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FOOD TECHNOLOGY

Microbial contamination in industrial tofu

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ABSTRACT: *This study aimed to evaluate the microbiological quality of tofu sold in supermarkets in Porto Alegre/Brazil. Bacteria counts were performed for **Bacillus cereus**, mesophilic, coliforms and **Staphylococcus** coagulase positive and negative. The presence of **Listeria** sp. was also evaluated. Two different brands of tofu (A and B) were collected, one lot per month, for six months. Five samples from each lot were analyzed. All lots presented mesophilic aerobic counts above $4.3x10^5$CFU g^{-1}. Four of the six lots from brand A and all lots from brand B showed **E. coli** and/or **Staphylococcus** coagulase positive counts above the Brazilian law accepted limits. The **Staphylococcus** coagulase negative counts were higher than those of coagulase positive in all lots. In all lots where **Staphylococcus** coagulase positive counts were above the legal limit, there were counts of coagulase negative above 10^4CFU g^{-1}. **B. cereus** and **Listeria** sp. were not found in either brand. The majority of lots of brand A and all lots of brand B were unsuitable for human consumption. Our results showed that there are problems in tofu manufacturing in both industries analyzed. There is a need of improvement on its microbial quality to avoid problems of food-borne illness, and finally the need of a better control by the Brazilian inspection services.*
Key words: *soy cheese, mesophile, coliforms, **Staphylococcus**, coagulase.*

Contaminação microbiana em tofu industrial

RESUMO: *Este estudo teve como objetivo avaliar a qualidade microbiológica de queijo tofu comercializados em um supermercado da cidade de Porto Alegre, RS. Foram realizadas contagens de **Bacillus cereus**, bactérias mesófilas, coliformes, **Staphylococcus** coagulase positive e negativo e, a pesquisa de **Listeria** sp. Foi realizada a coleta de duas diferentes marcas (A e B), com um lote por mês, durante seis meses; sendo analisadas cinco amostras de cada lote. Todos os lotes apresentaram contagens de mesófilos aeróbios acima de $4,3x10^5$UFC g^{-1}. Quatro dos seis lotes da marca A e todos os lotes da marca B apresentaram contagens de **E. coli** e/ou **Staphylococcus** coagulase positive acima do permitido pela legislação vigente. As contagens de **Staphylococcus** coagulase negativos foram superiores às contagens dos coagulase positivos em todos os lotes. Nos lotes em que foram encontradas contagens fora do padrão estabelecido para os **Staphylococcus** coagulase positivos, havia contagens dos coagulase negativos acima de 10^4UFC g^{-1}. Não foram isolados **B. cereus** e **Listeria** sp. Ambas as marcas apresentaram sérios problemas sanitários, pois a maioria dos lotes da marca A e todos os lotes da marca B estavam impróprios para o consumo mesmo na ausência dos outros patógenos pesquisados. Nossos resultados mostram que há problemas sérios na produção de tofu em ambas as indústrias analisadas. Existe a necessidade de melhorias no sistema de produção para evitar a ocorrência de doenças causadas por este alimento e, também, que deve haver um maior controle da qualidade deste alimento pelos serviços de fiscalização.*
Palavras-chave: *queijo de soja, mesófilos, coliformes, **Staphylococcus**, coagulase.*

INTRODUCTION

Brazil is the second largest producer of soybeans in the world (EMBRAPA SOJA, 2016). The consumption of soy and its products is still low in Brazil, although the consumption of soy grains and products has been encouraged since the 1980s due to its high protein content (BEHRENS & DA SILVA, 2004; EMBRAPA, 2015). Another incentive to its consumption has been the positive correlations between the consumption of soybased foods with a reduction in the risk of developing certain diseases (FRITZ et al., 2013; LAI & YEW, 2015; TAKAGI et al., 2015; JAMILIAN & ASEMI, 2016).

