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MICROBIOLOGICAL QUALITY OF MAYONNAISE-BASED SALADS IN BRAZILIAN FOOD-SERVICE ESTABLISHMENTS: A CASE STUDY FROM MONTES CLAROS

QUALIDADE MICROBIOLÓGICA DE SALADAS À BASE DE MAIONESE EM SERVIÇOS DE ALIMENTAÇÃO: ESTUDO DE CASO EM MONTES CLAROS, BRASIL

CALIDAD MICROBIOLÓGICA DE ENSALADAS A BASE DE MAYONESA EN SERVICIOS DE ALIMENTACIÓN: ESTUDIO DE CASO EN MONTES CLAROS, BRASIL

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Samilla Arruda Oliveira[1], **Helen Rodrigues Prudêncio**[2], **Luciana Caldeira de Paula Ricardo**[3], **Theles de Oliveira Costa**[4]

ABSTRACT

Ready-to-eat salads may be exposed to microbiological contamination during preparation, handling, storage, display, and service. This study characterized total and thermotolerant coliforms in mayonnaise-based salads from self-service restaurants, using a case study conducted in six restaurants at shopping centers in Montes Claros, Minas Gerais, Brazil, in 2013. One sample from each restaurant was analyzed by the three-tube, three-dilution most probable number method. Total coliform counts ranged from <3.0 to 4.6 × 10^2 MPN/g, and thermotolerant coliform counts ranged from <3 to 1.5 × 10^2 MPN/g. Under Brazilian Resolution RDC No. 12/2001, the historical criterion applicable to mayonnaise-based salads was based on coliforms at 45°C/g. One sample (17%) exceeded the indicative maximum of 10^2 MPN/g, whereas five samples (83%) were at or below this value. The dataset does not support national prevalence estimates. Its contribution is instead to document historical microbiological measurements from a Brazilian food-service setting and interpret them alongside evidence from other Brazilian locations and the national regulatory framework. The findings reinforce the importance of Good Practices, sanitation, prevention of cross-contamination, time-temperature control, and food-handler training in ready-to-eat food service.

Keywords: Mayonnaise-based Salad. Food Microbiology. Food Safety. Coliforms. Ready-to-eat Foods. Food-service Establishments.

RESUMO

Saladas prontas para consumo podem estar expostas à contaminação microbiológica durante o preparo, manuseio, armazenamento, exposição e serviço. Este estudo

[1] Master's student in Food Engineering and Health. Federal University of Minas Gerais (UFMG). E-mail: samilaarruda16@gmail.com Orcid: https://orcid.org/0009-0004-6063-8425
Lattes: http://lattes.cnpq.br/1065548362277004
[2] Bachelor of Science in Nutrition. Ibituruna Health College (FASI).
Lattes: http://lattes.cnpq.br/1704001021111436
[3] Bachelor of Science in Nutrition. Federal University of Viçosa (UFV). E-mail: lucianaricardo@gmail.com
Orcid: https://orcid.org/0000-0002-6200-802X Lattes: http://lattes.cnpq.br/1458551859165797
[4] PhD in Engineering. Federal University of Minas Gerais (UFMG). E-mail: thelescosta@ufmg.br
Orcid: https://orcid.org/0000-0003-0773-9839 Lattes: http://lattes.cnpq.br/763495 REG9210342552708

caracterizou coliformes totais e termotolerantes em saladas à base de maionese provenientes de restaurantes self-service, utilizando um estudo de caso realizado em seis restaurantes de shopping centers de Montes Claros, Minas Gerais, Brasil, em 2013. Uma amostra de cada restaurante foi analisada pelo método do número mais provável, com três tubos e três diluições. As contagens de coliformes totais variaram de <3,0 a 4,6 × 10² NMP/g, e as de coliformes termotolerantes, de <3 a 1,5 × 10² NMP/g. Conforme a Resolução RDC nº 12/2001, o critério histórico aplicável às saladas com maionese baseava-se em coliformes a 45°C/g. Uma amostra (17%) excedeu o limite indicativo máximo de 10² NMP/g, enquanto cinco amostras (83%) apresentaram valores iguais ou inferiores. O conjunto de dados não permite estimar a prevalência nacional. Sua contribuição consiste em documentar medições microbiológicas históricas de um serviço de alimentação brasileiro e interpretá-las em conjunto com evidências de outras localidades brasileiras e com o marco regulatório nacional. Os achados reforçam a importância das Boas Práticas, saneamento, prevenção da contaminação cruzada, controle de tempo e temperatura e capacitação de manipuladores de alimentos em serviços de alimentação de alimentos prontos para consumo.

