UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

JOYCE DE ALMEIDA CARMINATI

OCORRÊNCIA E SOBREVIVÊNCIA DE *Salmonella* EM AMENDOIM E PRODUTOS DERIVADOS DE AMENDOIM

OCORRENCE AND SURVIVAL OF *Salmonella* IN PEANUTS AND PEANUT CONFECTIONERY PRODUCTS

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Dissertação apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Mestra em Tecnologia de Alimentos.

Dissertation presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Food Technology.

Supervisor/ Orientador: Maristela da Silva do Nascimento

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RESUMO

Devido aos surtos de Salmonelose envolvendo produtos à base de amendoim, este passou a ser estudado como veículo de contaminação por *Salmonella.* O presente trabalho teve como objetivo avaliar a ocorrência e sobrevivência de *Salmonella* nesses produtos. Na primeira etapa do estudo foi avaliada a ocorrência de *Salmonella, Escherichia coli*, coliformes totais e enterobactérias totais durante a fabricação de produtos à base de amendoim. Nesta etapa foram avaliadas 30 amostras de matérias-primas (amendoim cru e torrado), 59 de produto final (drageados doce e salgado, paçoca, pé de moleque, pé de moça e doce de amendoim) e 116 de ambiente fabril (equipamentos, instalações e utensílios) coletadas em três diferentes plantas processadoras localizadas no Estado de São Paulo. Nas matérias-primas, o pH variou de 5,6 a 7,2 e a atividade de água (a_w) de 0,15 a 0,71. Os produtos processados apresentaram valores de 5,14 a 6,85 e de 0,20 a 0,77 para pH e a_w, respectivamente. Foram detectadas enterobactérias em três amostras de paçoca (1,5 a 1,6 log ufc/g), três amostras de doce de amendoim (1,3 a 1,5 log ufc/g), duas amostras de pé de moleque (1,0 a 1,3 log ufc/g) e em 82 amostras ambientais (0,0 a 7,0 log ufc/ml). Não foram isolados coliformes totais de amostras de matéria-prima e produto final. As amostras ambientais apresentaram contagens de coliformes totais entre <0 e 7,1 log ufc/ml. Seis amostras ambientais (uma de equipamento e cinco de instalações) apresentaram contaminação por *E. coli*, com contagens entre 0 e 2,3 log ufc/g. *Salmonella* foi isolada de uma amostra de ambiente (instalação) de processamento de doce (0,5%). Na segunda etapa do estudo foi avaliada a sobrevivência de *Salmonella* Typhimurium ATCC 14028 durante a estocagem de diferentes produtos de amendoim, utilizando dois níveis de inoculo. Para o estudo foram utilizados pé de moleque (a_w = 0,30), paçoca (a_w=0,42), pé de moça (a_w = 0,68), amendoim torrado (a_w= 0,39), amendoim cru (a_w = 0,54) e amendoim cru com casca (a_w = 0,29). Amostras de 500 g foram inoculadas com os dois níveis de inoculo (3,0 log ufc/g – experimento 1 e 6,0 log ufc/g – experimento 2) e armazenadas por 420 dias a 28 ± 1 °C. A sobrevivência de *Salmonella* foi influenciada pela a_w e composição do produto. No experimento 1, os maiores declínios nas contagens ocorreram nos primeiros 7 a 14 dias (exceto para a paçoca). Pé-de-moleque, Pé-de-moça, amendoim cru com pele e amendoim cru com casca apresentaram contagem

abaixo do limite de detecção (1 log ufc/ g) após 60, 21, 330 e 180 dias, respectivamente. No experimento 2, foi detectada *Salmonella* em todas as amostras após 420 dias de estocagem. A maior viabilidade do patógeno foi verificada na paçoca, com contagem final de 2,5 log ufc/g e no amendoim torrado, com 3,8 log ufc/g. Os resultados obtidos reforçam a importância do emprego de boas práticas de fabricação para garantir a segurança de produtos à base de amendoim.

Palavras-chave: *Salmonella*, amendoim, enterobactérias, segurança de alimentos, alimentos de baixa umidade, doces de amendoim.

ABSTRACT

Due to the outbreaks of salmonellosis involving products based on peanuts, this began to be studied as a contamination vehicle of *Salmonella.* The aim of this study was to evaluate the occurrence and survival of *Salmonella* in these products. In the first stage of the study, the occurrence of *Salmonella, Escherichia coli*, total coliforms and total *Enterobacteriaceae* during the manufacture of peanut confectionary products was evaluated. In this stage, 30 samples of raw material (raw peanut and roasted peanuts), 59 samples of final product (candy and crunch coasted peanuts, *paçoca*, peanut brittle, *pé de moça* and *doce de amendoim*) and 116 samples of processing environment (equipment, facilities and utensils) were collected in three different manufacturing plants located in São Paulo State. In the raw materials, pH ranged from 5.6 to 7.2 and water activity (a_w) ranged from 0.15 to 0.71. The end products had a range of values from 5.14 to 6.85 and from 0.20 to 0.77 for pH and a_w, respectively. *Enterobacteriaceae* were detected in three samples of *paçoca* (1.5 to 1.6 log cfu/g), three samples of *doce de amendoim* (1.3 to 1.5 log cfu/g), two samples of peanut brittle (1.0 to 1.3 log cfu/g) and in 82 environmental samples (0.0 to 7.0 log cfu/ml). Total coliforms were not isolated from samples of raw material and final product. Environmental samples showed total coliforms counts between <0 and 7.1 log cfu/ml. Six environmental samples also showed *E. coli* contamination (one – equipment and five – facilities), with counts between 0 and 2.3 log cfu/ ml. *Salmonella* was isolated from an environmental sample of sweet processing (0.5%). In the second stage of the study, the survival of *Salmonella* Typhimurium ATCC 14028 was evaluated during the storage of different peanut products, using two inoculum levels. The sample studied were peanut brittle (a_w = 0,30), *paçoca* (a_w = 0,42), *pé de moça* (a_w = 0,68), roasted peanuts (a_w = 0.39), unblanched peanut kernells (a_w = 0.54) and raw in-shell peanuts (a_w = 29). Samples of 500 g were inoculated with the two inoculum levels (3.0 log cfu/g - experiment 1 and 6.0 log cfu/g - experiment 2) and stored for 420 days at 28 ± 1 °C. *Salmonella* survival was influenced by water activity and product composition. In experiment 1, the greatest rate decline in counts occurred in the first 7 to 14 days (except for *paçoca*). Samples peanut brittle, *pé de moça* , unblanched peanut kernells and raw in-shell peanuts showed count below the detection limit (1 log cfu/ g) after 60, 21, 330 and 180 days, respectively. In experiment 2, *Salmonella* was

detected in all samples after 420 days of storage. The highest viability of the pathogen was verified in *paçoca*, with a final count of 2.5 log cfu/g and in roasted peanuts, with 3.8 log cfu/g. The results obtained reinforce the importance of using good manufacturing practices to ensure the safety of peanut confectionary products.

Keywords: *Salmonella*, peanuts, *Enterobacteriaceae*, food safety, low moisture food, peanut confectionary products..

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INTRODUÇÃO GERAL

O amendoim (*Arachis hypogaea L.*) é uma das principais oleaginosas cultivadas no Brasil e no mundo. É considerada, entre as leguminosas, uma das mais importantes culturas, juntamente com o feijão e a soja (EMBRAPA, 2006).

O Brasil é o terceiro maior produtor de amendoim das Américas, a produção se concentra na região Sudeste, principalmente no estado de São Paulo (maior produtor e exportador) (ABICAB, 2013; MARTINS, 2011).

A cadeia produtiva do amendoim pode ser dividida em três etapas: produção agrícola, beneficiamento e industrialização. De acordo com a ABICAB (2015) os amendoins são destinados principalmente à produção de óleo, consumo direto ou manufatura de produtos derivados como: paçoca, pé-de-moleque, manteiga e pastas.

A qualidade sanitária empregada na cadeia produtiva do amendoim é de grande importância, uma vez que a veiculação de patógenos e toxinas pode comprometer a saúde dos consumidores. A ação dos agentes microbianos pode ocorrer, praticamente, em todas as etapas da cadeia produtiva. Dentre estes agentes os fungos micotoxigênicos e as micotoxinas, em especial aflatoxina, têm sido amplamente estudados. Contudo, outros patógenos apresentam relevância do ponto de vista de saúde pública, como a *Salmonella.* Apesar dos poucos relatos encontrados na literatura, vários surtos envolvendo a contaminação de *Salmonella* em produtos de amendoim foram notificados, principalmente nos Estados Unidos pelo CDC nos anos de 2007, 2009 e 2013 (CDC 2013, 2009; 2007). Os resultados destes levantamentos apontaram baixa incidência e baixo nível de contaminação. A análise de amostras de amendoim com casca provenientes de um recolhimento do produto do mercado varejista norte-americano apontou contaminação de 38% das amostras, com concentração variando de <0,03 a 2 NMP/ g (KIRK *et al*., 2004).

Segundo PODOLAK *et al*. (2010) o amendoim possui como possíveis fontes de contaminação: matéria-prima, ingredientes, ambiente de processo e manipuladores. A capacidade de sobrevivência de *Salmonella* por longos períodos em produtos de baixa atividade de água já foi relatada em vários trabalhos (BEUCHAT *et al*., 2013; BEUCHAT e MANN, 2010; KOMITOPOULOU e PEÑALOZA, 2009; TAMMINGA *et al*., 1976) e seria um dos fatores contribuintes para a ocorrência de surtos neste tipo de produto. Uesugi *et al*. (2006) não observaram redução significativa

na população de *Salmonella* após 550 dias de estocagem de amêndoas a -20 °C e 4 ºC. Após o mesmo período a 23 ºC, reportaram taxa de redução de 0,3 log ufc/ g/ mês. Em um estudo realizado com manteiga de amendoim, Burnett *et al.* (2000), partindo de um inóculo inicial de 5,7 log ufc/g, obtiveram contagens de 1,0 e 2,0 log ufc/ g após 24 semanas de estocagem a 21 °C e 4 ºC, respectivamente. Park *et al* (2008) detectaram *Salmonella* Tennessee inoculada em manteiga de amendoim após 2 semanas de estocagem a 22 ºC.

A Comissão internacional sobre especificações microbiológicas(ICMSF) (ICMSF, 2011, 2000) considera essencial a verificação rotineira da presença de *Salmonella* na matéria-prima, no ambiente de processo e no produto final. Apesar de *Salmonella* ser o principal alvo bacteriano em uma investigação analítica, a presença de outras enterobactérias como *Escherichia coli* deve ser monitorada. Análises de *Enterobacteriaceae* totais e coliformes são consideradas ferramentas adicionais para fornecer informação sobre as condições higiênico-sanitárias de produtos e processos. Esses micro-organismos são amplamente utilizados na indústria de alimentos como indicadores de higiene, podendo ser monitorados em paralelo para verificação das condições gerais de eficácia de processo e programas de controle de qualidade. Entretanto, não podem substituir o monitoramento direto de *Salmonella*, uma vez que baixos níveis de *Enterobacteriaceae* totais ou coliformes não asseguram a ausência do patógeno (CORDIER, 2008).

Beuchat *et al*. (2013) afirmam que há a necessidade de conhecimento detalhado das possíveis fontes e rotas de contaminação de *Salmonella* em produtos de baixa atividade de água. Só assim poderão ser estabelecidas medidas de controle suficientemente robustas para gerenciar riscos. Os dados existentes a respeito de *Salmonella* em amendoim são escassos e predominantemente relacionados à investigação de surtos. Portanto, são necessários estudos mais detalhados sobre as possíveis fontes de contaminação e sobrevivência de *Salmonella* em produtos à base de amendoim (BEUCHAT *et al*., 2013).

REFERÊNCIAS BIBLIOGRÁFICAS

ABICAB - Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Amendoim**. 2013. Disponível em: <http://www.abicab.org.br/associados-3/>. Acesso em: 3 ago. 2015.

ABICAB- Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Cultivo de amendoim**. 2015. Disponível em: < http://www.abicab.org.br/cultivo-de-amendoim/ >. Acesso em: 13 out. 2015.

BEUCHAT, L. R.; MANN, D. A. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. **Journal of Food Protection,** v. 73, p.1257-1268, 2010.

BEUCHAT, L. R.; KOMITOPOULOU, E.; BECKERS, H.; BETTS, R. P., BOURDICHON, F.; FANNING, S.; JOOSTEN, H. M.; TER KUILE, B. H. Low-water activity foods: increased concern as vehicles of foodborne pathogens. **Journal of Food Protection**, v. 76, p. 150-172, 2013.

BURNETT, S. L.; GEHM, E. R.; WEISSINGER, W. R.; BEUCHAT, L. R. Survival of *Salmonella* in peanut butter and peanut butter spread. **Journal of Applied Microbiology**, v. 89, p. 472-477, 2000.

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006-2007. **MMWR Morbidity and Mortality Weekly Report,** v. 56, p. 521-524, 2007.

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. **MMWR Morbidity and Mortality Weekly Report**, v. 58, p. 85-90, 2009.

CDC - Center For Disease Control and Prevention. **Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland**. 2013**.** Disponível em: <http://www.cdc.gov/salmonella/bredeney-09-12/index.html>. Acesso em: 3 ago. 2015.

CORDIER, J. L. Production of powdered infant formula and microbiological control measures. In: FABER, J.M.; FORSYTHE, S. ***Enterobacter sakazakii***.. Washington, DC: American Society for Microbiology Press. p. 145-185. 2008.

EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária. **Cultivo do amendoim**. 2006. Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Amendoim/CultivodoAmendoim/>. Acesso em: 7 ago. 2015.

ICMSF - International Commission on Microbiological Specifications for Foods.Nuts, oilseeds and dried legumes. In: Microorganisms in Foods 6: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 356–378. 2000.

ICMSF - International Commission on Microbiological Specifications for Foods. Cocoa, Chocolate and Confectionery. In: Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 241–246. 2011.

KIRK, M. D.; LITTLE, C. L.; LEM, M.; FYFE, M.; GENOBILE, D.; TAN, A.; THRELFALL, J.; PACCAGNELLA, A.; LIGHTFOOT, D.; LYI, H.; MCINTYRE, L.; WARD, L.; BROWN, D.J.; SURNAM, S.; FISHER, I.S.T. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – Sharing moelcular information to solve internacional outbreak. **Epidemiology and Infection**, v. 132, p. 571-577, 2004.

KOMITOPOULOU, E.; PEÑALOZA, W. Fate of *Salmonella* in dry confectionery raw materials. **Journal of Applied Microbiology**, v. 106, p. 1892-1900, 2009.

MARTINS, R. Amendoim: produção, exportação e a safra 2011/2012**. Análises e Indicadores do Agronegócio – Instituto de Economia Agrícola**, v. 6, n. 11, 2011.

PARK, E. J.; OH, S. W.; KANG, D. H. Fate of *Salmonella* Tennessee in Peanut Butter at 4 and 22 ºC. **Journal of Food Science**, v. 73, n. 2, p. 82-86, 2008.

PODOLAK, R.; ENACHE, H.; STONE, W.; BLACK, D. G.; ELLIOT, P. Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low – Moisture Foods. **Journal of Food Protection,** v. 73, p. 1919-1936. 2010.

TAMMINGA, S. K.; BEUMER, R. R.; KAMPELMACHER, E. H.; VAN LEUSDEN, F. M. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. **Journal of Hygine,** v.76, n. 1, p. 41-47. 1976.

UESUGI, A. R.; DANYLUK, M. D.; HARRIS, L. J. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23 and 35 ºC. **Journal of Food Protection**, v**.** 69, p.1851-1857. 2006.

OBJETIVOS

Objetivo Geral

Este trabalho teve como objetivo avaliar a ocorrência e sobrevivência de *Salmonella* nos produtos à base de amendoim.

Objetivos específicos

- Avaliar a ocorrência de *Salmonella* e outras enterobactérias em indústrias processadoras de amendoim;
- Avaliar a sobrevivência de *S.* Typhimurium ATCC 14028 durante a estocagem de produtos à base de amendoim e amendoins com diferentes atividades de água e níveis de inoculo.

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

1. Amendoim

O amendoim (*Arachis Hypogaea* L.) é originário da América do Sul. Trata-se de uma planta herbácea, da família *Leguminosae*. A espécie é típica de climas quentes, adaptando-se a uma larga faixa climática, com exceção de regiões com alta umidade e/ou temperaturas mínimas inferiores a 15 °C. Na década de 70, o Brasil chegou a produzir 1 milhão de toneladas por ano, no entanto, a produção foi reduzida, devido a substituição do amendoim pela soja (ABICAB, 2016; EMBRAPA, 2009).

Os maiores produtores mundiais de amendoim são a China, Índia e EUA. (USDA, 2016).

O Brasil é o terceiro maior produtor das Américas, ficando atrás dos EUA e Argentina. O consumo médio per capita brasileiro é de 0,8 kg/habitante (ABICAB, 2013). A região Sudeste é responsável por 94,8 % da produção nacional, com 388,8 mil toneladas, destas 380,8 mil toneladas foram produzidas no Estado de São Paulo (CONAB, 2016). No Nordeste, o amendoim é cultivado basicamente por pequenos e médios produtores, utilizando poucos recursos tecnológicos e o consumo é local (EMBRAPA, 2006). A produção de amendoim é caracterizada por duas safras anuais, uma no verão denominada primeira safra, de maior produção e compreendendo os meses de janeiro a março, e a outra denominada segunda safra de menor produção ocorre no inverno entre maio e agosto, esta última de maior relevância no Nordeste (CONAB, 2016).

Os cultivares mais produzidos no Brasil, principalmente no Estado de São Paulo são o IAC Tatu ST, tipo Valência de porte pequeno e película vermelha, comercializado principalmente em casca ou descascado crus. E também o Runner IAC 886, de grande porte e película castanho claro, com sementes de tamanho médio destacando-se pelo alto teor lipídico (ABICAB, 2015).

De acordo com a Conab (2016) a área cultivada no Brasil na safra de 2015/2016 foi de 106,6 mil ha, com produtividade de 3312,5 kg/ ha.

A produtividade agrícola de leguminosas como amendoim, soja e feijão têm se destacado no mercado do Brasil e do mundo, tornando-se os principais commodities comercializados neste seguimento. Possui grande atrativo como alimento e excelentes propriedades nutricionais. Suas sementes possuem valores satisfatórios de vitaminas (principalmente E, Complexo B e ácido fólico) e minerais

(cálcio, fósforo, potássio e zinco), além de serem ricas em óleo (aproximadamente 50 %) e proteína (22 a 30 %) (EMBRAPA, 2006). Por apresentar um sabor agradável é consumido tanto *in natura* quanto processado, como aperitivos salgados, torrados, doces ou substituindo a castanha de caju em cobertura de sorvetes (MACÊDO, 2007).

A cadeia produtiva do amendoim abrange três grandes etapas: produção agrícola, beneficiamento e industrialização. Os processos de colheita e pós-colheita compreendem as operações de arranquio, enleiramento, secagem, despencamento das vagens, pré-limpeza e ensacamento (Figura 1). Estas etapas podem ser executadas manualmente ou mecanicamente. A colheita das sementes vai depender do tipo de cultivar. Após o arranquio, as plantas são levantadas e sacudidas para retirada do excesso de terra aderida as vagens. Em seguida as mesmas são enfileiradas (enleiradas) e deixadas ao sol para secagem natural por 3 a 10 dias, de acordo com as condições climáticas locais (EPA, 1995). O processo de secagem a campo deve reduzir a umidade inicial das vagens de 35-40 % para aproximadamente 10 %. (EMBRAPA, 2006). Com a finalização da secagem ocorre o despencamento, ou seja, desprendimento das vagens das raízes da planta. Nas indústrias beneficiadoras o amendoim com casca pode ser comercializado após as etapas de pré-limpeza e secagem artificial, esta consiste em submeter os grãos previamente a umidade entre 16 – 20 % em secadores com parâmetros controlados por 16 – 18 h, sendo 45 % de umidade e o limite de temperatura de 35 -38 °C, esta etapa tem por objetivo a redução da umidade final dos grãos para 8 % (BRASIL, 2009). De acordo com a legislação brasileira vigente, ao chegar na indústria beneficiadora o limite de umidade do amendoim cru descascado deve ser ≤ 8 %, enquanto que para o amendoim cru com casca este índice seria ≤ 11 % (BRASIL, 2003). Para a comercialização dos grãos são realizadas as operações de descascamento, seleção/classificação, torração, blancheamento, nova seleção e envase (Figura 1). O amendoim descascado pode ser comercializado cru, torrado e/ou blancheado, sendo destinado ao comércio varejista, à extração de óleo ou à indústria processadora. A torração é responsável pelo desenvolvimento do sabor característico do produto e pela redução da carga microbiana. O binômio tempo × temperatura utilizado varia de acordo com as características finais desejadas e o tipo de técnica utilizada. A torração a seco em estufas ou fornos emprega temperaturas ao redor de 160 °C por 25 a 60 min, já a torração por imersão em óleo utiliza temperatura entre 138 e 140 ºC por 3 a

10 min (WOODROOF, 1983). O blancheamento, etapa de retirada da película que recobre o grão, consiste na expansão do grão em temperatura a 100 °C para remoção da película. Posteriormente, os grãos são resfriados para voltar a forma original. Esta etapa pode ser realizada em associação a torração ou em etapa posterior por processo a seco (utilizando jatos de ar ou rotação em tambor) ou eventualmente por processo úmido (EPA, 1995).