Tofu, a soy based cheese, has a high water content which, combined with its high protein content, makes it susceptible to the growth of microorganisms, especially if proper manufacturing and/or storage procedures are not followed (CAI et al., 1999; PAULETTO & FOGAÇA, 2012). Microbiological quality of this product must be checked to avoid infections or food poisoning. But, few reports about the microbiological quality of tofu have been published (van KOOIJ & DE BOER, 1985; ASHENAFI, 1994; MALLET et al., 2007; DANIYAN et al., 2011; ANANCHAIPATTANA et al., 2012; ROSSI et al., 2016). Therefore the aim of this study was to evaluate the bacteriological quality of two different brands of industrialized tofu sold in a supermarket over a period of six months in 2015, at Porto Alegre city, Brazil. For this propos we searched for coliforms, mesophyles

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bacteria, ***Staphylococcus*** coagulase positive and negative, ***Bacillus cereus*** and ***Listeria*** sp.

MATERIALS AND METHODS

Collection and dilution of samples

Two traditional tofu brands (hereinafter referred to as brands A and B) were collected monthly. Every month five samples of the same lot of each brand were purchased in a supermarket. At the time of collection cooling conditions, the date of manufacture, expiry date and batch number were observed and recorded. Only samples within the legal expiry date were collected. Samples were immediately sent to the laboratory under refrigeration and analyzed within a maximum of 24 hours. Sampling and analyses were carried out over six months (November to April).

Counting of aerobic mesophilic bacteria, coliforms, ***Staphylococcus*** sp. *and* ***Bacillus cereus***

Each sample had the outermost layer of the product aseptically removed. An amount of 25g of the inner part of each tofu sample was first homogenised in 225mL of peptone saline. From this dilution subsequent dilutions (10^{-2} to 10^{-5}) were performed, all in duplicate (SILVA et al., 2007).

Counting of mesophilic aerobic bacteria

All dilutions were spread onto a plate counting agar (PCA-Kasvi) as described by SILVA et al. (2007). The counts were expressed in colony forming units per gram (CFU g^{-1}) of the product.

Coliform counting and ***Escherichia coli*** *identification*

Total coliform counting was performed with violet red bile agar (VRBA-Fluka Analytica). Confirmation of total and fecal coliforms and ***E. coli*** identification were carried out according to Silva and co-workers (2007) using brilliant green bile broth and EC broth (Himedia). Counts were expressed in CFU g^{-1} of the product.

Counting and identification of ***Staphylococcus*** *coagulase positive and negative*

All dilutions were spread in Baird Parker agar (Acumedia) supplemented with egg yolk solution and potassium tellurite according to SILVA et al. (2007). Typical and atypical colonies present were counted and expressed in CFU g^{-1} of the product. Four to five typical and atypical ***Staphylococcus*** colonies were isolated and tested by Gram staining, catalase test, glucose oxidation and fermentation, mannitol fermentation, thermonuclease production and coagulase test (MACFADDIN, 2000; SILVA et al., 2007). Isolates were identified as ***Staphylococcus*** coagulase positive (SCP) or ***Staphylococcus*** coagulase negative (SCN).

Counting of ***B. cereus***

The quantitative evaluation was performed by spreading all dilutions in cereus selective agar (Acumedia) (SILVA et al., 2007).

Detection of ***Listeria monocytogenes***

Detection was carried out according to HITCHINS (2014) consisting of the enrichment of the food in ***Listeria*** enrichment broth (Acumedia) and isolation in Oxford Agar (Acumedia) and Palcam agar (Himedia).

Statistical analysis

Results from all bacterial counts were expressed in CFU g^{-1} and analyzed using Tukey's test. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

A total of six lots of tofu from each manufacturer were collected, resulting in a total of 60 samples being analyzed. Distribution of the sampling over the different seasons was as follows: four (33%) in spring, six (50%) in summer and two (17%) in winter. The season in which the samples were obtained showed no effect on the bacterial counts that may indicate that probably no oscillation in the refrigeration temperature occurred during exposition of those cheeses on the shelves. All samples remained under refrigeration at the time of sampling. At the time of sampling, all samples had been on the shelf no more than three weeks and all were within three months of their expiry date. All samples showed the same characteristics: good–looking, white cheese with slightly yellowish liquid, sweet smell and soft texture.