Palavras-chave: Salada à Base de Maionese. Microbiologia de Alimentos. Segurança dos Alimentos. Coliformes. Alimentos Prontos para Consumo. Serviços de Alimentação.

RESUMEN

Las ensaladas listas para el consumo pueden estar expuestas a contaminación microbiológica durante la preparación, manipulación, almacenamiento, exhibición y servicio. Este estudio caracterizó los coliformes totales y termotolerantes en ensaladas a base de mayonesa de restaurantes de autoservicio, mediante un estudio de caso realizado en seis restaurantes de centros comerciales de Montes Claros, Minas Gerais, Brasil, en 2013. Se analizó una muestra de cada restaurante mediante el método del número más probable, con tres tubos y tres diluciones. Los recuentos de coliformes totales variaron de <3,0 a 4,6 × 10² NMP/g, mientras que los coliformes termotolerantes variaron de <3 a 1,5 × 10² NMP/g. Según la Resolución brasileña RDC nº 12/2001, el criterio histórico aplicable a las ensaladas con mayonesa se basaba en coliformes a 45°C/g. Una muestra (17%) superó el límite indicativo máximo de 10² NMP/g, mientras que cinco muestras (83%) presentaron valores iguales ou inferiores. El conjunto de datos no permite estimar la prevalencia nacional. Su contribución consiste en documentar mediciones microbiológicas históricas de un servicio de alimentación brasileño e interpretarlas junto con evidencias de otras localidades brasileñas y con el marco regulatorio nacional. Los hallazgos refuerzan la importancia de las Buenas Prácticas, el saneamiento, la prevención de la contaminación cruzada, el control del tiempo y la temperatura y la capacitación de los manipuladores de alimentos en los servicios de alimentos listos para el consumo.

Palabras clave: Ensalada a Base de Mayonesa. Microbiología de Alimentos. Inocuidad Alimentaria. Coliformes. Alimentos Listos para el Consumo. Servicios de Alimentación.

1 INTRODUCTION

Food safety remains a major public health concern because foods contaminated with pathogenic or indicator microorganisms can contribute to foodborne disease and impose health, social, and economic burdens. Ready-to-eat foods are particularly relevant because they are consumed without a subsequent lethality step and may retain contamination introduced during preparation, handling, storage, transport, display, or service. International and Brazilian evidence indicates that microbiological hazards remain relevant across food systems and that prevention depends on controls applied throughout the food chain (Compaore *et al.*, 2022; Makinde *et al.*, 2020).

In Brazil, the food-service sector includes restaurants, self-service establishments, cafeterias, buffets, bakeries, institutional kitchens, and other businesses that prepare, store, transport, display, or deliver ready-to-eat foods. Brazilian Health Regulatory Agency (ANVISA) Resolution RDC No. 216/2004 establishes Good Practices for Food Services and provides a national framework for food handling, preparation, storage, distribution, transportation, exposure for sale, and delivery. This framework makes food-service hygiene a nationally relevant food-safety issue, even though microbiological performance may differ among establishments and regions (ANVISA, 2004).

Microbiological quality may vary with raw materials, preparation practices, environmental conditions, equipment, water quality, handling, temperature control, storage time, and the effectiveness of food-safety management systems. Brazilian studies from different locations have documented microbiological concerns in self-service foods, salads, and other ready-to-eat products. Alves and Ueno (2010) reported coliform contamination in foods served in self-service restaurants, while Furlanetto *et al.* (1982) detected total coliforms and Escherichia coli in mayonnaise-based salads collected from food-service establishments in São Paulo. More recent Brazilian work has continued to identify microbiological hazards in ready-to-eat and minimally processed foods, including concerns involving Salmonella spp., Listeria spp., Escherichia coli, and hygiene indicators (Sant'Anna *et al.*, 2020; Finger *et al.*, 2024).

