UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

MICROBIOLOGIA NA ALTA GASTRONOMIA
AVALIAÇÃO DO COMPORTAMENTO DE *SALMONELLA* EM PREPARAÇÕES GASTRONÔMICAS À BASE DE OVOS

STEFANI MACHADO LOPES

PORTO ALEGRE
2019

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos na Universidade Federal do Rio Grande do Sul, como um dos requisitos para a obtenção do grau de Mestre em Ciência e Tecnologia de Alimentos.

Orientador: Eduardo Cesar Tondo

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Título da dissertação: Microbiologia na Alta Gastronomia - Avaliação do comportamento de *Salmonella* em preparações gastronômicas a base de ovos

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“Foi o tempo que dedicastes à tua rosa que a fez tão importante.”
Antoine de Saint-Exupéry

RESUMO

Ovos são amplamente utilizados em muitas preparações da alta gastronomia, uma vez que apresentam uma ampla gama de funções, dentre elas, de emulsificantes e espumantes ou mesmo como ingrediente principal de preparações reconhecidas. Entretanto, preocupações quanto à segurança dessas preparações ocorrem com frequência, uma vez que ovos podem estar contaminados por *Salmonella*, principalmente se servidos sem tratamento térmico adequado, como ocorre em algumas preparações da alta gastronomia. Com o objetivo de aumentar a segurança de muitas preparações, diferentes órgãos reguladores exigem o processamento térmico de todas as partes do alimento a 70 ºC ou mais, contudo nem sempre isso ocorre em preparações à base de ovos, os quais são servidos crus ou com insuficiente tratamento térmico. Nesse contexto, o presente estudo foi realizado para analisar a sobrevivência de *Salmonella* em três diferentes preparações à base de ovos: ovos moles processados em termocirculador a 62 ºC, *pisco sour* peruano e *spaghetti alla carbonara*. Para tanto, um *pool* de *Salmonella* foi inoculado nos ovos, os quais foram incubados *overnight,* atingindo 7 a 9 $\log_{10}$ UFC/g. Os ovos contaminados foram utilizados nas preparações culinárias e amostras foram coletadas para investigar a sobrevivência de *Salmonella* durante e após seus preparos. Os resultados indicaram que o processamento de ovos moles em termocirculador, aquecido a 62 °C, promoveu a completa inativação de 7.7 $\log_{10}$ UFC/g de *Salmonella*, após 30 minutos de preparo. O *pisco sour* peruano, com um teor alcoólico de 18 % v/v e pH 3,0, foi capaz de reduzir pelo menos 4,1 $\log_{10}$ UFC/mL de *Salmonella* após seis minutos de exposição, enquanto os resultados obtidos por modelos preditivos demonstraram que a bactéria foi completamente inativada após nove minutos de exposição (redução de 6,1 $\log_{10}$ UFC/mL). A preparação do *spaghetti alla carbonara* reduziu 4,7 $\log_{10}$ UFC/g de *Salmonella*, porém não inativou completamente a população inicial desse micro-organismo. Baseado nesses resultados, ovos moles processados a 62 °C, por 30 minutos ou mais, foram considerados seguros, mesmo que estejam contaminados por alta concentração de *Salmonella*. Por outro lado, as preparações do *pisco sour* peruano e do *spaghetti alla carbonara* não foram capazes de reduzir totalmente a população de *Salmonella*, sendo que a segurança dessas preparações depende, portanto, da

qualidade microbiológica inicial dos ovos ou do processamento adequado desses ovos, antes de serem utilizados.

Palavras-chave: Ovo perfeito; Pisco *sour*; *Spaghetti alla carbonara*; *Salmonella*; Inativação.

ABSTRACT

Eggs are widely used in many preparations of fine dining restaurants, once they present a wide range of functions in cooking, among them emulsifiers and foaming agents, or even used as the main ingredient of recognized preparations. However, concerns are frequently raised about the safety of these preparations, assuming the possibility of the eggs to be contaminated by *Salmonella*, especially if served with insufficient heat treatment as it happens in some fine dining preparations. To ensure the hygienic-sanitary quality of food, different regulatory agencies recommend or require the thermal processing of all parts of the food at least 70 °C or more. However, this does not always occur in egg-based preparations, which are commonly served raw or mild heat processed. In this context, the present study was performed to analyze the survival of *Salmonella* in three different egg-based preparations: soft-cooked eggs processing using temperature-controlled water circulator at 62 ºC, Peruvian pisco sour and spaghetti *alla carbonara*. A pool of *Salmonella* was inoculated in the eggs and incubated overnight reaching 7 to 9 $\log_{10}$ CFU/g. The contaminated eggs were used in the culinary preparations and samples were collected to investigate the survival of *Salmonella* during and after preparation. The results indicated that the preparation of soft-cooked eggs promoted complete inactivation of the bacteria (7.7 $\log_{10}$) after 30 minutes of preparing. Peruvian pisco sour, with an alcoholic content of 18 % v/v and pH 3.0, showed a reduction of at least 4.1 $\log_{10}$ CFU/ml of *Salmonella* after 6 minutes of exposure, while predictive results showed that the whole population (6.1 $\log_{10}$ CFU/ml) was completely inactivated in 9 minutes. Spaghetti *alla carbonara* showed a reduction of 4.7 $\log_{10}$ CFU/g after preparation, but did not promote complete inactivation of the initial population of this microorganism. Based on these results, soft-cooked eggs processing by temperature-controlled water circulator at 62 ºC for 60 minutes were considered safe, even if they were contaminated by *Salmonella*. On the other hand, Peruvian pisco sour and spaghetti *alla carbonara* preparations have not been able to completely reduce *Salmonella* population. Therefore, the safety of these preparations depends on the initial microbiological quality of the eggs or the appropriate processing of eggs before use.

Keywords: Perfect egg; Pisco *sour*; *Spaghetti alla carbonara*; *Salmonella*; Inactivation.

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1.1 INTRODUÇÃO

Mundialmente, *Salmonella* é um dos principais patógenos causadores de Doenças Transmitidas por Alimentos (DTA). No Brasil, na última década, 30 % dos agentes etiológicos de surtos alimentares notificados oficialmente foram identificados como *Salmonella* spp. (BRASIL, 2018). Nos Estados Unidos da América (EUA), em 2015, *Salmonella* foi a principal responsável por surtos, casos e hospitalizações relacionadas a alimentos, sendo que a incidência de salmoneloses, nesse mesmo país, foi de 16 casos em 100.000 habitantes, no ano de 2017 (MARDER et al., 2018). (CDC, 2017). Na União Europeia (UE) a salmonelose continua sendo a segunda maior zoonose em humanos, com taxa de notificação de 20,4 por 100.000 habitantes (EFSA, 2017).

A salmonelose está tradicionalmente associada ao consumo de produtos à base de frango e ovos e representa uma das relações de maior risco entre patógeno e alimentos. Ovos íntegros podem ser contaminados com *Salmonella,* através de duas vias: a primeira, através de fontes externas, devido à contaminação da superfície da casca do ovo com fezes e subsequente penetração através da casca. A segunda, através da contaminação durante a sua formação no interior do ovário ou durante a passagem pelos ovidutos das aves infectadas, antes que o conteúdo dos ovos seja coberto pela casca. Nestas duas rotas, a *Salmonella* pode contaminar tanto a gema quanto a clara do ovo (GANTOIS et al., 2009). A prevalência de *Salmonella* em ovos é normalmente baixa, 0,005 % nos EUA (EBEL; SCHLOSSER, 2000). Na UE, 0,29 % das 5.782 unidades de ovos de mesa testadas no ano de 2016, estavam contaminadas por *Salmonella* (EFSA, 2017). No Brasil, vários estudos relataram diferentes incidências desse patógeno no interior dos ovos, variando desde a ausência até 33 % (Baú, Carvalhal, & Aleixo, 2001; Kottwitz et al., 2013; Oliveira & Taham, 2011; Wolschick & Bosco, 2015). Ademais, ovos apresentam geralmente um baixo número de células de *Salmonella* (<20 / ovo) (Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991). Embora esses índices sejam baixos, o número de casos de salmonelose associados a ovos pode ser

grande, uma vez que o armazenamento de ovos à temperatura ambiente pode levar a multiplicação desse patógeno, especialmente devido às condições físico-químicas (pH, viscosidade e conteúdo nutricional) da gema de ovo (GUMUDAVELLI et al., 2007). Além disso, há o fato de que os ovos são altamente consumidos e utilizados em muitas preparações que frequentemente não recebem tratamento térmico suficiente (EFSA, 2017; WINDHORST; GRABKOWSKY; WILKE, 2013). Embora a clara de ovo possua características desfavoráveis à multiplicação microbiana quando comparado à gema, devido suas deficiências nutricionais, pH alcalino e presença de moléculas antimicrobianas, como lisozima e ovotransferrina, diferentes estudos demonstraram que *Salmonella* Enteritidis (SE) é capaz de sobreviver na clara de ovo, sendo possível contaminar alimentos preparados com produtos não suficientemente processados termicamente (BARON et al., 2016; CLAVIJO et al., 2006; GANTOIS et al., 2009; GUAN; GRENIER; BROOKS, 2006; KANG et al., 2006; MESSENS; GRIJSPEERDT; HERMAN, 2005).

O processamento térmico ainda é um dos métodos mais comuns e eficazes para inativar *Salmonella* presente nos ovos. Mundialmente, diferentes agências reguladoras recomendam ou exigem que ovos preparados e servidos devam ser cozidos a uma temperatura interna de pelo menos 70 ºC ou, alternativamente, que ovos pasteurizados sejam utilizados (BRASIL, 2004; CANADA, 2013; CDC, 2011; FDA, 2016). Alguns órgãos reguladores definem especificações mais restritas, exigindo que quando ovos pasteurizados não forem utilizados, tanto a gema quanto a clara de ovo devam estar sólidas, antes de serem servidas (FDA, 2016; RIO GRANDE DO SUL, 2009; SÃO PAULO, 2013). Entretanto, em muitos restaurantes, especialmente nos relacionados à alta gastronomia, muitas vezes, ovos são servidos sem tratamento térmico suficiente para inativar micro-organismos, com o intuito de modificar levemente a textura, manter a gema e a clara macias ou para formação de espumas (VEGA; MERCADÉ-PRIETO, 2011).

Diante do exposto, o objetivo desse trabalho foi avaliar o comportamento de *Salmonella* em três preparações gastronômicas à base de ovos, as quais não atendem os parâmetros estabelecidos pela legislação brasileira vigente (BRASIL, 2004).

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Avaliar o comportamento de *Salmonella* em ovo processado em termocirculador, *pisco sour* peruano e *spaghetti alla carbonara*.

1.2.2 Objetivos Específicos

- Identificar os modos de preparo de ovos em termocirculadores, *pisco sour* peruano e *spaghetti alla carbonara* em restaurantes de alta gastronomia;

- Analisar a sobrevivência de *Salmonella* durante e após a preparação de ovos em termocirculador, *pisco sour* e *spaghetti alla carbonara*;

- Avaliar fatores intrínsecos e extrínsecos durante e após o preparo das preparações e relacioná-los com a sobrevivência de *Salmonella;*

- Comparar os resultados microbiológicos experimentais com aqueles obtidos através de modelos matemáticos;

- Se necessário, propor alterações nas receitas, as quais garantam a segurança microbiológica das preparações;

1.3 REVISÃO BIBLIOGRÁFICA

1.3.1 Gastronomia e Alta Gastronomia

No início do século XIX, na França, a gastronomia deixou de ser vista apenas como fonte nutricional para converter-se em um fenômeno social. Esse fato esteve relacionado principalmente às mudanças nas condições sociais e culturais, a origem de restaurantes como locais específicos para a produção e o consumo de alimentos, e a criação de instituições que legitimaram a atuação dos profissionais dessa área (FERGUSON, 1998). Dentre as mudanças nas condições sociais e culturais, está o aumento gradativo da população que consome alimentos fora do lar. Aspectos como a progressiva urbanização, aumento da renda e as mudanças geradas nos hábitos de consumo aumentaram a demanda por esse tipo de alimentação (WHO, 2015). No Brasil, do total das despesas com a alimentação, aproximadamente 31 % são destinadas ao consumo fora do lar, sendo os maiores dispêndios com as refeições de almoço e jantar (62,7 %) seguido de bebidas (12,5 %) (IBGE, 2009). O mesmo comportamento é observado em outros países, como na China e nos Estados Unidos da América (EUA), onde o crescimento nas despesas com o consumo de alimentos fora do lar apresenta um papel importante na economia (LIU et al., 2015; USDA-ERS, 2016).

Em relação à origem de instituições específicas para a formação dos profissionais de gastronomia, essa demanda surgiu depois do estabelecimento dos primeiros restaurantes, com a valorização do profissional pelas classes urbanas, uma vez que, anteriormente as atividades referentes à cozinha eram deixadas para as mulheres no ambiente doméstico e para os homens, normalmente aqueles menos preparados para realizar outras atividades no ambiente profissional. Nessas circunstâncias, em Paris, em 1895, surgiu uma das mais famosas escolas de gastronomia do mundo, a *Le Cordon Bleu* (LE CORDON BLEU, 2019). Nos EUA, em 1946 foi fundada outra escola de referência no campo da gastronomia, a CIA – *Culinary Institute of America* (CIA, 2019). No Brasil, o SENAC foi a primeira instituição a lançar cursos relacionados à essa área, em 1951, com o curso de especialização para Garçom e, em 1964, os cursos de cozinheiro e *Barman*

(SENAC, 2019). Entretanto, somente em 1999 que surgiram os primeiros cursos superiores de Gastronomia no Brasil, nos estados Santa Catarina e São Paulo (Universidade do Sul de Santa Catarina, Universidade do Vale do Itajaí e Universidade Anhembi-Morumbi) (MIYAZAKI, 2006).

Atualmente, no país, há uma ascensão nos aspectos que envolvem a gastronomia: a formação de grandes profissionais em escolas renomadas, a propagação de restaurantes comandados por *Chefs*, e a vasta reprodução de conteúdo, através de livros de culinária, mídias sociais e televisivas, sobre o tema. Com isso, evidencia-se o cenário da alta gastronomia, que pode ser definida como a preparação de refeições de qualidade, executadas por um *Chef*, que objetivam oferecer uma experiência única aos clientes, não só através do sabor da comida, como também através de locais que prezam pelo atendimento e o ambiente (ZANONI, 2012). Nos EUA 1,4 % dos restaurantes são de alta gastronomia, com cerca de 5000 estabelecimentos (CHD, 2016). No Brasil, não há dados oficiais que apresentem o número de restaurantes de alta gastronomia, o que inclusive é dificultado pela ausência de classificação clara, desse tipo de restaurante. Entretanto, uma pesquisa que aborda as tendências em alimentos no Brasil, sugere que um dos principais aspectos a ser valorizado até o ano de 2020, no setor de sensorialidade e prazer é a sofisticação. No cardápio, é tendência a utilização de receitas exclusivas, menus degustação e o resgate de ingredientes tradicionais no desenvolvimento de novas receitas, e ademais, os sistemas de gestão exaltam a valorização dos *Chefs* de cozinha. Essas tendências se relacionam estritamente com o conceito da alta gastronomia e, portanto, podem ser indicativas do crescimento do setor no país (ITAL, 2010).