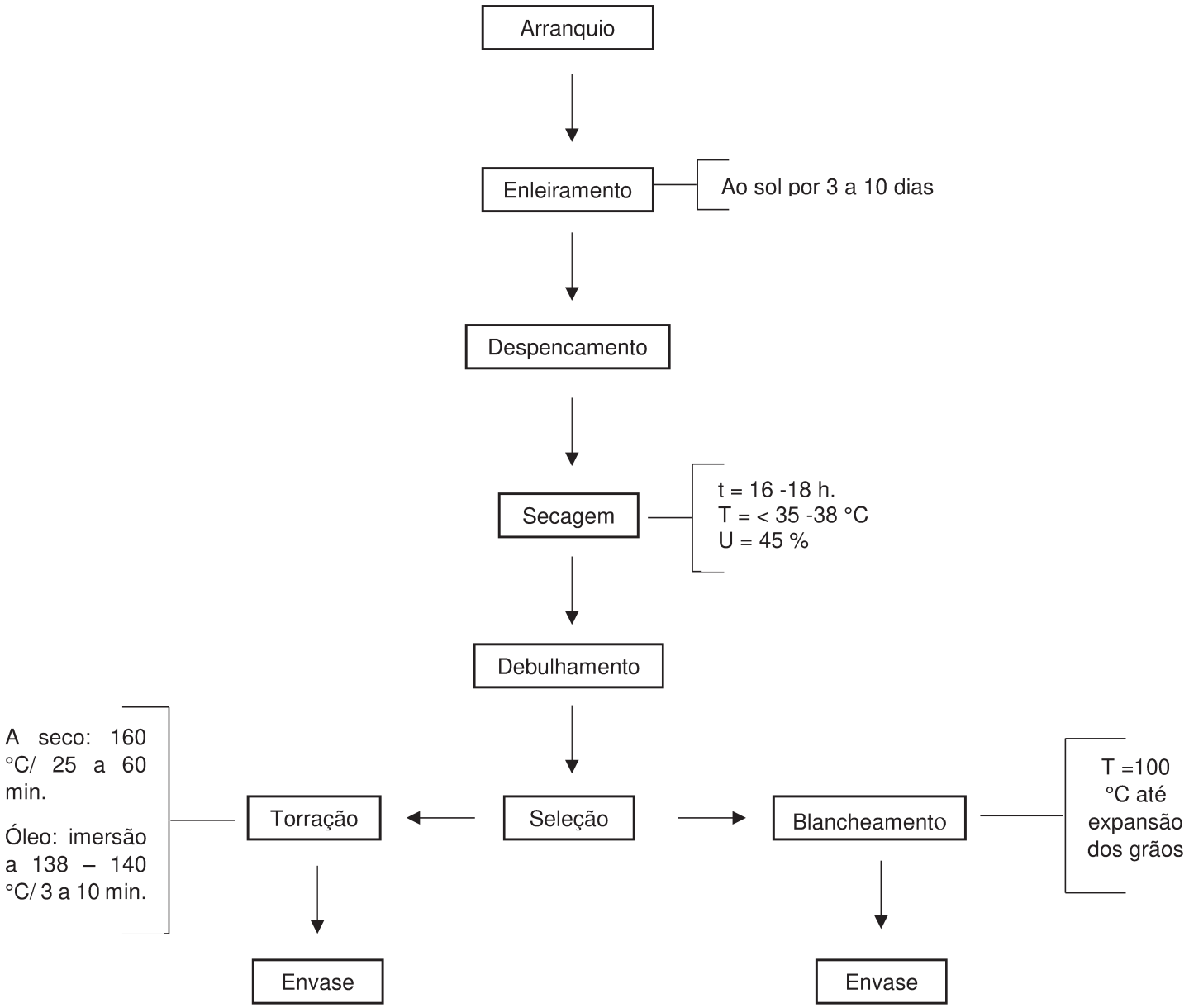

Figura 1: Cadeia produtiva do amendoim.

Atualmente no mercado brasileiro há diversos produtos industrializados a base de amendoim, dentre eles: manteiga, pasta, doces (doce de amendoim, paçoca, pé-de-moleque, torrone), confeitos doces e salgados tipo snack. Os processos variam de acordo com o produto final, podendo incluir as etapas de mistura, moagem em um ou dois estágios, pasteurização, com temperatura entre 71 e 77 ºC/20 min (MA *et al*., 2009), resfriamento, envase, desaeração.

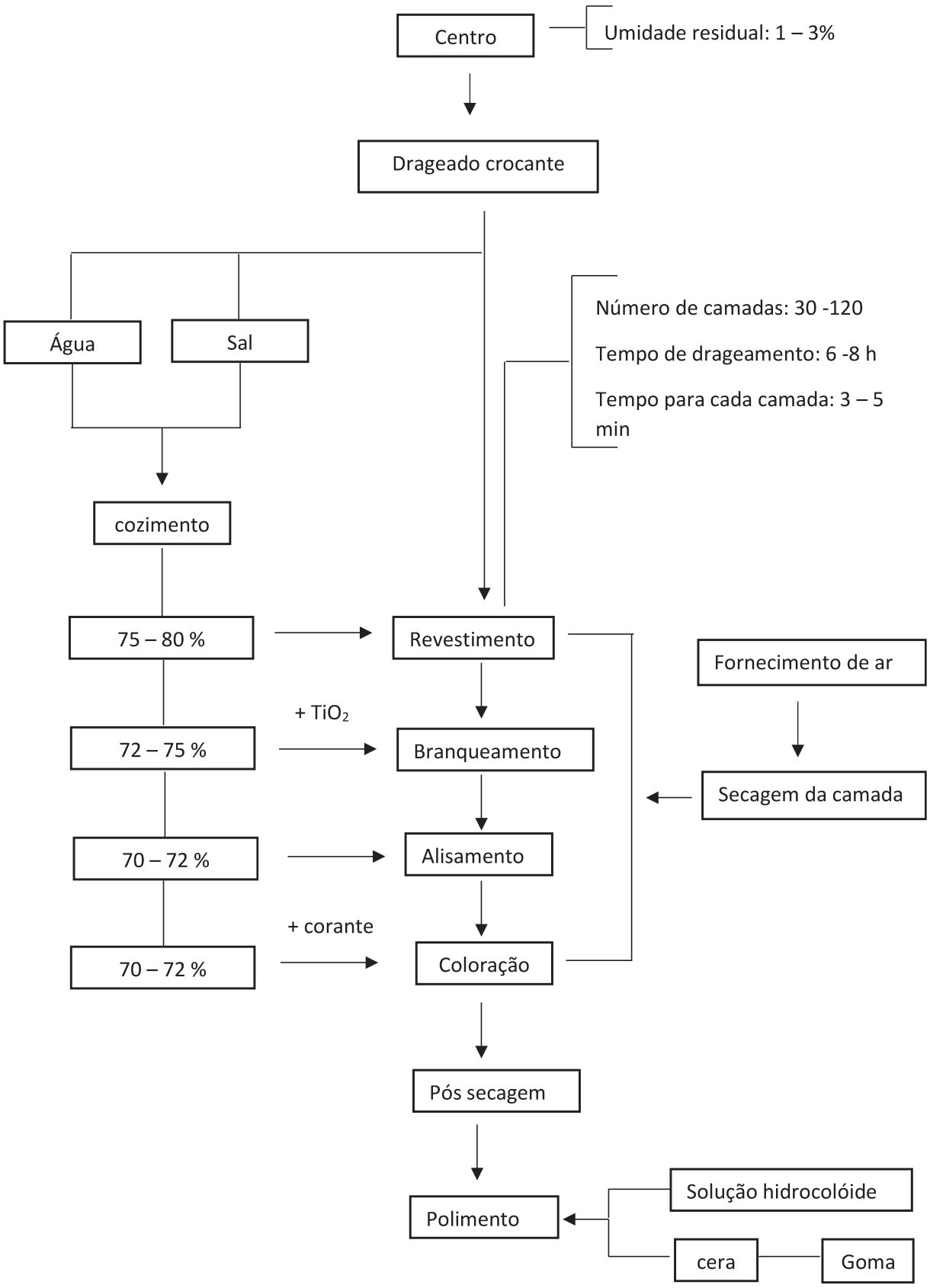

Figura 2: Fluxograma do processamento de amendoim drageado salgado

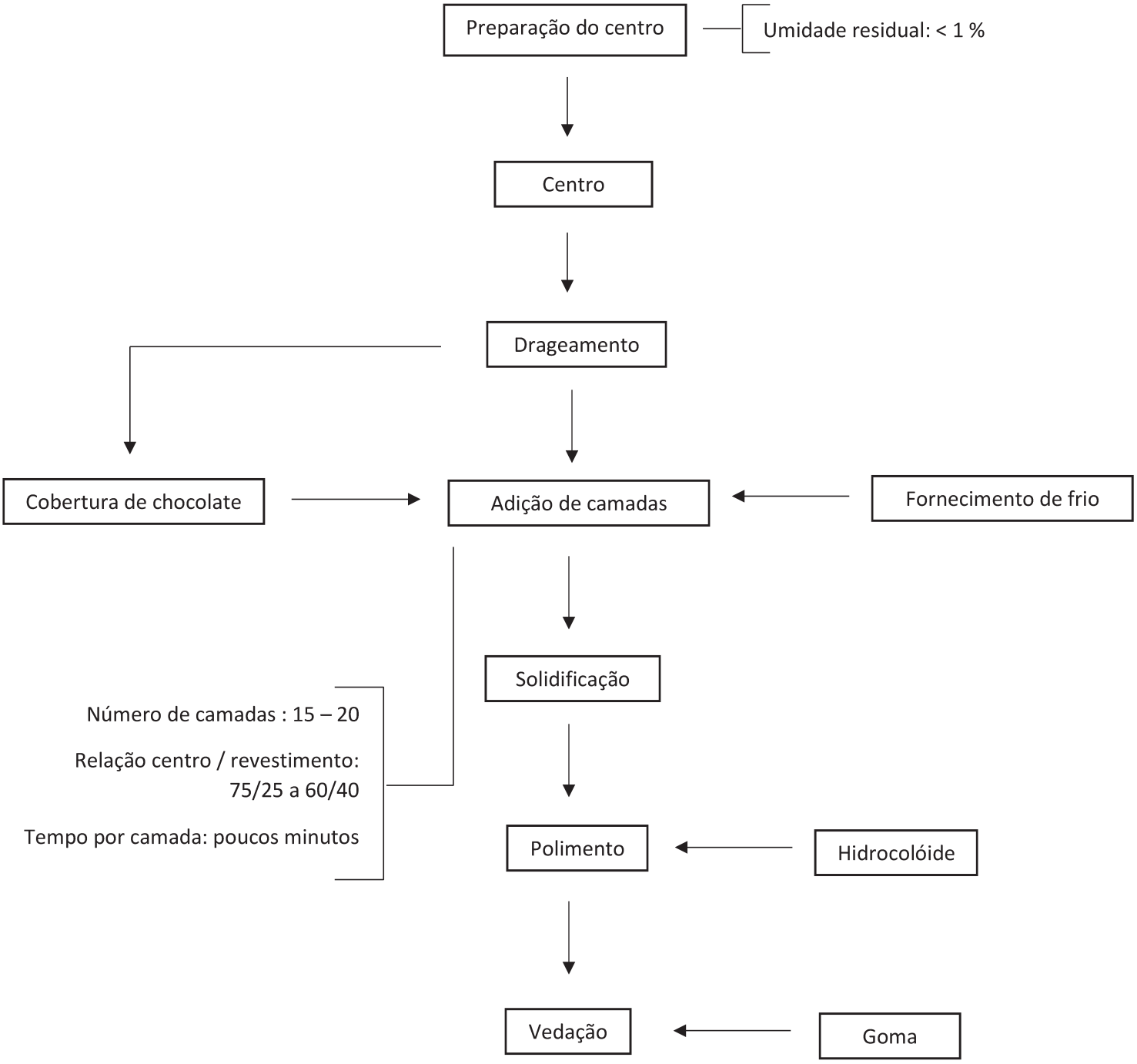

Figura 3: Fluxograma do processamento de amendoim drageado doce (cobertura de chocolate).

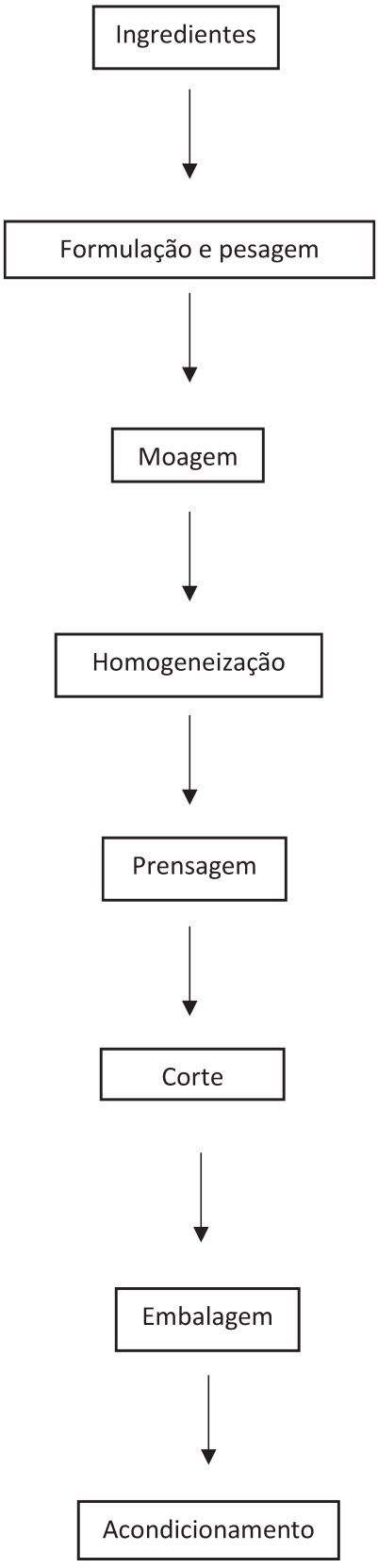

Figura 4: Fluxograma do processamento de paçoca.

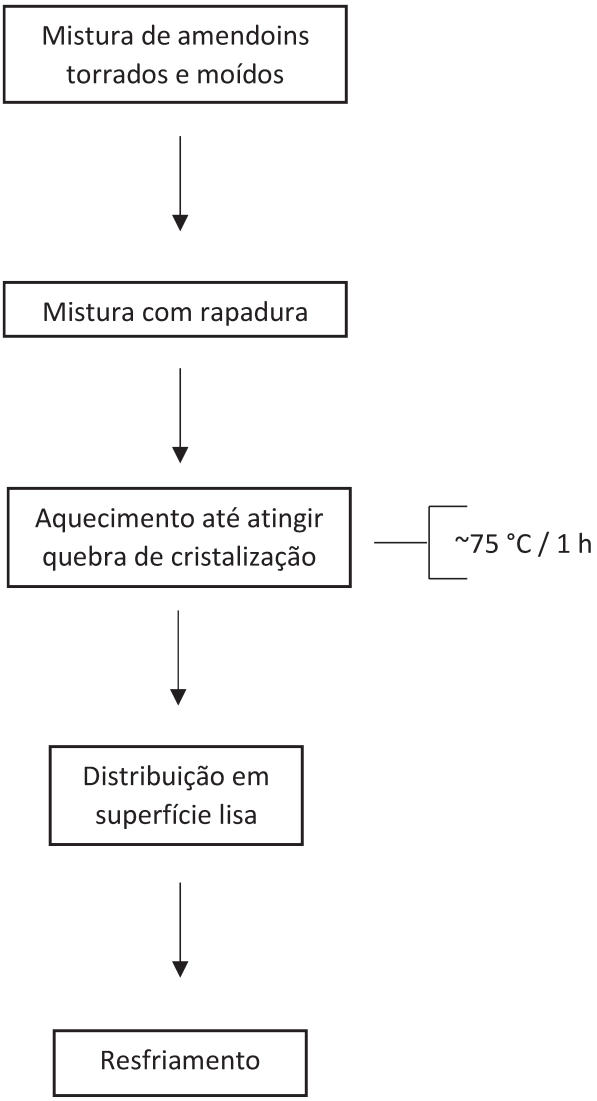

Figura 5: Fluxograma do processamento de pé de moleque.

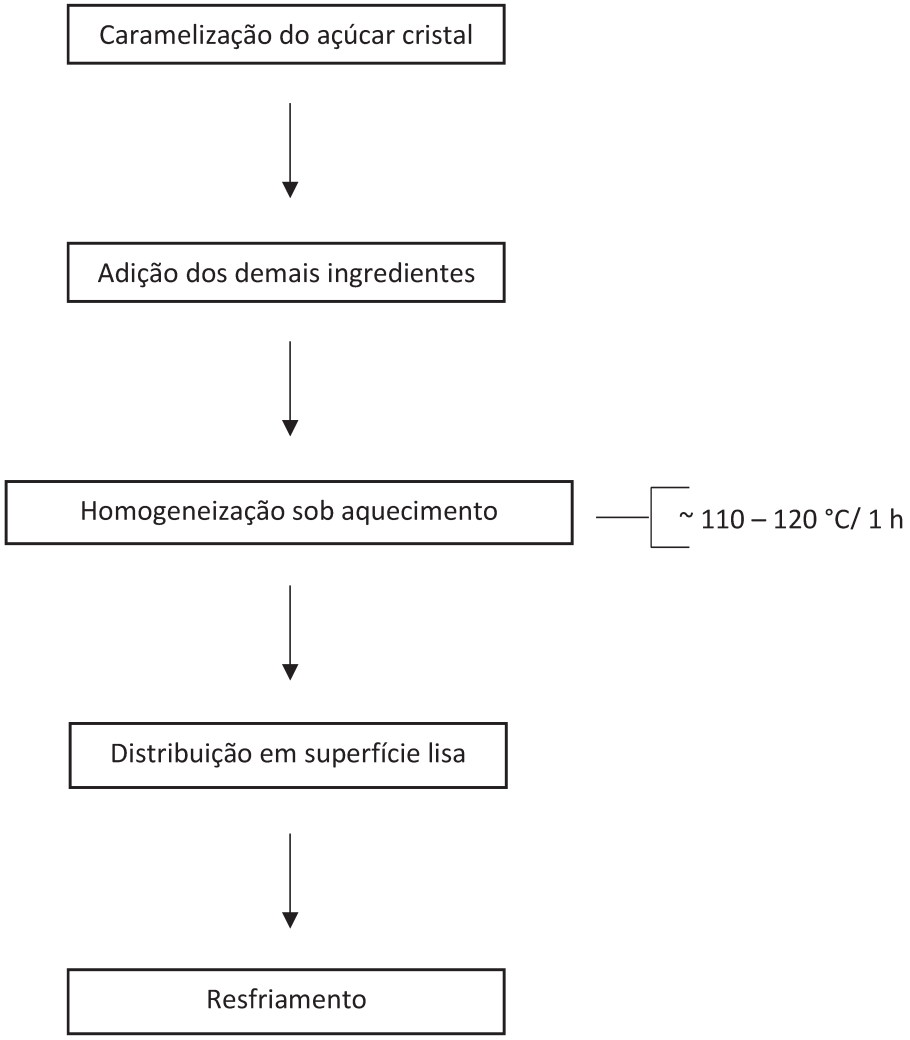

Figura 6: Fluxograma do processamento de pé de moça.

2. *Salmonella* spp.

Salmonella Typhi, agente etiológico da febre tifóide foi isolado pela primeira vez em 1880 por Eberth. Em 1900 ocorreu o primeiro caso confirmado de salmonelose alimentar, em que acometeu 57 pessoas após o consumo de carne bovina contaminada por *S.* Enteritidis. Atualmente o gênero *Salmonella* é um dos principais agentes causadores de doenças transmitidas por alimentos (ICMSF, 1996).

Salmonella é pertencente à família *Enterobacteriaceae*, apresenta forma de bastonetes curtos, Gram negativos, não esporogênicos, anaeróbio facultativo. As principais características bioquímicas são catalase positiva e oxidase e urease

negativas, capacidade de metabolizar nutrientes por vias respiratórias e fermentativas, com exceção da sacarose, descarboxilação da lisina e produção de sulfeto de hidrogênio (H_2S) (DOYLE e BEUCHAT, 2007).

O gênero *Salmonella* é composto por duas espécies *Salmonella enterica* e *Salmonella bongori*, sendo a primeira subdividida em seis subespécies (HAMMACK, 2012):

I - *Salmonella enterica* subsp*. enterica*
II - *Salmonella enterica* subsp*. salamae*
IIIa - *Salmonella enterica* subsp*. arizonae*
IIIb - *Salmonella enterica* subsp*. diarizonae*
IV - *Salmonella enterica* subsp*. houtenae*
VI - *Salmonella enterica* subsp*. indica*

Apesar do Comitê Internacional de Nomenclatura de bactérias não oficializar a inclusão da elevação da subespécie *bongori* à categoria de espécie, esta foi aceita por várias instituições de referências internacionais como CDC (US Center for Disease Control and Prevention), ASM (American Society for Microbiology) e OSM (Organização Mundial de Saúde) (POPOFF e Le MINOR, 2005; BRENNER *et al.*,2000).

A classificação de *Salmonella* mais usual é a nomenclatura por sorotipo que segue o sistema de Kauffmann-White baseado nas diferenças encontradas nas estruturas antigênicas de parede celular (antígeno somático), flagelar e capsular (LE MINOR, 1988; BRENNER, 1984) (Figura 7). Atualmente existem mais de 2500 sorotipos de *Salmonella*.

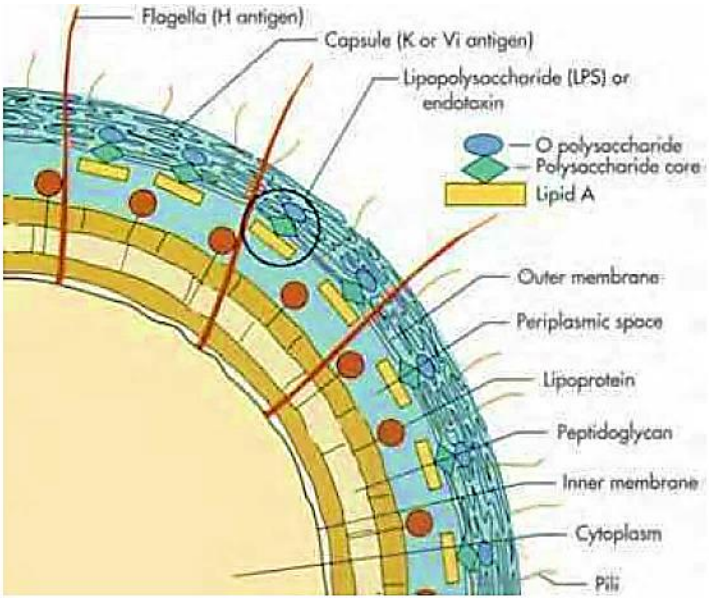

Figura 7: Estrutura celular de *Salmonella* spp. para identificação por sorotipagem.
Fonte: Instituto de Salud Pública de Chile, 2012.