Counting of mesophilic aerobic bacteria

All samples showed aerobic mesophilic bacteria above 4.3×10^5CFU g^{-1} (Tables 1 and 2). There was no significant difference between the means of the lots for the two brands. There is no specific legislation outlining the maximum allowable mesophilic bacteria counts in tofu in Brazil. But the amount of these bacteria obtained in many batches showed values around 10^6CFU g^{-1}, which is higher than the limits for many other foods. For example, the limits for mesophilic bacteria in banked breast

Table 1 - Bacterial counts of mesophilic bacteria, coliforms, coagulase positive and negative ***Staphylococci*** in tofu brand A.

Lot	Mesophilic Bacteria	Total coliform	Fecal coliform	SCP[b]	SCN[c]
	----------Colony forming units g^{-1} [a]----------				
1	3.3×10^6 (3×10^6 - 3.6×10^6)[d]	3.5×10^4 (3.1×10^4 – 3.7×10^4)	**0.2×10^4** (3.1×10^3 – 3.6×10^3)	(3.1×10^3 x 3.5×10^3) (3.1×10^3 x 3.5×10^3)	(5×10^3 - 5.5×10^3) (5×10^3 - 5.5×10^3)
2	4.3×10^5 (4×10^5 - 4.8×10^5)	3.2×10^1 (2.9×10^1 - 3.5×10^1)	NF[5]	3.4×10^3 (3.1×10^3 - 3.7×10^3)	6.2×10^3 (5.9×10^3 - 6.4×10^3)
3	4.5×10^5 (4.4×10^5 - 4.7×10^5)	3.7×10^1 (3.5×10^1 - 3.9×10^1)	NF	5.7×10^1 (5.5×10^1 - 5.9×10^1)	7.2×10^1 (6.9×10^1 - 7.3×10^1)
4	7.2×10^6 (7×10^6 - 7.6×10^6)	8.3×10^5 (8.1×10^5 - 8.5×10^5)	**6×10^5** (5.8×10^5 - 6.2×10^5)	**5.5×10^4** (5.4×10^4 - 5.7×10^4)	6.1×10^4 (6×10^4 - 6.3×10^4)
5	9.1×10^6 (9×10^6 - 9.3×10^6)	3.5×10^1 (3.4×10^1 - 3.7×10^1	NF	**5.2×10^4** (4.9×10^4 - 5.3×10^4)	6.8×10^4 (6.6×10^4 – 6.9×10^4)
6	7.2×10^6 (7×10^6 – 7.5×10^6)	7.9×10^5 (7.7×10^5 – 8×10^5)	**5.6×10^5** (5.4×10^5 – 5.8×10^5)	NF	3.4×10^1 (3.2×10^1 - 3.6×10^1)

a: Mean of five samples per lot. *b*: CPS: coagulase positive ***Staphylococci***. *c*: CNS: coagulase negative ***Staphylococci***. *d*: In brackets: minimal and maximal values. Bold type indicates lots that exceed the limits of Brazilian legislation to tofu. NF: not found.

milk is 10^2CFU mL^{-1}, and for pasteurized milk drinks is $1.5x10^5$CFU mL^{-1} (BRAZIL, 2001). Also, the values reported in our study are higher than those recommended by "The Soy Food Association of America" for soy milk, where counts of mesophilic bacteria should not exceed $2x10^4$CFU mL^{-1} (TSAA, 1996). Our results are in agreement with van KOOIJ & DE BOER (1985) and ASHENAFI (1994) who also reported high scores of mesophilic bacteria in tofu. ASHENAFI (1994) demonstrated a correlation between the high bacterial counts in tofu and the handling throughout its production. Working with tofu produced in a small industrial scale, ROSSI et al. (2016) reported mesophilic bacteria in all samples analyzed of fresh tofu, and observed that this counts were one to three log higher than those observed with soybean samples obtained in the same industry. This observation showed that along the process of tofu manufacturing, conditions are being created (addition of water, grinding, coagulation, molding, etc) that favour a naturally enhancement of the microbial load. Fails in the process certainly will favour the development of unsuitable microorganisms also.