Mayonnaise-based salads merit attention because they combine ready-to-eat ingredients with multiple handling steps. Their microbiological condition reflects the complete preparation system rather than mayonnaise alone: vegetables and cooked ingredients, utensils, food-contact surfaces, handlers, and storage conditions can all influence the final microbial load. Consequently, indicator microorganisms can provide information about

hygienic-sanitary conditions and possible post-preparation contamination, provided that their interpretation is linked to the analytical method and food category (Kamboj *et al.*, 2020; Martin *et al.*, 2016).

The specific contribution of the present study is the re-examination of a historical microbiological dataset from a Brazilian food-service setting using the regulatory criterion applicable when the samples were collected. The study also places the local findings alongside evidence from other Brazilian settings, allowing a national food-safety interpretation without converting a small local dataset into a national prevalence estimate. The objective was to characterize total and thermotolerant coliforms in mayonnaise-based salads served in self-service restaurants and to interpret the results within the Brazilian regulatory and food-safety context. The study is positioned at the intersection of food microbiology, public health, sanitary surveillance, and the organization of food-service activities, linking laboratory measurements to a nationally applicable regulatory framework and to the territorial context in which food is prepared and consumed.

2 MATERIALS AND METHODS

2.1 STUDY DESIGN AND SAMPLE COLLECTION

This was a descriptive observational case study based on quantitative microbiological data. The field component was conducted in 2013 in six self-service restaurants located in shopping centers in Montes Claros, Minas Gerais, Brazil. One mayonnaise-based salad sample was collected from each restaurant. The restaurants were selected randomly according to the source records. The sampling design was limited to the study setting and period and was not designed to estimate contamination prevalence among Brazilian food-service establishments.

After collection, the samples were placed in an insulated box containing ice and immediately transported to the a microbiology laboratory, where the analyses were performed. The source records do not specify the exact transport temperature or collection dates; therefore, no numerical temperature or date has been introduced. The source records also do not document replicate analytical runs, method blanks, positive or negative controls, or recovery experiments; these quality-assurance elements are therefore not claimed in the present report.

2.2 MICROBIOLOGICAL ANALYSIS

The analysis of total and thermotolerant coliforms was performed using the multiple-tube technique, with three series of three tubes for each dilution, according to the most probable number (MPN) approach described by Silva *et al.* (2010). Because the study endpoint was enumeration of indicator groups, no strain-level identification or isolate characterization was performed.

For total coliform analysis at 35°C, 25 g of each sample were aseptically weighed and homogenized in 225 mL of sterile 0.1% peptone water. From the 10−1, 10−2, and 10−3 dilutions, 1 mL aliquots were transferred to series of three tubes containing 9 mL of Lauryl Sulfate Tryptose (LST) broth with inverted Durham tubes. Tubes were incubated at 35°C for 48 h. Positive results were identified by microbial growth and gas production. Confirmation of total coliforms was performed by inoculation into 2% Brilliant Green bile broth, with growth and gas production after 24–48 h at 35°C considered confirmatory for total coliforms (Silva *et al.*, 2010).

For thermotolerant coliform analysis, material from LST tubes showing gas production was transferred with one loopful to tubes containing EC broth and incubated at 45°C for 24–48 h. Positive results were identified by microbial growth and gas production and used to determine MPN per gram according to the appropriate MPN reference table for the inoculated dilutions (Silva *et al.*, 2010). The study reports thermotolerant-coliform counts; no separate quantitative Escherichia coli results were reported and therefore no E. coli count has been inferred from the thermotolerant-coliform results.

