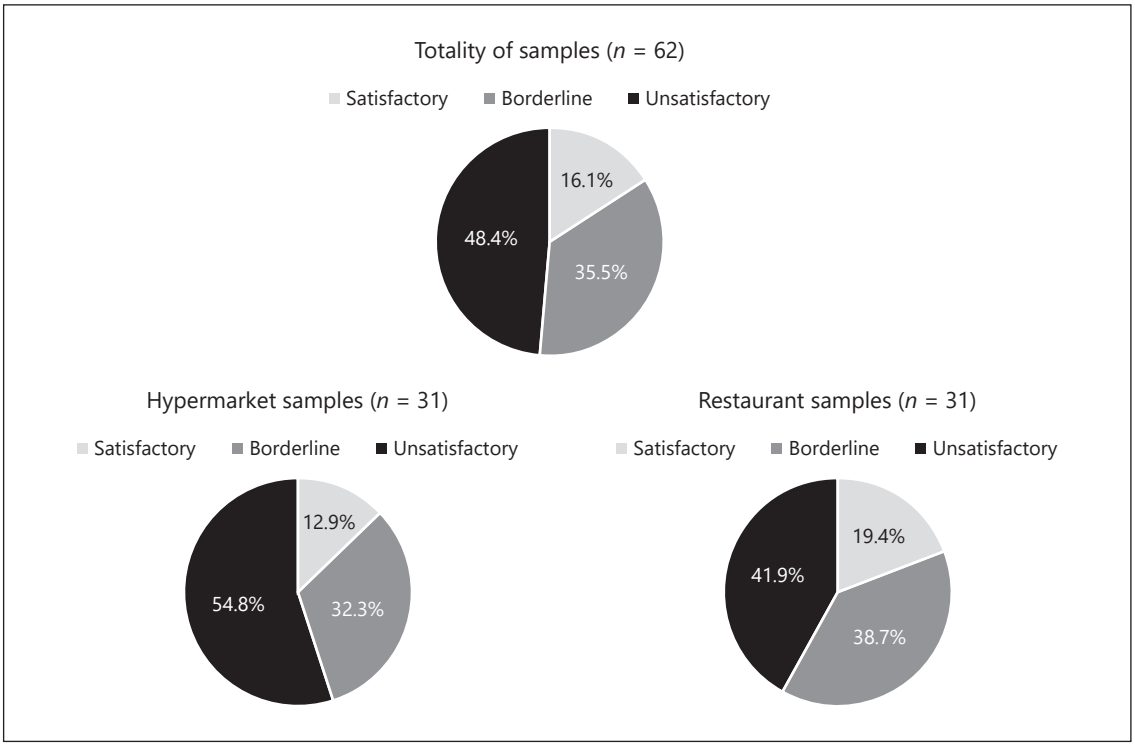

Fig. 1. Comparing the microbiological quality of total sushi samples (n = 62) analysed in different commercial areas (hypermarkets [*n* = 31] and restaurants [*n* = 31]).

tested for aerobic mesophilic microorganisms, Enterobacteriaceae, *E. coli*, coagulase-positive *Staphylococci*, presumptive *B. cereus* count, as well as for pathogenic microorganisms, such as *Salmonella* spp., *L. monocytogenes* and *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*. The samples were classified as either satisfactory, borderline or unsatisfactory according to the Portuguese guidelines [26]. Some bacteria species were not found in all samples, or any samples in some cases (see below). The average counts of aerobic mesophilic microorganisms obtained in the total of analysed samples (6.67 log cfu g^{-1}; Table 5) were higher than those obtained by other authors [6, 14, 30]. It is well known that the level of microbiological contamination of the final product is influenced by the microbiological quality of raw materials, compliance with strict personal hygiene practices, equipment, utensils, and storage of raw materials at appropriate temperatures, and high counts of aerobic mesophilic microorganisms may indicate failures during the process [31, 32]. For raw, ready-to-eat food products, such as vegetables and salads (often used in sushi preparations), expected values are between 10^6 and 10^8 cfu g^{-1}, and in raw fish between 10^6 and 10^7 cfu g^{-1} [32]. As a result, our counts are within the expected ranges.