Simultaneamente com a expansão da alta gastronomia no Brasil, a segurança de alimentos nessa área também tem ganhando maior importância nos últimos anos. Na alta gastronomia, a segurança de alimentos teve inicio no espaço hoteleiro, através do desenvolvimento do setor de alimentos e bebidas, que anteriormente eram vistos como uma área que gerava muitos custos e com poucos resultados para esses estabelecimentos. Entretanto, com a execução de preparações mais complexas, atração de um público mais exigente e consequentemente um maior valor agregado às refeições, tornou-se essencial uma maior preocupação com a distribuição de refeições seguras, além de sensorialmente prazerosas.

Nas últimas décadas, principalmente na alta gastronomia, iniciou-se a fusão da ciência e da culinária e, consequentemente, as colaborações entre cientistas e *Chefs*. Essas colaborações geraram avanços nos estudos referentes à gastronomia e estimularam as inovações culinárias com a finalidade de proporcionar o prazer durante a alimentação, principalmente associado ao aspecto sensorial. Com isso, pesquisadores transformaram a cozinha em local de estudo, detalhando as transformações físico-químicas envolvidas na culinária e promovendo inovações tecnológicas (HUMPHRIES, 2012). Com a aplicação dos princípios científicos na culinária, torna-se possível explorar novas áreas na gastronomia, como por exemplo, o uso de técnicas que fornecem texturas inéditas (BARHAM et al., 2010). Técnicas tais como, aquecimento com temperatura controlada em banho de água, cozimento a vácuo (*sous vide*), utilização de baixa temperatura por longos períodos, congelamento com uso de nitrogênio líquido, gelificação, formação de espumas e filmes comestíveis, são amplamente realizadas nas cozinhas de alta gastronomia (CASSI, 2011; RODGERS, 2007). Outra possibilidade explorada por essas cozinhas é a utilização de um único ingrediente para uma ampla gama de funções, como é o caso dos ovos (O 'DEA; HEWSON, 2015). Entretanto, em restaurantes de alta gastronomia, muitas vezes, esses ovos são servidos sem tratamento térmico suficiente para inativar toda sua eventual contaminação, principalmente com o intuito de manter a gema e a clara macias, ou para modificar levemente a textura dos componentes, ou então para formação de espumas (VEGA; MERCADÉ-PRIETO, 2011). E, portanto, preocupações quanto à segurança dessas preparações ocorrem com frequência, uma vez que ovos podem estar contaminados por *Salmonella*.

1.3.2 *Salmonella*

Salmonella são bastonetes Gram-negativos, anaeróbios facultativos, não formadores de esporos, não fermentadores de lactose, pertencentes à família *Enterobacteriaceae*. Maioritariamente são móveis, sendo exceções, *Salmonella* Gallinarum e *Salmonella* Pullorum. Multiplica-se em temperaturas entre 5,3 ºC a 45 °C, com temperatura ótima de 37 ºC. O pH ótimo de multiplicação encontra-se próximo a neutralidade (6,6 – 8,2), e a atividade de água (Aw) mínima em torno de

0,95. Os humanos e os animais, principalmente as aves, são reservatórios desses micro-organismos (JAY, 2005).

O gênero *Salmonella* está dividido em duas espécies (*S*. *enterica* e *S*. *bongori*), sendo os membros da espécie *S. enterica* os responsáveis pelas infecções em humanos. *S.* Enteritidis e *S*. Typhimirium são os dois principais sorovares relacionados com essas infecções alimentares, dentre aproximadamente 2600 existentes (FORSYTHE, 2013). A infecção causada pelas bactérias *S. enterica* é denominada salmonelose e está comumente associada à ingestão de alimentos e água contaminados com esses micro-organismos (CDC, 2014). Os sintomas relacionados à salmonelose surgem entre seis a 72 horas após a ingestão dos alimentos contaminados, sendo para maioria das pessoas saudáveis: diarreia, vômitos, dores abdominais, dor de cabeça e às vezes febre (HOFFMANN; MACULLOCH; BATZ, 2015). Entretanto, em imunodeprimidos, infecções mais severas podem ocorrer, onde a penetração da bactéria na corrente sanguínea pode ocasionar até a morte (JAY, 2005). Embora se considere uma dose infectante típica para humanos em torno de 10^6 a 10^8 Unidades Formadoras de Colônia (UFC), há relatos de surtos com doses infectantes menores (Humphrey, 2004).

Mundialmente, *Salmonella* é uma das principais causas de DTA. A incidência global de salmonelose é estimada em 80,3 milhões de casos, anualmente, e entre as DTA, salmonelose gera alguns dos maiores custos ao ano, causando um grande impacto econômico. Estima-se que cerca de 3,7 bilhões de dólares são gastos com atendimentos, hospitalizações e mortes, podendo chegar a 9,5 bilhões de dólares em anos com maior incidência de casos (MAJOWICZ et al., 2010; USDA-ERS, 2013). Nos EUA, um milhão de pessoas ao ano adoecem, devido à infecção alimentar por *Salmonella*, o que equivale a 11 % do total de DTA no país, e cerca de 380 pessoas chegam a óbito no país (HOFFMANN; MACULLOCH; BATZ, 2015). A incidência de infecções por *Salmonella,* em 2017, nos EUA, foi de 16 por 100.000 habitantes (MARDER et al., 2018), e em 2015, os números de surtos, casos e hospitalizações causados por *Salmonella* foram maiores que aqueles causados por outros patógenos (CDC, 2017). Na UE, a salmonelose é a segunda zoonose mais comum em humanos, com taxa de notificação de 20,4 por 100.000 habitantes (EFSA, 2017), só ficando atrás da campilobacteriose. No Brasil, na última década, 30 % dos agentes etiológicos de surtos alimentares notificados foram identificados

como *Salmonella,* e os ovos e produtos à base de ovos foram identificados entre os quatro principais alimentos envolvidos nos surtos, contabilizando 7,4 % (BRASIL, 2018).

1.3.3 Ovos

Ovos e preparações que contêm ovos são amplamente consumidos em todo o mundo. Em 2016, a produção mundial de ovos foi de 74 milhões de toneladas métricas. Mudanças nas dietas e o aumento da renda têm ocasionado um crescimento na demanda por ovos. O consumo de ovos previsto para o ano 2018, nos EUA, foi de 277,7 unidades por pessoa, tornando-se o maior índice de consumo de ovos, na última década ("Poultry Trends", 2018). Na maioria dos países, o consumo por pessoa varia de 104 a 208 ovos ao ano; no Brasil, em 2017, de acordo com a Associação Brasileira de Proteína Animal (ABPA), o consumo *per capita* foi de 192 unidades (ABPA, 2018).

Devido sua versatilidade, ovos são largamente utilizados na gastronomia, podendo ser utilizados como espessantes, emulsionantes, espumantes, clarificantes, coberturas, entre outros. Podem ser consumidos sozinhos ou utilizados como ingredientes em diferentes preparações e ser preparados em altas ou baixas temperaturas, por longos ou curtos períodos de tempo (O 'DEA; HEWSON, 2015). Um estudo nos EUA avaliou a utilização e a preparação de ovos e constatou que 80 % dos restaurantes analisados utilizavam ovos não pasteurizados e 78 % afirmaram que ocasionalmente serviam ovos pouco cozidos (ovos moles), assim como ovos fritos com a gema mole (CDC, 2011).

Os ovos e os produtos à base de ovos crus ou com insuficiente tratamento térmico são os principais alimentos transmissores de *Salmonella*, causadora de infecções em humanos (BARANCELLI; MARTIN; PORTO, 2012; BRADEN, 2006). Os ovos, mesmo que intactos, podem estar contaminados com *Salmonella*. A contaminação pode ocorrer através de duas rotas: a primeira, através de fontes externas, que contaminam à superfície da casca do ovo com fezes e subsequente penetração através da casca e a segunda durante a formação do ovo, dentro dos animais. Diferentes fatores, externos e internos, afetam a probabilidade de ocorrer a penetração bacteriana em ovos. Dentre os fatores externos, a influência da cepa bacteriana, o número de micro-organismos, a temperatura, a umidade e as

condições de armazenamento são os mais importantes. Por exemplo, quando o ovo é exposto a um ambiente mais frio do que a temperatura corporal da ave (42 °C), uma pressão negativa pode se desenvolver e as bactérias migram mais facilmente através da casca e das membranas (BOARD, 1966; BRUCE; DRYSDALE, 1994; GANTOIS et al., 2009). Com relação aos fatores internos estão: a presença de cutícula, as características da casca, dentre elas a porosidade e alguns defeitos, e as propriedades da membrana (MESSENS; GRIJSPEERDT; HERMAN, 2005). Por exemplo, logo após a postura do ovo, a cutícula recém-formada apresenta alguns poros que podem estar abertos que facilitam a penetração do micro-organismo. Por outro lado, a cutícula em ovos mais velhos apresenta-se desidratada, resultando em um encolhimento e uma maior exposição à penetração bacteriana (BOARD, 1966; BRUCE; DRYSDALE, 1994; GANTOIS et al., 2009).

A segunda rota de contaminação ocorre durante a formação do ovo no interior do ovário ou durante a passagem pelos ovidutos das aves infectadas, antes que o conteúdo dos ovos seja coberto pela casca. Nesse caso, pode ocorrer contaminação direta da gema, do albúmen, das membranas da casca e da casca, devido à infecção do ovário e oviduto. Nestas duas rotas, a *Salmonella* pode contaminar tanto a gema, caso o ovário esteja contaminado, quanto a clara do ovo, caso os ovidutos estejam contaminados (GANTOIS et al., 2009).

A prevalência de *Salmonella* em ovos é normalmente baixa, cerca de 0,005 % nos EUA (EBEL; SCHLOSSER, 2000). Na UE, 0,29 % das 5.782 unidades de ovos de mesa, testada em 2016, continham *Salmonella* (EFSA, 2017). No Brasil, vários estudos relataram diferentes prevalências desse patógeno no interior dos ovos, variando desde a ausência até 33 % (Baú, Carvalhal, & Aleixo, 2001; Kottwitz et al., 2013; Oliveira & Taham, 2011; Wolschick & Bosco, 2015). Ademais, ovos inteiros, quando contaminados, apresentam geralmente um baixo número de *Salmonella* (<20/ovo), principalmente se a contaminação estiver na clara do ovo, porém, valores de 10^3 células já foram encontrados. Em um estudo onde galinhas foram infectadas experimentalmente com dose oral de 10^9 células de *S*. Enteritidis, os ovos, logo após a postura, apresentaram 220 células dessa bactéria (HUMPHREY et al., 1989, 1991).

Embora a prevalência e o número de células sejam baixos, a quantidade de casos de salmonelose associados a ovos tem sido grande, uma vez que o

armazenamento de ovos à temperatura ambiente pode levar ao alto nível desse patógeno, atingindo 10^7 ou mais células por grama de ovo, em menos de 24 horas. Essa multiplicação considerável ocorre especialmente devido às condições físico-químicas como pH, viscosidade e conteúdo nutricional da gema de ovo (GUMUDAVELLI et al., 2007). Alguns estudos demonstraram que a clara do ovo é um meio não apropriado para a multiplicação microbiana. Condições físico-químicas, como deficiências nutricionais e pH alcalino próximo de 9,0, podem dificultar a multiplicação da bactéria. Além disso, moléculas antimicrobianas, como lisozima e ovotransferrina, podem desempenhar um papel importante na inibição da multiplicação bacteriana na clara (BARON et al., 2016; CLAVIJO et al., 2006; MESSENS; GRIJSPEERDT; HERMAN, 2005). Portanto, acredita-se que a clara do ovo não apresente tanto risco de multiplicação de *Salmonella.* Entretanto, diferentes estudos demostraram que, em contraste com outros sorovares de *Salmonella*, *S*. Enteritidis é capaz de sobreviver na clara de ovo, podendo colaborar no aumento dos casos de salmonelose (BARON et al., 2016; CLAVIJO et al., 2006; GANTOIS et al., 2009; GUAN; GRENIER; BROOKS, 2006; KANG et al., 2006; MESSENS; GRIJSPEERDT; HERMAN, 2005). Ademais, contribui para os casos de salmonelose o fato de os ovos serem altamente consumidos e utilizados em muitos pratos que frequentemente não recebem tratamento suficiente para a inativação de *Salmonella* (EFSA, 2017; WINDHORST; GRABKOWSKY; WILKE, 2013).

1.3.4 Inativação de *Salmonella*

Muitos métodos, tanto físicos quanto químicos, são conhecidos por promover a inativação de *Salmonella*. Dentre eles, estão o tratamento UHT, pasteurização, aquecimento em banhos térmicos e utilização de componentes químicos. *Salmonella* pode ser inativada, apesar de haver cepas resistentes, em pH acima de 9,0 e abaixo de 4,0, e em temperaturas acima de 55 °C (JAY, 2005; SPINKS et al., 2006).

Em relação aos ovos inteiros em casca, existe uma grande variedade de métodos utilizados para preservá-los e higienizá-los, como lavagem, resfriamento rápido, irradição e tratamento com ultrassom, entretanto, esses não são suficientes para inativar a *Salmonella* presente no interior dos ovos. Assim, o processamento

térmico ainda é um dos métodos mais eficazes e frequentemente utilizados para inativar a *Salmonella* dentro dos ovos.

A pasteurização de gemas e claras líquidas, misturadas ou separadas, é um tratamento térmico bem difundido, embora possa ocasionar algumas mudanças nas propriedades de seus componentes. Geralmente são comercializadas em embalagens de 500 ml a 1L e possuem, muitas vezes, prazo de validade inferior a quatro dias após a abertura. A utilização de pequenos volumes desses produtos pasteurizados suscita o descarte do mesmo, devido ao curto prazo de validade, e motiva o uso de ovos inteiros em casca, por exemplo, na preparação de drinques como o *pisco sour*. Estudos demonstram que ovos em casca imersos em água com temperaturas de 52 a 58 °C, juntamente com a utilização de vapor, reduzem a população de *Salmonella* presente no interior dos ovos, sem ocasionar grandes modificações nas suas propriedades, podendo ser utilizado na indústria para a pasteurização de ovos inteiros (GEVEKE et al., 2016; PARK; CHO, 2006). Entretanto, há poucos ovos inteiros em casca pasteurizados. Embora algumas indústrias já os produzam, nem sempre são encontrados facilmente em supermercados. Nos Estados Unidos, menos de 3% dos 74 bilhões de ovos frescos em casca produzidos a cada ano são pasteurizados (USDA, 2017). No Brasil, não há dados relacionados a essa categoria de ovos.