Table 1

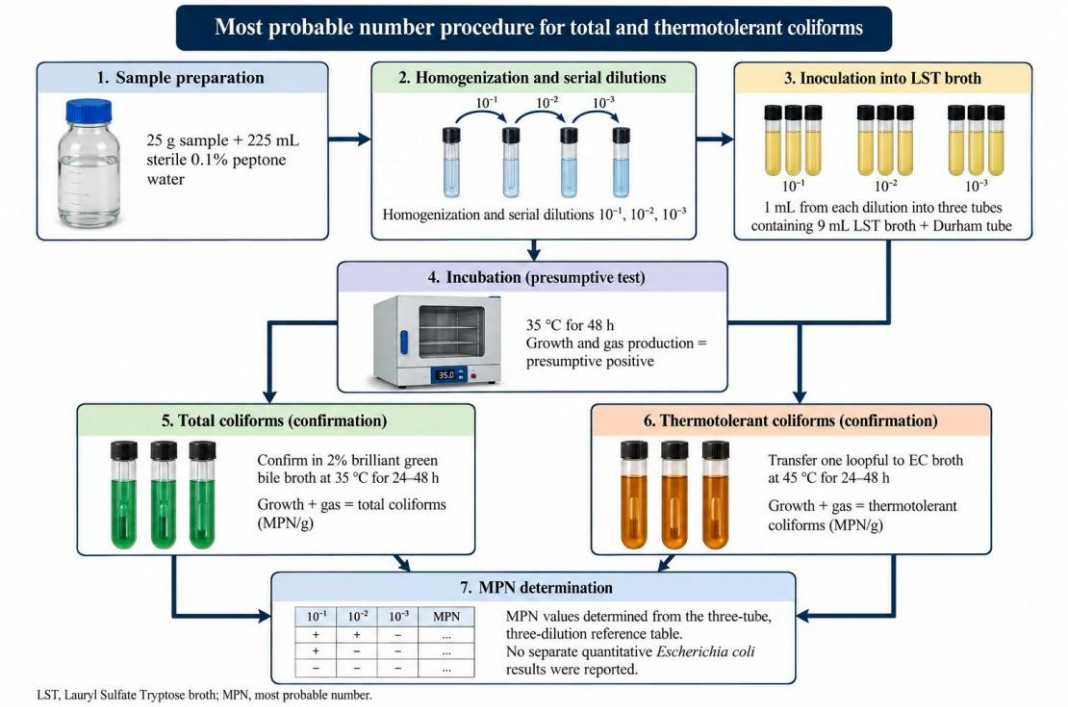
Most probable number reference values used for the three-tube, three-dilution method

Combination	MPN/g or mL	95% CI min	95% CI max	Combination	MPN/g or mL	95% CI min	95% CI max
0-0-0	<3	—	9.5	2-2-0	21	4.5	42
0-0-1	3.0	0.15	9.6	2-2-1	28	8.7	94
0-1-0	3.0	0.15	11	2-2-2	35	8.7	94
0-1-1	6.1	1.2	18	2-3-0	29	8.7	94
0-2-0	6.2	1.2	18	2-3-1	36	8.7	94
0-3-0	9.4	3.6	38	3-0-0	23	4.6	94
1-0-0	3.6	0.17	18	3-0-1	38	8.7	110
1-0-1	7.2	1.3	18	3-0-2	64	17	180
1-0-2	11	3.6	38	3-1-0	43	9	180
1-1-0	7.4	1.3	20	3-1-1	75	17	200
1-1-1	11	3.6	38	3-1-2	120	37	420
1-2-0	11	3.6	42	3-1-3	160	40	420
1-2-1	15	4.5	42	3-2-0	93	18	420
1-3-0	16	4.5	42	3-2-1	150	37	420
2-0-0	9.2	1.4	38	3-2-2	210	40	430
2-0-1	14	3.6	42	3-2-3	290	90	1.000
2-0-2	20	4.5	42	3-3-0	240	42	1.000
2-1-0	15	3.7	42	3-3-1	460	90	2.000
2-1-1	20	4.5	42	3-3-2	1,100	180	4.100
2-1-2	27	8.7	94	3-3-3	>1,100	420	—

MPN, most probable number; CI, confidence interval. The reference combinations, MPN values, and 95% confidence limits follow the standard three-tube, three-dilution table for inoculated quantities of 0.1, 0.01, and 0.001 g or mL. Reference values and confidence limits were verified against the standard table; the observed sample results were not changed.
Source: Prepared by the authors.

Figure 1

Scheme for analysis of total and thermotolerant coliforms by the most probable number method



The laboratory scheme used in the source project includes the presumptive LST step and confirmation in EC broth at 45°C. No separate quantitative Escherichia coli results were reported in the study.
Source: Prepared by the authors.