Salmonella é um micro-organismo mesófilo, com temperatura mínima de multiplicação de 5,2 °C e ótima de 35 a 43 ºC. A faixa de pH para multiplicação varia de 3,8 a 9,5, sendo o ideal próximo a 7,0. A atividade de água mínima para sua multiplicação é de 0,94 e a concentração de NaCl deve ser inferior a 9 % (ICMSF, 1996).

De acordo com o sorotipo, a *Salmonella* pode provocar três tipos de doenças, sendo a gastroenterite (Salmonelose) a mais disseminada e conhecida. A Salmonelose é provocada pela maioria dos sorotipos, sendo caracterizada por curto tempo de incubação, em torno de 6 a 72 horas. Os sintomas da Salmonelose são náuseas, vômitos e/ou diarreia, dores abdominais, febre e dor de cabeça e podem durar de 4 a 7 dias. No entanto, sorotipos específicos como a *S.* Typhi provoca a febre tifoide. A manifestação dos sintomas ocorrem entre 7 a 28 dias, em média 14 dias, sendo os principais sintomas mal estar, dor de cabeça, febre alta persistente, dores abdominais e corporais, os sentidos são entorpecidos e os pacientes podem sofrer delírios. O estado do portador pode ser prolongado por meses, e quando não tratada, a febre tifoide possui taxa de mortalidade de 10 %. Outra doença é a febre entérica, em que as características apresentadas são similares a febre tifoide, todavia é

acarretada pelo consumo de alimentos contaminados por *S.* Paratyphi (HAMMACK, 2012; ICMSF 1996).

3. *Salmonella* em produtos de baixa atividade de água

Produtos com alta atividade de água (> 0,90) são mais susceptíveis a multiplicação microbiana. No entanto, estudos recentes têm relatado a sobrevivência de *Salmonella* em produtos de baixa atividade de água, mesmo quando estocados por longos períodos (BEUCHAT *et al*., 2013; BEUCHAT e MANN, 2010; KOMITOPOULOU e PEÑALOZA, 2009; TAMMINGA *et al*., 1976). Isto seria um dos fatores contribuintes para a ocorrência de surtos neste tipo de produto.

Esses surtos têm sido relatados desde 1955, com diversos produtos, desde coco ralado (SEMPLE, PARRY, GRAHAM, 1961; WILSON e MACKENZIE, 1955), ração animal (CDC, 2007a), pimenta branca e preta (272 casos) (CDC, 2010), cereal de aveia (209 casos) (CDC, 1998), porém com maior frequência em amêndoas (242 casos) (ISAACS *et al*., 2005), pistaches (CDC 2009), chocolate (358 casos em 3 surtos) (HOCKIN *et al.,* 1989; GILL *et al.,* 1982; CRAVEN *et al*., 1975).

4. *Salmonella* em amendoim

Toda a cadeia produtiva do amendoim está susceptível a contaminação por patógenos ou toxinas, que podem acarretar em sérios problemas de saúde pública.

Durante a pré-colheita, fatores como a contaminação do solo, o uso de esterco ou composto não curtido de forma adequada e o uso de água contaminada e/ou pela adubação na irrigação, colheita, transporte e estocagem são algumas fontes importantes de contaminação da cultura (SWANSON, 2011; MATTICK *et al.,* 2000). Durante a pós-colheita, a presença de animais e vetores, a higiene inadequada de manipulação e dos equipamentos e utensílios seriam fontes adicionais para veiculação de patógenos. Já no beneficiamento, devido à baixa atividade de água do produto, o processo térmico ao qual o amendoim é submetido pode não eliminar ou reduzir a níveis seguros uma eventual contaminação ocorrida nas etapas anteriores. Durante a industrialização, as possíveis fontes de contaminação dos produtos são matéria-prima, ingrediente, ambiente de processo e manipuladores (PODOLAK *et al.*,

2010). A possibilidade de contaminação cruzada resultante de falhas em programas de controle de qualidade, entretanto, não é desprezível, podendo ser veiculada pelo ar, poeira, água, equipamentos e trânsito de pessoal (BEUCHAT *et al*., 2013; CHANG *et al.*, 2013; CHEN *et al*., 2009).

Os dados existentes envolvendo *Salmonella* em amendoim e produtos à base de amendoim são de amostras coletadas no mercado varejista ou envolvidas em surtos. Os resultados destes levantamentos apontaram baixa incidência e baixo nível de contaminação, como o relatado por Bansal *et al.* (2010), em que observaram *Salmonella* em 0,97 % da amostras de amendoim analisadas, com nível de contaminação entre 1,2 e 15,5 NMP/100 g. Little *et al.* (2010) isolaram *Salmonella* em 0,95 % de amostras de mix de castanhas com amendoim, o nível de contaminação encontrado foi <1 NMP/100 g.

Os baixos níveis de contaminação encontrados não excluem, contudo, a probabilidade de ocorrência de surtos, prova disto são os vários relatos de salmonelose envolvendo produtos à base de amendoim. Na maioria dos casos, os surtos foram epidêmicos, disseminados geograficamente e atingiram um grande número de pessoas, principalmente crianças. Em 1994, ocorreu um surto envolvendo o Reino Unido, Canadá, Estados Unidos e Israel, onde foram identificados 27 casos destas 26 eram crianças com faixa etária inferior a 10 anos, sendo snack de amendoim a fonte de contaminação. O primeiro surto relacionado a contaminação por manteiga de amendoim foi relado na Austrália em 1996, em que 15 casos foram reportados. (SCHEIL et *al.,* 1998). Em outro surto internacional ocorrido em 2001 com 109 casos registrados na Austrália, Canadá e Reino Unido a fonte de contaminação foi identificada como amendoim torrado (KIRK *et al*., 2004). Os surtos mais recentes ocorreram nos EUA e foram relacionados à manteiga de amendoim, a qual apresenta umidade de 1 % e atividade de água inferior a 0,30 (PARK *et al*., 2008). No surto ocorrido entre 2006 e 2007 foram reportados 425 casos, sem notificação de óbito. Neste surto, *Salmonella* Tennesse foi isolada do produto final e das instalações da indústria processadora (CDC, 2007b). Entre 2008 e 2009, ocorreu nos EUA, o maior surto de salmonelose veiculado por manteiga de amendoim. Foram notificados 714 casos, com 9 óbitos em 46 Estados e a possível fonte de contaminação apontada foi a matéria-prima a base de amendoim (CDC, 2009). Outro surto relatado ocorreu em 2012 e acometeu 42 pessoas em 20 Estados dos EUA. Durante a inspeção pós-surto,

as autoridades de saúde encontraram *Salmonella* em quatro amostras de produto acabado e uma amostra de amendoim cru sem casca. Além disso, o patógeno foi isolado de diferentes pontos da linha de processamento (CDC, 2013). O último surto ocorrido nos EUA envolvendo *Salmonella* em amendoim ocorreu em 2014, acometendo 6 pessoas em 5 estados após o consumo manteiga de amendoim (CDC, 2014).

Em um estudo realizado na Califórnia (EUA) por Uesugi *et al*. (2006) não se observou redução significativa na contagem de *Salmonella* após 550 dias de estocagem de amêndoas a -20 e 4 ºC. Após o mesmo período a 23 ºC, os autores reportaram taxa de redução de 0,3 log ufc/ g/ mês. Em um estudo realizado com manteiga de amendoim, Burnett *et al*. (2000), partindo de um inoculo inicial de *Salmonella* de 5,7 log ufc/ g, reportaram contagens de *Salmonella* de 1,0 e 2,0 log ufc/ g após 24 semanas de estocagem a 21 e 4 ºC, respectivamente. Park *et al*. (2008) detectaram *Salmonella* Tennessee em manteiga de amendoim após 2 semanas de estocagem a 22 ºC. Enquanto, amêndoas de nozes foram inoculadas com *Salmonella entérica* e sua presença foi detectada após 365 de estocagem em diferentes temperaturas (-20, 4 e 23 °C) (BLESSINGTON, MITCHAM, HARRIS, 2012). Em estudo recente, Brar *et al.* (2015) inocularam 5 sorotipos de *Salmonella entérica* em diferentes concentrações em amendoim cru e semente de nozes. Estes foram estocados em diferentes temperaturas, os resultados mostraram que na maioria das análises, não houve diferença nas contagens de *Salmonella* em função do inoculo e temperatura de estocagem em alimentos com baixa a_w.

5. Fatores que influenciam a sobrevivência de *Salmonella*

O levantamento epidemiológico de surtos de salmonelose envolvendo produtos de baixa atividade de água como castanhas, amendoim e chocolate confirma que a dose infectante de *Salmonella* nestes produtos é extremamente baixa, chegando a 0,04 NMP/ g (WERBER *et al*., 2005; LEHMACHER *et al*., 1995). Em ambientes inóspitos, como o estresse osmótico provocado pela baixa atividade de água, os micro-organismos como a *Salmonella* desencadeiam mecanismos de defesa, dentre eles a produção de solutos compatíveis como prolina, trealose, glutamina os quais podem aumentar sua resistência térmica (CÁNOVAS *et al*., 2001).

Outra estratégia observada em bactérias não esporuladas, como a *Salmonella* seria um mecanismo que permite que se mantenham em um estado latente até que as condições ambientais permitam a retomada de desenvolvimento (DODIER, 2015). Além disso, a gordura presente no amendoim (aproximadamente 50% da composição) também confere proteção contra o ácido gástrico, permitindo a colonização do intestino e a produção de sinais clínicos, mesmo que a dose infectante seja extremamente baixa (D'AOUST, 1977).

O alto teor de gordura associado à baixa atividade de água presente nestes produtos podem influenciar consideravelmente o aumento da resistência térmica da *Salmonella* (SWANSON, 2011; PODOLAK *et al.*, 2010). De acordo com Mazzotta (2001) seria necessário um tratamento térmico de 71 ºC/3 seg para redução de 5,0 log ufc/ g deste micro-organismo em suco de laranja. Contudo, em noz pecan o tratamento a 120 ºC/20 min promoveu redução de apenas 1 ciclo logarítmico da população de *Salmonella* (BEUCHAT e MANN, 2011). Estudos realizados nos EUA evidenciam o risco de sobrevivência do patógeno mesmo após tratamento térmico elevado (> 80 ºC). Shachar e Yaron (2006) verificaram redução de *Salmonella* de 3,2 log ufc/g após aquecimento de manteiga amendoim a 90 ºC/50 min. Ma *et al.* (2009) detectaram a presença do micro-organismo em manteiga de amendoim mesmo após tratamento térmico a 90 ºC/30 min. De acordo com Sirsat *et al.* (2011) o emprego de uma etapa de processo térmico sub-letal ao invés de reduzir a população inicial de *Salmonella* poderia induzir a mecanismos de resposta ao estresse aumentando assim a resistência do patógeno. O Food and Drug Administration (FDA) recomenda a validação de processo térmico em indústrias que utilizam amendoim ou derivados como ingredientes, estabelecendo como critério a capacidade do processo térmico em causar a redução de *Salmonella* em 5 ciclos logarítmicos (FDA, 2009).

6. Micro-organismos indicadores

Em 1985, o Conselho Nacional de Pesquisa da Academia Nacional de Ciências dos EUA (NAS) elaborou um texto propondo critérios microbiológicos para alimentos e seus ingredientes. Segundo o texto deveria ser estipulado limites críticos que determinado micro-organismo, grupo de micro-organismos ou toxinas produzidas por micro-organismos poderiam estar presente de acordo com o risco do alimento

(NRC, 1985). Dessa forma, foram estabelecidos planos amostrais, sendo divididos em duas categorias, as variáveis e os atributos. O plano por variável depende da distribuição de frequência que determinado micro-organismos ocorre no produto. O plano por atributo é adotado quando o micro-organismo se manifesta em baixos níveis no produto, porém com alto grau de patogenicidade, e também pode ser usado para monitoramento. Outra forma de classificação são planos com duas classes ou três classes, o primeiro divide o lote entre alimentos aceitáveis e inaceitáveis para consumo, já no segundo é possível aceitar algum limite do micro-organismo no produto final, dependo do tipo de alimento e do micro-organismos contaminante (DOYLE e BEUCHAT, 2007).

No Brasil, a RDC 12 de 02/01/01, a atual legislação de parâmetros microbiológicos para alimentos preconiza que para nozes, amêndoas, amendoim e similares, cruas, inteiras ou descascadas a tolerância de coliformes a 45 °C para amostra indicativa deve ser de 10^3, e *Salmonella* deve estar ausente em 25 g de produto (BRASIL, 2001).

Os micro-organismos indicadores podem ser usados como critério microbiológico para determinar a qualidade do produto bem como sua vida de prateleira. Normalmente é realizada contagem de micro-organismos mesófilos aeróbios totais, no entanto, por considerar apenas células viáveis este método não seria suficiente para fornecer dados sobre a qualidade de matérias primas utilizadas (DOYLE e BEUCHAT, 2007). Portanto, a Comissão Internacional de Especificações Microbiológicas para Alimentos (ICMSF 2011) considera essencial para assegurar a qualidade microbiológica e segurança do amendoim, o monitoramento da presença de *Salmonella*, assim como de outras *Enterobacteriaceae*, por exemplo *Escherichia coli* ou outros micro-organismos indicadores na matéria-prima, no ambiente de processo e no produto final para predizer desvios nas medidas de controle de higiene (ICMSF, 2011; SWANSON, 2011).

Stott *et al*. (1975) observaram a existência de correlação entre a presença de *Enterobacteriaceae* e *Salmonella* em alimentos granulados. Jones e Richardson (2004) verificaram maiores contagens de *Enterobacteriaceae* em amostras positivas para *Salmonella* do que em amostras negativas.

Ao realizar um levantamento de *Salmonella* em amêndoas no Reino Unido Little *et al.* (2010) não encontraram uma correlação entre a presença de *E. coli* e

Salmonella. No entanto, o autor afirma que o uso de micro-organismos indicadores pode fornecer evidencias de que o produto foi contaminado e que pode haver contaminação de outros agentes patogênicos, e baixos níveis da contagem de *Salmonella* pode não ser detectada em 25 g.

7. REFERÊNCIAS BIBLIOGRÁFICAS

ABICAB - Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Amendoim**. 2013. Disponível em: <http://www.abicab.org.br/associados-3/>. Acesso em: 3 ago. 2016.

ABICAB- Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Cultivo de amendoim**. 2015. Disponível em: < http://www.abicab.org.br/cultivo-de-amendoim/ >. Acesso em: 13 out. 2016.

ABICAB – Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Amendoim – História**, 2016. Disponível em: < http://www.abicab.org.br/historia-3/>. Acesso em: 10 set. 2016.

BANSAL, A.; JONES, T. M.; ABD, S. J.; DANYLUK, M. D.; HARRIS, L. J. Most-Probable-Number determination of *Salmonella* levels in naturally contaminated raw almonds using two samples preparation methods. **Journal of Food Protection**, v. 73, p. 1986-1992, 2010.

BEUCHAT, L. R.; MANN, D. A. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. **Journal of Food Protection,** v. 73, p.1257-1268, 2010.

BEUCHAT, L. R.; MANN, D. A. Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. **Journal of Food Protection**, v. 74, p. 1441-1450, 2011.

BEUCHAT, L. R.; KOMITOPOULOU, E.; BECKERS, H.; BETTS, R. P.; BOURDICHON, F.; FANNING, S.; JOOSTEN, H. M.; TER KUILE, B. H. Low-water activity foods: increased concern as vehicles of foodborne pathogens. **Journal of Food Protection**, v. 76, p. 150-172, 2013.

BLESSINGTON, T.; MITCHAM, E. J.; HARRIS, L. J. Survival of *Salmonella enterica, Escherichia coli 0157:H7,* and *Listeria monocytogenes* on inoculated walnut kernels during storage. **Journal of Food Protection,** v. 75, n.2, p. 245 – 254. 2012.

BRAR, P. K.; PROANO, L. G.; FRIEDRICH, L. M.; HARRIS, L. J.; DANYLUK, M. D. Survival of *Salmonella, Escherichia coli 0157:H7*, and *Listeria monocytogenes* on raw peanuts and pecan kernels stored at -24, 4, and 22 °C. **Journal of Food Protection**, v. 78, p. 323 – 332. 2015.

BRASIL. ANVISA. Agência Nacional de Vigilância Sanitária. **Resolução RDC n° 12/2001 de 02 de janeiro de 2001** - Aprova regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União. Disponível em: < http://portal.anvisa.gov.br/documents/33880/2568070/RDC_12_2001.pdf/15ffddf6-3767-4527-bfac-740a0400829b>. Acesso em: 16 set. 2016.

BRASIL. ANVISA. Agência Nacional de Vigilância Sanitária. **Resolução RDC n° 172/2003, de 04 de julho de 2003** - Dispõe sobre o Regulamento Técnico de Boas Práticas de Fabricação para Estabelecimentos Industrializadores de Amendoins Processados e Derivados e a Lista de Verificação das Boas Práticas de Fabricação para Estabelecimentos Industrializadores de Amendoins Processados e Derivados. Diário Oficial da União. Disponível em: <

http://portal.anvisa.gov.br/wps/wcm/connect/503f2c00474579428651d63fbc4c6735/RDC_172_2003.pdf?MOD=AJPERES>. Acesso em: 11 ago. 2016.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento – MAPA. Instrução Normativa nº 3, de 28 de Janeiro de 2009.

BRENNER, D. J. Facultatively anaerobic Gram- negative rods, In: Krieg, N. R. e Holt, J. C. **Bergey's Manual of Systematic Bacteriology**. Baltimore: Williams and Wilkins, 1984. p. 408-516.

BRENNER, F. W.; VILLAR, R. G.; ANGULO, F. J.; TAUXE, R. SWAMINATHAN, B. *Salmonella* Nomenclature. **Journal Clinical Microbiology**, v. 38, n. 7, p. 2465-2467, 2000.

BURNETT, S. L.; GEHM, E. R.; WEISSINGER, W. R.; BEUCHAT, L. R. Survival of *Salmonella* in peanut butter and peanut butter spread. **Journal of Applied Microbiology**, v. 89, p. 472-477, 2000.

CÁNOVAS, D.; FLETCHER, S. A.; HAYASHI, M.; CSONKA, L. Role of trehalose in growth at high temperature of *Salmonella enterica* Serovar Typhimurium. **Journal of Bacteriology**, v. 183, n. 11, p. 3365 – 3371. 2001.

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of Salmonella Serotype Agona Infections Linked to Toasted Oats Cereal -- United States, April-May, 1998. 1998. Disponível em: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00053368.htm>.Acesso em: 6 out. 2016.

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of *Salmonella* Schwarzengrund Infections Linked to Dry Pet Food**.** 2007 a. Disponível em: <http://www.cdc.gov/salmonella/2007/pet-food-9-4-2007.html>. Acesso em: 05 abr. 2016).

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006-2007 b. **MMWR Morbidity and Mortality Weekly Report,** v. 56, p. 521-524, 2007.

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. **MMWR Morbidity and Mortality Weekly Report**, v. 58, p. 85-90, 2009.

CDC. Multistate Outbreak of Human *Salmonella* Montevideo Infections. 2010. Disponível em: <http://www.cdc.gov/salmonella/2010/montevideo-5-4-2010.html>. Acesso em: 6 out. 2016.

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland. 2013**.** Disponível em: <http://www.cdc.gov/salmonella/bredeney-09-12/index.html>. Acesso em: 3 ago. 2015.

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of *Salmonella* Braenderup Infections Linked to Nut Butter Manufactured by Spired Natural Foods**.** 2014. Disponível em: http://www.cdc.gov/Salmonella/braenderup-08-14/. Acesso em: 05 de set de 2016.

CHANG, A.S.; SREEDHARAN, A.; SCNEIDER, K.R. Peanut and peanut products: a food safety perspective. **Food Control**, v. 32, p. 296-303, 2013.

CHEN, Y.; SCOTT, V. N.; FREIER, T. A. Control of *Salmonella* in low-moisture foods. III: process validation and environmental monitoring. **Food Protection Trends**, v. 29, p. 493-508, 2009.

CONAB – COMPANHIA NACIONAL DE ABASTECIMENTO, **Maio/2016. Acompanhamento da Safra Brasileira, Grãos - V. 3 - SAFRA 2015/16- N. 8 - Oitavo levantamento** – Disponível em:<http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_05_10_09_03_26_boletim_graos_maio_2016.pdf>. Acesso em: 08 abr. 2016.

CRAVEN, P. C.; MACKEL, D. C.; BAINE, W. B.; BARKER, W. H.; GANGAROSA, E. J.; GOLDFIELD, M.; ROSENFELD, H.; ALTMAN, R.; LACHAPELLE, G.; DAVIES, J. W.; SWANSON, R. C. International outbreak of *Salmonella* eastbourne infection traced to contaminated chocolate. **Lancet**, v. 1, p. 788–792. 1975.

D'AOUST, J. Y. *Salmonella* and the chocolate industry. A review. **Journal of Food Protection,** v. 40, p. 718-727, 1977.

DODIER, D. Comparative Study Of The D-values of *Salmonella* spp. and *Enterococcus faecium* in Wheat Flour. Faculty of The Graduate College at the University of Nebraska Major. **Food Science and Technology**. 2015.

DOYLE, M. P.; BEUCHAT, L. R. **Food Microbiology**: Fundamentals and Frontiers. 3 ed. Washington: American Society for Microbiology Press. 2007.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Cultivo do amendoim**. 2006. Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Amendoim/CultivodoAmendoim/>. Acesso em: 7 ago. 2016.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária, **Amendoim: o produtor pergunta, a Embrapa responde**. Brasília, DF: Embrapa Informação Tecnológica: Campina Grande: Embrapa Algodão, 2009. 240 p. il. (Coleção 500 perguntas, 500 respostas).

EPA - United State Environmental Protection Agency. Food and Agricultural Industries. In: **Confectionary products: peanut processing**. 5. Ed, v. 1. 1995. Disponível em: <http://www.epa.gov/ttnchie1/ap42/ch09/>. Acesso em: 6 ago. 2015.