Tendo em vista a importância da inativação de *Salmonella* em produtos à base de ovos e o fácil acesso ao tratamento térmico, diferentes agências reguladoras, em todo o mundo, recomendam ou, muitas vezes exigem que os ovos que serão preparados e servidos devem ser cozidos a uma temperatura interna de pelo menos 70 ºC (BRASIL, 2004; Canada, 2013; CDC, 2011; FDA, 2016; WHO, 2015). O Codex Alimentarius recomenda que o tempo e a temperatura dos alimentos cozidos oferecidos em serviços de alimentação devam ser suficientes para garantir a destruição de micro-organismos patógenos não produtores de esporos (CODEX ALIMENTARIUS, 1993). No Brasil, os alimentos preparados devem ser submetidos ao tratamento térmico que garanta que todas as suas partes atinjam, no mínimo, 70 °C. Temperaturas inferiores podem ser utilizadas, desde que as combinações de tempo e temperatura sejam suficientes para assegurar a qualidade higiênico-sanitária dos alimentos (BRASIL, 2004). No geral, recomenda-se que caso não atingida a temperatura recomendada durante a preparação de ovos, devem ser

utilizados ovos pasteurizados (RIO GRANDE DO SUL, 2009). Alguns órgãos reguladores definem especificações mais restritas, exigindo que quando ovos pasteurizados não forem utilizados, tanto a gema quanto a clara de ovo devem estar sólidas, antes de serem servidas (FDA, 2016; RIO GRANDE DO SUL, 2009; SÃO PAULO, 2013). Embora não estejam de acordo com as legislações, muitas preparações da alta gastronomia seguem sendo preparadas devido a motivos históricos, culturais e/ou sensoriais.

1.3.5 Ovos Moles Processados em Termocirculador

Conhecido também como “Ovo Perfeito”, essa nova técnica de preparação de ovos utilizada nos restaurantes de alta gastronomia consiste em cozinhar ovos inteiros, com casca, em temperatura constante, em torno de 60 °C, por períodos de pelo menos 1h, com o intuito de manter a clara e a gema macias e com as texturas ligeiramente modificadas (VEGA; MERCADÉ-PRIETO, 2011).

O controle da temperatura é realizado com a utilização de termocirculadores de água e os ovos cozidos através dessa técnica também são conhecidos como “ovo 6X °C”, o qual indica a temperatura da água utilizada para o cozimento desses ovos. As temperaturas utilizadas geralmente variam entre 60 a 65 °C, mas podem chegar até os 69 ºC. A escolha da temperatura parte do C*hef* do restaurante, de acordo com a textura desejada. Quanto maior for o tempo de tratamento térmico, maior a consistência da gema e clara dos ovos. Entretanto, o mais comum é a utilização de temperaturas a partir de 62 °C, uma vez que o uso de temperaturas abaixo de 61 °C requerem longos períodos de cozimento para proporcionar um aumento na viscosidade da clara e da gema, tornando-se pouco usual nos restaurantes. Cozinhar os ovos em temperatura constante por diferentes tempos, ou em diferentes temperaturas em tempos fixos pode formar texturas que vão do líquido ao viscoso até a gema gelificada (VEGA; MERCADÉ-PRIETO, 2011).

1.3.6 *Pisco Sour* Peruano

O *pisco sour* peruano é um coquetel alcoólico, originário do Peru, em torno dos anos de 1916 – 1929, e considerado uma bebida nacional nesse país. No geral, os *sours* são bebidas basicamente compostas de uma bebida alcoólica, como suco de limão, açúcar e clara de ovo. O *pisco sour* peruano é preparado com pisco, uma bebida obtida a partir da destilação de mostos fermentados de uvas peruanas de teor alcoólico de aproximadamente 40 %, suco de limão, xarope de açúcar, gelo, Angostura e clara de ovo crua. O uso da clara de ovo concede uma textura cremosa e espumosa para o coquetel, e é obtida através da agitação vigorosa da mistura, que pode ser realizada através de um liquidificador ou uma coqueteleira (JORDÁN; RAMOS, 2015).

1.3.7 *Spaghetti alla carbonara*

Spaghetti alla carbonara é um prato tradicional italiano, apreciado e preparado mundialmente. A receita inclui a combinação de macarrão com bacon, queijo ralado, pimenta preta e ovos crus batidos. Devido ao fato desta preparação ter se tornado popular, muitas variações desta receita são encontradas em diferentes países. Dentre as variações, há alteração na quantidade dos ovos usados para formar o molho, e no uso de ovos inteiros ou apenas gemas. No entanto, embora ocorram algumas variações na preparação dessa receita, é consensual a textura que o molho deve apresentar. Os ovos misturados com queijo devem formar, através de cozimento suave, um molho cremoso e sedoso e não devem apresentar textura semelhante a ovos mexidos e grumos, que ocorre se os ovos são aquecidos a aproximadamente 70 ºC, temperatura a qual a gema começa a coagular e formar grumos (BALDWIN, 2012; HOSKING, 2007).

Nas receitas mais tradicionais de *spaghetti alla carbonara*, as gemas cruas são batidas e misturadas com queijo ralado e pimenta, e então essa mistura é incorporada à massa recém-cozida, escorrida e ainda quente, longe da fonte direta de calor. Assim, o processamento térmico do molho de ovos crus nesta receita ocorre apenas através da transferência de calor da massa (HOSKING, 2007).

1.3.8 Microbiologia Preditiva

A microbiologia preditiva possibilita predizer a resposta ao comportamento do micro-organismo frente às variações de fatores extrínsecos e intrínsecos como temperatura, pH e outras condições de armazenamento, através da utilização de modelos matemáticos. Com a microbiologia preditiva, é possível prever a evolução quantitativa da população microbiana ao longo do tempo, através da construção da cinética de multiplicação, inativação ou sobrevivência, de acordo com o comportamento do micro-organismo, em determinadas condições ambientais (HABERBECK, 2011). É um elemento essencial à microbiologia de alimentos, pois permite analisar riscos, avaliar a vida de prateleira, desenvolver novos produtos e processos, auxiliar tomadas de decisões e, portanto possibilita melhorias na qualidade e na segurança dos alimentos (OLIVEIRA, A. P., REZENDE, C. S. M. SOLA, J. C. F.; OLIVEIRA, 2013).

Os modelos matemáticos podem ser classificados como modelos primários, secundários e terciários. Os modelos primários descrevem a dinâmica da população microbiana em função do tempo, sob determinadas condições ambientais e de cultivo, e são utilizados como parâmetros do modelo, o número inicial de células, taxa de multiplicação/inativação, tempo da fase de adaptação (*lag*) e densidade populacional. Diferentes modelos preditivos primários foram desenvolvidos e descritos, como, Função de Gompertz, Modelo Baranyi, Modelo Monod, Modelo Logístico, valores D de inativação térmica, Modelo linear de três fases, entre outros (MCDONALD; SUN, 1999). Como exemplo da utilização dos modelos de nível primário, eles podem ser utilizados para descrever a redução populacional, através da contagem de unidades formadoras de colônia, durante um tratamento térmico ao longo tempo (NAKASHIMA; ANDRÉ; FRANCO, 2000).

Os modelos secundários descrevem a variação dos parâmetros cinéticos em função da variação de uma condição ambiental. São exemplos modelos secundários: Modelo Ratkowsky, Modelo Arrhenius, Modelos probabilísticos, Valores Z, Polinomiais ou resposta, Modelos de superfície, Modelo Belehradek, entre outros (MCDONALD; SUN, 1999). A identificação dos principais parâmetros ambientais que influenciam o comportamento microbiano é essencial para o desenvolvimento e utilização desses modelos. Dentre estes parâmetros, a temperatura é um dos fatores

predominantes, pois a determinação da fase *lag* e a velocidade de crescimento/inativação estão estritamente relacionadas com a temperatura. Ademais, fatores como pH e atividade de água também devem ser pontuados.

Por fim, os modelos terciários utilizam um ou mais modelos secundários e primários, os quais fazem parte ou possibilitam gerar um programa computacional (*software*). Através deles é possível calcular e comparar as respostas microbianas em diferentes condições, e também comparar com o comportamento de outros micro-organismos (KAJAK; KOŁOŻYN-KRAJEWSKA, 2006; MARKS, 2008; MCDONALD; SUN, 1999; MCMEEKIN et al., 2002; WHITING, 1995). Esses programas facilitam a modelagem das curvas de multiplicação e inativação microbiana sob diferentes condições. Muitos desses *softwares* estão disponíveis de forma gratuita, e fornecem automaticamente predições do comportamento microbiano, além da taxa de crescimento/inativação e tempo de fase *lag*, sob condições definidas. Os softwares mais utilizados são: *ComBase Pathogen Modeling Program, Predictive Models* e *GInaFiT (Geeraerd e Van Impe Inactivation Model Fitting Tool)* (GEERAERD; HERREMANS; VAN IMPE, 2000; ROSS; MCMEEKIN, 2003).

O *software GInaFit* versão 1.6, é uma ferramenta gratuita utilizada como uma extensão do Microsoft®Excel. Atualmente é muito utilizado para modelar e avaliar a cinética de inativação de micro-organismos através de ferramentas avançadas de regressão não linear (MARESCA; FERRARI, 2017; NG et al., 2016; WANG et al., 2016). A ferramenta possui dez modelos diferentes de inativação microbiana: Regressão Log-Linear; Modelo Log-Linear + Ombro; Modelo Log-Linear + Cauda; Modelo Log-Linear + Ombro + Cauda; Modelo de Weibull; Modelo de Weibull com parâmetro p fixo; Modelo de Weibull + Cauda; Modelo de Weibull Duplo, Modelo Bifásico e Modelo Bifásico + Ombro. O *software* permite testar os modelos e é possível verificar qual modelo melhor se ajusta aos dados coletados e, portanto qual o mais adequado para ser utilizado, através dos parâmetros gerados: soma das médias da raiz dos erros quadráticos (RMSE), soma das médias dos erros ao quadrado, coeficiente de determinação (R^2) e coeficiente de determinação ajustado (R^2_{adj}). Neste estudo foram utilizados modelos matemáticos gerados pelo *software GInaFit* para predizer a inativação microbiana nas preparações à base de ovos contaminados artificialmente com um pool de *Salmonella*.

Os materiais e métodos, resultados e discussão dessa dissertação serão apresentados na forma de artigos científicos, os quais são apresentados a seguir.

2 CAPÍTULO 2

Salmonella survival during soft-cooked eggs processing by *t*emperature-controlled water circulator

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Abstract

Soft-cooked eggs have been cooked and served worldwide, however concerns frequently raise about the safety of these preparations, assuming the possibility of eggs be contaminated by *Salmonella*. Temperature-controlled water circulators at low temperature (62 °C – 65 °C) for long periods (at least 1 h) has been used to thermally process eggs, aiming to modify its textures. However, time and temperature patterns are not in agreement with some recommendations for processing food preparations at least 70 °C. This study was undertaken to analyze the survival of *Salmonella* spp. during soft-cooked eggs processing by temperature-controlled water circulator. A pool of *Salmonella* spp. was inoculated in egg yolks and were incubated at 37 °C, for 18 h, reaching 7.7 ± 0.1 $\log_{10}$ CFU/g. Contaminated eggs were processed at 62 °C for 60 minutes and samples were collected in order to investigate Salmonella survival. Results indicated that the egg center temperature reached 61.7 ± 0.4 °C after 30 minutes, completely inactivating 7.7 log of Salmonella spp. After 30 minutes of cooking, yolk remained liquid and the egg white slightly opaque, demonstrating that the *Salmonella* inactivation was not related with the solidification of egg white or yolk. The survival curve did not follow first order kinetic and Double Weibull model was used to estimate inactivation kinetic parameters. In summary, the

results of this study can be used by food processors in order to validate soft-cooked eggs processing by temperature-controlled water circulator.

Keywords: gastronomy; food processing; cooking; *sous-vide*

1. Introduction

Salmonella has caused an expressive impact on foodborne illnesses worldwide. In the United States (U.S), the incidence of *Salmonella* infection was 16 per 100,000 in 2017 (MARDER et al., 2018). In 2015, *Salmonella* outbreaks, cases and hospitalizations were all ranked the highest compared to food diseases caused by other pathogens (CDC, 2017). Salmonellosis remains the second most common zoonosis in humans in the Europe Union (EU), being that the number of confirmed cases was 94.53, with a notification rate of 20.4 per 100,000 population (EFSA, 2017). In Brazil, 30 % of the pathogens identified in foodborne outbreaks were associated with *Salmonella* in the last decade (BRAZIL, 2018).

Salmonellosis has traditionally been associated with egg consumption and represents one of the highest risk agent/food combinations. The prevalence of *Salmonella* in eggs is normally low, 0.005 % in the US (EBEL; SCHLOSSER, 2000). In the EU, 0.29 % of the 5,782 tested table egg units were *Salmonella* positive, in 2016 (EFSA, 2017). In Brazil, several studies reported different incidences of this pathogen inside eggs and ranged between absence and 33 % (Baú, Carvalhal, & Aleixo, 2001; Kottwitz et al., 2013; Oliveira & Taham, 2011; Wolschick & Bosco, 2015). Although the low prevalence, the number of Salmonellosis human cases associated with eggs can still be large, especially because eggs are highly consumed and used in many dishes that frequently are not well heat-treated (EFSA, 2017; WINDHORST; GRABKOWSKY; WILKE, 2013).

Thermal processing is still one of the most common and effective methods to inactivate *Salmonella* inside eggs. Based on this fact, different regulatory agencies worldwide recommend or require that eggs that will be prepared and served should be cooked to an internal temperature of at least 70 °C (BRAZIL, 2004; CANADA, 2013; CDC, 2011; FDA, 2016). Some regulation bodies set higher specifications, requiring both yolk and egg white should be solid before serving (FDA, 2016; RIO GRANDE DO SUL, 2009; SÃO PAULO, 2013). When the temperature reaches 70 °C

the egg yolk coagulates and the protein ovomucoid denatures, the consistency of the egg white becoming harder (BALDWIN, 2012; THIS, 2016). However, in several restaurants around the world, especially those linked to fine gastronomy, eggs are cooked at relatively low temperatures, around 60 °C (VEGA; MERCADÉ-PRIETO, 2011), in order to keep the white and yolk soft or slightly modifying their texture.

Actually, different textures can be created using the slow cooking of the eggs. The new approach used in restaurants consists of cooking eggs at constant low temperature for long periods of time (at least 1 h), through the use of temperature-controlled water circulators. Eggs cooked through this technique are described as “6X °C egg”, the “X” usually varies from 0 to 5 °C, depending on the restaurant. However, the most common thermal processing pattern is the use of temperatures starting from 62 °C, because the use of temperatures below 61 °C requires longer cooking periods to increase the viscosity, becoming unusual. Cooking an egg using the same temperature at different times, or different temperatures with a fixed time can form textures going from viscous fluid to gelled yolk (VEGA; MERCADÉ-PRIETO, 2011). Therefore, the “6X °C egg” disagrees with official recommendations of texture and temperature. Thus, restaurants may need to validate this cooking practice for eggs.