2.3 REGULATORY COMPARISON AND DATA TREATMENT

Because the field study was conducted in 2013, microbiological conformity was assessed using Brazilian Resolution RDC No. 12 of January 2, 2001, which was the applicable regulatory standard at that time. For salads added with mayonnaise sauce and similar products, RDC No. 12/2001 established a criterion for coliforms at 45°C/g of 10^2 MPN/g for an indicative sample, with n = 5, c = 2, m = 5×10^1, and M = 10^2 for a representative sampling plan (ANVISA, 2001). The historical criterion was used only for contemporaneous interpretation of the 2013 dataset.

RDC No. 12/2001 was subsequently revoked as part of the later consolidation of Brazilian food regulations. The current framework includes RDC No. 724/2022 and IN No. 161/2022. These later provisions were not applied retrospectively to reclassify the 2013 samples; they are cited only to document regulatory evolution (ANVISA, 2022).

Results were expressed as MPN/g. No inferential statistical test was applied because the study comprised six descriptive samples, one from each restaurant, and the sampling

design was not intended for population estimation. Descriptive percentages were calculated directly from the six observations.

3 RESULTS

The six mayonnaise-based salad samples yielded total-coliform counts from <3.0 to 4.6 × 10² MPN/g and thermotolerant-coliform counts from <3 to 1.5 × 10² MPN/g (Table 2). Thermotolerant coliforms were detected in five of the six samples. Sample 6 had 1.5 × 10² MPN/g and was the only sample above the historical indicative maximum of 10² MPN/g under RDC No. 12/2001. The other five samples were at or below this value. Thus, 1/6 samples (16.7%) exceeded the historical indicative maximum and 5/6 (83.3%) were at or below it.

Table 2

Most probable number of total and thermotolerant coliforms in mayonnaise-based salads sampled in self-service restaurants in Montes Claros, Brazil

Sample	Total coliforms (MPN/g)	Thermotolerant coliforms (MPN/g)
1	<3.0	2.3 × 10¹
2	7.5 × 10¹	4.3 × 10¹
3	4.3 × 10¹	<3
4	3.5 × 10¹	9.3 × 10¹
5	2.0 × 10¹	2.7 × 10¹
6	4.6 × 10²	1.5 × 10²

MPN, most probable number. Values are reproduced from the source study; scientific notation has been standardized typographically.
Source: Prepared by the authors.

Total-coliform counts ranged from <3.0 to 4.6 × 10² MPN/g. These results are presented as descriptive hygienic indicators and were not used as a legal pass/fail criterion because the historical standard applicable to this food category specified coliforms at 45°C rather than a separate total-coliform limit.

The historical regulatory parameters used for interpretation are summarized in Table 3. The representative sampling plan in RDC No. 12/2001 specified n = 5, c = 2, m = 5 × 10¹, and M = 10² for coliforms at 45°C/g. The present study, however, comprised one sample per establishment and therefore did not reproduce the representative sampling plan.

Table 3

Historical Brazilian microbiological criterion applicable to mayonnaise-based salads under Resolution RDC No. 12/2001

Food group	Microorganism	Indicative tolerance	n	c	m	M
Salads with mayonnaise sauce and similar products	Coliforms at 45°C/g	10^2	5	2	5×10^1	10^2

MPN, most probable number; n, number of sample units in the representative sampling plan; c, maximum acceptable number of units between m and M; m, lower limit; M, upper limit. For an indicative sample, the maximum tolerance was 10^2 MPN/g.
Source: Brazilian Health Regulatory Agency (ANVISA), RDC No. 12/2001.

4 DISCUSSION

The detection of thermotolerant coliforms in five of six samples indicates that indicator microorganisms were measurable in most of the sampled mayonnaise-based salads. One sample exceeded the historical indicative maximum used for the 2013 dataset. These findings should be interpreted as observations from a small case study rather than as an estimate of contamination prevalence in Brazilian food-service establishments.