*Coliform counting and **E. coli** identification*

Tables 1 and 2 show the averages of fecal and total coliforms, and ***E. coli*** identification in all lots tested. Of the six lots analyzed for brand A, all presented total and fecal coliforms and three lots showed fecal coliform counts above the legal limit of 10^2CFU g^{-1}. All samples of brand B showed total and fecal coliform levels above the established limits (BRAZIL, 2003). The presence of ***E. coli*** was confirmed in the three (50%) lots of brand A and in the six lots (100%) of brand B. These results demonstrated that there were failures in the production of tofu. ANANCHAIPATTANA et al. (2012) also reported coliforms and ***E. coli*** in 67% and 28%; respectively, in tofu samples, and highlighted the need of the improvement of hygienic practices during tofu production. ***E. coli,*** besides having toxigenic strains (not specifically searched in this research), is an indicator of possible contamination by other harmful microorganisms as ***Salmonella*** **sp.** and ***Campylobacter*** sp. (ANANCHAIPATTANA et al., 2012).

*Counting and identification of **Staphylococcus** coagulase positive and negative*

Two lots of brand A and four lots of brand B showed levels of SCP above the official limits of $5.3x10^3$CFU g^{-1} (Tables 1 and 2). These results show, once again, the likely inadequate hygiene in the production process of those cheeses or in the cooling conditions during storage. Contamination with SCP is a significant indicator of improper handling of the product during manufacturing. ***Staphylococcus*** sp. can be found in the microbiota of the handlers and in the absence of good hygiene practices may be transferred to food (SOARES et al., 2012). SCP counts as high as $5.5x10^4$CFU g^{-1} were observed, which are similar to the levels demonstrated by van KOOIJ & De BOER (1985) in Nigeria and by MALLET et al. (2007) in the city of Lavras, Brazil. The presence of SCP was also confirmed in tofu by DANIYAN et al. (2011).

SCN counts ranged from $3.4x10^1$ to $6.8x10^4$CFU g^{-1} in brand A and from $3.7x10^1$ to $6.3x10^4$CFU g^{-1} in brand B. Levels of SCN were higher than those of SCP in all samples. In all lots where SCP

Table 2 - Bacterial counts of mesophilic bacteria, coliforms, coagulase positive and negative ***Staphylococci*** in tofu brand B.

	----------Colony forming units g^{-1} [a]----------				
Lot	Mesophilic Bacteria	Total coliform	Fecal coliform	SCP[b]	SCN[c]
1	5.3×10^6 (5×10^6 - 5.7×10^6)[d]	6.2×10^5 (5.9×10^5 - 6.5×10^5)	**4.4×10^5** (4×10^5 - 4.7×10^5)	**4.5×10^4** (4.2×10^4 - 4.8×10^4)	6.3×10^4 (6×10^4 - 6.6×10^4)
2	6.3×10^5 (6×10^5 - 6.6×10^5)	7.1×10^4 (6.7×10^4 - 7.3×10^4)	**4.2×10^4** (3.9×10^4 - 4.4×10^4)	**3.6×10^3** (3.3×10^3 - 3.9×10^3)	4.1×10^3 (3.9×10^3 - 4.3×10^3)
3	4.8×10^6 (4.5×10^6 - 5.2×10^6)	7.3×10^5 (7×10^5 - 7.5×10^5)	**6.5×10^5** (6.3×10^5 x 6.7×10^5)	**4.1×10^4** (3.8×10^4 - 4.3×10^4)	5.1×10^4 (4.9×10^4 - 5.3×10^4)
4	5.3×10^6 (5.1×10^6 - 5.5×10^6)	5.7×10^5 (5.3×10^5 - 5.8×10^5)	**3.3×10^5** (3.1×10^5 - 3.5×10^5)	**4×10^4** (3.8×10^4 - 4.2×10^4)	4.7×10^4 (4.5×10^4 - 4.9×10^4)
5	7.4×10^6 (7.2×10^6 - 7.8×10^6)	8.2×10^5 (7.9×10^5 - 8.4×10^5)	**3.7×10^5** (3.5×10^5 - 3.8×10^5)	NF	3.7×10^1 (3.5×10^1 - 3.8×10^1)
6	8.2×10^6 (8×10^6 - 8.4×10^6)	7.9×10^5 (7.7×10^5 - 8.2×10^5)	**3.7×10^5** (3.5×10^5 - 3.9×10^5)	**3.5×10^4** (3.3×10^4 - 3.7×10^4)	3.9×10^4 (3.8×10^4 - 4.1×10^4)