Studies have demonstrated that shell eggs immersed in water at low temperatures (52 – 58 °C) reduced the *Salmonella* population, therefore this method is particularly useful for the egg industry (Geveke, Gurtler, Jones, & Bigley, 2016; Park & Cho, 2006). However, no study has reported the use of this technique as a method to control *Salmonella* in foodservices. Thus, the objective of this study was to evaluate the inactivation of *Salmonella* spp. in eggs cooked at low temperature (62 °C) through the use of temperature-controlled water circulator, in order to validate the safety of these eggs served in restaurants.

2. Material and methods

2.1 Bacterial strains and inoculum preparation

Five strains of *Salmonella* were used as a pool: *S.* Enteritidis SE86, S. Enteritidis 55507, *S.* Typhimurium L12031 (isolated from food outbreaks reported in Rio Grande do Sul State, Brazil), *S.* Minnesota and *S*. Heidelberg, isolated from poultry products. All strains were grown separately on 5 ml of Brain Heart Infusion

broth (BHI; Merck, Darsmtadt, Germany) at 37 °C, for 18 - 24 h. After incubation, 2 ml of BHI broth containing each strain were used in order to compose 10 ml of *Salmonella* pool. The pool was centrifuged (3500 RPM, 10 minutes, 4 °C) (CIENTEC CT-5000 R, Brazil), the supernatant was discharged and pallet was washed three times with 0.1 % peptone water (w/w) (Merck, Darsmtadt, Germany). Finally, cells were re-suspended 0.1 % in peptone water (w/w) and final cell concentration was adjusted through optical density (OD_{630nm}) and plate counts at 10^8 CFU/ml. Decimal serial dilutions in 0.1% peptone water (w/w) were prepared and used as inoculum in eggs as described below.

2.2 Growth of *Salmonella* pool in eggs

Eggs were inoculated according to the method report by (DE PAULA; MARIOT; TONDO, 2005) with some modifications. Eggs (Filippsen Eggs, Brazil), weighing 60 – 78 g were chosen and the yolk was inoculated using an egg candler and a sterile needle of 25 mm × 0.80 mm (Descarpack, Jiangsu Jichun Medical Devices Co., China) attached to a 10 ml syringe (Plastipak TM Becton Dickinson). A hole was punched through the shell of each egg and the needle was inserted about 2.5 cm until reached the yolk. After the yolk inoculation with approximately 100 cfu of *Salmonella* pool, a drop of quick-drying glue (Scotch, 3M, Brazil) was used to close the hole of the shell egg. The eggs were incubated at 37 °C for 18 – 24 h. After incubation *Salmonella* spp. population was counted and ranged from 7 – 8 log CFU/g.

2.3 Egg Preparation

After 18 to 24 h incubation, artificially contaminated eggs were stored for 60 minutes at room temperature, and then were immersed in a temperature-controlled water circulator (TermoHobby, H2heater, Brazil) at 62 °C for 60 minutes. Three eggs were removed from random positions at each set time points (5, 8, 10, 12, 14, 15, 16, 20, 25, 30 and 60 minutes) in order to quantify *Salmonella*. Post-treated eggs were immediately immersed into ice-water bath to stop the cooking process. Inoculated and non-heat treated shell eggs were used as controls. The experiment was performed three times and all counts were done in triplicate.

2.4 Internal Temperature

The temperature of the center of eggs was monitored using egg samples (not inoculated) previously stored at room and refrigeration temperature. A hole was punched through the shell egg and K-type thermocouples were inserted through *sous vide* foam tape and the temperature was recorded using a data logger (Tenmars, Taiwan).

2.5 Microbiological analysis

Each egg was aseptically opened and the content was placed in a sterile plastic bag and then mixed using a stomacher (Stomacher® 400, Seward, England) for one minute. To obtain the 10^{-1} dilution, 25 g were blended with 225 ml of sterile 0.1 % peptone water (w/w) for two minutes in a sterile plastic bag using a stomacher. Subsequent dilutions were obtained by mixing 1 ml aliquots with 9 ml of 0.1 % peptone water (w/w), and 100 µl these dilutions were spread on agar plates. Agar plates were prepared using Lysine Desoxycholate (XLD, Merck, Darsmtadt, Germany) plus a thin layer of Tryptic Soy Agar (TSA, KASVI, Italy) according to the one-step TAL method report by Kang & Fung (2000). The plates were incubated at 37 °C for 24 h and typical *Salmonella* colonies were counted. The detection limit of plate counts was 100 CFU/g. When increased sensitivity was required, 1000 µl of the undiluted suspension were plated on four XLD-TSA agar plates (250 µl for plate).

2.6 Complete inactivation test

Complete inactivation test was conducted to detect ≤ 1 CFU/g of *Salmonella*. Three post-treated eggs removed from random positions at each set time points (20, 25, 30 and 60 minutes) were immediately immersed into ice-water bath and then incubated at 37 °C for 24 h. Presence/absence testing per 25 g was performed as described above (item 2.5).

2.7 Data analysis

The inactivation kinetics was modeled with GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool) version 1.6, a freeware Tool for Microsoft®Excel. The Root Mean Sum of Squared Errors (RMSE), Mean Sum of Squared Errors, coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) were

evaluated and the *Double Weibull* model was chosen as best fit. In addition, the time needed for a 4 log reduction of the initial microbial population (4D) was also automatically estimated (COROLLER et al., 2006).

3. Results and Discussion

3.1 Temperature profile

Raw eggs have been stored in two ways before cooking, kept them at room temperature or refrigerated. In order to validate eggs submitted at different storage conditions, both methods of storage were analyzed.

The temperature profile of the center of eggs previously stored at room and refrigeration temperature, during cooking at 62 °C is presented in Figure 1. The heating profile did not show differences between room and refrigeration temperature. The come-up time (time that the center of an egg reaches within 1 °C of the final temperature) for both storing conditions was 21 ± 2 minutes (Figure 1). This result is in agreement with others studies. For example, according to Geveke et al. (2016) eggs placed inside a circulating hot water bath at 56.6 °C presented come-up time of yolk center of 21 minutes. Similarly, Stadelman et al. (1996) indicated that the time for the yolk to reach within 1 °C of the final temperature (56 °C) was approximately 20 minutes. However, other studies have reported higher come-up times. Cox et al. (1995) conducted a research and determined the come-up time of eggs previously stored at refrigeration temperature. The center-of-yolk temperature placed in a controlled water bath at 57 °C took approximately 45 minutes to achieve a temperature of 56 °C. Correspondingly, Park and Cho (2006) immersed room temperature eggs in 58.5 °C water and found come-up time of 45 minutes.

The differences in the come-up times observed in this study and others are probably related with the efficiency of circulating water bath in transferring properly heat from the water to the eggs.

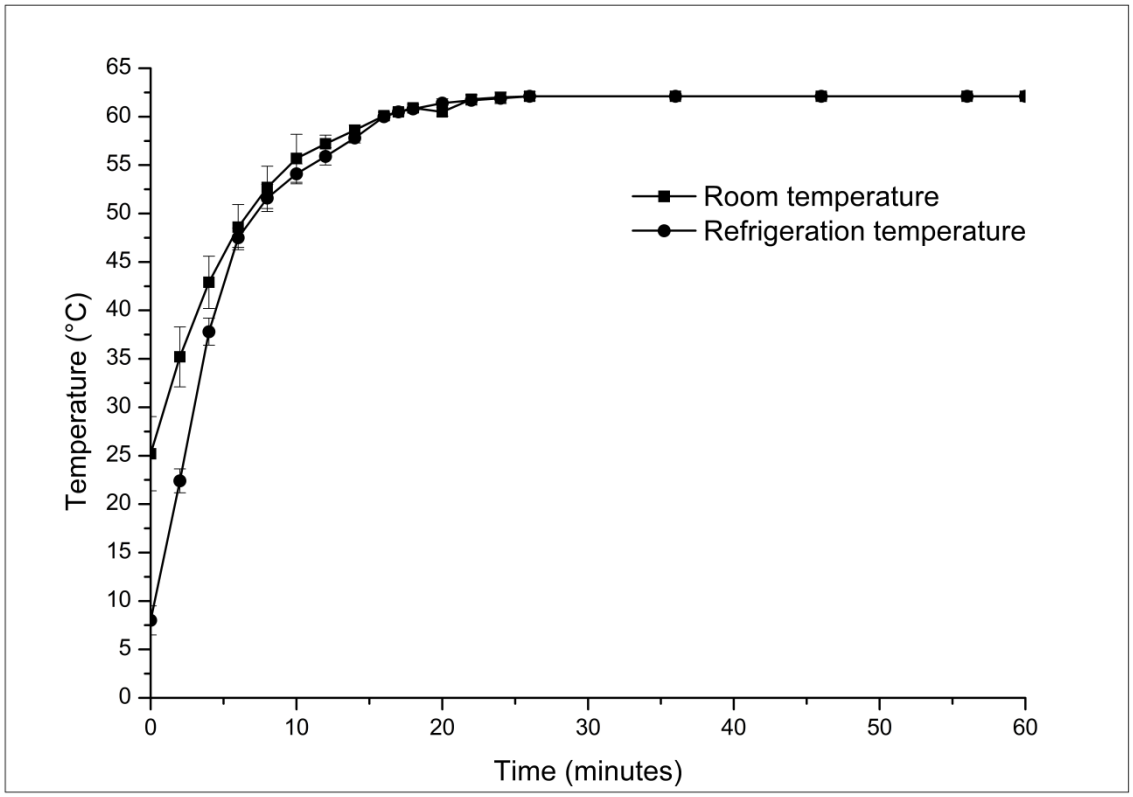

Figure 1 Temperature profile of the center of yolk in a shell egg placed in temperature-controlled water circulator at 62 °C

3.2 Thermal inactivation of *Salmonella*

The mean initial concentration of *Salmonella* spp. in the raw eggs was 7.7± 0.1 $\log_{10}$ CFU/g. The microbiological inactivation results (Table 1) indicated that processing at 62 °C for 30 minutes resulted in a complete inactivation of *Salmonella* spp. Correspondingly, Schuman et al. (1997) demonstrated that water bath at 58 °C for 57.5 minutes was sufficient for total inactivation of 6 - 7 $\log_{10}$ CFU/g of *Salmonella* Enteritidis (SE) inside shell eggs.

In the present study, *Salmonella* populations inside eggs decreased 4 $\log_{10}$ CFU/g in 15 minutes, when the mean temperature of yolks was 59 ± 0.4 °C (Table 1). Geveke et al. (2016) inoculated eggs with approximately 7 $\log_{10}$ CFU/mL of SE and placed them in a circulating hot water bath at 56.7 °C. The authors reported that it was necessary 60 minutes to produce an inactivation of 4.5 $\log_{10}$ CFU.

In our experiment, viable *Salmonella* was present in eggs at the end of 25 minutes of cooking at population levels of 1.1 ± 0.4 $\log_{10}$ CFU/g (6 $\log_{10}$ CFU/g

reduction), this time resulted in a final temperature of 61.1 ± 0.5 °C in the center of the yolk (Table 1). In other study, the treatment of shell eggs in circulating water bath at 57 °C for 25 minutes reduced approximately 3 log of SE (HOU et al., 1996). Differences in times and levels of reductions obtained in our study compared with the works cited above are probably due to the use of a higher temperature for thermal processing. In our research, the experiments were performed at 62 °C, while others used egg pasteurization temperatures, ranging from 57 to 59 °C. As expected, there is a negative correlation between temperature and time for reduction bacteria. The higher temperature took less time to reach the same reduction of *Salmonella*.

Davis et al. (2008) prepared soft-cooked eggs in a pan with water on hot plate. In that study, the initial concentration of *Salmonella* spp. was 3.5 $\log_{10}$ CFU/g and after approximately 15 minutes of cooking no viable bacteria was found. The authors indicated that soft-cooked eggs by this method were safe. Although, the number of *Salmonella* cells found in eggs are generally quite low (<20/egg) (Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991), the storage of eggs at environmental temperatures can leads to grow up to 7 $\log_{10}$ CFU/g in less than 24 h (GUMUDAVELLI et al., 2007). Moreover, in many cases the infective dose of *Salmonella* can be substantially lower (< 10 CFU) (Humphrey, 2004). Therefore, a reduction of 3.5 $\log_{10}$ CFU/g may be insufficient to produce safe eggs.

In the present study, total inactivation of *Salmonella* (7.7 $\log_{10}$ reduction) occurs at 30 minutes, when the mean temperature of yolk reached 61.7 ± 0.4 °C (Table 1). At this temperature and time, the yolks remained liquid and the egg white slightly opaque (Figure 2). Our results show that the complete inactivation of *Salmonell*a in eggs has no relation with the solidification of the yolk. Perhaps the only factors that can insure the inactivation of *Salmonella* are the internal temperature that the egg yolk reaches and the time which that temperature is maintained.

In restaurants, the use of water circulators for soft-cook eggs generally range from 62 °C to 65 °C, during periods longer than 60 minutes. Our results demonstrated that the use of this method using 62 °C for 60 minutes is safe.

Table 1 Thermal inactivation of Salmonella spp. in shell eggs immersed in temperature-controlled water circulator at 62 °C.

Total process time (min)	Mean egg center temperature (°C ± SD)	Mean survivors (log_{10} CFU/g ± SD)	Samples *Salmonella*-positive by complete inactivation test
0	25.2 ± 3.8	7.7 ± 0.1	-
5	46.2 ± 2.7	7.5 ± 0.1	-
8	52.7 ± 2.2	7.2 ± 0.6	-
10	55.7 ± 2.5	6.7 ± 0.8	-
12	57.2 ± 0.9	6.1 ± 0.1	-
14	58.6 ± 0.5	5.2 ± 0.1	-
15	59.1 ± 0.5	2.9 ± 0.3	-
16	60.1 ± 0.4	2.8 ± 0.3	-
20	60.5 ± 0.4	1.1 ± 0.4	2/3
25	61.1 ± 0.5	ND	1/3
30	61.7 ± 0.4	ND	0/3
60	62.1 ± 0.2	ND	0/3

SD, standard deviation; ND, Not detected (<10 CFU/g).

Figure 2 Visual texture of an egg processed at 62 °C for 30 minutes in a temperature-controlled water circulator

3.3 Curve fitting

The survival curve of *Salmonella* in eggs processed in water circulator was fitted. The *Double Weibull* model was selected and used to represent this curve. The fit was good with an R^2 (0.98), R^2_{adj} (0.98), RMSE (0.42) and Mean Sum of Squared Errors (0.18).