FDA - Food and Drug Administration. **Guidance for industry: measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as an ingredient.** 2009. Disponível em: <http://www.fda.gov/guidancecomplianceregulatoryinformation/guidancedocuments/produceandplanproducts/ucm115386.htm>. Acesso em: 10 ago. 2016.

GILL, O. N.; BARTLETT, C. L. R.; SOCKETT, P. N.; VALLE, M.S.B.; ROWE, B.; GILBERT, R.J.; DULAKE, C.; MURRELL, H.C.; SALMASO, S. **Outbreak of Salmonella napoli chocolate bars**. Public Health Laboratory , Dulwich Summary in England and Wales in 1982 was detected Epidemiological Investigations Extensive inquiries were made by telephone and by visits to Epidemiology. p. 574–577, 1982.

HAMMACK, T. M. S. *Salmonella spp*. In: Lampel, K. A., **Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins**. Washington, DC: U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, 2012. p. 9 – 13.

HOCKIN, J. C.; D'AOUST, J. Y.; BOWERING, D.; JESSOP, J. H.; KHANNA, B.; LIOR, H.; MILLING, M. E. An international outbreak of *Salmonella* nima from imported chocolate. **Journal of Food Protection**, v. 52, n. 1, p. 51–54, 1989.

ICMSF - International Commission on Microbiological Specifications for Foods. Salmonellae. In: **Microorganisms in Foods 5**: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 217–264. 1996.

ICMSF - International Commission on Microbiological Specifications for Foods. Cocoa, Chocolate and Confectionery. In: **Microorganisms in Foods 8**: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 241–246. 2011.

INSTITUTO DE SALUD PÚBLICA DE CHILE (ISP). **Ministerio de Salud**. Disponível em: <http://www.ispch.cl/lab_amb/serv_lab/salmonella.html#uno>. Acesso em 31 de ago de 2016.

ISAACS, S.; ARAMINI, J.; CIEBIN, B.; FARRAR, J. A.; AHMED, R.; MIDDLETON, D.; CHANDRAN, A. U.; HARRIS, L. J.; HOWES, M.; CHAN, E.; PICHETTE, A. S.; CAMPBELL, K.; GUPTA, A.; LIOR, L. Y.; PEARCE, M.; CLARK, C.; RODGERS, F.; JAMIESON, F.; BROPHY, I.; ELLIS, A. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. **Journal of Food Protection**, v. 68, p. 191–198. 2005.

JONES, F. T.; RICHARDSON, K. F. *Salmonella* in commercially manufactured feeds. **Poultry Science**, v. 83, p. 384-391. 2004.

KIRK, M. D.; LITTLE, C. L.; LEM, M.; FYFE, M.; GENOBILE, D.; TAN, A.; THRELFALL, J.; PACCAGNELLA, A.; LIGHTFOOT, D.; LYI, H.; MCINTYRE, L.; WARD, L.; BROWN, D. J.; SURNAM, S.; FISHER, I. S. T. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – Sharing moelcular information to solve internacional outbreak. **Epidemiology and Infection**, v. 132, p. 571-577, 2004.

KOMITOPOULOU, E.; PEÑALOZA, W. Fate of *Salmonella* in dry confectionery raw materials. **Journal of Applied Microbiology**, v. 106, p. 1892-1900, 2009.

LE MINOR, L. Typing *Salmonella* species. **European Journal of Clinical Microbiology & Infectious Diseases**, v. 7,n. 2, p. 214-218. 1988.

LEHMACHER, A.; BOCKEMÜHL, J.; ALEKSIL, S. Nationwide outbreak of human salmonelosis in Germany due to contaminated Paprika and Paprika-powdered potato chips. **Epidemiology and Infection,** v. 115, p. 501-511, 1995.

LITTLE, C. L.; RAWAL, N.; PINNA, E.; MCLAUCHLIN, J. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. **Food Microbiology,** v. 27, p. 171-174, 2010.

MA, L.; ZHANG, G.; GERNER-SMIDT, P. Thermal inactivation of *Salmonella* in peanut butter. **Journal of Food Protection,** v. 72, p. 1596-1601, 2009.

MACÊDO, M. H. G. de. **Amendoim**. In: CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. 2007. Disponível em: < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/83e31b69fc4c1f45a1cee5eb53797f41..pdf >. Acesso em: 26 out. 2016.

MATTICK, K. L.; JORGENSEN, F.; LEGAN, J. D.; LAPPIN-SCOTT, H. M.; HUMPHREY, T. J. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. **Applied and Environmental. Microbiology**, v. 66, p. 4921–4925. 2000.

MAZZOTTA, A. S. Thermal Inactivation of Stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in fruit juices. **Journal of Food Protection**, v. 64, p. 315-320, 2001.

NRC - NATIONAL RESEARCH COUNCIL. **An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients**. National Academic Press, Washington, D.C. 1985.

PARK, E. J.; OH, S. W.; KANG, D. H. Fate of *Salmonella* Tennessee in Peanut Butter at 4 and 22 ºC. **Journal of Food Science**, v. 73, n. 2, p. 82-86, 2008.

PODOLAK, R.; ENACHE, H.; STONE, W.; BLACK, D. G.; ELLIOT, P. Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low – Moisture Foods. **Journal of Food Protection,** v. 73, p. 1919-1936. 2010.

POPOFF, M. Y.; Le MINOR, L. E. The genus *Salmonella*. In: Brenner D.J.; Krieg, N.R.; Staley, J.T. **Bergey's manual of systematic bacteriology**. 2. ed. New York, USA: Springer, 2005. v. 2. p.764-799.

SCHEIL, W.; CAMERON, S.; DALTON, C.; E WILSON, D. A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. **Australian and New Zealand Journal of Public Health**, v. 22, n 5, p. 536–539. 1998.

SEMPLE, A. B.; PARRY, W. H.; GRAHAM, A. J. Paratyphoid fever traced to desiccated coconut. **Lancet London, England**, v. 2, n. 7198, p. 364–365, 1961.

SHACHAR, D.; YARON, S. Heat tolerance of *Salmonella enteric* serovars Agona, Enteritidis, Typhimurium in peanut butter. **Journal of Food Protection**, v. 11, p. 2687-2691, 2006.

SIRSAT, S. A.; BURKHOLDER, K. M.; MUTHAIYAN, A.; DOWD, S. E.; BHUNIA, A. K.; RICKE, S.C. Effect of sublethal heat stress on *Salmonella* Typhimurium virulence. **Journal of Applied Microbiology**, v. 110, p. 813 – 822. 2011.

STOTT, J. A; HODGSON, J. E.; CHANEY, J. C. Incidence of Salmonellas in animal feed and the effect of pelleting on content of Enterobacteriaceae. **Journal f Applied Bacteriology**, v. 39, p. 41-46. 1975.

SWANSON, K. M. J. Nuts, oilseeds, dried legumes and coffee. In: ICMSF – International Commission on Microbiological Specifications for Food. **Microorganisms in Food 8**. New York: Springer, p. 227 – 237. 2011.

TAMMINGA, S. K.; BEUMER, R. R.; KAMPELMACHER, E. H.; VAN LEUSDEN, F. M. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. **Journal of Hygine,** v.76, n. 1, p. 41-47. 1976.

UESUGI, A. R.; DANYLUK, M. D.; HARRIS, L. J. 2006. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23 and 35 ºC. **Journal of Food Protection**, v**.** 69, p.1851-1857.

USDA – United States Departament of Agriculture. PSD – Production, Supply and Distribution Online, 2016. Disponível em: <http://apps.fas.usda.gov/psd online/psdQuery.aspx>. Acesso em: 30 maio 2016.

WERBER, D.; DREESMAN, J.; FEIL, F.; van TREECK, U.; FELL, G.; ETHELBERG, S.; HAURI, A. M.; ROGGERNTIN, P.; PRAGER, R.; FISHER, I. S. T.; BEHNKE, S. C.; BARTELT, E.; WEISE, E.; ELLIS, A.; SIITONEN, A.; ANDERSSON, Y.; TSCHÄPE, H.; KRAMER, M. H.; AMMN, A. International outbreak of *Salmonella* Oranienburg due to German chocolate. **BioMed Central Infectious Diseases,** v. 5, p. 7-17, 2005.

WILSON, M. M.; MACKENZIE, E. F. Typhoid Fever and Salmonellosis Due To the Consumption of Infected Desiccated Coconut. **Journal of Applied Bacteriology**, v. 18, n. 3, p. 510–521, 1955.

WOODROOF, J. G. **Peanut: production, processing, products**, 3 ed, Westport, CN: The Avi Publishing Company. 1983.

CAPÍTULO 2

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Microbiological contamination in peanut confectionery processing plants

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Running headline: *Salmonella* in peanuts

Abstract

Aims: In order to investigate *Enterobacteriaceae*, coliforms, *Escherichia coli,* and *Salmonella* contamination a survey was conducted at three peanut confectionery processing companies (A, B, and C) in Brazil.

Methods and Results: Samples of different peanut confectionery products (n=59), peanut raw material (n=30), manufacturing environment (n=116) were analyzed. *Salmonella and E. coli* were not detected in any final product or raw material analyzed. *Enterobacteriaceae* was isolated from 15 % of final products. Coliforms were detected in only one sample. Referring to the raw material, six samples showed contamination by *Enterobacteriaceae* and three samples by coliforms. For the process environment, 19 % and 11 % of samples presented *Enterobacteriaceae* and coliforms. *E. coli* was detected in 5 % of samples, and one of these samples tested positive for *Salmonella*; this strain was serotyping as *S.* Yoruba.

Conclusion: Significance and Impact of Study: This has been the first study to investigate the occurrence of *Salmonella* and other *Enterobacteriaceae* throughout peanut confectionery processing lines. The results might be used to assist risk assessment studies and to establish more effective control measures.

Key-words: *Salmonella, Escherichia coli*, peanuts, food safety, risk assessment, food surveillance.

1. Introduction

Peanuts may be commercialized in-shell or shelled as well as be consumed as snacks after roasting or frying. They are also used as an ingredient in a range of confectionery products, including peanut butter, coated peanuts, and peanut candies.

During the primary production, environmental conditions (temperature, rainfall, and humidity), contaminated soil, contaminated irrigation water, inadequate equipment hygiene, presence of animals, and mechanical damage caused by insects could influence the load and kind of microbiological contamination, such as *Salmonella* (Horn and Dorner 1999; Schatzki and Ong 2001; ICMSF 2011).

Several outbreaks of salmonellosis involving peanut-based products have been reported in literature (Kirk *et al.* 2004; Isaacs *et al.* 2005; CDC 2007, 2009). In an international outbreak that occurred in 2001, 109 people were infected with *Salmonella* by eating roasted peanuts (Kirk *et al.* 2004). In the largest outbreak, which occurred in the USA between 2008 and 2009, 714 cases and 9 deaths were linked to the consumption of peanut butter (CDC 2009). In 2012, 42 cases were linked to the consumption of peanut butter. In this case, *Salmonella* were isolated from four samples of end product, one sample of raw shelled peanuts, and from several processing line points (CDC 2013).

Roasting is a crucial point in peanut processing to control microbiological contamination. Temperature varies according to the process and equipment used, and can reach up to 160 °C for 60 min (Woodroof 1983). However, high fat content and the low water activity (a_w) in peanuts result in an increase in the heat resistance of *Salmonella* (Podolak *et al*. 2010; ICMSF 2011). Shachar and Yaron (2006) and Ma *et al.* (2009) detected *Salmonella* in peanut butter even after a heat treatment at 90 °C. Furthermore, several studies have reported *Salmonella* survivability for long periods in low water activity products (Tamminga *et al.* 1976; Komitopoulou and Peñaloza 2009; Beuchat and Mann 2010; Beuchat *et al.* 2013). In peanut butter, *Salmonella* can persist for at least 12 months of storage (Burnett *et al.* 2000; Nummer *et al.* 2012; Kataoka *et al.* 2014).

Aiming to assure the microbiological quality and safety of peanuts, the International Commission on Microbiological Specifications for Foods (ICMSF 2011) considers it essential to monitor *Salmonella* in raw material, processing environment,

and end products. Even though *Salmonella* is the main target bacteria in a microbiological investigation, other *Enterobacteriaceae* should also be monitored. The monitoring of *Enterobacteriaceae* or other indicator microorganisms can be used to predict deviations in the hygiene control measures (ICMSF 2011, Doyle and Buchanan 2013).

Although *Salmonella* has been recognized as a potential hazard for the peanut industry (ICMSF 2011), there are no survey reports on the occurrence of *Salmonella* and other *Enterobacteriaceae* throughout the peanut confectionery processing line. Even greater is the lack of information on the points of entrance for this pathogen to these end products. The few studies available are associated with outbreak surveys (Kirk *et al*. 2004, CDC 2007, 2009). For that reason, the aim of this study was to investigate the presence of *Enterobacteriaceae*, coliforms, *E. coli,* and *Salmonella* in the processing environment, peanut-based ingredients, and peanut confectionery products in three peanut manufacturing companies.

2. Material and methods

2.1. Sampling

Three peanut manufacturing companies (A, B, and C), located in Brazil, were evaluated for *Enterobacteriaceae*, coliforms, *Escherichia coli,* and *Salmonella* contamination. Two visits to collect samples were conducted in each processing plant over 18 months. Samples of peanut confectionery products (n=59), raw and roasted peanuts used as ingredients (n=30), environment (equipment, utensils, and facilities, n=116) were analyzed.

Samples of 500 g of raw, roasted peanuts, and end products from different batches were collected. The preparation of these samples and their decimal dilutions are described in the next item. Environmental sampling was carried out according to Midura and Bryant (2001) on product contact surfaces (equipment and utensils) and non-contact surface (wall, floor, and drain) at different processing steps. From each surface, five areas of 100 cm^2 (total of 500 cm^2) were chosen randomly and sampled using a swab or a sponge. After that, the swab was homogenized in 500 mL of buffered peptone water (BPW, Acumedia, Pennsylvania, USA), and the obtained solution was

divided into two portions of 250 mL - one for *Salmonella* test and the other for the remaining microbiological determinations. In the case of smaller utensils, the entire surface was sampled. Swab samples from the environment surfaces were maintained below 4 °C until the time of analysis.

2.2. Microbiological analyses

For the end products and raw material, *Salmonella* determination was performed in samples of 250 g by a single dilution series of the Most Probable Number (MPN) technique (Blodgett 2010). The analysis was carried out by Vidas *Salmonella* assay (Biomerieux, France). Initially, peanut samples were transferred to sterile plastic bags and manually crushed using a hammer. For each sample, 10 subsamples of 25-g crushed peanuts or peanut-containing product were transferred to 10 sterile containers and homogenized for 2 min with 225 ml of Buffered Peptone Water (BPW, Acumedia, MI, USA). After that, the broth was supplemented with selective reagents (Biomerieux) and incubated at 41.5 °C for 20-24 h. Then, 500 µl of each 10 portion were transferred to Vidas Up *Salmonella* kit (SPT BioMérieux), and heated at 131 °C for 5 min. After this period, the barrets were cooled to room temperature for 10 min and placed in Mini-Vidas assay equipment (AOAC 2013). The results were expressed as Most Probable Number per gram (MPN g-1), and the detection limit was 0.004 MPN g-1 (Thomas 1942). In the environmental samples, the *Salmonella* detection was performed by presence/absence method. For pre-enrichment step, a dilution of 1:1 was prepared using 250 mL of double-strength BPW and 250 mL of the samples collected as mentioned in item 2.1. The following steps were carried out according to the same Vidas *Salmonella* assay protocol described for the end product and raw material samples. The results were expressed as present or absent in 250 cm^2 and 250 mL. The positive result was confirmed using the same pre-enriched sample by the culture method (ISO 6579, 2007). *Salmonella* isolate was serotyped by agglutination method using specific O and H antisera (Difco, Sparks, MD) according to the Kauffmanne-White scheme (Grimont & Weill, 2007)

For all other microbiological determinations, the samples were prepared and diluted according to ISO 6887 (1999). For *Enterobacteriaceae* counts, pour plate method with overlay was carried out using Violet Red Bile Agar supplemented with

Glucose (VRBG, Acumedia), with incubation at 35 °C for 24 h (Kornacki and Johnson 2001). Coliforms and *E. coli* were determined using Petrifilm 6404 (3M, Minnesota, USA), with incubation at 35 °C for 24-48 h (AOAC 2010). The results were expressed in Colony Forming Unit (cfu) per gram, cm^2, or mL, and the detection limit was 1.0 log cfu g^{-1} and 0.0 log cfu cm^{-2} or mL^{-1}, for end products/raw materials and environmental sample, respectively.

2.3. Determination of water activity and pH

Water activity of the raw materials and final products was determined in duplicate at 25 °C using a hygrometer (Decagon Device, Washington, USA). For pH analysis, samples of 10 g were milled and homogenized with 100 mL of deionized water for 20 min. Then, pH values were determined using calibrated pHmeter (Hanna instruments, HI 9110, Singapore) (IAL 2005).

2.4. Statistical analysis

The analysis of variance (ANOVA) and the Tukey test at the 5 % level of significance were performed using SAS software (version 9.4, SAS Institute, Cary, NC).

3. Results

3.1. Final products

The water activity varied according to the kind of product. The lowest a_w media value was found in peanut brittle (0.275), whereas *doce de amendoim* showed the highest a_w (0.746). The pH media values varied between 5.9 and 6.6 (Table 1). *E. coli* and *Salmonella* were not detected in any of the 59 analyzed samples. In Company A, *Enterobacteriaceae* was isolated in only one sample of crunchy-coated peanuts (1.7 log cfu g^{-1}) as observed in Table 2. In Company B, both analyzed products (*paçoca* and peanut brittle) showed *Enterobacteriaceae*. Three samples of *doce de amendoim*

of Company C presented *Enterobacteriaceae,* and in one of them, coliforms were detected (1.4 log cfu g^{-1}) (Table 2).

3.2. Raw materials

The a_w of raw and roasted peanut samples used as raw material ranged from 0.534 to 0.641 and from 0.267 to 0.316, respectively. The pH values were between 6.3 and 6.8. *E. coli* and *Salmonella* were not isolated in any analyzed sample. Only raw peanuts showed contamination by *Enterobacteriaceae* and coliforms. In Company A, 60 % of the samples were positive for *Enterobacteriaceae* and 20 % for coliforms. In Company B, *Enterobacteriaceae* was detected in all the three analyzed samples, whereas coliforms were isolated from only one sample. In Company C, *Enterobacteriaceae* and coliforms were observed in 50 % of the raw peanuts samples, with maximum counts of 3.6 and 2.9 log cfu g^{-1}, respectively (Table 2).

3.3. Manufacturing environment

In Company A, samples showed high counts for aerobic mesophilic, ranging from 0.3 to 8.5 log cfu cm^{-2} or mL^{-1}. Twenty-two out of 38 samples were positive for *Enterobacteriaceae,* with counts up to 6.6 log cfu cm^{-2} or mL^{-1}. While coliforms were isolated in 19 samples (50 %), *E. coli* was recovered from only one sample - the manual peanut sorting equipment placed in the dirty area. *Salmonella* was not found in environmental samples collected in the processing plant of Company A (Table 3).

In Company B, all samples were positive for aerobic mesophilic. Contamination by *Enterobacteriaceae* ranged from < 0 to 5.8 log cfu cm^{-2} or mL^{-1}, and 17 samples (45 %) showed coliforms. Further, *E. coli* was isolated in four samples, and one of these samples tested positive for *Salmonella* (Table 3), which was identified as *Salmonella* Yoruba.

In Company C, contamination by aerobic mesophilic was present in all the collected samples. Thirty-three samples were positive for *Enterobacteriaceae,* and in 68 % (27/40) coliforms were isolated. *E. coli* (0.7 log cfu cm^{-2}) was detected in only one sample, the floor of clean area. *Salmonella* was not isolated in the processing environment of Company C (Table 3).

4. Discussion

This is the first study carried out that has investigated *Enterobacteriaceae*, coliforms, *E. coli,* and *Salmonella* contamination throughout different peanut confectionery processing lines. Three plants that process different types of peanut products were surveyed. Compositions of the peanut confectionery products analyzed are presented in Table 1. Company A is a large company that manufactures coated peanuts. The main stages of crunchy coated peanuts manufacturing are peanut roasting at 150 °C/1 h, coating with a salty mixture (flour, salt, and spices), and heating at 90 °C. For candy coated peanuts manufacturing, a sweet coating (sugar syrup, pigments, or chocolate) is used. The average water activity (a_w) of crunchy and candy coated peanuts samples were 0.543 and 0.348, respectively (Table 1). Company B is a midsize company that processes peanut brittle and *paçoca* – a traditional Brazilian candy made of pressed peanut crumbs. For peanut brittle manufacturing, peanuts are heated with a caramel sauce to 75 °C/1 h. The average water activity of this product was 0.275 (Table 1). *Paçoca* does not suffer any thermal process after the peanut roasting step. Roasted peanuts are milled, mixed with sugar and salt, pressed, and packed. This product showed an average water activity of 0.299 (Table 1). Company C is a small company that processes two products with similar characteristics, *pé de moça* and *doce de amendoim*. Both products are heated at temperature around 110-120 °C for one hour. The main difference between these products is that whole peanut beans and condensed milk are used to manufacture *pé de moça*, whereas *doce de amendoim* is made with crushed peanuts, without addition of condensed milk. The average water activity is 0.643 for *pé de moça* and 0.746 for *doce de amendoim* (Table 1).

Salmonella and *E. coli* were not detected in any of the analyzed raw material and peanut confectionery products (Table 2). Therefore, they are in accordance with the microbiological criteria established by the Brazilian National Health Surveillance Agency (ANVISA) and ICMSF (ANVISA 2001, ICMSF 2011). However, the presence of both microorganisms has already been reported worldwide in peanut products (Eglezos *et al.* 2008; Bansal *et al.* 2010). Due to the absence of *Salmonella* and *E. coli*, the authors opted to use the contamination data of the indicator microorganisms to evaluate the hygienic condition of the products. *Enterobacteriaceae* was isolated in

5 % of the end products from Company A, in 25 % from Company B, and in 16 % from Company C (Table 2). In general, these microorganisms are not heat resistant; their presence in final products would indicate post-process contamination. However, the presence of high concentration of sucrose in the formulation of the great majority of peanut confectionery products raises an additional concern for food safety. This is because sucrose stands out among sugars as the greatest heat-protecting agent, supporting *Salmonella* thermal resistance (Corry 1974). According to Ma *et al.* (2009) 120 min at 90 °C were necessary to reduce 7.0 log cfu of *Salmonella* in peanut butter.

