

ACT HEALTH PROTECTION SERVICE

**MICROBIOLOGICAL
QUALITY OF
TOFU**



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BACKGROUND/OBJECTIVE

Tofu has become a popular food product that now has its own section in most ACT supermarkets and Asian grocers. A study in the U.S. (Ashraf, HR et al 1999) has found that most tofu was displayed at unsafe temperatures (>5 degrees C) and one in eight contained Coliform bacteria. There is little known microbiological data about tofu in the ACT and whether it is stored appropriately in supermarkets and Asian grocers. The tofu food survey is intended to provide information on the microbiological status of this food group in the ACT. These foods are defined for this survey as tofu that is prepared to be eaten after purchase (such as added to salads) or may have further processing (ie cooking etc). The tofu survey was under taken for the following reasons:

- determine the bacteriological status of tofu products available in the ACT
- obtain data regarding the storage conditions of tofu in supermarkets
- determine the compliance of these products to Food Standards Australia New Zealand (FSANZ) Guidelines for the Microbiological Examination of Ready-to-Eat Foods 2001
- compliment Health Protection Service (HPS) audits of high-risk food producing/handling establishments.

STANDARDS

The FSANZ Ready-to-Eat (RTE) Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guideline value. This table not only reflects both the high level of microbiological quality that is achievable for RTE foods in Australia and New Zealand but also indicates the level of contamination that is considered to be a significant risk to public health.

Table 1¹

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count (SPC)				
Level 1*	<10 ⁴	<10 ⁵	≥10 ⁵	
Level 2*	<10 ⁶	<10 ⁷	≥10 ⁷	
Level 3*	N/A	N/A	N/A	
Indicators				
<i>Escherichia coli</i>	<3	3-100	>100	**
Pathogens				
Coagulase positive staphylococci	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴ SET +ve
<i>Bacillus cereus</i>	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
Salmonella spp.	not detected in 25g			detected
<i>Listeria monocytogenes</i>	not detected in 25g	detected but <10 ² #		≥10 ² ##

NOTE:

*see below "Standard Plate Counts" for definition of level.

** Pathogenic strains of *E. coli* should be absent.

Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.

The detection of *L. monocytogenes* in ready-to-eat-foods prepared specifically for "at risk" population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous.

SET +ve: Staphylococcus enterotoxin positive.

N/A – SPC testing not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls).

Level 1 – applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process/preparation of the final food product and, as such, microbial counts should be low.

Level 2 – applies to ready-to-eat foods which contain some components which have been cooked and then further handled (stored, sliced or mixed) prior to preparation of the final food or where no cooking process has been used.

Level 3 – SPC not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls). It would be expected that these foods would have an inherent high SPC because of the normal microbial flora present.

Note: An examination of the microbiological quality of a food should not be based on SPC alone. The significance of high (unsatisfactory) SPC cannot truly be made without identifying the predominant microorganisms or other microbiological testing.

SURVEY

The survey was conducted between 2 April and 30 June 2012. During this period 52 samples from 12 ACT retail outlets were collected randomly by HPS Officers and processed by the HPS Laboratory. The samples were collected in such a manner as to cover a wide range of the available tofu products including plain and marinated tofu. All of the samples were tested for the hygiene indicators SPC, *Escherichia coli* (*E.coli*) and the food pathogens *Salmonella* spp. and *Listeria monocytogenes*. The survey collected multiple samples from single outlets and in general outlets were only tested once.

Where the HPS identifies non-compliance issues in food businesses, corrective actions are addressed through a graduated and proportionate response. Unsatisfactory results, excluding those for SPC are resampled. Marginal results may be resampled; this is dependent on resources as these foods are still considered compliant. Unsatisfactory SPC results are not resampled unless pathogens are also isolated.

MICROBIOLOGICAL METHOD OF ANALYSIS

Samples were tested for the presence of:

- *Salmonella* species using AS 5013 (modified);
- SPC using AS 5013.5
- *Bacillus cereus* using AS 5013
- *Escherichia coli* using AS 5013
- *Listeria monocytogenes* using AS 5013 (modified).

The sample preparation for SPC, *E. coli*, coagulase positive *Staphylococci* and *Bacillus cereus* consisted of 25g of sample being homogenised with 225mL of 0.1% peptone diluent; with subsequent serial dilutions prepared for enumeration.

***B. cereus* enumeration:** Spread plates (using a 100µl of each dilution) on a solid selective medium containing egg yolk and mannitol. Typical large, pink colonies, with or without lecithinase action were counted and a proportion of the colonies confirmed by a haemolysis test and spore staining. *B. cereus* cells are rods 4-5 µm long and 1-1.5 µm wide and stain red. The cells contain black-stained lipid globules. The spores stain green, are ellipsoidal in shape, central to sub central in position, and do not swell the sporangium.