As presented in Figure 3, the survival curve is concave ($p > 1$), indicating that the inactivation of *Salmonella* did not follow a first order kinetic. This result is different compared to other authors that found first-orders kinetics curves of the inactivation of

Salmonella within intact shell eggs immersed in water-bath at temperatures ranged from 54.4 °C to 58.0 °C (GEVEKE et al., 2016; SCHUMAN et al., 1997).

In agreement to our results, other studies have demonstrated that survival curves of heat inactivation are generally not governed by classical first order kinetics, especially for mild heat treatments (Oliveira et al., 2018; Scanlon et al., 2015; Van Boekel, 2002; Wang et al., 2016). Moreover, the curvature of survival curves is commonly attributed to the presence of mixed populations and also a high spectrum of heat resistance, as the pool of *Salmonella* used in this study (COROLLER et al., 2006; PELEG; COLE, 1998).

In the Double Weibull model three important parameters are estimated, i.e., α (5.9 ± 0.8 $\log_{10}$), δ_1 (10.7 ± 1.0 minutes) and δ_2 (21.9 ± 3.6 minutes). The α value is the fraction of first subpopulation remaining in total population and the δ values are, respectively, the time for first and second decimal reduction. In this model δ_1 is lower than δ_2, representing that the subpopulation_1 is more sensitive to stress and subpopulation_2 more resistant (COROLLER et al., 2006).

The 4D reduction automatically provided by Double Weibull model was 15.25 minutes. While 4D is a value generated in non-log-linear curves, D-value is generated in linear microbial survivor curves. For comparison purposes, the parameter δ_1 and D-value were considered similar, since both of them represent the time for first decimal reduction. The value of the parameter δ_1 generated in this study (10.7 ± 1.0 minutes) was similar with the D-value data reported by Geveke et al. (2016) for shell eggs inoculated with a pool of SE. They pasteurized the eggs using a water immersion process at 56.7 °C and reported a mean D-value of 9.33 minutes. A D-value lower than parameter δ1 was found by Schuman et al. (1997). They also inoculated shell eggs with SE, placed the eggs in water circulator at 58 °C and obtained a decimal reduction time of 4.5 minutes. An explanation for the higher value of decimal reduction obtained in our present research (even using higher temperatures compared with other studies) can be attributed to the high heat resistance of the different strains used.

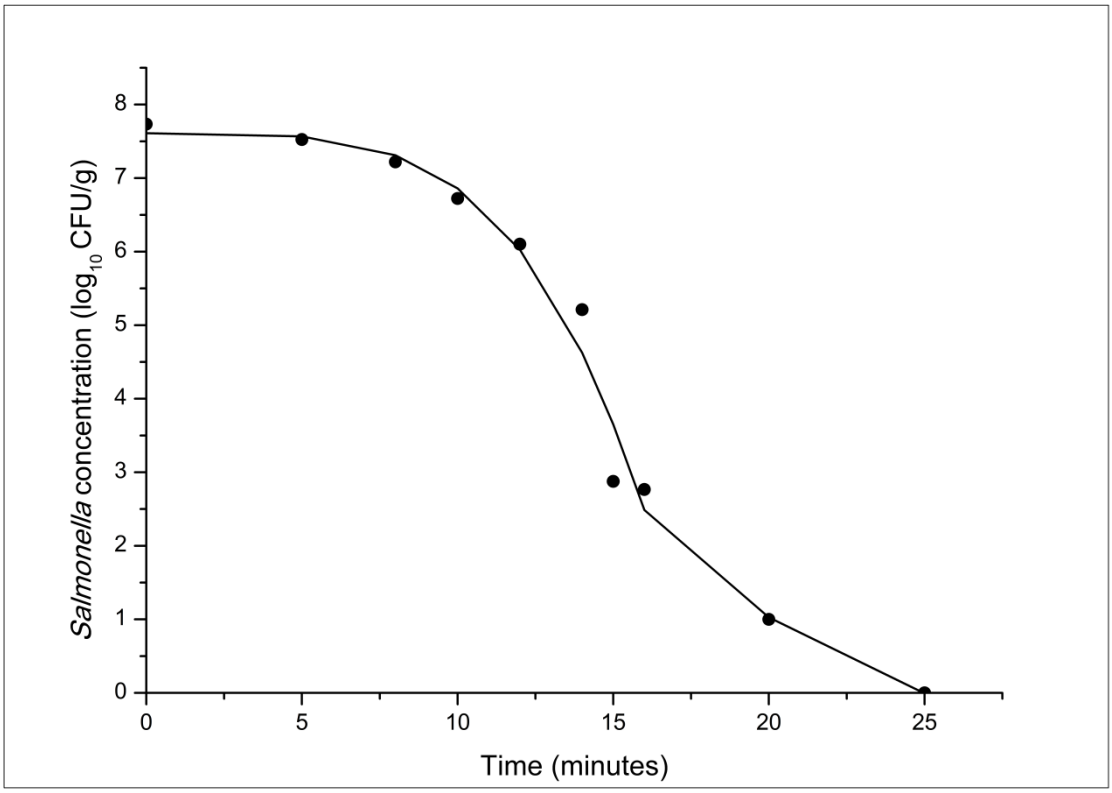

Figure 3 Inactivation curve of Salmonella spp. at 62 °C, obtained by Double Weibull model. ●: observed curve. -: estimated curve by the inactivation model.

4. Conclusion

This study demonstrated that soft-cooked eggs processed by temperature-controlled water circulator at 62 °C for at least 30 minutes was able to inactivate 7.7 $\log_{10}$ CFU/g of *Salmonella* spp. Based on these result, we considered eggs processed at this temperature but for 1 hour safe.

Our findings demonstrated that the soft-cooked eggs processed by this method had the yolks liquid, what can be a desirable characteristic for eggs in many restaurants. Indifferently if eggs were stored at room or refrigeration temperature, the come-up time of the coldest part of eggs immersed in circulating water bath at 62 °C was 21 ± 2.5 minutes. The survivor curves at the temperature studied indicating that the inactivation of *Salmonella* inside shell eggs did not follow the first-order kinetics. Double Weibull model provided the best fit to data, showing a decimal reduction time of 10.7 ± 1.0 minutes and a 4D reduction of 15.25 minutes.

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3 CAPÍTULO 3

Survival of *Salmonella* in Peruvian pisco sour drink

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The article was formatted according to the Food Microbiology - Journal

Abstract

Peruvian pisco sour is an alcoholic cocktail prepared with egg white in order to form a creamy texture. This drink has been served worldwide, however concerns about its safety have been raised because of the possibility of egg whites be contaminated by *Salmonella.* This study was undertaken to analyze the survival of *Salmonella* in pisco sour drink. A pool of *Salmonella* was inoculated in egg white and then mixed during 30 seconds with Quebranta pisco, syrup, lemon juice and ice cubes, reaching 6.1 ± 0.1 $\log_{10}$ CFU/ml. Samples were collected to investigate the *Salmonella* survival. Microbiological results demonstrated that *Salmonella* was not detected (<100 UFC/ml) after 6 minutes of exposure to pisco sour (18 % vol., pH 3.0), while predictive results showed that the whole population (6.1 $\log_{10}$ CFU/ml) was completely inactivated after 9 minutes. The alcohol content associated with a low pH of pisco sour was responsible for *Salmonella* reduction, however, phenolic compounds present in Quebranta pisco (3.5 ± 0.3 µgGAE/ml) can also contributed to the *Salmonella* inactivation. In conclusion, it is advisable to wait 9 minutes between preparation and consumption of pisco sour when raw egg white is used or prepare pisco sour using thermal processed egg whites.

Keywords: Gastronomy; Cocktail; Food processing; Alcoholic beverage;

1. Introduction

Peruvian pisco sour is a well-known cocktail originated from Peru and served worldwide in bars, pubs and restaurants. This drink is prepared with pisco, a beverage obtained from the distillation of fermented Peruvian grape musts, lemon juice, syrup, ice, Angostura and egg white. The use of egg white forms a creamy and frothy texture obtained from the vigorously shake of the mixture (Jordán and Ramos, 2015).

The consumption of eggs has been associated with salmonellosis and represents one expressive impact on foodborne illnesses worldwide. Intact eggs can be contaminated with *Salmonella* by external sources due to contamination of the eggshell surface with feces and subsequent penetration through the shell. Also, eggs can be contaminated during their formation inside the ovary or during the passage through oviducts of infected hens, before the eggs contents are covered by shell. In these two possible routes, *Salmonella* can contaminate both the yolk and the egg white (EFSA, 2014).

Although contaminated whole eggs present generally a quite low number of *Salmonella* (<20/egg) (Humphrey et al., 1991), the storage of eggs at environmental temperatures can lead to high numbers of this pathogen, specially due to the physicochemical conditions (pH, viscosity and nutritional) of the egg yolk (Gumudavelli et al., 2007). In opposite, some studies have demonstrated that physicochemical conditions of egg white, like nutritional deficiencies and alkaline pH, can difficult the bacterial growth. Moreover, antimicrobial molecules such as lysozyme and ovotransferrin can play an important role in defense of egg white against *Salmonella* (Baron et al., 2016; Clavijo et al., 2006; Messens et al., 2005).

However, different studies showed that *Salmonella* Enteritidis is able to survive in egg white (Clavijo et al., 2006; Gantois et al., 2009; Guan et al., 2006; Kang and Fung, 2000), being possible to contaminate foods and cause salmonellosis after the intake of inadequately thermal-processed food preparations.

Thermal processing is the most common method to inactivate *Salmonella* in eggs. Based on this fact, regulatory agencies of different countries require the use of pasteurized eggs in preparations that will not be cooked to an internal temperature of at least 70 °C (BRAZIL, 2004; Canada, 2013; CDC, 2011; FDA, 2016). Even being recommended, the use of pasteurized eggs are not very common worldwide, because they are not always easily found in supermarkets and people are not used to looking for them to prepare foods or drinks. In the United States, less than 3 % of the 74 billion fresh eggs produced each year are pasteurized (USDA, 2017).

Generally, pasteurized liquid whole eggs, yolks or egg whites are commercialized in 500 ml to 1 L packages, with shelf-life frequently shorter than 4 days after opening. In order to prepare one dose of Peruvian pisco sour, approximately 10 ml of egg white are used. If high volumes of this drink is not prepared and sold, pasteurized egg whites have to be discarded, and this fact have motivated bars, pubs and restaurants to prefer the use of raw egg whites to prepare pisco sour instead of pasteurized ones. When pisco sour is prepared with raw white eggs the risk of salmonellosis is not insignificant and concerns about the safety of this drink have been raised even though this drink has high content of alcohol. Thus, the present study aimed to evaluate the survival of *Salmonella* in Peruvian pisco sour in order to evaluate its safety in case of preparation using contaminated egg whites.

2. Material and methods

2.1 Bacterial strains and inoculum preparation

Five strains of *Salmonella* were used to compose a pool: *S*. Enteritidis SE86, *S*. Enteritidis 55507, *S*. Typhimurium L12031 *S*. Minnesota and *S*. Heidelberg, being the three first strains isolated from salmonellosis outbreaks and the last two isolated from poultry products. All the strains were isolated in the State of Rio Grande do Sul, Southern Brazil. The strains were grown individually on 5 ml of Brain Heart Infusion broth (BHI; Merck, Darsmtadt, Germany) at 37 °C, for 18 - 24 h. After incubation, 2 ml of BHI broth containing each strain were used in order to form 10 ml of *Salmonella* pool. The pool was centrifuged (3500 RPM, 10 minutes, 4 °C) (CIENTEC CT-5000 R, Brazil), the supernatant was disposed and pallet was washed three times with 0.1 % peptone water (w/w) (Merck, Darsmtadt, Germany). Finally, cells were re-suspended 0.1 % in peptone water (w/w) and final cell concentration was adjusted through optical density (OD_{630nm}) and plate counts at 10^8 CFU/ml. The inoculum was used in egg whites as described below.

2.2 *Salmonella* pool inoculation in egg whites

Eggs (Filippsen Eggs, Brazil) weighing 60 – 73 g were chosen and egg whites were aseptically separated from the yolks by hands wearing sterilized gloves inside bacteriological chamber. Ten milliliters (10 ml) of egg white was filled into sterile plastic bags and then artificially inoculated with a *Salmonella* pool to reach a final concentration of approximately 10^7 CFU/ml.

2.3 Peruvian pisco sour preparation

Artificially contaminated egg whites (10ml) were added to 100 ml of Quebranta variety of Peruvian pisco (Santiago Queirolo, Lima, Peru, with an alcohol content of

42 % volume and pH 3.9) 36 ml of syrup, 36 ml of lemon juice and five ice cubes (corresponding to 50 ml of potable water) in a cocktail shaker (Jordán and Ramos, 2015). The drink was mixed by hand during 30 seconds, reaching 18 % of final alcohol content and 6.1 $\log_{10}$ CFU/ml. Among a variety of pisco sour recipes on the literature, the one studied by Jordan and Ramos at the College of Culinary Arts, Le Cordon Bleu, was chosen for this study due to its international recognition.

In order to evaluate the effect of Quebranta pisco on the survival of *Salmonella*, a drink prepared without this ingredient was used as control. All the experiments were performed three times and all counts were done in triplicate.

2.4 Microbiological analysis

After the drink preparation, aliquots at each set time points (1, 2, 3, 5, 6, 7, 8 and 10 minutes) were collected and *Salmonella* quantification was carried out. Before each sampling, the drink was slowly homogenized for 5 seconds. Decimal dilutions were obtained by mixing aliquots of 1 ml of contaminated pisco sour with 9 ml of 0.1 % peptone water (w/w), and 100 µl of each dilution were spread on Xylose Lysine Desoxycholate agar (XLD, Merck, Darsmtadt, Germany). XLD plates were incubated at 37 °C for 24 h and typical *Salmonella* colonies were counted after incubation. The detection limit of plate counts was 100 CFU/ml.

Salmonella was also researched according to the International Standard Organization protocol (ISO 6579, 2007) with the intention to detect ≤ 100 CFU/ml (presence/absence), which was not possible by traditional plate counts. For this research, samples were collected at 7, 8, 9, 10 and 14 minutes.

2.5 Antibacterial test

The antibacterial effect of different variants of the pisco sour was evaluated. Three different solutions were tested (ethanol 18 %, water pH 3.0 and ethanol 18 % + pH 3.0). The tests were conducted according to the method reported by Marimón et al. (1998) with some modifications. Absolute ethanol was diluted in sterilized water in order to obtain a final ethanol concentration of 18 % (vol.) and the pH 3.0 was adjusted with hydrochloric acid (HCl) solution. Sterilized water pH 3.0 was also adjusted using HCl. An aliquot of 200 µl of the *Salmonella* pool was added to 3.8 ml of the three different solutions, reaching a final concentration of 10^6 CFU/ml. At 7 and 30 minutes of exposure, aliquots were collected and *Salmonella* was quantified. The analysis was carried out as described in item 2.4.