Raw material is a common means by which *Salmonella* enters the food supply chain. As previously mentioned, *Salmonella* and *E. coli* were not detected either in raw or roasted peanuts used as ingredients (Table 2). ICMSF (2011) recommends analysis of *Salmonella* only in raw material that does not suffer heat treatment before being used as ingredients in processing of confectionery products. *Enterobacteriaceae* and coliforms were isolated only from raw peanuts samples, with contamination rate of 65 % and 29 %, respectively. After the roasting phase, 100 % of samples were contamination free (Table 2). These results indicate that the roasting process was efficient to reduce the initial microbial load. Guidance from the U.S. Food and Drug Administration for peanuts and pistachios recommends validation of thermal processes in place to ensure 5.0 log reductions of *Salmonella* (FDA 2009a, 2009b). Therefore, the roasted peanuts used as raw material were not a direct contamination source to the final product. However, because only peanut raw materials were evaluated, it is not possible to rule out the possibility that some other ingredients played an important role in contaminating the end product.

In this current study, the manufacturing environment seemed to be the most probable critical point for the introduction of microbial contamination in peanut confectionery products. *Enterobacteriaceae* and coliforms with counts above 3.0 log cfu cm^{-2} in 19 % and 11 % of samples were detected (data not shown). In addition to the presence of *E. coli* and especially *Salmonella*, these data indicate serious failures in the hygiene control measures. Company B showed the most critical situation during the survey; four samples were positive for *E. coli*, and *Salmonella* was detected in one of them (Table 3). This pathogen was isolated from the floor nearby the *paçoca* mixer, which presents a potential risk for cross contamination of the final product and, hence, a risk to the consumer health, because *paçoca* does not undergo any kind of heat

treatment after the mixing step. The isolated strain was identified as *Salmonella* Yoruba; this is the first report in the literature on the presence of this serotype in peanut processing environment. Investigations of *Salmonella* outbreak associated with peanut products have indicated poor sanitation practices as the main source of this pathogen in the end product (CDC 2009, 2013). In the three manufacturing plants surveyed, wet cleaning was carried out daily as standard protocol to sanitize equipment and facilities. Moisture control is one of the most critical points to control the growth of pathogens in processing environment of low a_w food (ICMSF 2011), because the introduction of water in the facilities will promote microbial growth, increasing the contamination risk to the final product.

In conclusion, this study examined a significant number of samples throughout the peanut confectionery product manufacturing. The data of this surveillance may contribute to risk assessment studies and to establish more effective control measures. The results show that the processing environment should be considered a potential source of contamination of end product. The low incidence of *Salmonella* contamination found does not exclude the possibility of outbreaks in peanut confectionery products, since low infectious doses have been described in previously published reports. Therefore, *Salmonella* control in peanut products requires strict adherence to good manufacture practices (GMP), especially of hygiene and working practices.

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Conflict of interest

No conflict of interest declared.

References

Agência Nacional de Vigilância Sanitária (ANVISA) (2001) Resolução RDC nº 12, de 02 de janeiro de 2001. Aprova regulamento técnico sobre padrões microbiológicos para alimentos. *Diário Oficial*, Brasília, 10 jan. 2001. Seção 1.

Association of Official Analytical Chemists (AOAC) (2010) Rapid Methods Adopted as AOAC Official Methods[SM]. Available online: http://www.aoac.org/vmeth/oma_testkits.pdf. Accessed on 18.08. 2015.

Association of Official Analytical Chemists (AOAC) (2013) Rapid Methods Adopted as AOAC Official Methods[SM]. Available online: http://www.aoac.org/vmeth/oma_testkits.pdf. Accessed on 18.08. 2015.

Bansal, A., Jones, T.M., Abd, S.J., Danyluk, M.D., and Harris, L.J. (2010) Most-Probable-Number determination of *Salmonella* levels in naturally contaminated raw almonds using two samples preparation methods. *J Food Prot* 73, 1986-1992.

Beuchat, L.R. and Mann, D.A. (2010) Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. *J Food Prot 73*, 1257–1268.

Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M. and Kuile, B.H.T. (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. *J Food Prot 76*, 150-172.

Blodgett, R. (2010) Appendix 2: Most Probable Number from serial Dilutions. In Bacteriological Analytical Manual. Silver Spring: Food and Drug Administration. Available online: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm . Accessed on 18.08. 2015.

Burnett, S.L., Gehm, E.R., Weissinger, W.R. and Beuchat, L.R. (2000) Survival of *Salmonella* in peanut butter and peanut butter spread. *J Appl Microbiol 89*, 472-477.

Centers For Disease Control and Prevention (CDC) (2007) Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006–2007. *Morb Mortal Wkly Rep (MMWR) 56*, 521–524.

Centers For Disease Control and Prevention(CDC) (2009) Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. *Morb Mortal Wkly Rep (MMWR) 58*, 85-90.

Centers For Disease Control and Prevention (CDC) (2013) Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland. Available online: http://www.cdc.gov/salmonella/bredeney-09-12/index.html. Accessed on 3.08.2015.

Corry, J. (1974) The effect of sugars and polyols on the heat resistance of salmonellae. *J Appl Bacteriol 37*, 31-43.

Doyle, M.P. and Buchanan, R.L. (2013) *Salmonella* Species. In Food microbiology: fundamentals and frontiers 4th ed. pp. 225-261. Washington: American Society for Microbiology (ASM Press).

Eglezos, S., Huang, B. and Stuttard. E. (2008) A Survey of the Bacteriological Quality of Preroasted Peanut, Almond, Cashew, Hazelnut, and Brazil Nut Kernels Received into three Australian Nut-Processing Facilities over a Period of 3 Years. *J Food Prot 71*, 402-404.

Food and Drug Administration (FDA) (2009a) Recall of products containing peanut butter: *Salmonella* Typhimurium. Available online: http://www.fda.gov/oc/opacom/hottopics/salmonellatyph.html. Accessed on 3.08.15.

Food and Drug Administration (FDA) (2009b) Peanut Corporation of America (PCA), Blakely, GA:FDA form 483 (inspectional observations). Available online: http://www.fda.gov/ora/frequent/default.html. Accessed on 3.08.2015.

Grimont, P.A.D. and Weill, F.X. (2007) Antigenic formulae of the *Salmonella* serovars. In World Health Organization Collaborating Center for Reference and Research on *Salmonella* 9th ed. Paris: Institute Pasteur. Available online: http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf. Accessed on 19.08.2016.

Horn, B.W. and Dorner, J.W. (1999) Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. *Appl Environ Microbiol 65*, 1444-1449.

IAL - Instituto Adolfo Lutz (2005) Métodos Físico-Químicos para Análise de Alimentos: Normas Analíticas do Instituto Adolfo Lutz 4th ed. Brasilia: ANVISA.

International Commission on Microbiological Specifications for Foods (ICMSF) (1988) *Microrganisms in Foods 6 – Microbiological Ecology of Food Commodities*. Gaithersburg, Maryland: Aspen Publishers, Inc.

International Commission on Microbbiological Specifications for Foods (ICMSF) (2011) *Microrganisms in Foods 8 – Use of data for assessing process control and product acceptance.* New York: Springer.

International Organization for Standardization (ISO) 6887 – 1:1999 (1999) General rules for the preparation of the initial suspension and decimal dilutions. In Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Geneva: ISO.

International Organization for Standardization (ISO) 6579 – 1:2007 (2007) Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. Geneva: ISO.

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J.A., Ahmed, R., Middleton, D., Chandran, A. U., Harris, L.J., Howes, M., Chan, E., Pichette, A.S., Campbell, K., Gupta, A., Lior, L. Y., Pearce, M., Clark, C., Rodgers, F., Jamieson, F., Brophy, I. and Ellis, A. (2005)

An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J Food Prot 68*, 191–198.

Kataoka, A.I., Enache, E. and Black, D.G. (2014) Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. *J Food Prot 77*, 1252-1959.

Kirk M.D., Little, C.L., Lem, M., Fyfe, M., Genobile, D., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D., Lyi, H., Mcintyre, L., Ward, L, Brown, D.J., Surnam, S. and Fisher, I.S.T. (2004) An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – Sharing molecular information to solve international outbreak. *Epidemiol Infect 132*, 571-577.

Komitopoulou, E. and Peñaloza, W. (2009) Fate of *Salmonella* in dry confectionery raw materials. *J Appl Microbiol 106*, 1892–1900.

Kornacki, J.L. and Johnson, J.L. (2001) *Enterobacteriaceae*, coliforms and *Escherichia coli* as quality and safety indicatiors. In Compendium of *Methods for the Microbiological Examination of Foods* 4th ed. Downes, F.P. and Ito, K. pp.69-82. Washington: American Public Health Association (APHA).

Ma, L., Zhang, G., Gerner-Smidt, P., Matripragada, V., Ezeoke, I. and Doyle, M.P. (2009) Thermal inactivation of *Salmonella* in peanut butter. *J Food Prot 72*, 1596-1601.

Midura, T.F. and Bryant, R.G. (2001) Sampling plants, sample collection, shipment, and preparation for analysis. In *Compendium of Methods for the Microbiological Examination of Foods* 4th ed. Downes, F.P. and Ito, K. pp.13-23. Washington: American Public Health Association (APHA).

Nummer, B.A., Shrestha, S. and Smith, J.V. (2012) Survival of *Salmonella* in a high sugar, low water- activity, peanut butter flavored candy fondant. *Food Control 27*, 184–187.

Podolak, R., Enache, H., Stone, W., Black, D.G. and Elliot, P. (2010) Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low – Moisture Foods. *J Food Prot 73*, 1919-1936.

Reij, M.W., Den, A.E.D. and ILSI Europe Risk Analysis in Microbiology Task Force. (2004). Recontamination as a source of pathogens in processed foods. *Inter J Food Microbiol 91*, 1-11.

Schatzki, T.F. and Ong, M.S. (2001) Dependence of aflatoxin in almonds on the type and amount of insect damage. *J Agric Food Chem 49*, 4513-4519.

Scott, V.N., Chen, Y., Freier, T.A., Kuehm, J., Moorman, M., Meyer, J., Morille-Hinds T., Post L., Smoot L., Hood, S., Shebuski, J. and Banks, J. (2009) Control of *Salmonella* in low-moisture foods. I: minimizing entry of *Salmonella* into a processing facility. *Food Prot Trends 29*, 342-353.

Shachar, D. and Yaron, S. (2006) Heat tolerance of *Salmonella enterica* serovars

Agona, Enteritidis, and Typhimurium in peanut butter. *J Food Prot 69*, 2687–91.

Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H. and Van Leusden, F.M. (1976) Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. *The J Hyg 76* (1), 41-47.

Thomas, H.A. (1942) Bacterial densities from fermentation tube tests. *J Am Water Works Assoc* 34, 572-576.

Woodroof, J.G. (1983) *Peanut: production, processing, products* 3rd ed. Westport: The Avi Publishing Company.

Table 1 Composition of different peanut confectionary products collected from processing plants in Brazil

Company		Sample	a_w*	pH*	Nutritional information (in 100g)**			
					Total Fat (g)	Sodium (mg)	Carbohydrate (g)	Protein (g)
A	Raw Material	Raw peanuts	0.550 ± 0.034^{b}	6.5 ± 0.2^{a}	43.9	-	20.3	27.2
	End product	Candy coated peanuts	0.543 ± 0.069^{C}	5.9 ± 0.5^{B}	10.0	66.4	78.0	7.6
		Crunchy coated peanuts	0.348 ± 0.061^{D}	6.4 ± 0.1^{A}	26.4	800.0	42.0	19.2
B	Raw Material	Raw peanuts	0.534 ± 0.030^{b}	6.3 ± 0.6^{a}	43.9	-	20.3	27.2
		Roasted peanuts	0.267 ± 0.067^{a}	6.6 ± 0.5^{a}	54.0	-	18.7	22.5
	End product	*Paçoca*	0.299 ± 0.085^{D}	6.3 ± 0.6^{B}	23.8	125.0	54.4	18.8
		Peanut brittle	0.275 ± 0.021^{D}	6.2 ± 0.6^{B}	31.9	13.1	40.6	20.0
C	Raw Material	Raw peanuts	0.641 ± 0.054^{a}	6.8 ± 0.0^{a}	43.9	-	20.3	27.2
		Roasted peanuts	0.316 ± 0.022^{a}	6.7 ± 0.3^{a}	54.0	-	18.7	22.5
	End product	*Pé de moça*	0.643 ± 0.120^{B}	6.5 ± 0.2^{A}	15.2	12.0	44.0	12.0
		Doce de amendoim	0.746 ± 0.022^{A}	6.6 ± 0.1^{A}	17.7	91.4	62.9	9.4

* Media value determined at our laboratory. ** Values are listed on the product label.

a,b,c: Values marked with different lower case letters in the same column are significantly different ($p<0.05$).

A,B,C: Values marked with different upper case letters in the same column are significantly different ($p<0.05$).

Table 2 Analysis results of raw material and peanut confectionery products collected from processing plants in Brazil

Company		Sample	No. of sample	*Enterobacteriaceae* (log cfu g^{-1})*		Total coliform (log cfu g^{-1})*		*E. coli* (log cfu g^{-1})*		*Salmonella* (MPN g^{-1})**	
				No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)
A	Raw Material	Raw peanuts	10	6 (60)	1.0 to 2.0 (1.4)^{b}	2 (20)	1.0 to 1.3 (1.2)^{a}	0	-	0	-
	End product	Candy coated peanuts	10	0	-	0	-	0	-	0	-
		Crunchy coated peanuts	10	1 (10)	1.7^{A}	0	-	0	-	0	-
B	Raw Material	Raw peanuts	3	3 (100)	3.0 to 3.2 (3.1)^{a}	1 (33)	1.7 ^{a}	0	-	0	-
		Roasted peanuts	7	0	-	0	-	0	-	0	-
	End product	*Paçoca*	10	3 (30)	1.5 to 1.6 (1.6)^{A}	0	-	0	-	0	-
		Peanut brittle	10	2 (20)	1.0 to 1.3 (1.2)^{A}	0	-	0	-	0	-
C	Raw Material	Raw peanuts	4	2 (50)	2.8 to 3.6 (3.2)^{a}	2 (50)	2.3 to 2.9 (2.6) ^{a}	0	-	0	-
		Roasted peanuts	6	0	-	0	-	0	-	0	-
	End product	*Pé de moça*	9	0	-	0	-	0	-	0	-
		Doce de amendoim	10	3 (30)	1.3 to 1.5 (1.4)^{A}	1 (10)	1.3^{a}	0	-	0	-

*cfu g^{-1} - Colony Forming Unit per gram of sample. **MPN g^{-1} – Most Probable Number per gram.

a,b,c: Values marked with different lower case letters in the same column are significantly different ($p<0.05$).

A,B,C: Values marked with different upper case letters in the same column are significantly different ($p<0.05$).

Table 3 Analysis results of environment in processing plants of peanut confectionery products in Brazil

Company	Sample	No of samples	Aerobic plate count (log cfu cm^{-2} or mL^{-1})*		*Enterobacteriaceae* (log cfu cm^{-2} or mL^{-1})*		Total coliform (log cfu cm^{-2} or mL^{-1})*		*E. coli* (log cfu cm^{-2} or mL^{-1})*		*Salmonella* (in 250 cm^2 or mL)
			No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)	No. of positive (%)	Count in positives (mean)	No. of positive (%)
A	Equipment	19	19 (100)	0.3 to 8.5 (3.3)[a]	10 (53)	0.0 to 6.6 (2.5)[a]	8 (42)	0.0 to 5.9 (1.9)[a]	1 (5)	2.3	0
	Utensils	10	10 (100)	0.3 to 8.5 (3.2)[a]	4 (40)	1.8 to 3.3 (2.9)[a]	3 (30)	0.0 to 2.4 (1.3)[b]	0	-	0
	Facilities	9	9 (100)	1.9 to 8.5 (5.0)[a]	8 (89)	0.7 to 5.8 (3.0)[a]	8 (89)	0.3 to 5.7 (2.4)[a]	0	-	0
B	Equipment	18	18 (100)	1.0 to 4.6 (2.4)[a]	11 (61)	0.0 to 4.1 (1.7)[a]	5 (28)	0.5 to 3.0 (1.4)[a]	0	-	0
	Utensils	11	11 (100)	0.3 to 5.0 (2.6)[a]	7 (64)	0.0 to 4.5 (2.3)[a]	3 (27)	2.3 to 4.2 (3.1)[a]	0	-	0
	Facilities	9	9 (100)	5.1 to 7.8 (6.3)[a]	9 (100)	1.8 to 5.8 (4.0)[a]	9 (100)	2.3 to 5.0 (3.7)[a]	4 (44)	0.0 to 2.3 (0.9)[a]	1 (11)
C	Equipment	18	18 (100)	1.2 to 5.8 (2.6)[a]	14 (78)	0.0 to 3.0 (1.6)[a]	12 (67)	0.0 to 2.8 (1.2)[a]	0	-	0
	Utensils	15	15 (100)	0.3 to 3.9 (2.1)[a]	12 (80)	0.0 to 2.3 (1.2)[a]	8 (53)	0.0 to 2.3 (1.0)[b]	0	-	0
	Facilities	7	7 (100)	3.2 to 8.2 (5.1)[a]	7 (100)	2.0 to 7.0 (3.7)[a]	7 (100)	1.7 to 7.1 (2.7)[a]	1 (14)	0.7[a]	0

*cfu - Colony Forming Unit.

a,b,c: Means with different letters in the same type of sample are significantly different ($p<0.05$).

CAPÍTULO 3

Long-term kinetics of Salmonella Typhimurium ATCC 14028 survival on peanuts and peanut confectionery products

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Abstract

This study evaluated the long-term kinetics of *Salmonella* survival on different peanut products under storage at 28 ºC for 420 days. Samples of roasted peanuts (a_w=0.39), shelled peanuts (a_w=0.54), in-shell peanuts (a_w=0.29), peanut brittle (a_w=0.30), *paçoca* (a_w=0.40), and *pé-de-moça* (a_w=0.68) were inoculated with *Salmonella* Typhimurium ATCC 14028 at two inoculum levels (3 and 6 log cfu/ g). In most cases for both inoculum levels, the greatest reductions were seen after the first two weeks of storage, followed by a slower decline phase. The Weibull model provided a satisfactory fit for the data, with δ value ranging from 0.38 to 148.82 days. The lowest reductions were verified in *paçoca* and roasted peanuts, with counts of 1.01 and 0.87 log cfu/ g in low inoculum level and 2.53 and 3.82 log cfu/ g in high inoculum level after 420 days of storage. The greatest reductions were observed in *pé-de-moça*, with counts below the limit of detection after 21 and 60 days in low and high inoculum level, respectively. Therefore, the results of this study indicate that *Salmonella* survives longer in peanut products with a_w around 0.40.

Keywords: *Salmonella*, peanuts, food safety, low moisture food, storage.

1 Introduction

Traditionally low moisture foods, such as peanuts, have been considered as low risk for foodborne illness. Despite this, *Salmonella* outbreaks linked to this kind of product have been reported worldwide. Nine salmonellosis outbreaks in peanuts or peanut products have been reported in the literature since 1994, with a total of 1791 cases and 10 deaths (CDC, 2007, 2009, 2012; Killalea et al., 1996; Kirk et al., 2004; Scheil et al., 1998). The most recent outbreaks occurred in the USA, associated with peanut butter. The last report was in 2014 and had six cases (CDC, 2014).

Peanuts may become contaminated by *Salmonella* at any point throughout the supply chain (do Nascimento et al. unpublished data). In primary production, soil, water, insects, birds, handlers and equipment are possible sources of contamination (Mattick et al., 2000). After heat treatment, the principal causes of cross-contamination are environmental processing and handling (Kimber et al., 2012; Carminati et al., 2016).

The shelf life of peanuts and peanut confectionary products range from six months to one year or more, depending on the composition of the food and the storage condition. Although *Salmonella* does not grow in water activity (a_w) below 0.94, it can often persist for long periods in low moisture foods and in a dry processing environment (Beuchat et al., 2013). This characteristic may be one of the main factors that contribute to the occurrence of outbreaks in products with low a_w. Burnett et al. (2000) noted the presence of *Salmonella* in peanut butter for at least 168 days at 5 and 21 ºC. Uesugi et al. (2006) observed the presence of *S.* Enteritidis in samples of shelled almonds after 550 days of storage. In addition, *Salmonella* was recovered for at least 365 days on pecans, walnut kernels, almonds, and pistachios during storage at different temperatures (Beuchat and Mann, 2010; Blessington et al., 2012; Kimber et al. 2012).

It is known that osmotic stress caused by low a_w promotes the activation of defense mechanisms in the microbial cell, such as the production of solutes (proline, trehalose and glutamine). The increase in the intracellular concentration of these compounds results in higher thermal and osmotic resistance (Cánovas et al., 2001). Ma et al. (2009) detected *Salmonella* in peanut butter even after treatment at 90 ºC for 30 min. Furthermore, the high fat content

of peanuts also protects the pathogen against gastric acidity, resulting in a reduction dose-response curve with a low infectious dose. A very small number of *Salmonella* viable cells (0.5 to 5 MPN/g) was associated with an outbreak caused by *Salmonella* in almonds (Danyluk et al., 2007).

Therefore, for the adoption of preventive or control measure and for the implementation of risk management strategies, more studies are necessary to know the behavior of the microorganism in this product category. However, there is no data published on *Salmonella* survivability in peanut confectionary products, with exception of peanut butter, and only one study on peanut kernels (Brar et al, 2015). This is the first study that evaluated the survival of *Salmonella* Typhimurium ATCC 14028 during long-term storage of different kind of peanut based products.