***E.coli* enumeration:** Pour plates of 1ml of 10⁻¹ dilution were prepared in triplicate on TBX medium and incubated at 37°C/4 h followed by 44°C/20 h. Confirmed *E. coli* colonies appear blue/green after incubation.

***Salmonella* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL buffered peptone water (non-selective enrichment) and incubated at 37°C/16-20h. Aliquots were then transferred into Brain Heart Infusion broth (BHI) and incubated for 4h. DNA was extracted from 200uL of enriched BHI. This was screened for the presence of *Salmonella* using a BAX System *Salmonella* PCR Assay. No confirmation testing was performed as there were no samples that screened positive.

SPC: Pour plates (using a 100ml of each dilution) of plate count agar where incubated at 30 °C/72h. Plates from the dilution on which there are greater 15 and less than 300 colonies visible were counted. Counts outside this range were considered estimate counts only.

***Listeria monocytogenes* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL Half Fraser broth (selective enrichment) and incubated at 30°C/24h. Aliquots were then transferred into a single tube of Fraser broth incubated for 37°C/48h and MOPS BLEB broth incubated for 37°C/24h. DNA was extracted from 200uL of enriched MOPS BLEB broth. This was screened for the presence of *Listeria monocytogenes* using a BAX System *Listeria monocytogenes* PCR Assay. No confirmation testing was performed as there were no samples that screened positive.

RESULTS / DISCUSSION

SPC

All 52 samples were tested for SPC. The results for the samples ranged between <50 and 260,000,000 colony forming units per gram (cfu/g). All 52 samples were assessed as applying to Level 2 with satisfactory counts <10⁶. A total of 34 (65.4%) of the food samples met satisfactory levels while 5 (9.6%) were marginal. A total of 13 (25%) samples were in the unsatisfactory category. Unsatisfactory SPC results were not re-sampled due to the difficulty in determining whether or not the product was fermented.

Escherichia coli

All 52 samples were tested for *E. coli*. The presence of *E. coli* in RTE foods is undesirable because it indicates that the food has possibly been prepared under poor hygienic conditions. Ideally *E. coli* should not be detected and as such a level of <3 cfu/g (the limit of the Most Probable Number test) has been set for satisfactory samples. All 52 (100%) samples had <3 cfu/g of *E. coli* and met the satisfactory criterion.

Salmonella spp.

Salmonella spp. was not detected in any of the 52 samples tested. RTE foods should be free of *Salmonella* spp. as consumption of food containing this pathogen may result in food borne illness.

Listeria monocytogenes

Listeria monocytogenes was not detected in any of the 52 samples tested. Foods in which all components have been cooked in the final food preparation should be free of *Listeria monocytogenes*. The detection of *Listeria monocytogenes* in such foods indicates the food was inadequately cooked or the food was contaminated post preparation.

Bacillus cereus

All 52 samples were tested for *B. cereus*. 48 (92.3%) were considered satisfactory having less than 50 cfu/g. Two samples (3.8%) reported counts of 100cfu/g each and are considered marginal. Two results have been reported as <5000 cfu/g due to the growth of high background flora. These two results have not been categorised due to the significant estimation involved. Follow-up on marginal results was not performed are still considered as compliant.

CONCLUSION

The microbiological quality of the tofu surveyed in the ACT is very good. Raw results of analysis are attached at Appendix A. There was generally a good level of compliance with

the Food Standards Australia New Zealand Guidelines for the Microbiological Examination of Ready-to-Eat Foods December 2001. However compliance to SPC counts were difficult to determine as tofu may or may not be a fermented product. The survey did encounter some difficulty in determining which tofu was fermented. During the survey it was discovered that some varieties were advertised as being able to be eaten fresh while others required further cooking or processing. Future surveys should focus on only the ready to eat variety only.

BIBLIOGRAPHY

1. Guidelines for the microbiological examination of ready-to-eat foods FSANZ Dec 2001.
2. Microbiological Survey of Tofu Sold in a Rural Illinois County, Ashraf, HR et al 1999.

Appendix A

Assessment: S = satisfactory, M = marginal, U = unsatisfactory and * = estimate count only.