2.6 pH

The pH of ingredients, pisco sour and control was measured using a pH meter model Q400A (Quimis®, São Paulo, Brazil). The analysis was performed in triplicate.

2.7 Total phenolic content

The total phenolic content of the Quebranta pisco was determined by the Folin–Ciocalteu method. Briefly, a 200 µl aliquot of Quebranta pisco was added to 1.58 ml of distilled water, mixed thoroughly with 100 µl of Folin–Ciocalteu reagent for 3 min, followed by the addition of 300 µl of sodium carbonate 1N. The mixture was mixed well and allowed to stand for 2 hours in the dark. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). The total phenolic content was calculated from the calibration curve prepared using concentrations of gallic acid ranging from 20 to 500 mg/l. The results were expressed in µg of gallic acid equivalents (GAE) per ml.

2.8 Data analysis

The inactivation kinetics was modeled using GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool) version 1.6, Microsoft®Excel. The Root Mean Sum of Squared Errors (RMSE), Mean Sum of Squared Errors, coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) were evaluated and the Geeraerd Log-Linear + Shoulder was chosen as best fit (Geeraerd et al., 2000). Complete inactivation of *Salmonella* was predicted using the predictive model (Geeraerd Log-Linear + Shoulder) because it was not possible by traditional microbiological methods due the detection limit of 100 CFU/ml.

3. Results and discussion

3.1 pH

Ingredients, pisco sour and control showed acid pH values as demonstrated in Table 1, while egg white showed alkaline pH (9.1± 0.1). The egg white pH was considered normal because egg white from a freshly laid egg generally has pH values ranging from 7.6 to 7.9, however during storage, it increases to around 9.0, due to CO_2 solubilization through the shell (Belitz et al., 2009).

Table 1. pH value of ingredients, pisco sour and control.

Ingredients/Beverage	pH ± SD
Egg white	9.1 ± 0.1
Syrup	4.4 ± 0.3
Quebranta pisco	3.9 ± 0.1
Lemon juice	2.3 ± 0.5
Pisco Sour	3.0 ± 0.3
Control	2.4 ± 0.1

SD, standard deviation; Control: drink prepare without Quebranta pisco (0 % ethanol (vol.))

3.2 Inactivation of *Salmonella*

The initial mean concentration of *Salmonella* in the pisco sour was 6.1 ± 0.1 $\log_{10}$ CFU/ml. The microbiological inactivation results (Table 2) indicated that, after 6 minutes of contact with pisco sour (18 % vol., pH 3.0), *Salmonella* was reduced below to the detection limit (100 CFU/ml), resulting in at least 4.1 $\log_{10}$ CFU/ml of *Salmonella* inactivation. In addition, was detected the absence of *Salmonella* in 25 ml from 7 minutes. This result is in agreement with others studies, for example, Marimón et al. (1998) who studied the antibacterial activity of red wine (12.5 % vol., pH 3.5) against *Salmonella* Enteritidis and demonstrated that no viable bacteria were found after 5 minutes of exposure, representing 6 $\log_{10}$ CFU/ml of *S*. Enteritidis reduction. Similarly, Gaglio et al. (2017) inoculated three different enteric bacteria in ice cubes (60 ml) and added to 100 ml of whisky (40 % vol., pH 4.2) and in Martini (14.4 % vol., pH 3.8). The authors concluded that after one hour of exposure bacterial counts decreased consistently in both drinks, completely disappearing from Martini (2.6 $\log_{10}$ CFU/100 ml reduction). In other study, ice cubes (20 ml) inoculated with *S.*

Typhimurium (3 $\log_{10}$ CFU/100 ml) in contact with 100 ml of tequila (43 %) and scotch (40 %) showed 5 % and 11 % of remaining viable bacteria after one hour of exposure, respectively. Differences in the reduction levels obtained in our study compared with these studies are probably due to different types of beverages with different alcohol contents, pH and ingredients.

The association of ingredients of pisco sour drink can play different roles in the bacterial reduction and physicochemical properties. For example, although Quebranta pisco has an acid pH, the presence of lemon juice contributes to the pH reduction. Both, associated with alcohol, resulted in the inactivation of *Salmonella* observed in our study, as demonstrated below.

Salmonella populations in the control beverage (without alcohol and pH 2.4) showed no reduction during 30 minutes of exposure (Figure 1). Corroborating these results, Menz et al. (2011) evaluated the effects of ethanol on the survival of *S.* Typhimurium in beer (pH 4.3). The authors reported that beers with alcohol (2.7 % vol. and 5.0 % vol.) were able to reduce *S.* Typhimurium counts, while alcohol-free beer (0.5 % vol.) increased *Salmonella* counts after more than 10 days of lag phase. Our results indicated that although the control beverage has a low pH due to the lemon juice presence, the inactivation of *Salmonella* in the pisco sour was mainly due to the presence of Quebranta pisco.

In restaurants, bars and pubs, the preparation of pisco sour usually takes few minutes before serving to the consumers. Our results demonstrated that in 3 minutes of exposure to pisco sour, *Salmonella* was present at population levels of 4.3 ± 0.1 $\log_{10}$ CFU/ml. This result indicated that the rapid distribution of this beverage may not be safe to the consumers if egg white is highly contaminated.

Table 2. Inactivation of *Salmonella* in pisco sour (18 % vol., pH 3.0).

Total exposure time (min)	Mean survivors (log_{10} CFU/g ± SD)
0	6.1 ± 0.1
1	6.1 ± 0.6
2	5.1 ± 0.1
3	4.3 ± 0.1
5	3.3 ± 0.6
6	2.1 ± 0.1
7	ND
8	ND
10	ND

SD, standard deviation; ND, Not detected (<100 CFU/ml).

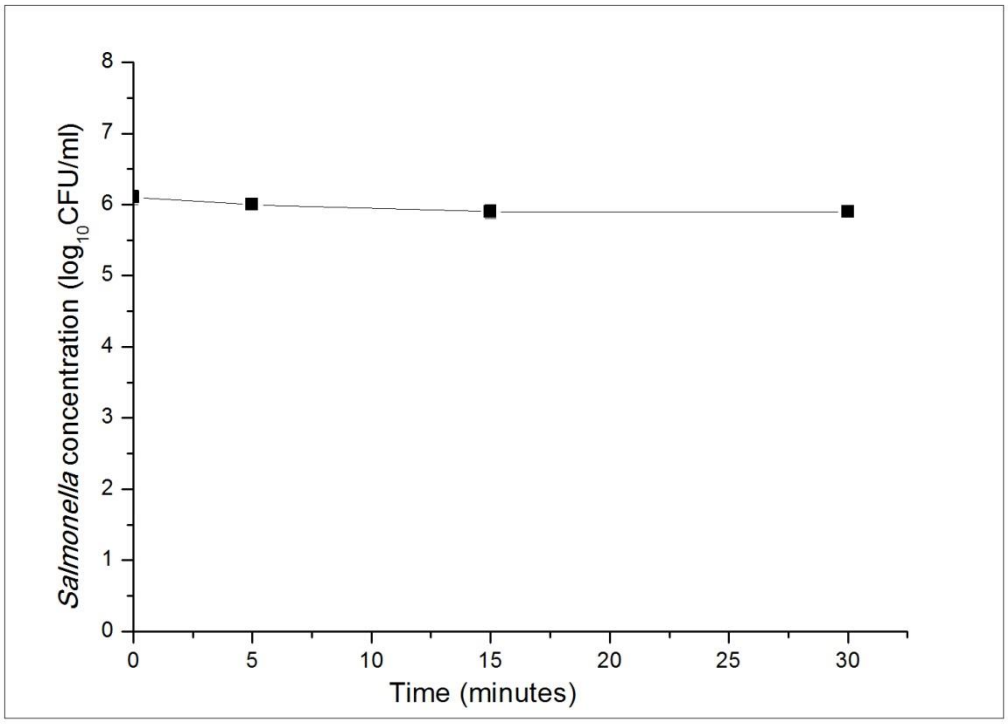

Figure 1. *Salmonella* survival in control beverage (0 % ethanol vol., pH 2.4).

3.3 Curve fitting

The survival curve of *Salmonella* in pisco sour drink was fitted to the Geeraerd Log-Linear + Shoulder model. The fit was considered adequate with R^2 (0.98), R^2_{adj} (0.97), RMSE (0.28) and Mean Sum of Squared Errors (0.08).

As presented in Figure 2, the survival curve exhibited an initial constant phase (shoulder) followed by a log-linear phase. In the Log-Linear + Shoulder model three parameters were estimated, i.e., Sl, k_{max} and N_0. The SI represents the time of shoulder length. A shoulder of 0.7 ± 0.6 minutes was predicted, which means that no *Salmonella* inactivation occurred before this period. The initial microbial population (N_0) was 6.2 ± 0.3 $\log_{10}$ CFU/ml and the maximum inactivation rate of the microbial population (k_{max}) was 1.7 ± 0.2 $minutes^{-1}$.

No studies concerning non-thermal survival kinetics in beverages were found to compare the results found in the present research. However, the model structure has been successfully applied to survival data of different non-thermal treatments and microorganisms, such as the Acid Tolerance Response (ATR) of *Salmonella* (Greenacre et al., 2003) and the inactivation of *Listeria monocytogenes* in a pH-modified chicken salad during refrigerated storage (Guentert et al., 2005).

Based on the model used by us, it was possible to predict the complete inactivation of Salmonella in pisco sour, which was not possible by traditional methods that have the detection limit of 100 CFU/ml. The predictive results demonstrated that 6.1 $\log_{10}$CFU/ml of *Salmonella* was completed inactivated in 9 minutes of exposure to pisco sour.

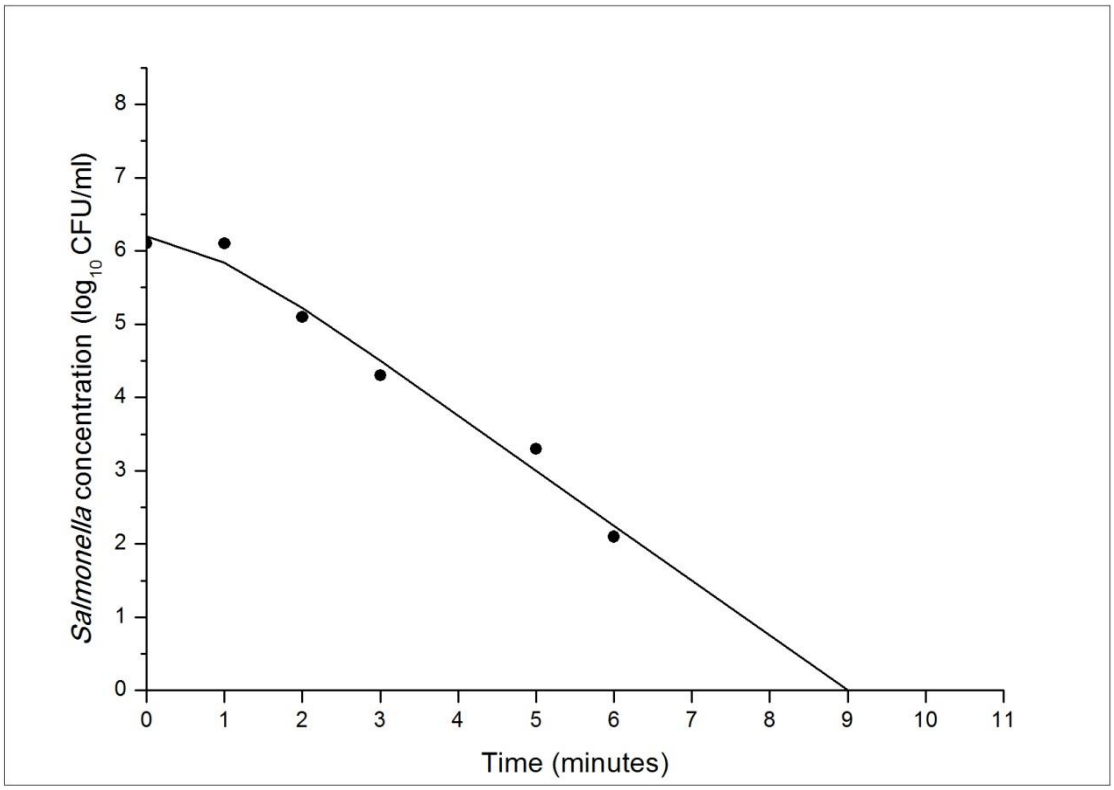

Figure 2. Inactivation curve of *Salmonella* in pisco sour, obtained by Geeraerd Log-Linear + Shoulder model. ●: observed curve. -: estimated curve by the inactivation model.

3.4 Effect of pH, Ethanol and Ethanol + pH

In order to elucidate which variant of the pisco sour was responsible for *Salmonella* inactivation, three different solutions with the beverage parameters were tested (ethanol 18 % vol., water with pH 3.0 and ethanol 18 % + pH 3.0).

The microbiological inactivation results (Figure 3) showed that the ethanol 18 % + pH 3.0 solution was the most effective solution against *Salmonella*. Isolated solutions (pH 3.0 and ethanol 18 %) were not able to significantly reduce *Salmonella*

counts at both exposure times (7 and 30 minutes). Corroborating this result, Malheiros, Brandelli, Noreña and Tondo (2009) found that acid non-adapted *S.* Typhimurium and *S.* Enteritidis exposed to pH 3.5 in nutrient broth, by 30 minutes, were reduced approximately 1 $\log_{10}$ CFU/ml.

In the present study, *Salmonella* was undetected after 30 minutes of exposure to ethanol + pH solution. According to Gaglio et al. (2017), there is a possible correlation between alcohol and pH of beverages affecting the survival of bacteria. In their study, vodka with higher alcohol content (38 %) and a pH 6.0 facilitated a higher survival of enteric bacteria when compared to whisky (40 %, pH 4.2). The authors concluded that a higher survival observed in vodka could be partly attributable to the neutral pH (6.0).

The results of our experiments suggested that *Salmonella* inactivation occurred in pisco sour mainly because of the alcohol content associated with a low pH. However, in the pisco sour, *Salmonella* was undetected after 6 minutes of exposure, while 3.6 ± 0.1 $\log_{10}$ CFU/ml of viable bacteria remained detected after the same exposure time in the ethanol 18 % + pH 3.0 solution. This result indicated that other compounds may contribute to the inactivation of *Salmonella* in pisco sour. Corroborating this finding, Marimón et al. (1998) evaluated the effect of red wine (12.5 % vol., pH 3.5), pH (3.5) and ethanol (12.5 % vol.) against *S.* Enteriditis. This bacterium was not detected after 5 minutes of exposure to red wine, while the other solutions did not show significant bacterial reduction in 30 minutes of exposure. The authors concluded that red wine bactericidal effect does not exclusively depended on the pH and ethanol concentration. The polyphenols contained in red wines contributed to the bactericidal effect against *S.* Enteriditis.