2 Material and methods

2.1 Peanut-based products

Six types of peanut-based products obtained in the Brazilian retail market were used: roasted peanuts (a_w=0.39), unblanched peanut kernels (a_w=0.54), raw in-shell peanuts (a_w=0.29) and peanut brittle (a_w=0.30), *paçoca* (a_w=0.42) and *pé-de-moça* (a_w=0.68). All samples used were previously tested for *Salmonella*.

The peanut confectionary products used for this study are traditionally consumed in Brazil. For peanut brittle manufacturing, peanuts are heated with a caramel sauce to 75 °C for 1 h. *Paçoca* is a candy made of ground peanuts that is milled, mixed with sugar and salt, pressed and packed. *Pé-de-moça* is prepared with whole peanut kernels and condensed milk, which is heated at 110-120 °C for 1 h.

2.2 *Salmonella* strain and Inoculum preparation

The strain used as inoculum was *Salmonella* Typhimurium ATCC 14028. It was stored at -80 ºC in trypticase soy broth (TSB, Difco, MD, USA)

supplemented with 15% glycerol. *Salmonella* strain was cultivated twice in trypticase soy broth (TSB) at 37 ºC for 18-24 h. The culture was streaked on trypticase soy agar (TSA, Difco, MD, USA) and incubated at 37 °C for 24 h. The inoculum was prepared by transferring a cell culture loop to 0.85 % saline tubes up to a turbidity of 5.0 MacFarland scale (10^9 cells/ml). Then, decimal dilutions were performed in 0.1% peptone water (Difco, MD, USA) and cell numbers in each suspension were determined by plating appropriate dilutions on TSA.

2.3 Inoculation of the samples

Initially, the samples were divided into 500 g portions and the samples of confectionary products were crushed. Then, the samples were inoculated by spraying with 0.2% (1 ml) of *Salmonella* suspension, plus 2% Tween 80 (Merck, DA, Germany) to reduce the surface tension (do Nascimento et al., 2012). The initial concentration of the inoculum in the samples was *ca.* 3 log cfu/ g (low inoculum level) and 6 log cfu/g (high inoculum level). After homogenization by hand for 2 min, the samples were transferred to aluminum screen trays and kept in a biosafety cabinet (Vecco, Brazil) for 10 to 20 min, with the purpose of ensuring a maximum adherence of the inoculum and bring back the a_w closer to the original level (do Nascimento et al., 2012). Three 10g-portions from different locations in the same bag of each inoculated sample were taken to confirm the uniformity of the initial inoculum level. The inoculated samples were transferred to sterile bags. Peanut brittle and raw in-shell peanuts were stored in desiccators containing saturated solution of magnesium chloride (a_w=0.32), and *paçoca* and roasted peanuts in desiccators containing saturated solution of potassium carbonate (a_w=0.42) in order to keep the a_w stable throughout the storage. *Pé-de-moça* and unblanched peanut kernels and the desiccators containing the other products were stored in BOD at 28 ± 1 °C for 420 days.

For both experiments, the *Salmonella* count was determined after 0, 7, 14, 21, 28, 45, 60 days, and then every 30 days for up to 420 days. Temperature and humidity of the BOD were monitored throughout the storage by a Testo 615 portable thermometer (Testo, Germany). The experiments were replicated four times.

2.4 Enumeration of *Salmonella*

The *Salmonella* enumeration was performed by plate counting method. At each time point, portions of each inoculated sample were taken from different locations in the bag to totalize 11 g and homogenized by hand for 2 min with 99 ml of Buffered Peptone Water (BPW, Acumedia, MI, EUA). Then the samples were maintained at room temperature for 60 min to repair injured cells, but without favoring the growth. After, 10 ml of this first dilution were used to prepare subsequent serial dilutions in 0.1% peptone. Specific volume of each dilution (3x0.25 or 0.1 ml) were spread-plated onto Xylose Lysine Deoxycholate agar (XLD, Acumedia, MI, USA) and incubated at 37 ºC for 24 h. When the counts were close to 10 cfu/g, 10 ml of the first dilution were pour-plated onto XLD (2.5 ml per plate) to improve the limit of detection (1 cfu/g). Presumptive-positive colonies were subjected to confirmation by biochemical and serological tests (ISO, 2007). The results were expressed in log of colony forming unit (cfu) per gram. The remaining volume of the first dilution (100 ml) was also incubated at 37 ºC for 24 h (pre-enrichment step). When counts decreased to below the limit of detection, this pre-enrichment broth was used to determine the presence/absence of *Salmonella* in 10 g, according to ISO 6579 method (ISO, 2007).

In preliminary studies the *Salmonella* count was carried out on TSA and XLD agar. The results showed that there was no significant difference ($p>0.05$) between TSA and XLD agar performance. Therefore, to avoid the interference of the background microflora in *Salmonella* determination throughout the storage time, XLD agar was used for all the subsequent experiments.

2.5 Water activity

Water activity of the uninoculated and inoculated samples was measured in duplicate at 25 °C with a water activity meter - hygrometer (Aqualab CX2, Decagon Device, Pullman, WA).

2.6 Chemical composition

The chemical composition of peanuts and peanut confectionary products was carried out in triplicate using milled samples. Moisture content was determined in an oven at 105 °C to constant weight (IAL, 2005). The Kjeldahl method was used to estimate the nitrogen content and the result was converted to protein percentage by using the conversion factor 5.76 (Horwitz, 1984). The fat content was determined according to the method of Bligh and Dyer (1959). Ash analysis was carried out in muffle at 550 °C for 8 hours (IAL, 2005). Carbohydrate content was calculated by the difference between total dry matter and contents of ash, fat and protein, and the results were expressed as percent dry weight.

2.7 Modeling and statistical analyses

Geeraerd and Van Impe Inactivation Model Fitting Tool (GinaFiT, Excel version 2010) was used to fit the Weibull model to the data (Mafart et al., 2002):

$$\log\left(\frac{N}{No}\right) = -\left(\frac{t}{\delta}\right)^{p}$$

where N is the population at time t (cfu/g), N_0 is the population at time 0 (cfu/g), t is the time (days), δ is the time required for first decimal reduction (days) and p is a fitting parameter that defines the shape of the curve (dimensionless). Mean sum of squared error (MSE) and regression coefficient (R^2) were used to access appropriateness of fit. T_{3d} was also calculated based on Weibull model.

The data were analyzed with one-way analysis of variance (ANOVA) and Tukey test to determine whether there were significant differences ($p<0.05$) on *Salmonella* population using SAS software (version 9.4, SAS Institute, Cary, NC).

3 Results and Discussion

In this study *Salmonella* survivability in roasted peanuts, unblanched peanut kernels and raw peanuts in-shell, and three peanut confectionary products widely consumed in Brazil (peanut brittle, *paçoca* and *pé-de-moça*) was evaluated. Storage was performed at 28 ± 1 °C for 420 days, which is longer than the shelf life of these products. The temperature chosen for the study was based on meteorological data indicating that this is the average temperature of the spring and summer months in most of the country (INMET, 2016).

3.1 Water activity of the samples during storage

There was no significant difference ($p > 0.05$) among the samples stored outside the desiccators (*pé-de-moça* and unblanched peanut kernels) and the samples stored inside the desiccators. In the experiment using low inoculum level, the media a_w value was 0.42 ± 0.01 for roasted peanuts, 0.53 ± 0.04 for unblanched peanut kernels, 0.31 ±0.02 for raw peanuts in-shell, 0.31 ± 0.02 for peanut brittle, 0.41 ± 0.01 for *paçoca* and 0.69 ± 0.02 for *pé-de-moça*. In the experiment using high inoculum level, the media a_w values were 0.41 ± 0.02, 0.53 ± 0.03, 0.31 ± 0.02, 0.32 ± 0.02, 0.42 ± 0.01, 0.68 ± 0.04 for roasted peanuts, unblanched peanut kernels, raw peanuts in-shell, peanut brittle, *paçoca* and *pé-de-moça*, respectively. The variation of a_w observed over time occurred mainly due to the opening of the BOD and the desiccators to collect samples for *Salmonella* enumeration.

3.2 Influence of inoculum level

During most of the long-term storage no significant difference ($p>0.05$) was noted in the reduction of *Salmonella* counts between the two inoculum levels (3 and 6 log cfu/g). Significant differences ($p<0.05$) were observed only in raw in-shell peanuts at 7 days, peanut brittle at 7, 14 and 45 days and *paçoca* at 14 days (data not shown). It corroborates with other reports that did not obtain a difference in *Salmonella* reduction using different inoculum levels (1 to 10 log cfu/g) during

short or long storage of almonds (Kimber et al., 2012; Uesugi et al., 2006), walnut kernels (Blessington et al., 2012) and peanut kernels (Brar et al., 2015). In contrast, greater declines were observed using low inoculum level (3 log) on pecan halves and pieces (Beuchat and Mann, 2010).

3.3 Fate of *Salmonella* during long-term storage of peanut-based products

Figure 1 shows the reduction of *Salmonella* artificially inoculated in different peanut products during storage for 420 days at 28 °C. The results indicate that a_w significantly influenced ($p<0.05$) the survival of *Salmonella* in peanut or peanut confectionary products. The highest survival rate was seen in a_w around 0.40 (roasted peanuts and *paçoca*), followed by 0.54 (unblanched peanut kernels) and 0.30 (raw peanuts in-shell and peanut brittle). Greater rate of decline was observed at the highest a_w (0.68 - *pé-de-moça*). Similar findings were reported by Juven et al. (1984) who evaluated the survival of *S.* Montevideo in different products. They found a greater reduction in the pathogen population at a_w 0.75 when compared to 0.52 and 0.43. Kataoka et al. (2014) when inoculated *S.* Typhimurium in peanut butter with the same percentage of fat, but with different a_w (0.30 and 0.60) detected the pathogen only in the product with the lowest a_w. The lipid and carbohydrate content did not directly affect the behavior of *Salmonella* during the storage of the peanut based products. Since products with similar aw and different lipid and carbohydrate contents (peanut brittle vs. raw peanuts in shell and *paçoca* vs. roasted peanuts) most of the time did not show significant difference ($p > 0.05$) in the *Salmonella* reduction rate (Table 1).

For peanut samples, in experiment 1, after 28 days of storage, reductions of 0.7, 1.5 and 3.4 log cfu/g were obtained for roasted peanuts, unblanched peanut kernels and raw peanuts in-shell, respectively. After that, raw in-shell peanuts followed the trend of greater loss of *Salmonella* viability; from 240 days onwards, the pathogen was only recovered in this product by enrichment of 10-g samples. The same fact was verified for unblanched peanut kernels after 330 days. At the end of the storage time, the lowest reduction in *Salmonella* counts was found in roasted peanuts, 3.4 log cfu/g (Figure 1).

However, no significant difference ($p>0.05$) was observed between counts in roasted peanuts and unblanched peanut kernels until the 90th day. In experiment 2 (high inoculum level), reduction of 1-log was observed after less than 7 days in raw in-shell peanuts, 21 days in unblanched peanut kernels and 120 days in roasted peanuts. After 420 days, reductions of 2.6, 4.6 and 6.1 log cfu/g were observed for the roasted peanuts, unblanched peanut kernels and raw peanuts in-shell, respectively (Figure 1). Blessington et al. (2012) noted reduction in *Salmonella* count of *ca.* 5 log cfu/g after 1.5 years in walnut kernels stored at room temperature. Slow but significant reductions have been reported for nuts stored at room temperature (Brar et al., 2015; Beuchat and Mann, 2010; Kimber et al. 2012). Uesugi et al. (2006) studied the survival of *S.* Enteritidis PT30 in almonds stored at 35 °C. The authors observed a reduction of 1.1 log cfu/ g between 0 and 59 days, whereas no reduction was noted between 59 and 171 days. *Salmonella* levels remained above the limit of detection for 12 months on almonds and pistachios (Kimber et al., 2012) and peanuts and pecans (Brar et al., 2015) stored at 24 and 22 ºC, respectively.

In regard to confectionary products, in experiment 1, counts of *Salmonella* decreased sharply in peanut brittle and *pé-de-moça*, and were significantly different ($p<0.05$) from counts obtained in *paçoca*. *Salmonella* population approached the limit of detection (1 cfu/ g) after 21 days in *pé-de-moça* and 60 days in peanut brittle. From 180 days onwards, the pathogen could not be recovered by enrichment from any of the four 10g-samples analyzed for *pé-de-moça* (Table 2). At the end of the storage, only 50 % of the sample of peanut brittle had viable cells after enrichment. Meanwhile, in *paçoca Salmonella* population remained above the limit of detection throughout the study (Figure 1). It is a period higher than that reported by Burnett et al. (2000) who observed viability of *Salmonella* in peanut butter for 6 weeks at 21 °C. These authors observed a rapid decline in counts in the first 24-36 h after inoculation and a gradual slow decline after one week, with reduction of 4.1 to 4.5 log cfu/g after 24 weeks. In experiment 2, *pé-de-moça* showed the greatest reduction, with counts below the limit of detection from the 60th day. However, the pathogen remained viable in at least 50 % of the samples of this product until 420 days. *Paçoca* had the lowest *Salmonella* rate of decline: 1.1 and 3.1 log cfu/ g after 14 and 420

days, respectively. After these same periods, reductions of 1.6 and 5.8 log cfu/g were observed in peanut brittle (Figure 1). Park et al. (2008) reported reductions of *Salmonella* in peanut butter (a_w *ca.* 0.2) between 0.3 and 1.3 log cfu/ g after 14 days at 22 °C.

The shelf life declared on the manufacturers' labels ranges between 180 and 360 days at room temperature. According to the results *Salmonella* viability extended beyond the shelf life of the products investigated. The only exception was observed for *pé-de-moça* (a_w 0.68) when inoculated with low inoculum level (Table 2). These results bring up a concern from a public health point of view, since the presence of *Salmonella* even at low levels (1 cfu/g) over the shelf life of these products could cause illness (Kirk, 2004; Scheil, 1998).

3.4 Weibull model

Salmonella survival curves obtained during storage of peanuts and peanut confectionary products are shown in Figure 1. Weibull model includes data up to the point the samples fell below the limit of detection (1 cfu/g, minimum of six points). The model fitted well the data ($r^2 \geq 0.889$), which indicates that Weibull model was appropriate to describe the survival kinetic of *Salmonella* in peanuts and confectionary products (Table 3).

Increasing a_w to 0.68 or decreasing to around 0.30 resulted in curves with a more pronounced concavity (Figure 1). A similar behavior was noted in different kind of nuts and confectionary products (Beuchat and Mann, 2010; Blessington et al., 2012; Burnett et al., 2000; Keller et al., 2012). On the other hand, for roasted peanuts, which has a_w of 0.40, a log-linear decline rate was observed. It was also reported for almonds and pistachios stored at 24 ºC, 0.2 and 0.15 log cfu/g/month (Kimber et al., 2012), and raw peanut kernels stored at 22 ºC, 0.22 log cfu/g/month (Brar et al., 2015). However, Uesugi et al. (2006) observed a biphasic survival curve for *Salmonella* survival during storage of almonds at 35 ºC.

According to Mossel and Koopman (1965) and Tamminga et al. (1976) a rapid decline in bacteria counts shortly after inoculation can be caused by

osmotic shock. The remaining cells would be able to adapt to the inhospitable environment, creating conditions to persist for long time in low moisture products.

The predicted data are consistent with the results obtained in the experiments. The time required for the first decimal reduction (delta) ranged from 0.38 (*pé-de-moça*) to 148.82 days (roasted peanuts). The Weibull model was also used to calculate t_{3d} values of *Salmonella* in peanut products (Table 3). The predicted data indicate that it would take 450 to 843 days to reach three decimal reductions of *Salmonella* in *paçoca* or roasted peanuts. On the other hand, for *pe-de-moça* it would happen after 10 days of storage at 28 °C. Intermediate values were found for the other samples: around 30 days for peanut brittle, between 51 and 137 days for raw in-shell peanuts and 200 days for unblanched peanut kernels.

4 Conclusion

The long-term *Salmonella* survivability in and peanuts and peanut-based products was influenced by the water activity. For both inoculum levels analyzed, the lowest reductions were verified in roasted peanuts and *paçoca*, between 2 and 3.4 log cfu/g. These data can be used to risk assessment studies on *Salmonella* in peanuts and peanut confectionary products.

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References

Beuchat, L.R., Komitopoulou, E., Beckers, H., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M., Ter Kuile, B.H., 2013. Low-water activity foods: increased concern as vehicles of foodborne pathogens. J. Food Prot. 76, 150-172.

Beuchat, L.R., Mann, D.A., 2010. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. J. Food Prot. 73, 1257-1268.

Blessington, T., Mitcham, E.J., Harris, L.J., 2012. Survival of *Salmonella enterica, Escherichia coli 0157:H7,* and *Listeria monocytogenes* on inoculated walnut kernels during storage. J. Food Prot. 75, 245 – 254.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911 – 917.

Brar, P.K., Proano, L.G., Friedrich, L.M., Harris, L.J., Danyluk, M.D., 2015. Survival of *Salmonella, Escherichia coli 0157:H7*, and *Listeria monocytogenes* on raw peanuts and pecan kernels stored at -24, 4, and 22 °C. J. Food Prot. 78, 323 – 332.

Burnett, S.L., Gehm, E.R., Weissinger, W.R., Beuchat, L.R., 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. J. Appl. Microbiol. 89, 472-477.

Cánovas, D., Fletcher, S.A., Hayashi, M., Csonka, L., 2001. Role of trehalose in growth at high temperature of *Salmonella enterica* Serovar Typhimurium. J. Bacteriol. 183, 3365 – 3371.

Carminati, J. de A., Amorim Neto, D.P., Morishita, K.N., Takano, L.V., Bernardi, A.O., Copetti, M.V., do Nascimento, M. da S., 2016. Microbiological contamination in peanut confectionery processing plants. J. Appl. Microbiol. 121, 1071- 1078.

CDC - Center For Disease Control and Prevention, 2007. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006-2007. MMWR Report, 56, 521-524.

CDC - Center For Disease Control and Prevention, 2009. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. MMWR Report, v. 58, p. 85-90.

CDC - Center For Disease Control and Prevention, 2012. Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland.. http://www.cdc.gov/salmonella/bredeney-09-12/index.html (accessed 20.07.16).

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of *Salmonella* Braenderup Infections Linked to Nut Butter Manufactured by Spired Natural Foods. 2014. http://www.cdc.gov/*Salmonella*/braenderup-08-14/ (accessed 20.07.16).

Danyluk, M.D., Jones, T.M., Adb, S.J., Schlitt – Dittrich, F., Jacobs, M., Harris, L.J., 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. J. Food Prot. 70, 820–827.

Do Nascimento, M. da S. do, Brum, D.M., Pena, P.O., Berto, M.I., Efraim, P., 2012. Inactivation of *Salmonella* during cocoa roasting and chocolate conching. Int. J. Food Microbiol**.** 159, 225–229.

Horwitz, W., 2006. Kjeldahl method, method no. 2001.14, in: Official Methods of Analysis of the Association of Official Analytical Chemists, eighteenth ed. AOAC International, Gaitherburg. pp. 72-73.

IAL - Instituto Adolfo Lutz. 2005. Métodos Físico-Químicos para Análise de Alimentos: Normas Analíticas do Instituto Adolfo Lutz. fourth ed. ANVISA, Brasília.

INMET – Instituto Nacional de Meteorologia. Estações Automáticas – Gráficos. 2016. http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_auto_graf (accessed 16.07.16).

ISO - International Organization for Standardization 6579 – 1:2007, 2007. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. Geneva: ISO.

Juven, B.J., Cox, N.A., Bailey, J.S., Thomson, J.E., Charles, O.W., Shutze, J.V., 1984. Survival of *Salmonella* in dry food and feed. J. Food Prot. 47, 445-44.

Kataoka, A.I., Enache, E., Black, D.G., 2014. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. J. Food Prot. 77, 1252-1959.

Keller, S., Grasso, E., Halik, L., Fleischman, G., Chirtel, S., Grove, S., 2012. Effect of growth on the thermal resistance and survival of *Salmonella* Tennessee and Oranienburg in peanut butter, measured by a new thin-layer thermal death time device. J. Food Prot. 75, 1125 - 1130.

Killalea, D., Ward, L.R., Roberts, D., de Louvois ,J., Sufi ,F., Stuart, J.M., Wall, P.G., Susman, M., Schwieger, M.P., Sanderson, J.I., Fisher, S.T., Mead, P.S., Gill, O.N. Bartlett, C.L.R., Rowe, B, 1996. International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack—I: England and Wales and the United States. BMJ. 313, 1105–1107.

Kimber, M.A., Kaur, H., Wang, L., Danyluk, M.D., Harris, L.J., 2012. Survival of *Salmonella, Escherichia coli 0157:H7,* and *Listeria monocytogenes* on inoculated almonds and pistachios stored at – 19, 4, and 24 °C. J. Food Prot. 75, 1394 – 1403.

Kirk, M.D., Little, C.L., Lem, M., Fyfe, M., Genobile, D., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D., Lyi, H., Mcintyre, L., Ward, L., Brown, D.J., Surnam, S., Fisher, I.S.T., 2004. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – Sharing moelcular information to solve international outbreak. Epidemiol. Infect. 132, 571-577.

Ma, L., Zhang, G., Gerner-Smidt, P., 2009. Thermal inactivation of *Salmonella* in peanut butter. J. Food Prot. 72, 1596-1601.

Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. Int. J. Food Microbiol. 72, 107 -113.

Mattick, K.L., Jorgensen, F., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2000. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl. Environ. Microbiol. 66, 4921–25.

Mossel, D.A.A., Koopman, M.J., 1965. Losses in viable cells of salmonellae upon inoculation into dry animal feeds of various types. Poult. Sci. 44, 890-892.

Park, E.J., Oh, S.W., Kang, D.H., 2008. Fate of *Salmonella* Tennessee in Peanut Butter at 4 and 22 ºC. J. Food Sci. 73, 82-86.

Scheil, W., Cameron, S., Dalton, C., Murray, C., Wilson, D., 1998. A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. Aust N Z J Public Health. 22, 536–539.

Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H., van Leusden, F.M., 1976. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. J. Hyg-Cambridge. 76, 41-47.