On the other hand, the mean count of Enterobacteriaceae (4.16 log cfu g^{-1}; Table 5) was also higher than the average obtained by some authors, for example by Hoel et al. [1] or Tirloni et al. [33]; however, the average counts obtained in restaurant samples were similar to those obtained in sashimi by Miguéis et al. [15, 16] in samples from restaurants in Northern Portugal. This supports the notion that despite the recent revision of food safety regulations, the bacteriological quality in sushi products needs to be more thoroughly supervised. Nevertheless, it should be noted that many genera of Enterobacteriaceae are part of the raw vegetable and fish microbiota and, therefore, are expected to have high counts in sushi. However, Enterobacteriaceae count is used to assess the general hygiene status of a food product; its presence in food can be suggestive of environmental contamination and poor hygiene practices, such as, for example, incorrect hygiene of horticultural products [1]. In food production, Enterobacteriaceae are inactivated through the thermal processes used [32], but in sushi, the lack of thermal pro-

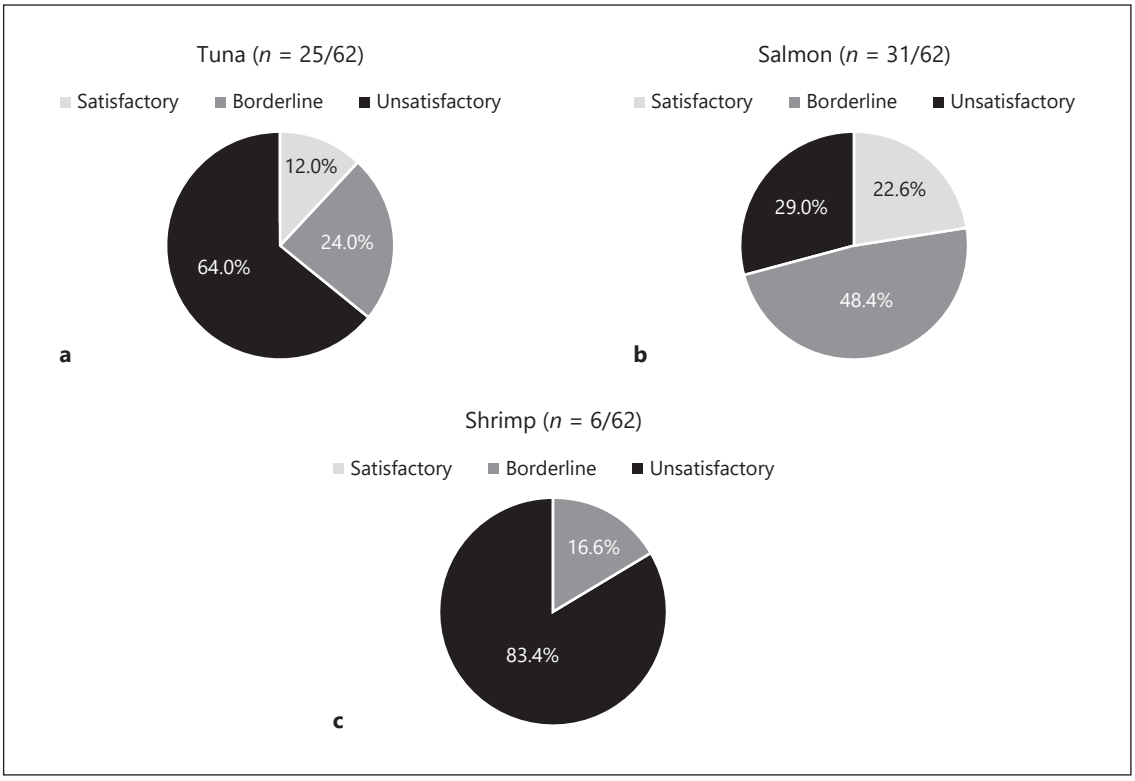

Fig. 2. Comparing the microbiological quality of sushi samples by a variety of fish (tuna, salmon and shrimp).

cesses makes it extremely important that the raw materials used are of high quality and that strict hygiene and manufacturing procedures are followed throughout all stages.

Nonetheless, regarding the numbers of aerobic mesophilic microorganisms and Enterobacteriaceae obtained in this work, it should be noted that the presence of competitive microbiota could partly contribute to reducing the number of pathogenic bacteria, which indeed were found reduced in the present work (see below). Some recent studies reflect that dirty conditions or low-grade food premises do not necessarily harm consumers because of the protective effect of indigenous microbiota, which can help reduce the growth of pathogens through antagonistic effects, including direct and indirect competition for nutrients, competition for physical attachment sites, and production of antimicrobial compounds. This is the case for Clostridiales, Flavobacteriales, Enterobacteriales and Lactobacillales, which have been reported to interact to ensure survival and impair the growth of pathogenic bacteria [34–36].

In terms of food safety, *E. coli* is the most commonly used indicator to assess the hygiene status of a product since it reflects faecal contamination and the possible presence of pathogenic microorganisms in food. In the present work, *E. coli* was detected in only one restaurant sample, hence the results obtained in the present study were quite satisfactory compared to the results obtained by other authors [13, 14]. On the other hand, in this work, coagulase-positive *Staphylococci* were detected at unsatisfactory levels, although not posing a potentially high health risk. Still, the mean coagulase-positive *Staphylococcus* count (1.44 log cfu g^{-1}) was more satisfactory when compared to the averages obtained in other published studies [6, 30]. Hoel et al. [1] analysed the ingredients used in the preparation of sushi before processing in the factory; they did not detect *S. aureus* in any of the analysed raw materials. Likewise, Basti et al. [37], when analysing freshly caught fish, also did not isolate *S. aureus*, indicating that this microorganism is not part of the microbiota of these products and, therefore, is introduced through handling and preparation.