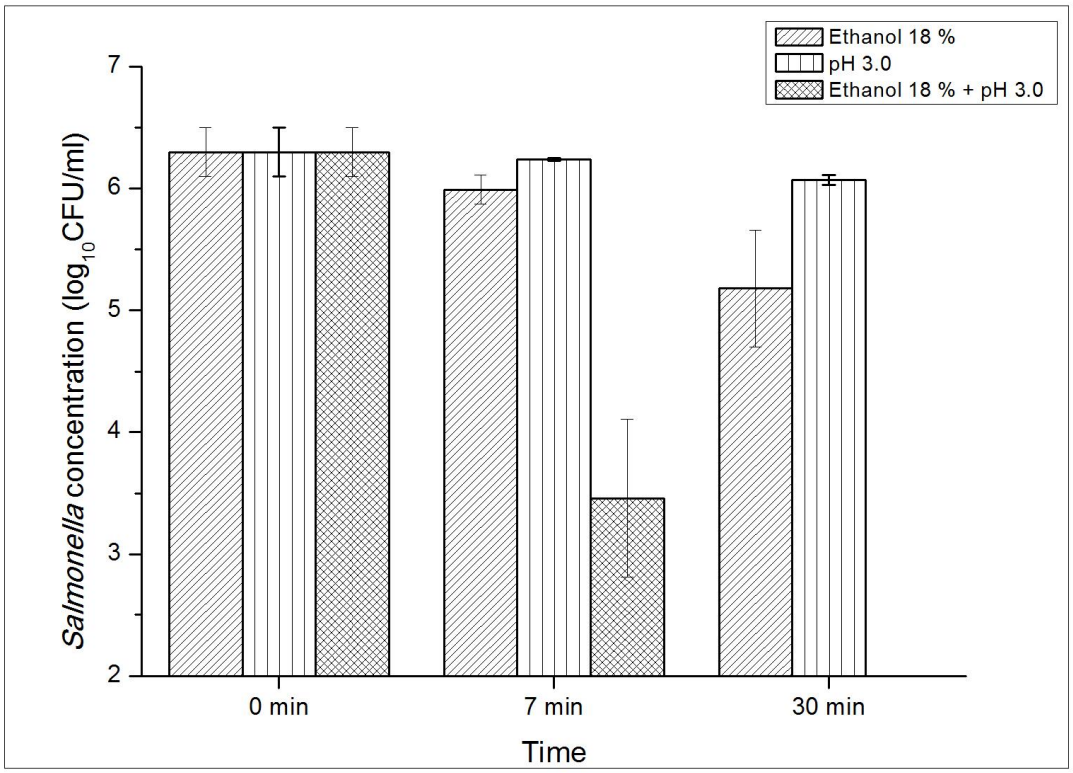

Figure 3. *Salmonella* survival in different solutions: ethanol 18 % vol., water with pH 3.0 and ethanol 18 % + pH 3.0.

3.5 Phenolic Compounds

The concentration of total phenolic compounds of Quebranta pisco was 3.5 ± 0.3 µgGAE/ml. No study about phenolic compounds of pisco was found to compare the result found in the present research. However, different studies showed the presence of phenolic compounds in distilled beverages (Goldberg et al., 1999; Nie and Kleine-Benne, 2012; Otsuka et al., 1965). The antimicrobial properties of phenolic compounds have been studied by different authors (Cetin-Karaca and Newman, 2015; Fu et al., 2016; Vaquero et al., 2007). Certain phenolic compounds exhibited great antimicrobial potential properties. For example, Cetin-Karaca and Newman (2015) evaluated the antimicrobial efficacy of natural phenolic compounds

against *Salmonella* and concluded that although these compounds showed varied degrees of antimicrobial activity, some compounds tested were very effective in concentrations <5 µg/ml. Similar results were demonstrated by Vaquero et al. (2007) who analysed the antimicrobial properties of polyphenols present in red wine against pathogens and determined that the inhibition of bacteria increased according to the polyphenols concentration of wines. In their study, few compounds with concentrations less than 2 µg/mL were effective against some pathogens.

Therefore, due to the content of phenolic compounds found in our research, the results suggested that polyphenol compounds also contributed to the *Salmonella* inactivation in the Peruvian pisco sour.

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4. Conclusion

The plate count results of the present study demonstrated that at least 4.1 $\log_{10}$ CFU/ml of *Salmonella* were inactivated after 6 minutes of exposure to Peruvian pisco sour, while the predictive results showed that *Salmonella* was completely inactivated at 9 minutes of exposure (6.1 $\log_{10}$ CFU/ml reduction). Based on these results, if pisco sour is prepared with highly *Salmonella* contaminated egg white, the rapid distribution of this beverage, may not be safe to the consumers. To improve the safety of this drink, it is advisable the use of thermo-processed egg whites or let raw egg whites in contact to pisco sour ingredients for periods of 9 minutes or more, before serving this drink.

Our findings also demonstrated that the presence of Quebranta pisco contributed for *Salmonella* reduction. The inactivation in pisco sour was related mainly to alcohol content associated with a low pH, which was reduced even more in

presence of lemon juice. However, phenolic compounds present in the Quebranta pisco can also contributed to the *Salmonella* inactivation.

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4 CAPÍTULO 4

Survival of *Salmonella* in spaghetti *alla carbonara*

Stefani Machado Lopes, Eduardo César Tondo

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Abstract

Spaghetti *alla carbonara* is a traditional Italian dish, which the sauce made of raw egg yolks and other ingredients is heated using only the heat of cooked pasta. *C*oncerns about the safety of this preparation have been raised due the possibility of egg yolks be contaminated by *Salmonella* and the heat treatment may not be sufficient for total *Salmonella* inactivation. This study was undertaken to analyze the survival of *Salmonella* spp. in spaghetti *alla carbonara* in which the only thermal processing of egg yolks was the heat transfer from the pasta. A pool of *Salmonella* spp. was inoculated in egg yolks reaching 8.8 ± 0.1 $\log_{10}$ CFU/g. Contaminated egg yolks were mixed with grated cheese and black pepper, and then added to the *cooked* spaghetti, away from the heat source. Samples were collected and *Salmonella* was quantified. Results indicated that immediately after cooking and draining, the pasta reached 86.0 ± 1.7 ºC. After 4.5 minutes of contact with the egg yolks, the mean temperature of spaghetti *alla carbonara* rapidly decreased to lower than 60 ºC. The preparation method was able to inactivate approximately 4.7 $\log_{10}$ CFU/g of *Salmonella* spp. and the spaghetti *alla carbonara* processed by this method had a creamy and silky sauce formed by yolks. In conclusion, based on the results, it should be advisable the use of thermo-processed eggs to ensure the safety of this preparation.

Keywords: gastronomy; food processing; cooking; pasta.

1. Introduction

Spaghetti *alla carbonara* is a traditional Italian dish, appreciated and prepared worldwide. In summary, this recipe includes the combination of pasta with cured pork, grated cheese, black pepper and beaten raw eggs (Hosking, 2007). Due to the popularity of this preparation, many variants of this recipe were created around the world based on original recipe. For example, the amount of eggs used to form the sauce and the use of whole eggs or only egg yolks are variations of the original recipe. Even though there are different forms to prepare spaghetti *alla carbonara,* the texture of the sauce seems to be a consensus among them. The eggs mixed with cheese should form, through gentle cooking, a creamy and a silky sauce and not the curd-like texture of scramble eggs (Hosking, 2007).

The consumption of spaghetti *alla carbonara* with the creamy eggs sauce commonly raises concerns about the safety of this preparation due the possibility of the presence of *Salmonella* in egg yolks. Worldwide, *Salmonella* has caused a large number of foodborne illnesses and generally is ranked as the largest cause of food diseases when compared to other pathogens (BRAZIL, 2018; CDC, 2017; EFSA, 2017). The relationship between the consumption of eggs and the salmonellosis infection is recognized worldwide. In the majority of the cases, salmonellosis is linked to dishes made with raw eggs or that are not well heat-treated (EFSA, 2017; Godoy et al., 2000; Reynolds et al., 2010).

The use of thermal processing is the most effective method for *Salmonella* inactivation in preparations containing eggs. Consequently, according to regulatory agencies, it is generally mandatory that these preparations must be completely cooked to a temperature of at least 70 °C. Otherwise, pasteurized eggs must be used (BRAZIL, 2004; Canada, 2013; CDC, 2011; FDA, 2016).

In the most traditional recipes of spaghetti *alla carbonara,* the beaten yolks mixed with grated cheese and pepper are incorporated with the just cooked, drained and still hot pasta, away from the direct heat source. Thus, the only thermal processing received by the yolks in this recipe is the heat transfer from the pasta (Hosking, 2007). Many food processors believe that this heat coming from the pasta is enough for the safety of this preparation. However, it is unclear if in this preparation the heat received from the pasta is capable to completely inactivate *Salmonella* populations.

Studies have demonstrated that at 70 °C the egg yolk coagulates and the texture of the egg white becomes harder (Baldwin, 2012; This, 2016). These characteristics are not desirable in the spaghetti *alla carbonara,* indicating that the sauce does not reach this temperature. On the one hand, different studies reported that mild heat treatments (temperatures lower than 70 °C) can reduce *Salmonella* population (Geveke et al., 2016; Lopes et al., 2018; Stadelman et al., 1996). On the other, outbreaks were documented linking *Salmonella* to spaghetti *alla carbonara* (Gelbíčová and Karpíšková, 2016; Godoy et al., 2000). Thus, the objective of this study was to evaluate the *Salmonella* spp. inactivation during the preparation of traditional spaghetti *alla carbonara.*

2. Material and methods

2.1 Bacterial strains and inoculum preparation

Five strains of *Salmonella* were used to compose a pool: *S.* Enteritidis SE86, *S.* Enteritidis 55507, *S.* Typhimurium L12031 *S.* Minnesota and *S.* Heidelberg. The three first strains were isolated from foods involved in foodborne outbreaks occurred in the State of Rio Grande do Sul and the last two were isolated from poultry products in the State of Mato Grosso and Santa Catarina, respectively. The bacterial cultures and inoculum preparations

were conducted according to the method reported by Lopes, Batista, & Tondo (2018). The inoculum was used to contaminate the eggs as described below.

2.2 Growth of *Salmonella* pool in eggs

Eggs (Filippsen Eggs,Brazil), weighing 70–77 g were chosen and the yolks were inoculated according to the method reported by Lopes et al. (2018). After inoculation, eggs were incubated at 37 °C for 18 – 24 h. *Salmonella* spp. final population in the eggs ranged from 8 – 9 $\log_{10}$ CFU/g.

2.3 Spaghetti *alla carbonara* preparation

After 18 to 24 h of incubation, three artificially contaminated eggs were stored for 30 minutes at room temperature, and then the yolks were aseptically separated from the egg whites by gloved hands. The three yolks were placed into a disinfected bowl and 40 g parmesan grated cheese plus 0.5 g black pepper were incorporated. This blend was mixed at room temperature. In a pan with boiling salted water (2 liters water with 10 g salt), 200 g of spaghetti were cooked for 11 minutes. Meanwhile spaghetti was cooking, 12 g extra virgin olive oil were placed in a pan and one (5 g) pressed garlic clove was cooked at medium heat for 1 min. After, the garlic was removed and 150 g chopped bacon were added and cooked in high heat for 4 min. Immediately after cooking, the spaghetti was drained (50 ml of cooking water was reserved) and placed into the pan with bacon. The spaghetti was mixed and the pan removed from the heat. Subsequently, the yolk's mixture was added to the spaghetti and the cooking water previously reserved was incorporated and then everything was well mixed (Contaldo, 2015).

Three spaghetti *alla carbonara* portions (25 g) were placed inside sterile plastic bags at each set time points (1, 3, 5, 8, 10 and 15 minutes) in order to quantify *Salmonella*. Plastic

bags were closed and immediately immersed into ice-water bath in order to stop the cooking process. The experiment was repeated three times and all counts were done in triplicate.

2.4 Temperature

The temperature of the preparation was monitored using K-type thermocouples and recorded using a data logger (Tenmars, Taiwan).

2.5 Microbiological analysis

Each plastic bag containing one portion of spaghetti was aseptically opened and 25 g were collected and blended with 225 ml of sterile 0.1 % peptone water (w/w) for two minutes, using a stomacher (Stomacher® 400, Seward, England). Subsequent decimal dilutions were obtained by adding 1 ml aliquot into 9 ml of 0.1 % peptone water (w/w), and 100 µl of each dilution were spread on Xylose Lysine Desoxycholate agar (XLD, Merck, Darsmtadt, Germany) added of a thin layer of Tryptic Soy Agar (TSA, KASVI, Italy), according to the one-step TAL method report by Kang & Fung (2000). The plates were incubated at 37 °C for 24 h and typical *Salmonella* colonies were counted. The detection limit of plate counts was 100 CFU/g.

2.6 Data analysis

The analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$) were applied using SAS software (SAS Institute Inc., Cary, USA).

3. Results and Discussion

The temperature profile and the concentration of *Salmonella* spp. in spaghetti *alla carbonara* during the preparation are presented in Figure 1. The mean initial concentration of *Salmonella* spp. in the raw egg yolks and in the mixture of yolks added of grated cheese and pepper were 8.8 ± 0.1 $\log_{10}$ CFU/g and 8.7 ± 0.1 $\log_{10}$ CFU/g, respectively. The microbiological counts indicated that, after 15 minutes of spaghetti *alla carbonara* preparation, *Salmonella* spp. was reduced to 4.3 ± 0.2 $\log_{10}$ CFU/g, resulting in approximately 4.5 $\log_{10}$ CFU/g of *Salmonella* spp. inactivation.

Immediately after cooking for 11 minutes in boiled water and drained, the pasta presented a temperature of 86.0 ± 1.7 ºC. When the mixture of yolks with grated cheese and pepper was incorporated into the cooked spaghetti, during 1 minute, the mean temperature of the spaghetti *alla carbonara* was reduced to 71.3 ± 3.4 °C and *Salmonella* populations decreased to 5.1 ± 0.1 $\log_{10}$ CFU/g. After the first minute of contact, no more reduction was observed ($p \leq 0.05$). Viable *Salmonella* was present in spaghetti *alla carbonara* at the end of 15 minutes of preparation at mean population levels of 4.1 ± 0.3 $\log_{10}$ CFU/g. At this time, the temperature of spaghetti was 43.2 ± 1.2 °C. In our experiment, the pasta was mixed with yolks far from the heat source and the yolks remained liquid and formed a creamy and a silky sauce according recommendations of recipe (Figure 2).