Sample Description	SPC result cfu/g	Asses-ment	<i>E. coli</i> count in food cfu/g	Coag Pos Staph cfu/g	<i>B. cereus</i> result cfu/g	<i>Salmonella</i> in food P/A in 25g	<i>L. monocytogene</i> s in food P/A in 25g
Tofu Soy Bean Curd Firm	2400	S	<3	<50	<50	Absent	Absent
Tufu Nuggets (Sweet Chili)	23000000 0	U	<3	<50	<50	Absent	Absent
Nigari Hard Tofu (Stir Fry Tofu)	240000	S	<3	<50	<50	Absent	Absent
Firm Tofu	620000	S	<3	<50	<50	Absent	Absent
Soyco Tufu Malaysian Peanut Satay	40000000 *	U	<3	<50	<50	Absent	Absent
Soyco Tofu	<50	S	<3	<50	<50	Absent	Absent
Silk firm Tofu	550*	S	<3	<50	<50	Absent	Absent
Fortune press silken tofu	<50	S	<3	<50	<50	Absent	Absent
Momen Tofu	7800000*	M	<3	<50	<50	Absent	Absent
Hard Tofu	20000	S	<3	<50	<50	Absent	Absent
Five Spice hard Tofu	250*	S	<3	<50	<50	Absent	Absent
Extra Firm Tofu	68000000	U	<3	<50	<5000 *	Absent	Absent
Original Tofu	560000	S	<3	<50	<50	Absent	Absent
Hard Tofu	26000000 0	U	<3	<50	<50	Absent	Absent
Silken egg Tofu	2700000	M	<3	<50	<5000 *	Absent	Absent
Soyco Chinese Honey Soy	<50	S	<3	<50	<50	Absent	Absent
Soyco Malaysian Peanut Satay	50*	S	<3	<50	<50	Absent	Absent
Soyco Japanese	<50	S	<3	<50	<50	Absent	Absent
Soyco Thai	50*	S	<3	<50	<50	Absent	Absent
Pureland Organic	12000000	U	<3	<50	<50	Absent	Absent
Malaysian Peanut Tofu	<50	S	<3	<50	<50	Absent	Absent
Organic Tofu (Silken)	2700000	M	<3	<50	<50	Absent	Absent
Silken Japanese Tofu	3800	S	<3	<50	100*	Absent	Absent
Vegies Burger	<50	S	<3	<50	<50	Absent	Absent
Chinese Honey Soy Tofu	<50	S	<3	<50	<50	Absent	Absent
Kingland Firm Tofu	2800000	M	<3	<50	<50	Absent	Absent
Pureland Tofu	1100000	M	<3	<50	<50	Absent	Absent
Nutrisoy Tofu Orgnaic	<50*	S	<3	<50	<50	Absent	Absent
Nutrisoy Tofu Tempeh	<50	S	<3	<50	<50	Absent	Absent
Sanitarium Vegetarian Sausages	<50	S	<3	<50	<50	Absent	Absent
Dau Hu Silkun Tofu	<50	S	<3	<50	<50	Absent	Absent
Uni Green Fried Tofu Puff	14000000 *	U	<3	<50	<50	Absent	Absent
Se Aroy Fish Tofu	<50	S	<3	<50	<50	Absent	Absent

Sample Description	SPC result cfu/g	Asses-ment	<i>E. coli</i> count in food cfu/g	Coag Pos Staph cfu/g	<i>B. cereus</i> result cfu/g	<i>Salmonella</i> in food P/A in 25g	<i>L. monocytogenes</i> in food P/A in 25g
Fortune Hard Tofu	20000000 0	U	<3	<50	<50	Absent	Absent
Tofu (Pureland Organic)	33000000	U	<3	<50	<50	Absent	Absent
Pureland Organic Tofu	11000000 0	U	<3	<50	<50	Absent	Absent
Kingland Organic firm Tofu	19000000	U	<3	<50	<50	Absent	Absent
Super Soy Tofu	<50	S	<3	<50	<50	Absent	Absent
Organic Smoked Tofu	800*	S	<3	<50	<50	Absent	Absent
Silken Japanese Tofu	600*	S	<3	<50	<50	Absent	Absent
Sweet Chilli Tofu Nuggets	10000000 0	U	<3	<50	<50	Absent	Absent
Niutri Soy Silken Tofu	85000*	S	<3	<50	<50	Absent	Absent
Pureland Organic Tofu	54000000	U	<3	<50	100**	Absent	Absent
Nutri Soy Organic Tofu	250*	S	<3	<50	<50	Absent	Absent
Nutri Soy Tofu Burger	150*	S	<3	<50	<50	Absent	Absent
Soya King Hard Tofu	23000000 0	U	<3	<50	<50	Absent	Absent
Soyco Tofu Chinese	13000	S	<3	<50	<50	Absent	Absent
Soyco Tofu Japanese	360000	S	<3	<50	<50	Absent	Absent
Soyco Tofu Thai	1500*	S	<3	<50	<50	Absent	Absent
Tofutti Soy Mozzarella Slices	1100*	S	<3	<50	<50	Absent	Absent
Silken Tofu	<50	S	<3	<50	<50	Absent	Absent
Firm Tofu	100000*	S	<3	<50	<50	Absent	Absent

*indicates results that were an estimate due to analytical processes. **These have been deemed marginal results due to the large estimation present.