As a rule, the presence of *B. cereus* in food indicates improper processing and failures in temperature control. Inadequate temperature control can allow spore survival and growth to unsatisfactory levels [32]. In sushi, the presence of *B. cereus* usually reflects incorrect rice acidification or cross-contamination by other foods such as vegetables and fish [38]. However, in general, the presence of *B. cereus* is not very frequent in sushi. Other studies have obtained similar results: Martins [39] detected *B. cereus* in only 1 of 8 samples acquired in a specialized sushi establishment and 2 of 12 samples acquired in non-specialized establishments with values <3 log CFU g^{-1}, while Tirloni et al. [33] did not observe any growth of *B. cereus* in most of the analysed samples, having detected it in just one sample with a value of 2.0 log CFU g^{-1}.

Moreover, for *Salmonella* spp., *L. monocytogenes* and *Vibrio* spp., all samples were considered to have a satisfactory microbiological level according to INSA Portuguese guidelines [23]. Given these results, we can consider that the samples analysed do not compromise the product's food safety.

In this study, significant differences were observed between hypermarket and restaurant samples with typical sushi restaurant samples presenting significantly ($p<0.05$) more favourable results when compared to hypermarket samples.

Interestingly, Miguéis et al. [16] also found that non-typical restaurants had the majority of cases of unacceptable/potentially hazardous sashimi samples when compared to traditional establishments, as a result of high values of pathogenic bacteria such as *L. monocytogenes* and *S. aureus*. The high percentage of samples classified with an unsatisfactory and borderline level of microbiological quality in the present study is in agreement with flaws in one or more stages of the production chain of ready-to-eat foods, corroborating the need to control these types of operations, particularly in hypermarket takeaways.

Regarding the bacterial quality of the sushi samples according to fish variety, in this work, sushi containing shrimp presented a significantly higher percentage of samples classified with an unsatisfactory microbiological level ($p<0.05$), followed by tuna and salmon ($p<0.05$). This may be because shrimp needs to be peeled and deveined, increasing the likelihood of contamination by the human manipulator or by internal contamination from the gut. In addition, as the surface of the shrimp is not smooth, it may allow the formation of niches where microorganisms can lodge. On the other hand, sushi prepared with salmon had a greater number of borderline-level samples than other fish types ($p<0.05$). Interestingly, in previous works with sashimi, no significant differences were observed in microbiota counts from different fish species [16], which suggests that it is not the type of fish per se, but the addition of raw fruits and vegetables that might increase the potential contamination of fish. We may infer that the observed differences substantiate the fact that throughout the food processing chain, more handling and more cross-contamination, coupled with failures in good hygiene and manufacturing practices by food handlers, can exponentiate the potential that the type of fish may become a risk factor. So, overall, in the case of sushi preparation, it is worth noting the importance of the type of fish as well as the type of commercial surface origin when implementing microbiological surveillance programs, and alerting consumers that the consumption of these products should be monitored, especially by specific risk groups, such as immunocompromised individuals, pregnant women, children, and the elderly, among others.

Although there are few previous studies on ready-to-eat sushi in Portugal, the present work provides a necessary and important general perception of the quality of takeaway sushi marketed in this country, showing that 48.4% of the samples analysed presented at least one microbiological parameter higher than the maximum allowed value. Even though we did not detect potentially pathogenic microorganisms in sushi, *B. cereus* and coagulase-positive *Staphylococci* were detected at unsatisfactory levels, although not posing a potentially high health risk. In conclusion, our results indicate the need to improve good practices in takeaway sushi preparation, and since the worst microbiological results were obtained in hypermarkets, this work further suggests that there is an essential need to improve food safety plans in these establishments, to obtain a final product with the desired quality level.

Overall, we believe this study could be of great value for food operators as it alerts to the urgent need to perform a risk analysis of their Food Safety Systems, such as HACCP. It is also a warning to the authorities to improve vigilance and appropriate levels of protection of the consumer, as already suggested by other previous Portuguese studies, since it shows no evolution in food hygiene levels during the past years despite authorities' efforts to establish new regulations.

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Statement of Ethics

Ethics approval was not required for this work.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.J.C. Alegria was responsible for conceiving the idea, carrying out the experiments and writing the manuscript; M.I.S. Santos corrected and edited the manuscript; R.M.S. Furtado conceived the idea and supervised the work; C.B. Correia supervised the work; A.I.G. Lima wrote the manuscript; L.R. Pedroso corrected the manuscript; S.C.D.S. Ramos supervised the work and edited the manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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