Although our results demonstrated approximately 4.7 $\log_{10}$ CFU/g of *Salmonella* reduction, the preparation method was considered unsafe, considering the possibility of using egg yolks highly contaminated with *Salmonella*. Even though this possibility is low when eggs came from industries following Good Hygiene Practices (GHP), the risk of *Salmonella* contamination still existing. For example, the storage of eggs at environmental temperatures can lead to *Salmonella* counts, ranging from quite low numbers (<20/egg) in egg whites to 7-8 $\log_{10}$ CFU/g of yolk in less than 24 h (Gumudavelli, Subbiah, Thippareddi, Velugoti, &

Froning, 2007; Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991). Moreover, in many cases, a low dosage of *Salmonella* (< 10 CFU) can be enough to cause disease in humans (Humphrey, 2004). Corroborating our results, salmonellosis outbreaks were reported involving spaghetti *alla carbonara*, where eggs contaminated by *S*. Enteritidis were the source of infection (Gelbíčová and Karpíšková, 2016; Godoy et al., 2000).

It is already known that cooking at temperatures of ≥70 °C can contribute to the safety of eggs and egg preparation (BRAZIL, 2004; Canada, 2013; CDC, 2011; FDA, 2016), however studies have demonstrated that at 70 °C the egg yolks coagulates (Baldwin, 2012; This, 2016), what is not a desirable characteristic for traditional spaghetti *alla carbonara*. The creamy and silky sauce formed by yolks is a very important characteristic for this preparation and increasing the temperature of egg yolks to ensure the safety, can lead to a significant undesired change in the texture of yolk sauce. Based on this, spaghetti *alla carbonara* should be prepared using high quality and safe eggs or, alternatively, if the origin of eggs cannot guarantee these characteristics, it is recommended the use of thermo-processed eggs, such as eggs prepared inside temperature-controlled water circulators or pasteurized eggs.

Studies reported that whole eggs immersed in water at temperatures lower than 70 °C, around 58 to 62 ºC, can reduce 4.5 to 7.7 $\log_{10}$ of *Salmonella* population inside eggs, with a minimal modification in the yolk textures (Geveke et al., 2016; Lopes et al., 2018). The factors that can promote the inactivation of *Salmonella* in mild heat treatment are the temperature that the egg yolk reaches and the time in which that temperature is maintained (Lopes et al., 2018). In our experiment, the yolks reached higher temperatures when compared with the other studies cited above. However, it was not observed further reductions, as expected, when higher temperatures were used. This fact can be attributed to the contact of pasta with the mixture of yolks that led to a rapidly decrease of temperature. After 4.5 minutes of contact between pasta and sauce, the mean temperature of spaghetti *alla carbonara* was

lower than 60 ºC, and this temperature was not kept for sufficient time to completely inactive *Salmonella* population.

In our previously study, we have prepared soft-cooked eggs, immersing whole eggs in temperature-controlled water circulator at 62 °C and found that 30 minutes of processing led to a complete inactivation of *Salmonella* spp (7.7 ± 0.1 $\log_{10}$ CFU/g). In this study, at 30 minutes the egg center temperature reached 61.7 ± 0.4 °C and the yolk remained liquid (Lopes et al., 2018). This method can be used by restaurants as a pre-treatment before the preparation of spaghetti *alla carbonara*. Thus, the sum of the reductions caused by both methods can promote a safe spaghetti *alla carbonara* without modifications in the desirable creamy and silky sauce formed by yolks.

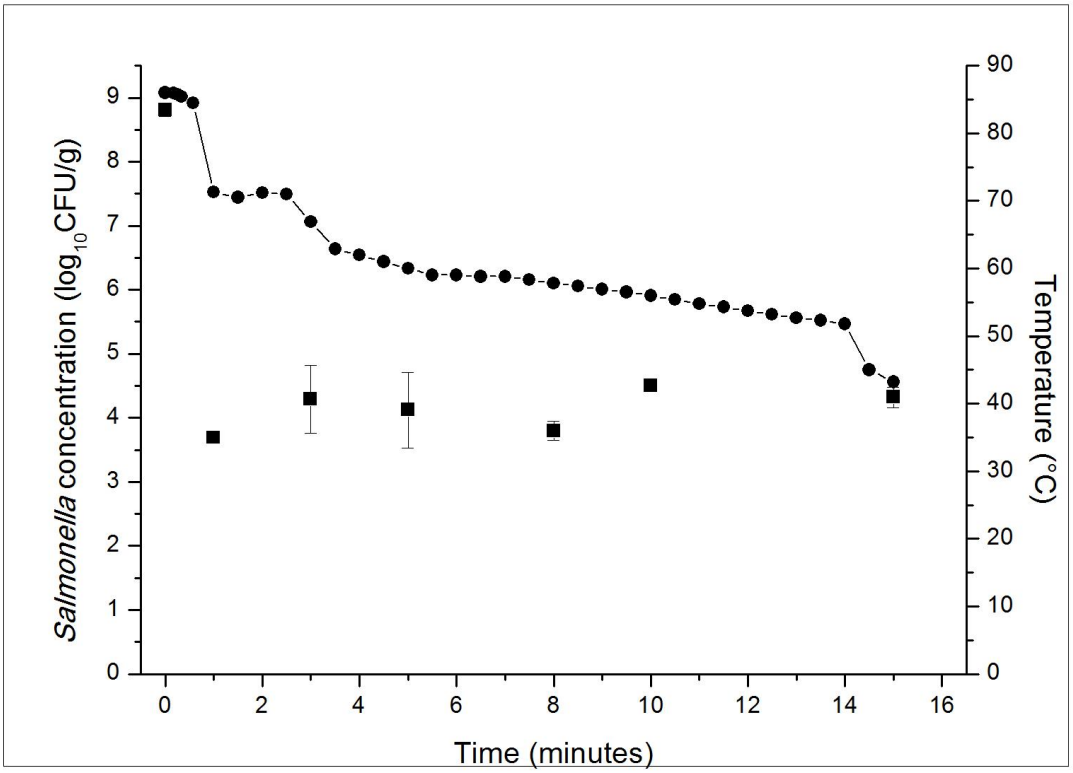

Figure 1. Temperature profile and concentration of *Salmonella* spp. in spaghetti *alla carbonara*. ●: temperature profile. ■: concentration of *Salmonella* spp.

Figure 2. Visual texture of a spaghetti *alla carbonara* with a creamy and a silky sauce.

4. Conclusion

This study demonstrated that spaghetti *alla carbonara* prepared according to a traditional method was able to reduce 4.7 $\log_{10}$ CFU/g of *Salmonella* spp., but around 4.0 $\log_{10}$ CFU/g of *Salmonella* still viable. Based on these results, if spaghetti *alla carbonara* is prepared with highly *Salmonella* contaminated egg yolk, it may not be safe to the consumers. Our findings demonstrated that the spaghetti *alla carbonara* processed by this method had a creamy and silky sauce formed by yolks, what is an essential and desirable characteristic for this preparation in many restaurants.

To improve the safety of this preparation without resulting in significant modifications of the desirable characteristics of yolk sauce it is advisable the use of thermo-processed eggs,

which can be prepared by restaurants through the use of temperature-controlled water circulator at 62 °C for 30 minutes (Lopes et al., 2018) or the adoption of pasteurized eggs.

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5 CAPÍTULO 5

5.1 DISCUSSÃO GERAL

Este trabalho foi desenvolvido com o objetivo de avaliar o comportamento de *Salmonella* em três preparações gastronômicas à base de ovos, sendo elas: ovos moles preparados em termocirculador a 62 º C, também conhecido como “ovo perfeito” na alta gastronomia, *pisco sour* peruano e *spaghetti alla carbonara*, uma vez que essas preparações não atendem os parâmetros estabelecidos pela legislação brasileira vigente (BRASIL, 2004).

No capítulo 2, foi investigado o comportamento de *Salmonella* em ovos moles processados em termocirculador a temperatura constante de 62 º C, durante 60 minutos. Os resultados indicaram que a temperatura do centro do ovo atingiu 61,7 ± 0,4 °C, após 30 minutos, inativando completamente 7,7 $\log_{10}$ de *Salmonella* presente na gema dos ovos. Após esse tempo de cozimento, a gema permaneceu líquida e a clara de ovo ligeiramente opaca, demonstrando que a inativação da *Salmonella* não está necessariamente relacionada com a solidificação da gema e da clara do ovo, como preconizado em algumas legislações. Com base nesses resultados, foi considerado que os ovos processados a esta temperatura, por 30 minutos ou mais, são seguros. Ademais, indiferentemente se os ovos estavam armazenados à temperatura ambiente ou sob refrigeração (previamente ao início do processo), ambos apresentaram o mesmo comportamento em relação ao aquecimento, sugerindo que os dois tipos de armazenamento da matéria-prima podem ser utilizados. Com a utilização da microbiologia preditiva, o modelo Weibull Duplo forneceu o melhor ajuste aos dados, apresentando um tempo de redução decimal de 10,7 ± 1,0 minutos e uma redução de 4D em 15,25 minutos.

No capítulo 3, foi investigado o comportamento de *Salmonella* na bebida alcoólica *pisco sour* peruano (18% vol., pH 3,0). Clara de ovo artificialmente contaminada foi misturada durante 30 segundos com pisco Quebranta (bebida alcoólica com 40 % vol.), xarope de açúcar, suco de limão e cubos de gelo. Os resultados microbiológicos demonstraram que, após 6 minutos de exposição, o *pisco sour* não apresentou células viáveis de *Salmonella*. Entretanto, devido ao limite de

detecção da contagem em placas (100 UFC) não possibilitar a definição do tempo da completa inativação, foi realizado, através da microbiologia preditiva, o ajuste a um modelo para a definição deste tempo de inativação total. O modelo Geeraerd Log-Linear + Shoulder forneceu o melhor ajuste de dados e verificou-se que a população total (6,1 $\log_{10}$ UFC / ml) de *Salmonella* seria completamente inativada em 9 minutos. O teor de álcool associado ao baixo pH da bebida *pisco sour* foi o principal responsável pela redução de *Salmonella*, entretanto, é possível que os compostos fenólicos presentes na bebida alcoólica pisco Quebranta (3,5 ± 0,3 µgGAE/ml) também tenham contribuído para a inativação da *Salmonella*.

No capítulo 4, foi investigado o comportamento de *Salmonella* no *spaghetti alla carbonara* preparado por um método tradicional. O método consistiu em adicionar a mistura de gemas sobre a massa recém-cozida e escorrida, longe da fonte de calor. O tratamento térmico recebido pelas gemas foi devido apenas à transferência de calor da massa recém-cozida em água fervente por 11 minutos. Gemas de ovos contaminadas com 8,8 ± 0,1 $\log_{10}$ UFC/ml foram misturadas com queijo ralado e pimenta preta, e depois adicionadas ao espaguete cozido. Os resultados demonstraram que, imediatamente após ser cozida e escorrida a água, a massa atingiu a temperatura de 86,0 ± 1,7 ºC. No entanto, após 4,5 minutos de contato com as gemas, a temperatura média do *spaghetti alla carbonara* diminuiu rapidamente para menos de 60 ºC. O método de preparo tradicional foi capaz de reduzir apenas 4,7 $\log_{10}$ UFC/g de *Salmonella*, não sendo suficiente para garantir a segurança da preparação, uma vez que aproximadamente 4,0 $\log_{10}$ UFC/g de *Salmonella* permaneceram viáveis. O molho de gemas apresentou uma textura cremosa e sedosa, que é uma característica essencial e desejável em muitos restaurantes na elaboração do *spaghetti alla carbonara,* porém não estava seguro.

5.2 CONCLUSÃO

O principal modo de preparo de ovos moles em termocirculador de água refere à manutenção dos mesmos a 62 ºC por ao menos 1 hora. Desse modo, o processamento de ovos moles em termocirculador, aquecido a 62 °C, promoveu a completa inativação de 7,7 $\log_{10}$ UFC/g de *Salmonella*, após 30 minutos de preparo. O *pisco sour* peruano, elaborado de acordo com publicação da renomada escola de gastronomia *Le Cordon Bleu*, com um teor alcoólico de 18 % v/v e pH 3,0, foi capaz de reduzir pelo menos 4,1 $\log_{10}$ UFC/mL de *Salmonella* após seis minutos de exposição. No *pisco sour*, o teor de álcool associado ao baixo pH da bebida foi o principal responsável pela redução do micro-organismo. A preparação do *spaghetti alla carbonara,* executada conforme descrito em tradicionais livros italianos, reduziu 4,7 $\log_{10}$ UFC/g de *Salmonella*, logo após o molho de ovos ter contato com a massa quente a 86 °C, porém não ocorreu inativação completa da população inicial de *Salmonella*.

Ao comparar os resultados microbiológicos experimentais com os obtidos através de modelos matemáticos, na preparação de ovos moles em termocirculador o modelo Weibull Duplo forneceu o melhor ajuste aos dados, com R^2 e R^2_{adj} de 0.98 e RMSE de 0.42. Esse modelo apresentou um tempo de redução decimal de 10,7 ± 1,0 minutos e uma redução de 4D em 15,25 minutos para *Salmonella*. No *pisco sour* peruano, o modelo Geeraerd Log-Linear + Shoulder forneceu o melhor ajuste de dados, com R^2 e R^2_{adj} de 0.98 e 0.97, respectivamente, e RMSE de 0.28. Com a utilização desse modelo foi possível predizer a completa inativação de *Salmonella* que não foi possível verificar pelo método microbiológico tradicional devido ao limite de detecção de 100 UFC/ml. Os resultados preditivos demonstraram que a bactéria foi completamente inativada após nove minutos de exposição ao *pisco sour* (redução de 6,1 $\log_{10}$ UFC/mL). Na preparação do *spaghetti alla carbonara* não foi possível o ajuste aos modelos matemáticos devido à rápida redução microbiológica que impossibilitou a coleta de pontos na inflexão da curva de inativação.

Das três preparações avaliadas, somente os ovos moles processados em termocirculador a 62 °C, durante ao menos 30 minutos, foram considerados seguros, sem que fosse necessária qualquer modificação na receita tradicional, mesmo que

os ovos utilizados estejam altamente contaminados com *Salmonella*. O *pisco sour* peruano e o *spaghetti alla carbonara*, caso sejam preparados com ovos altamente contaminados com *Salmonella*, podem não ser seguros para os consumidores. Portanto para promover a segurança dessas duas preparações, é aconselhável o uso de ovos termoprocessados ou, no caso do pisco sour peruano deixar as claras cruas em contato com os ingredientes do pisco sour por períodos de 9 minutos ou mais, antes de servir a bebida. Na preparação *spaghetti alla carbonara*, o termoprocessamento dos ovos pode ser obtido através da utilização do método de obtenção dos ovos moles validado nesse estudo (62 °C durante 30 minutos), uma vez que esse processo inativa de 7,7 $\log_{10}$ UFC/g de *Salmonella* e mantem a gema com textura líquida necessária para a elaboração da receita.

Em resumo, os resultados deste estudo podem ser usados por processadores de alimentos, a fim de validar a receita, no caso dos ovos moles em termocirculador, ou, no caso das outras preparações, avaliar um modo seguro de servi-las.

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