Uesugi, A.R., Danyluk, M.D., Harris, L.J., 2006. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23 and 35ºC. J. Food Prot. 69,1851-1857.

Table 1 Effect of maturity on chemical composition of peanuts.

Sample	pH	Moisture content (%)	Dry Basis (%)			
			Oil	Protein	Ash	Carbohydrates
Peanut brittle	6.52 ± 0.01 ^{E}	3.41 ± 0.13 ^{C}	35.45 ± 0.13 ^{D}	17.47 ± 0.16 ^{D}	1.43 ± 0.17 ^{C}	42.24 ± 0.17 ^{C}
Paçoca	6.40 ± 0.00 ^{F}	1.60 ± 0.08 ^{D}	27.24 ± 1.04 ^{E}	11.35 ± 0.30 ^{E}	1.67 ± 0.11 ^{C}	58.14 ± 1.36 ^{A}
Pé-de-moça	6.65 ± 0.00 ^{C}	8.88 ± 0.13 ^{A}	25.68 ± 0.70 ^{E}	11.90 ± 0.64 ^{E}	1.56 ± 0.05 ^{C}	51.98 ± 0.48 ^{B}
Roasted peanut	7.13 ± 0.00 ^{A}	1.57 ± 0.20 ^{D}	55.01 ± 0.71 ^{A}	26.83 ± 0.41 ^{A}	2.30 ± 0.03 ^{B}	14.29 ± 0.85 ^{F}
Unblanched peanut kernell	6.79 ± 0.00 ^{B}	5.35 ± 0.05 ^{B}	47.88 ± 0.25 ^{C}	25.11 ± 0.30 ^{B}	2.14 ± 0.04 ^{B}	19.52 ± 0.37 ^{E}
Raw in-shell peanuts	6.57 ± 0.00 ^{D}	1.55 ± 0.17 ^{D}	51.33 ± 0.52 ^{B}	20.36 ± 0.22 ^{C}	2.59 ± 0.07 ^{A}	24.17 ± 0.30 ^{D}

Means in column followed by common superscript not significantly different (P > 0.05).

Table 2 Frequency of positive samples for *Salmonella* below the limit of detection.

Inoculum level	Sample	Nº of positive samples (time in days)																	
		7	14	21	28	45	60	90	120	150	180	210	240	270	300	330	360	390	420
3 log	Peanut brittle			1 (1)	3 (3)	2 (2)	4 (4)	4 (3)	4 (4)	4 (3)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
	Paçoca																		
	Pé-de-moça			4 (4)	4 (3)	4 (2)	4 (1)	4 (1)	4 (0)	4 (2)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)
	Roasted peanut																		
	Unblanched peanut kernell												1 (1)	1 (1)	3 (3)	4 (4)	4 (4)	4 (4)	4 (4)
	Raw in-shell peanuts						1 (1)	2 (2)	1 (1)	3 (3)	4 (3)	4 (3)	4 (4)	4 (4)	4 (4)	4 (4)	4 (1)	4 (4)	4 (4)
6 log	Peanut brittle											1(1)		1 (1)	1 (1)	1 (1)	1 (0)	1 (0)	1 (0)
	Paçoca																		
	Pé-de-moça					3 (3)	4 (4)	4 (4)	4(4)	4 (4)	4 (3)	4 (3)	4 (4)	4 (1)	4 (2)	4 (2)	4 (1)	4 (2)	4 (2)
	Roasted peanut																		
	Unblanched peanut kernell																		
	Raw in-shell peanuts														1 (1)	2 (2)	3 (3)	4 (4)	4 (4)

[a] Number of samples below the limit of detection (number of positive samples in 10 g after enrichment). Limit of detection in plate count method: 1 cfu/ g.

Table 3 Parameters of the Weibull model fit for *Salmonella* survival in peanuts products with two inoculum level during storage at 28 ºC.

Inoculum level	Sample	a_w[a]	Weibull model						
			δ (days)[b]	SE δ[c]	P[d]	SE p[e]	T_{3d}[f]	R^2 [g]	MSE[h]
3 log	Peanut brittle	0.31±0.02	1.04	2.01	0.32	0.15	30.84	0.889	0.340
	Paçoca	0.41±0.01	99.57	37.47	0.51	0.10	842.92	0.931	0.036
	Pé-de-moça	0.69±0.02	0.38	0.81	0.33	0.15	10.66	0.919	0.422
	Roasted peanut	0.42±0.01	148.82	22.22	0.99	0.13	450.23	0.964	0.038
	Unblanched peanut kernell	0.53±0.04	17.45	7.69	0.45	0.06	200.18	0.969	0.043
	Raw in-shell peanuts	0.31±0.02	1.03	1.14	0.28	0.05	51.27	0.942	0.121
6 log	Peanut brittle	0.32±0.02	0.84	0.60	0.30	0.03	34.63	0.972	0.094
	Paçoca	0.42±0.01	20.92	15.85	0.35	0.07	474.34	0.912	0.073
	Pé-de-moça	0.68±0.04	0.76	0.93	0.44	0.12	9.11	0.941	0.496
	Roasted peanut	0.41±0.02	144.03	25.22	0.72	0.10	663.23	0.968	0.019
	Unblanched peanut kernell	0.53±0.03	35.81	7.42	0.62	0.05	213.20	0.990	0.026
	Raw in-shell peanuts	0.31±0.02	12.02	9.66	0.45	0.09	137.09	0.926	0.199

[a] Average measured water activity± sd; [b] time required for first decimal reduction (days); [c] standard error of delta parameter value; [d] fitting parameter that defines the shape of the curve; [e] standard error of p parameter value; [f] time required for three decimal reductions; [g] regression coefficient; [h] mean sum of square error.

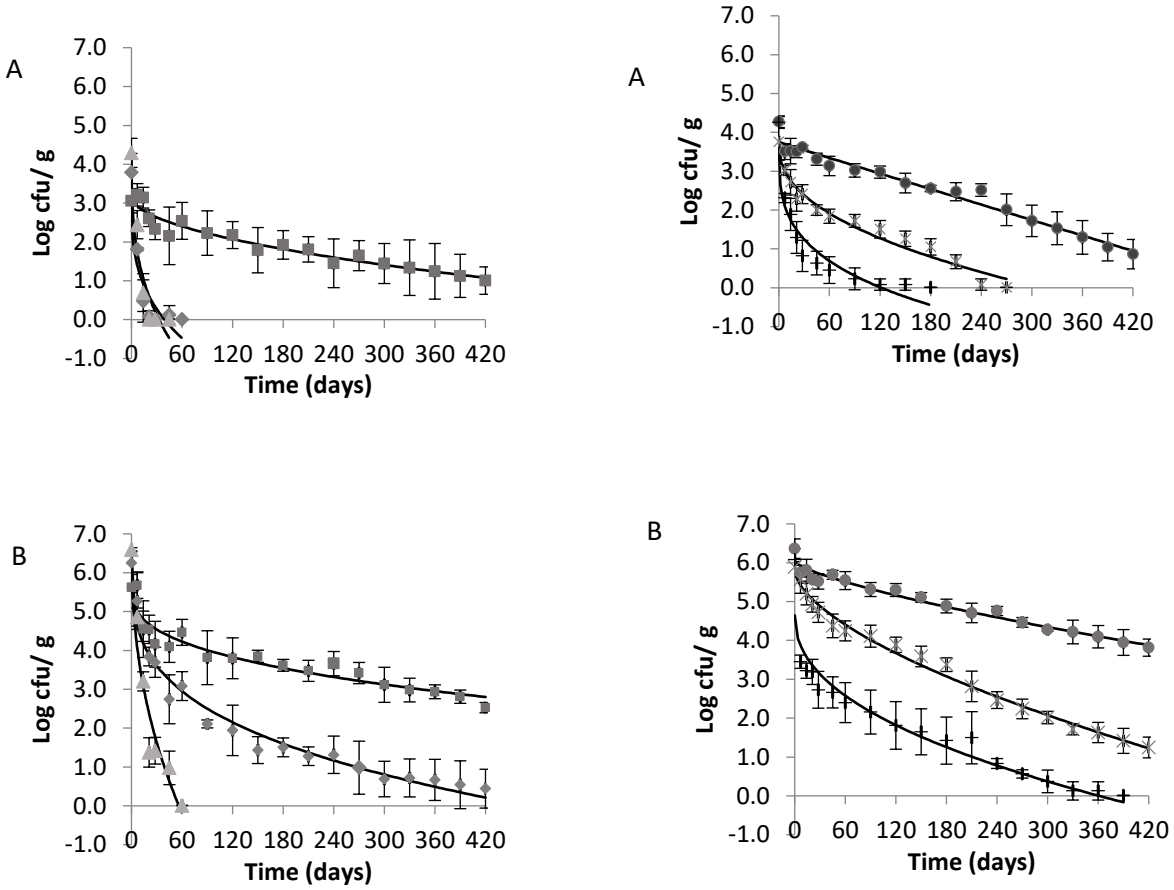

Figure 1 *Salmonella* survival curve artificially inoculated in peanut stored at 28 ± 1 ° C. Peanut brittle (◆), *Paçoca* (■), *Pé-de-moça* (▲), Roasted peanut (●), Unblanched peanut kernell (✳) and Raw in-shell peanuts (+). Experiment 1: initial inoculum level 3 log cfu/ g (A); Experiment 2: initial inoculum level 6 log cfu/ g (B).

DISCUSSÃO GERAL

O presente estudo apresentou a prevalência e sobrevivência de *Salmonella* em amendoim e produtos derivados de amendoim, sendo o primeiro estudo a investigar a ocorrência de contaminação de *Enterobacteriaceae*, coliformes totais, *E. coli* e *Salmonella* em indústrias processadoras de produtos à base de amendoim. Foram coletadas amostras de três indústrias localizadas no Estado de São Paulo, sendo uma de grande porte (indústria A), uma de médio porte (indústria B) e uma de pequeno (indústria C) porte. Na indústria A são produzidos drageados salgados e doces, onde passam por processo de torração a 150 °C/ 1 h e posterior aquecimento a 90 °C, o que os diferencia são os tipos de cobertura. A atividade de água dos drageados salgados foi de 0,543 e 0,348 para os drageados doces. A indústria B produz pé de moleque e paçoca, no primeiro os amendoins são aquecidos a 75 °C em calda de caramelo, a atividade de água deste produto foi de 0,275. Para a fabricação de paçoca não ocorre o emprego de tratamento térmico, os ingredientes utilizados apenas são prensados e apresentaram a_w de água de 0,299. A indústria C processa produtos com características semelhantes, pé de moça e doce de amendoim, em que ambos são aquecidos a 110 – 120 °C por uma hora em tachos, o que os diferencia é a forma do amendoim (pé de moça – grão inteiro e o doce de amendoim - grãos moídos) e o leite condensado utilizados como ingredientes. As atividades de água desses produtos foram de 0,643 e 0,746 para pé de moça e doce de amendoim, respectivamente.

A legislação brasileira (BRASIL, 2001) e o “International Commission on Microbiological Specifications for Foods” (ICMSF, 2011) estabelecem como critérios microbiológicos ausência de *Salmonella* nesses tipos de produtos. Dessa forma, como não foram detectados, os produtos analisados estavam de acordo com as legislações vigentes no país. No entanto, a presença desse patógeno já foi relata em outros trabalhos ou outras pesquisas na área *(*BANSAL *et al*., 2010; EGLEZOS *et al.,* 2008*).*

Apesar da ausência de *Salmonella* e *E. coli*, micro-organismos indicadores como *Enterobacteriaceae* foram isolados em 5 % dos produtos finais da indústria A, em 25 % da indústria B e em 16 % da indústria C. Em geral, estes micro-organismos não são termorresistentes, e sua presença em produtos finais que passaram por tratamento térmico, indica que pode ter ocorrido contaminação pós-processo. O alto

teor de açúcar presente nestes produtos pode conferir efeito protetor, potencializando a resistência térmica de *Salmonella* (Corry, 1974). De acordo com Ma *et al.* (2009) foram necessários 120 min a 90 °C para reduzir 7,0 log ufc de *Salmonella* em manteiga de amendoim, neste estudo o tratamento térmico conduzido foi banho de água com agitação, em que foi utilizado termopar para monitorar a temperatura.

A presença de *Salmonella* no produto final também pode ser oriunda de matérias-primas contaminadas. Neste estudo, *Enterobacteriaceae* e coliformes totais foram isolados de amendoim crus, porém suas presenças não foram constatadas nas amostras de amendoim torrado. Isto pode indicar que o tratamento térmico empregado nas indústrias foi eficiente para a redução da carga microbiana inicial.

A maior fonte potencial de contaminação do produto durante o processamento foi o ambiente fabril (instalações e equipamentos). *Enterobacteriaceae* e coliformes totais apresentaram contagens superiores a 3,0 log ufc/g. Além disso foi detectado presença de *E. coli* e *Salmonella*. Este cenário indica falhas no controle higiênico – sanitário utilizados nas indústrias. A indústria mais crítica foi a indústria B, que apresentou quatro amostras positivas pra *E. coli* e uma para *Salmonella*, que foi isolada do piso próximo ao misturador de paçoca. Este produto não sofre nenhum tratamento térmico após a mistura dos ingredientes, portanto caso ocorra contaminação cruzada nesta etapa, isto colocaria em risco a saúde dos consumidores. A cepa isolada foi identificada como *Salmonella* Yoruba, sendo o primeiro relato sobre a presença deste sorotipo no ambiente de processamento de amendoim.

Devido aos dados coletados nas indústrias e aos surtos envolvendo *Salmonella* em amendoim, este começou a ser estudo como fonte de veiculação para contaminação do patógeno. Assim sendo, permite conhecer o comportamento de *Salmonella* neste tipo de produto e em sua matéria prima. A influência da concentração do inoculo e da composição do produto na sua sobrevivência foi realizada a segunda parte deste estudo onde avaliou-se o comportamento de *Salmonella* em seis tipos de amostras pé de moleque (a_w=0,30), paçoca (a_w=0,42), pé de moça (a_w=0,68), amendoim torrado (a_w=0,39), amendoim com pele (a_w=0,54) e amendoim cru com casca (a_w=0,29),, com dois níveis de inoculo (3,0 log ufc/ g para o experimento 1 e 6,0 log ufc/ g para o experimento 2) durante 420 dias de estocagem a 28 ± 1 °C

Ao térmico de 420 dias, as amostras B e D ainda apresentavam contagens, sendo para amostra B de 1,0 log ufc/ g no experimento 1 e 2,5 log ufc/ g no experimento 2. Amostra D apresentou contagem de 0.87 e 3,8 log ufc/ g no experimento 1 e 2, respectivamente. A amostra C foi a primeira a apresentar perda de viabilidade do inoculo em ambos os experimentos, ausência de *Salmonella* em 100 % das amostras após 180 dias para o experimento 1 e 50 % após 300 dias para o experimento 2. As amostras A, E e F apenas apresentaram contagem abaixo do limite de detecção, porém *Salmonella* estava presente em 10 g de amostra em pelo menos 50 % das 4 repetições avaliadas, no entanto, baixos níveis não excluem a probabilidade de causar a doença, Kirk *et. al.* (2004) encontraram níveis de contaminação de *Salmonella* variando de <0,03 a 2 NMP/ g ao analisar amendoim com casca proveniente de um recolhimento do mercado varejista, que ocorreu em julho de 2001 após as agências de saúde da Austrália e do Canadá registrarem um aumento nos casos de infecção provocados por *S.* Stanley, as investigações realizadas reportaram que a fonte de contaminação era amendoim provenientes da Ásia.

Dessa forma, as amostras com a_w em torno de 0,40 e 0,55 (B, D e E) apresentaram melhores condições para a sobrevivência de *Salmonella*. Juven *et al.* (1984) ao estudar diferentes produtos como leite e cacau em pó, ração para aves, entre outros, com a_w variando entre 0,40 e 0,70, observaram que os produtos que apresentavam a_w de 0,43 e 0,52 *Salmonella* foi capaz de sobreviver por um período maior de tempo.

Os teores de lipídeos e carboidratos não apresentaram diretamente efeito sobre o comportamento de *Salmonella* durante a estocagem

A composição do produto não influenciou significativamente o comportamento de *Salmonella* durante a estocagem, uma vez que produtos com aw similares e teores diferentes de lipídeos e carboidratos (pé-de-moleque vs. amendoim cru com casca e paçoca vs. amendoim torrado) na maior parte do tempo não apresentaram diferença significativa ($p > 0,05$) na taxa de redução de *Salmonella*.

A sobrevivência de *Salmonella* em amendoim, deve-se ao fato desta possuir mecanismos de defesa que quando em ambientes inadequados para o seu desenvolvimento são liberados, como por exemplo a prolina e betaína, o que associada ao alto teor de gordura confere proteção contra o suco gástrico, permitindo

que a *Salmonella* chegue viável ao intestino onde encontra condições favoráveis para o seu crescimento (SWANSON, 2011; PODOLAK et al, 2010; CÁNOVAS, 2001).

CONCLUSÕES GERAIS

O estudo do ambiente fabril indica que a alta contaminação de micro-organismos indicadores (enterobactérias e coliformes totais) encontrada evidencia que este é o principal sítio de contaminação nas indústrias analisadas.

Apesar de ter sido encontrada baixa ocorrência de contaminação de *Salmonella* no presente estudo, não deve ser descartada a possibilidade de ocorrência de surtos envolvendo amendoim e produtos à base de amendoim, uma vez que doses extremamente baixas já foram descritas (0,004 ufc/g).

Ao estudar o comportamento de *Salmonella* em amendoim e produtos à base de amendoim, o patógeno apresentou maior sobrevivência nas amostras com atividade de água em torno de 0,40 e 0,55, indicando que a atividade de água, e a composição do produto influenciam a sobrevivência de *Salmonella*.

Os resultados obtidos no trabalho reforçam a importância do emprego de boas práticas de fabricação em produtos à base de amendoim, afim de evitar que ocorra contaminação por *Salmonella* e assim minimizar os riscos a saúde do consumidor.

REFERÊNCIAS BIBLIOGRÁFICAS

ABICAB - Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Amendoim**. 2013. Disponível em: <http://www.abicab.org.br/associados-3/>. Acesso em: 3 ago. 2015.

ABICAB- Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Cultivo de amendoim**. 2015. Disponível em: < http://www.abicab.org.br/cultivo-de-amendoim/ >. Acesso em: 13 out. 2016.

ABICAB – Associação Brasileira da Indústria de Chocolate, Cacau, Amendoim, Balas e Derivados. **Amendoim – História**, 2016. Disponível em: <http://www.abicab.org.br/historia-3/>. Acesso em: 10 set. 2016.

AOAC - Association of Official Analytical Chemists. Rapid Methods Adopted as AOAC Official Methods^{SM}. 2010. Disponível em : <http://www.aoac.org/vmeth/oma_testkits.pdf>. Acesso em: 18 ago 2015.

AOAC - Association of Official Analytical Chemists. Rapid Methods Adopted as AOAC Official Methods^{SM}. 2013. Disponível em: <http://www.aoac.org/vmeth/oma_testkits.pdf>. Acesso em: 18 ago. 2015.

BANSAL, A.; JONES, T. M.; ABD, S. J.; DANYLUK, M. D.; HARRIS, L. J. Most-Probable-Number determination of *Salmonella* levels in naturally contaminated raw almonds using two samples preparation methods. **Journal of Food Protection**, v. 73, p. 1986-1992, 2010.

BEUCHAT, L. R.; MANN, D. A. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. **Journal of Food Protection,** v. 73, p.1257-1268, 2010.

BEUCHAT, L. R.; MANN, D. A. Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. **Journal of Food Protection**, v. 74, p. 1441-1450, 2011.

BEUCHAT, L. R.; KOMITOPOULOU, E.; BECKERS, H.; BETTS, R. P., BOURDICHON, F.; FANNING, S.; JOOSTEN, H. M.; TER KUILE, B. H. Low-water activity foods: increased concern as vehicles of foodborne pathogens. **Journal of Food Protection**, v. 76, p. 150-172, 2013.

BLESSINGTON, T.; MITCHAM, E.J.; HARRIS, L.J. Survival of *Salmonella enterica, Escherichia coli 0157:H7,* and *Listeria monocytogenes* on inoculated walnut kernels during storage. **Journal of Food Protection,** v. 75, n. 2, p. 245 – 254. 2012.

BLIGH, E. G., DYER, W. J. A rapid method of total lipid extraction and purification.

Canadian Journal of Biochemistry and Physiology, v. 37, p. 911 – 917. 1959.

BLODGETT, Robert. Appendix 2: Most Probable Number from serial Dilutions. In Bacteriological Analytical Manual. Silver Spring: Food and Drug Administration. 2010.Dispinível em: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm>. Acesso em: 18 ago. 2015.

BRAR, P. K.; PROANO, L. G.; FRIEDRICH, L. M.; HARRIS, L. J.; DANYLUK, M. D. Survival of *Salmonella, Escherichia coli 0157:H7*, and *Listeria monocytogenes* on raw peanuts and pecan kernels stored at -24, 4, and 22 °C. **Journal of Food Protection**, v. 78, p. 323 – 332. 2015.

BRASIL. ANVISA. Agência Nacional de Vigilância Sanitária. **Resolução RDC n° 12/2001 de 02 de janeiro de 2001** - Aprova regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União. Disponível em: < http://portal.anvisa.gov.br/documents/33880/2568070/RDC_12_2001.pdf/15ffddf6-3767-4527-bfac-740a0400829b>. Acesso em: 16 set. 2016.

BRASIL. ANVISA. Agência Nacional de Vigilância Sanitária. **Resolução RDC n° 172/2003, de 04 de julho de 2003** - Dispõe sobre o Regulamento Técnico de Boas Práticas de Fabricação para Estabelecimentos Industrializadores de Amendoins Processados e Derivados e a Lista de Verificação das Boas Práticas de Fabricação para Estabelecimentos Industrializadores de Amendoins Processados e Derivados. Diário Oficial da União. Disponível em: < http://portal.anvisa.gov.br/wps/wcm/connect/503f2c00474579428651d63fbc4c6735/RDC_172_2003.pdf?MOD=AJPERES>. Acesso em: 11 ago. 2016.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento – MAPA. Instrução Normativa nº 3, de 28 de Janeiro de 2009.