a: Mean of five samples per lot. *b*: CPS: coagulase positive ***Staphylococci***. *c*: CNS: coagulase negative ***Staphylococci***. *d*: In brackets: minimal and maximal values. Bold type indicates lots that exceed the limits of Brazilian legislation to tofu. NF: not found.

counts were above the legal limit, there were counts of SCN above 10^4CFU g^{-1}. In a comparison between packaged and unpackaged tofu ANANCHAIPATTANA et al. (2012) showed a high contamination rate, of both kinds of samples, contaminated with SCN, but no bacterial counts were discriminated. FLEMING et al. (2010), studying bovine cheese, reported higher SCN counts in 50% of those samples where counts of SCP were also above the limits of legislation. The SCN may also hold enterotoxin genes and many reports of food poisoning from SCN through ingestion of raw cow's milk have been made (CARMO et al., 2002; CUNHA et al., 2006; PODKOWIK et al., 2013; MELLO et al., 2014). Despite the possible ability of SCN to produce enterotoxins, most reports of staphylococcal poisoning have been predominantly related to the presence of SCP. However, future studies should examine a possible role of SCN in staphylococcal food poisoning.

*Counting of **B. cereus** and Detection of **Listeria monocytogenes***: ***B. cereus*** and ***Listeria*** sp. were not isolated in any of the tofu samples analyzed. Both bacteria are ubiquitously distributed, and can be present even in the food-processing environments (RYSER & MARTH, 2007; LOGAN, & De VOS, 2009). The search of these bacteria are important because they may cause food borne illness. Presence of ***L. monocytogenes*** may cause death in immunologically deficient persons. Unexpectedly there are no obligation to search ***Listeria*** sp. in tofu, despite their higher nutritive and water contends, conditions that favour bacterial growth, ***Listeria*** sp. particularly, by its ability of growing at cold temperatures (BRAZIL, 2001). But, the presence of some microorganisms in food at high counts is able to inhibit the growth of other microorganisms. For example, the presence of ***E. coli*** in high counts inhibits the growth of ***L. monocytogenes*** in food (DAILEY et al., 2014). In our study, the presence of high counts of mesophilic aerobic bacteria, coliforms and ***Staphylococcus*** sp. may have been an obstacle to the growth of ***B. cereus*** and ***Listeria*** sp. Other authors have been describing ***B. cereus*** and ***L. monocytogenes*** presence in tofu and recalls of contaminated tofu have been published (SCHAEFER, 2007; ANANCHAIPATTANA et al., 2012; UFDA, 2015).

CONCLUSION

Based on the levels of coliforms and SCP presented in the analyzed tofu, four of the six lots of brand A and all six lots of brand B were unsuitable for human consumption because of the high counts of these microorganisms. Contamination of fecal coliforms and SCP were most likely due to the absence of good manufacturing practices during the production of these cheeses, taking into account that these microorganisms are natural human microbiota. The high counts of mesophilic bacteria and SCN are also the reflection of the lack of hygiene in the process. Concluding, it seems that there are fails in the control of the good practices in the industries and that, this food, is not receiving attention by the Brazilian inspection services.

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