The Brazilian literature provides useful context. Guerra and Miguel (2011), in an analysis of 15 cold dishes containing mayonnaise sauce, reported no occurrence of thermotolerant coliforms, whereas the present study detected these indicators. Junqueira *et al.* (2008) reported coliforms at 45°C above the maximum permitted by the legislation used in their study in 32 of 37 salad samples (83.8%). Mallet *et al.* (2017), in Volta Redonda, Rio de Janeiro, reported coliforms at 45°C and coagulase-positive Staphylococcus in all analyzed raw-salad samples. Furlanetto *et al.* (1982) reported total coliforms in 80% and Escherichia coli in 40% of 20 mayonnaise-based salads collected in São Paulo. Together, these studies show that microbiological findings in ready-to-eat salads have varied across Brazilian settings and periods.

The differences among studies should not be interpreted as direct evidence of geographic effects. Food matrices, sample sizes, sampling strategies, analytical procedures, regulatory criteria, and study periods differ substantially. The present dataset is also more limited than studies designed to estimate prevalence. Its value therefore lies in documenting a historical measurement set and in allowing comparison with other Brazilian evidence under an explicitly stated regulatory framework.

The broader food-safety relevance is consistent with evidence that ready-to-eat foods can be exposed to contamination during handling, preparation, storage, and service. Research on Brazilian minimally processed vegetables has highlighted the potential occurrence of pathogens such as Salmonella spp. and Listeria monocytogenes, while recent Brazilian studies continue to report hygiene indicators and pathogenic microorganisms in foods marketed for direct consumption (Sant'Anna *et al.*, 2020; Finger *et al.*, 2024; Paiva *et al.*, 2023). These studies are not directly comparable with the present mayonnaise-based salad dataset, but they reinforce the broader relevance of microbiological control in ready-to-eat and food-service systems. The present study did not evaluate these pathogens and therefore should not be interpreted as evidence of their absence or presence in the sampled salads.

From a food-safety management perspective, the findings reinforce the importance of Good Practices, sanitation of food-contact surfaces and utensils, hand hygiene, prevention of cross-contamination, and control of time and temperature. These measures are consistent with ANVISA Resolution RDC No. 216/2004 and with the broader literature on food-handler practices and food-safety management systems (Al-Kandari *et al.*, 2019; Maiberger & Sunmola, 2023; Young *et al.*, 2019). This interdisciplinary perspective is relevant to food-service systems because microbiological quality is shaped by interactions among biological hazards, sanitary regulation, management practices, workers, infrastructure, and the local organization of food preparation and service.

The regulatory correction is important for interpretation. The criterion applicable to the relevant food category in RDC No. 12/2001 was coliforms at 45°C/g, not a quantitative E. coli criterion. The present thermotolerant-coliform results can therefore be compared with the historical regulatory threshold without inferring E. coli results that were not reported. Later Brazilian standards were not used retrospectively.

The principal limitations are the small sample size, one sample per restaurant, the single study period in 2013, the geographic concentration in one municipality, and the absence of additional pathogen testing or documented quality-control experiments in the source records. These limitations preclude estimation of national prevalence, assessment of temporal trends, or statistical generalization to the Brazilian food-service sector. The national relevance of the study is consequently contextual rather than epidemiological: the local measurements are interpreted against a national regulatory framework and compared with evidence from other Brazilian settings.

5 CONCLUSION

Mayonnaise-based salads sampled in six self-service restaurants in Montes Claros showed detectable total and thermotolerant coliforms, and one sample exceeded the historical indicative maximum of 10^2 MPN/g for coliforms at 45°C under Brazilian RDC No. 12/2001. The study does not provide a national prevalence estimate. Its contribution is a documented historical case study that can be interpreted alongside evidence from other Brazilian settings and the national food-safety regulatory framework. The findings support continued implementation of Good Practices, sanitation, cross-contamination prevention, time-temperature control, and food-handler training in food-service establishments handling ready-to-eat foods.

6 DECLARATIONS AND EDITORIAL COMPLIANCE

6.1 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

6.2 DATA AVAILABILITY STATEMENT

The underlying microbiological results are contained in the study records and source tables used to prepare this manuscript. No public data repository or dataset DOI was identified in the source materials.

6.3 FUNDING INFORMATION

No external funding information was reported in the source documents.

6.4 ETHICS APPROVAL STATEMENT

Ethics approval was not applicable because the study involved food samples and did not involve human participants, animals, or identifiable personal data.

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