BRENNER, D. J. Facultatively anaerobic Gram- negative rods, In: Krieg, N. R. e Holt, J. C. **Bergey's Manual of Systematic Bacteriology**. Baltimore: Williams and Wilkins, 1984. p. 408-516.

BRENNER, F. W.; VILLAR, R. G.; ANGULO, F. J.; TAUXE, R. SWAMINATHAN, B. *Salmonella* Nomenclature. **Journal Clinical Microbiology**, v.38, n.7, p. 2465-2467, 2000.

BURNETT, S. L.; GEHM, E. R.; WEISSINGER, W. R.; BEUCHAT, L. R. Survival of *Salmonella* in peanut butter and peanut butter spread. **Journal of Applied Microbiology**, v. 89, p. 472-477, 2000.

CÁNOVAS, D.; FLETCHER, S.A.; HAYASHI, M.; CSONKA, L. Role of trehalose in growth at high temperature of *Salmonella enterica* Serovar Typhimurium. **Journal of Bacteriology**, v. 183 (11), p. 3365 – 3371. 2001.

CARMINATI, J. de A.; AMORIM NETO, D. P.; MORISHITA, K. N.; TAKANO, L. V.; BERNARDI, A. O.; COPETTI, M. V.; DO NASCIMENTO, M. da S. Microbiological contamination in peanut confectionery processing plants. **Journal of Applied Microbiology**, v. 121, p. 1071- 1078. 2016.

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of Salmonella Serotype Agona Infections Linked to Toasted Oats Cereal -- United States, April-May, 1998. 1998. Disponível em: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00053368.htm>.Acesso em: 6 out. 2016.

CDC - Centers for Disease Control and Prevention. Multistate Outbreak of *Salmonella* Schwarzengrund Infections Linked to Dry Pet Food**.** 2007 a. Disponível em: <http://www.cdc.gov/salmonella/2007/pet-food-9-4-2007.html>. Acesso em: 05 abr. 2016).

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006-2007 b. **MMWR Morbidity and Mortality Weekly Report,** v. 56, p. 521-524, 2007.

CDC - Center For Disease Control and Prevention. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008-2009. **MMWR Morbidity and Mortality Weekly Report**, v. 58, p. 85-90, 2009.

CDC - **Multistate Outbreak of Human *Salmonella* Montevideo Infections**. 2010. Disponível em: <http://www.cdc.gov/salmonella/2010/montevideo-5-4-2010.html>. Acesso em: 6 out. 2016.

CDC - Center For Disease Control and Prevention. **Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland**. 2012**.** Disponível em: <http://www.cdc.gov/salmonella/bredeney-09-12/index.html>. Acesso em: 20 jul. 2015.

CDC - Center For Disease Control and Prevention. **Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland**. 2013**.** Disponível em: <http://www.cdc.gov/salmonella/bredeney-09-12/index.html>. Acesso em: 3 ago. 2015.

CDC - Centers for Disease Control and Prevention. **Multistate Outbreak of *Salmonella* Braenderup Infections Linked to Nut Butter Manufactured by Spired Natural Foods.** 2014. Disponível em: http://www.cdc.gov/Salmonella/braenderup-08-14/. Acesso em: 05 de set de 2016.

CHANG, A.S.; SREEDHARAN, A.; SCNEIDER, K.R. Peanut and peanut products: a food safety perspective. **Food Control**, v. 32, p. 296-303, 2013.

CHEN, Y.; SCOTT, V. N.; FREIER, T. A. Control of *Salmonella* in low-moisture foods. III: process validation and environmental monitoring. **Food Protection Trends**, v. 29, p. 493-508, 2009.

CONAB – COMPANHIA NACIONAL DE ABASTECIMENTO, **Maio/2016. Acompanhamento da Safra Brasileira, Grãos - V. 3 - SAFRA 2015/16- N. 8 - Oitavo levantamento** – Disponível em:<http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_05_10_09_03_26_boletim_graos_maio_2016.pdf>. Acesso em: 08 de Abril de 2016.

CORDIER, J. L. Production of powdered infant formula and microbiological control measures. In: FABER, J.M.; FORSYTHE, S. ***Enterobacter sakazakii***.. Washington, DC: American Society for Microbiology Press. p. 145-185. 2008.

CORRY, J. The effect of sugars and polyols on the heat resistance of salmonellae. **Journal of Applied Bacteriology**, v*. 37*, p. 31-43. 1974

CRAVEN, P. C.; MACKEL, D. C.; BAINE, W. B.; BARKER, W. H.; GANGAROSA, E. J.; GOLDFIELD, M.; ROSENFELD, H.; ALTMAN, R.; LACHAPELLE, G.; DAVIES, J. W.; SWANSON, R. C. International outbreak of *Salmonella* eastbourne infection traced to contaminated chocolate. **Lancet**, v. 1, p. 788–792. 1975.

D'AOUST, J. Y. *Salmonella* and the chocolate industry. A review. **Journal of Food Protection,** v. 40, p. 718-727, 1977.

DANYLUK, M. D.; JONES, T. M.; ADB, S. J.; SCHLITT – DITTRICH, F.; JACOBS, M.; HARRIS, L. J. Prevalence and amounts of *Salmonella* found on raw California almonds. **Journal of Food Protection**, v. 70, p. 820–827. 2007.

DODIER, D. Comparative Study Of The D-values of *Salmonella* spp. and *Enterococcus faecium* in Wheat Flour. Faculty of The Graduate College at the University of Nebraska Major: **Food Science and Technology**. 2015

DOYLE, M. P.; BEUCHAT, L. R. **Food Microbiology**: Fundamentals and Frontiers. 3 ed. Washington: American Society for Microbiology Press. 2007.

DOYLE, M. P.; BUCHANAN, R. L. ***Salmonella* Species**. In Food microbiology: fundamentals and frontiers 4th ed. Washington: American Society for Microbiology (ASM Press). 2013. pp. 225-261

EGLEZOS, S.; HUANG, B.; STUTTARD. E. A Survey of the Bacteriological Quality of Preroasted Peanut, Almond, Cashew, Hazelnut, and Brazil Nut Kernels Received into three Australian Nut-Processing Facilities over a Period of 3 Years. **Journal of Food Protection,** v. 71, p. 402 - 404. 2008.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária, **Amendoim: o produtor pergunta, a Embrapa responde**. Brasília, DF: Embrapa Informação Tecnológica: Campina Grande: Embrapa Algodão, 2009. 240 p. il. (Coleção 500 perguntas, 500 respostas).

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Cultivo do amendoim**. 2006. Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Amendoim/CultivodoAmendoim/>. Acesso em: 7 ago. 2016.

EPA - United State Environmental Protection Agency. Food and Agricultural Industries. In: **Confectionary products: peanut processing**. 5. Ed, v. 1. 1995. Disponível em: <http://www.epa.gov/ttnchie1/ap42/ch09/>. Acesso em: 6 ago. 2015.

FDA - Food and Drug Administration. **Guidance for industry: measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as an ingredient.** 2009. Disponível em: <http://www.fda.gov/guidancecomplianceregulatoryinformation/guidancedocuments/produceandplanproducts/ucm115386.htm>. Acesso em: 10 ago. 2016.

FDA - Food and Drug Administration. Recall of products containing peanut butter: *Salmonella* Typhimurium. 2009a. Disponível em: http://www.fda.gov/oc/opacom/hottopics/salmonellatyph.html. Acesso em: 3 ago. 15.

FDA - Food and Drug Administration. Peanut Corporation of America (PCA), Blakely, GA:FDA form 483 (inspectional observations). 2009b. Disponível em: http://www.fda.gov/ora/frequent/default.html. Acesso em: 3 ago. 2015.

GILL, O. N.; BARTLETT, C. L. R.; SOCKETT, P. N.; VALLE, M. S. B.; ROWE, B.; GILBERT, R. J.; DULAKE, C.; MURRELL, H. C.; SALMASO, S. **Outbreak of *Salmonella* napoli chocolate bars**. Public Health Laboratory , Dulwich Summary in England and Wales in 1982 was detected Epidemiological Investigations Extensive inquiries were made by telephone and by visits to Epidemiology. p. 574–577, 1982.

GRIMONT, P. A. D.; WEILL, F. X. **Antigenic formulae of the *Salmonella* serovars**. In World Health Organization Collaborating Center for Reference and Research on *Salmonella* 9th ed. Paris: Institute Pasteur. 2007.Disponível em: <http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf>. Acesso em: 19 ago. 2016.

HAMMACK, T. M. S. *Salmonella spp*. In: Lampel, K. A., **Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins**. Washington, DC: U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition, 2012. p. 9 – 13.

HOCKIN, J.C.; D'AOUST, J.Y.; BOWERING, D.; JESSOP, J.H.; KHANNA, B.; LIOR, H.; MILLING, M.E. An international outbreak of *Salmonella* nima from imported chocolate. **Journal of Food Protection**, v. 52, n. 1, p. 51–54, 1989.

HORN, B. W.;DORNER, J. W. Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. **Applied and Environmental Microbiology**, v. 65, 1444-1449. 1999

HORWITZ, W. Kjeldahl method, method no. 2001.14, in: Official Methods of Analysis of the Association of Official Analytical Chemists, eighteenth ed. AOAC International, Gaitherburg. pp. 72-73. 2006.

IAL - Instituto Adolfo Lutz. **Métodos Físico-Químicos para Análise de Alimentos**: Normas Analíticas do Instituto Adolfo Lutz 4th ed. Brasilia: ANVISA. 2005.

ICMSF - International Commission on Microbiological Specifications for Foods. Salmonellae. In: **Microorganisms in Foods 5**: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 217–264. 1996.

ICMSF - International Commission on Microbiological Specifications for Foods.Nuts, oilseeds and dried legumes. In: **Microorganisms in Foods 6:** Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 356–378. 2000.

ICMSF - International Commission on Microbiological Specifications for Foods. Cocoa, Chocolate and Confectionery. In: **Microorganisms in Foods 8**: Use of Data for Assessing Process Control and Product Acceptance. Springer, New York, p. 241–246. 2011.

INMET – Instituto Nacional de Meteorologia. **Estações Automáticas – Gráficos**. 2016. Disponível em: <http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_auto_graf>. Acesso em: 16 jul. 2016.

ISAACS, S.; ARAMINI, J.; CIEBIN, B.; FARRAR, J. A.; AHMED, R.; MIDDLETON, D.; CHANDRAN, A. U.; HARRIS, L. J.; HOWES, M.; CHAN, E.; PICHETTE, A. S.; CAMPBELL, K.; GUPTA, A.; LIOR, L. Y.; PEARCE, M.; CLARK, C.; RODGERS, F.; JAMIESON, F.; BROPHY, I.; ELLIS, A. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. **Journal of Food Protection**, v. 68, p. 191–198. 2005.

ISO - International Organization for Standardization (ISO) 6887 – 1:1999 (1999) General rules for the preparation of the initial suspension and decimal dilutions. In

Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Geneva: ISO.

ISO - International Organization for Standardization 6579 – 1:2007, 2007. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp. Geneva: ISO.

ISP - INSTITUTO DE SALUD PÚBLICA DE CHILE. **Ministerio de Salud**. Disponível em: <http://www.ispch.cl/lab_amb/serv_lab/salmonella.html#uno>. Acesso em: 31 de ago de 2016.

JONES, F. T.; RICHARDSON, K. F. *Salmonella* in commercially manufactured feeds. **Poultry Science**, v. 83, p. 384-391. 2004.

JUVEN, B. J.; COX, N. A.; BAILEY, J. S.; THOMSON, J. E.; CHARLES , O. W.; SHUTZE, J.V. Survival of *Salmonella* in dry food and feed. **Journal of Food Protection**, v. 47, p. 445-44. 1984.

KATAOKA, A. I.; ENACHE, E.; BLACK, D. G. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. **Journal of Food Protection**, v. 77, p. 1252-1959. 2014.

KELLER, S.; GRASSO, E.; HALIK, L.; FLEISCHMAN, G.; CHIRTEL, S.; GROVE, S. Effect of growth on the thermal resistance and survival of *Salmonella* Tennessee and Oranienburg in peanut butter, measured by a new thin-layer thermal death time device. **Journal of Food Protection**, v. 75, p. 1125 - 1130. 2012

KILLALEA, D.; WARD, L. R.; ROBERTS, D.; DE LOUVOIS ,J.; SUFI ,F.; STUART, J. M.; WALL, P. G.; SUSMAN, M.; SCHWIEGER, M. P.; SANDERSON, J. I.; FISHER, S. T.; MEAD, P. S.; GILL, O. N.; BARTLETT, C. L. R.; ROWE, B. International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack—I: England and Wales and the United States. **British Medical Journal**, v. 313, p. 1105–1107. 1996.

KIMBER, M. A.; KAUR, H.; WANG, L.; DANYLUK, M. D.; HARRIS, L. J. Survival of *Salmonella, Escherichia coli 0157:H7,* and *Listeria monocytogenes* on inoculated almonds and pistachios stored at – 19, 4, and 24 °C. **Journal of Food Protection**, v. 75, n. 8, p. 1394 – 1403. 2012.

KIRK M. D.; LITTLE, C. L.; LEM, M.; FYFE, M.; GENOBILE, D.; TAN, A.; THRELFALL, J.; PACCAGNELLA, A.; LIGHTFOOT, D.; LYI, H.; MCINTYRE, L.; WARD, L.; BROWN, D. J.; SURNAM, S.; FISHER, I. S. T. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – Sharing molecular information to solve international outbreak. **Epidemiology & Infection**, v. 132, p. 571-577. 2004.

KOMITOPOULOU, E.; PEÑALOZA, W. Fate of *Salmonella* in dry confectionery raw materials. **Journal of Applied Microbiology**, v. 106, p. 1892-1900, 2009.

KORNACKI, J. L.; JOHNSON, J. L**.** *Enterobacteriaceae*, coliforms and *Escherichia coli* as quality and safety indicatiors. In: DOWNES, F.P.; ITO, K. **Compendium of Methods for the Microbiological Examination of Foods** 4. ed. Washington: American Public Health Association (APHA), 2001. p.69-82.

KRAPF, T.; GANTENBEIN-DEMARCHI, C. Thermal inactivation of *Salmonella* spp. During conching. **LWT Food Science Technology**, v. 43, p. 720–723. 2010.

LE MINOR, L. **Typing *Salmonella* species**. European Journal of Clinical Microbiology & Infectious Diseases, v. 7, n. 2, p. 214- 218. 1988.

LEHMACHER, A.; BOCKEMÜHL, J.; ALEKSIL, S. Nationwide outbreak of human salmonelosis in Germany due to contaminated Paprika and Paprika-powdered potato chips. **Epidemiology and Infection,** v. 115, p. 501-511, 1995.

LITTLE, C. L.; RAWAL, N.; PINNA, E.; MCLAUCHLIN, J. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. **Food Microbiology,** v. 27, p. 171-174, 2010.

MA, L.; ZHANG, G.; GERNER-SMIDT, P. Thermal inactivation of *Salmonella* in peanut butter. **Journal of Food Protection,** v. 72, p. 1596-1601, 2009.

MACÊDO, M. H. G. de. **Amendoim**. In: CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. 2007. Disponível em: < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/83e31b69fc4c1f45a1cee5eb53797f41..pdf >. Acesso: 26 out. 2016

MAFART, P.; COUVERT, O.; GAILLARD, S.; LEGUERINEL, I. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. Int**. Journal of Food Microbiology**, v. 72, p. 107 -113. 2002

MARTINS, R. Amendoim: produção, exportação e a safra 2011/2012**. Análises e Indicadores do Agronegócio – Instituto de Economia Agrícola**, v. 6, n. 11, 2011.

MATTICK, K. L.; JORGENSEN, F.; LEGAN, J. D.; LAPPIN-SCOTT, H. M.; HUMPHREY, T. J. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. **Applied and Environmental. Microbiology**, v. 66, p. 4921–25. 2000.

MAZZOTTA, A. S. Thermal Inactivation of Stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in fruit juices. **Journal of Food Protection**, v. 64, p. 315-320, 2001.

MIDURA, T. F.; BRYANT, R. G. **Sampling plants, sample collection, shipment, and preparation for analysis**. In *Compendium of Methods for the Microbiological Examination of Foods* 4th ed. Downes, F.P. and Ito, K. pp.13-23. Washington: American Public Health Association (APHA). 2001

MOSSEL, D. A. A.; KOOPMAN, M.J. Losses in viable cells of salmonellae upon inoculation into dry animal feeds of various types. **Poultry. Science**, v. 44, p. 890 - 892.1965.

NASCIMENTO, M. da S. do; Brum, D.M.; Pena, P.O.; Berto, M.I. e Efraim, P. Inactivation of *Salmonella* during cocoa roasting and chocolate conching. **International Journal of Food Microbiology**, v**.** 159, n. 3, p. 225 – 229. 2012.

NASCIMENTO, M. S.; SILVA, I. F.; SILVA, J. C.; REOLON, E. M.; SANTOS, A. R. B.; SILVA, N. Enteropathogens in cocoa pre-processing. **Food Control**. v. 24, n. 4, p. 408-411. 2010.

NRC - NATIONAL RESEARCH COUNCIL. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academic Press, Washington, D.C. 1985.

NUMMER, B. A.; SHRESTHA, S.; SMITH, J. V. Survival of *Salmonella* in a high sugar, low water- activity, peanut butter flavored candy fondant. **Food Control**, v. 27, p. 184–187. 2012.

PARK, E. J.; OH, S. W.; KANG, D. H. Fate of *Salmonella* Tennessee in Peanut Butter at 4 and 22 ºC. **Journal of Food Science**, v. 73, n. 2, p. 82 - 86, 2008.

PODOLAK, R.; ENACHE, H.; STONE, W.; BLACK, D. G.; ELLIOT, P. Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low – Moisture Foods. **Journal of Food Protection,** v. 73, p. 1919 - 1936. 2010.

POPOFF, M. Y.; Le MINOR, L. E. The genus *Salmonella*. In: Brenner D.J.; Krieg, N.R.; Staley, J.T. **Bergey's manual of systematic bacteriology**. 2. ed. New York, USA: Springer, 2005. v. 2. p.764-799.

REIJ, M. W.; DEN, A. E. D.;ILSI Europe Risk Analysis in Microbiology Task Force. Recontamination as a source of pathogens in processed foods. **International Journal of Food Microbiology**, v. 91, p. 1-11. 2004.

SCHATZKI, T. F.; ONG, M. S. Dependence of aflatoxin in almonds on the type and amount of insect damage. **Journal of Agricultural and Food Chemistry**, v. 49, p. 4513 - 4519. 2001

SCHEIL, W.; CAMERON, S.; DALTON, C.; E WILSON, D. A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. **Australian and New Zealand Journal of Public Health**, v. 22, n. 5, p. 536 – 539. 1998.

SCOTT, V. N.; CHEN, Y.; FREIER, T. A.; KUEHM, J.; MOORMAN, M.; MEYER, J.; MORILLE-HINDS, T.; POST L.; SMOOT, L.; HOOD, S., SHEBUSKI, J.; BANKS, J. Control of *Salmonella* in low-moisture foods. I: minimizing entry of *Salmonella* into a processing facility. **Food Protection Trends**, v. 29, p. 342-353. 2009.

SEMPLE, A. B.; PARRY, W. H.; GRAHAM, A. J. Paratyphoid fever traced to desiccated coconut. **Lancet London, England)**, v. 2, n. 7198, p. 364–5, 1961.

SHACHAR, D.; YARON, S. Heat tolerance of *Salmonella enteric* serovars Agona, Enteritidis, Typhimurium in peanut butter. **Journal of Food Protection**, v. 11, p. 2687-2691, 2006.

SIRSAT, S. A.; BURKHOLDER, K. M.; MUTHAIYAN, A.; DOWD, S.E.; BHUNIA, A.K.; RICKE, S.C. Effect of sublethal heat stress on *Salmonella* Typhimurium virulence. **Journal of Applied Microbiology**, v. 110, p. 813 – 822. 2011.

STOTT, J. A; HODGSON, J. E.; CHANEY, J. C. Incidence of Salmonellas in animal feed and the effect of pelleting on content of Enterobacteriaceae. **Journal f Applied Bacteriology**, v. 39, p. 41 - 46. 1975.

SWANSON, K. M. J. Nuts, oilseeds, dried legumes and coffee. In: ICMSF – International Commission on Microbiological Specifications for Food. **Microorganisms in Food 8**. New York: Springer, p. 227 – 237. 2011.

TAMMINGA, S. K.; BEUMER, R. R.; KAMPELMACHER, E. H.; VAN LEUSDEN, F. M. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. **Journal of Hygine,** v.76, v. 1, p. 41 - 47. 1976.

THOMAS, H. A. Bacterial densities from fermentation tube tests. **Journal of the American Water Works Association**, v. 34, p. 572-576. 1942

UESUGI, A. R.; DANYLUK, M. D.; HARRIS, L. J. 2006. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23 and 35 ºC. **Journal of Food Protection**, v**.** 69, p. 1851-1857.

USDA – United States Departament of Agriculture. PSD – Production, Supply and Distribution Online, 2016. Disponível em: http://apps.fas.usda.gov/psd online/psdQuery.aspx. Acesso em: 30 maio 2016.

WERBER, D.; DREESMAN, J.; FEIL, F.; van TREECK, U.; FELL, G.; ETHELBERG, S.; HAURI, A. M.; ROGGERNTIN, P.; PRAGER, R.; FISHER, I. S. T.; BEHNKE, S. C.; BARTELT, E.; WEISE, E.; ELLIS, A.; SIITONEN, A.; ANDERSSON, Y.; TSCHÄPE, H.; KRAMER, M. H.; AMMN, A. International outbreak of *Salmonella* Oranienburg due to German chocolate. **BioMed Central Infectious Diseases,** v. 5, p. 7 - 17, 2005.

WILSON, M. M.; MACKENZIE, E. F. Typhoid Fever and Salmonellosis Due To the Consumption of Infected Desiccated Coconut. **Journal of Applied Bacteriology**, v. 18, n. 3, p. 510–521, 1955.

WOODROOF, J. G. **Peanut: production, processing, products**, 3 ed, Westport, CN: The Avi Publishing Company. 1983.

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