**LEONARDO DO PRADO SILVA**

# USE OF META-ANALYSIS AS A TOOL TO ASSES DATA AVAILABLE IN THE LITERATURE ON MICROBIAL INACTIVATION BY PHYSICAL AND CHEMICAL METHODS

***USO DA META-ANÁLISE COMO FERRAMENTA NA AVALIAÇÃO DE DADOS DISPONÍVEIS NA LITERATURA SOBRE A INATIVAÇÃO DE MICRO-ORGANISMOS POR MÉTODOS FÍSICOS E QUÍMICOS***

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**Universidade Estadual de Campinas**

**Faculdade de Engenharia de Alimentos**

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*DISSERTAÇÃO APRESENTADA À FACULDADE DE ENGENHARIA DE ALIMENTOS DA UNIVERSIDADE ESTADUAL DE CAMPINAS COMO PARTE DOS REQUISITOS EXIGIDOS PARA OBTENÇÃO DO TÍTULO DE MESTRE EM CIÊNCIA DOS ALIMENTOS.*

**Orientador: Prof. Dr. Anderson de Souza Sant'Ana**

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**ABSTRACT**

Fruits and vegetables have their consumption widely recommended because of their importance in disease prevention. Fruit juices and minimally processed vegetables (MPV) are two alternative widely accepted by the population for consumption derived from fruits and vegetables. So, to ensure microbiological stability and safety of these products becomes essential. For pasteurized juices and fruits stable at room temperature because of its acidic pH and pasteurization temperature, sporulated microorganisms, acidophilus and spoilage as *Alicyclobacillus acidoterrestris* are the cause of great concern and often the limiting of it's shelf-life. In the case of MPV, the major concern is the increasingly frequent outbreaks of diseases caused by pathogenic microorganisms (*Salmonella* spp, *Escherichia coli* 0157:H7, *Listeria monocytogenes*) and washing with sanitizers is the single step during the processing of these products, able to inactivate potentially pathogenic microorganisms. Both the pasteurization of fruit juices, as washing and disinfection of fruits and vegetables, have their efficiency affected by various parameters, and thereby inactivate the target microorganisms could result in a large variability. In the literature there are several studies on the thermal inactivation of *A. acidoterrestris* in fruit juices and about the use of different sanitizers for cleaning fruits and vegetables in order to inactivate bacterial pathogens. If observed individually, these studies can not provide consistent results on the studied situations. However, if evaluated together, can enable obtaining new and more consistent information about the studied phenomena and may even result in the appointment of new topics to be searched or that deserve further elaboration. A meta-analysis constitutes the application of statistical methods in order to integrate the results of different studies in the literature on the same issue. In this study, meta-analysis techniques were used aiming to: (*i*) integrating different *D* values (time at a fixed temperature required to cause the reduction of 1-log cycle in a microbial population) and the z value (variation of temperature needed to result in decrease of one log-cycle in the *D* value) of *A. acidoterrestris* in fruit juices and (*ii*) compiling results logarithmic reductions (CFU $ml^{-1}$) log on *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* caused by sanitizers applied during washing of vegetables and fruits. For thermal inactivation, a total of 55 papers were obtained in the scientific literature. Based on specific criteria, 11 studies were selected, resulting in 142 *D* values in wide temperature ranges (70-105ºC) and pH (2.28 to 4.00). For sanitizers efficiency evaluation, were collected 55 studies, and 40 studies that met the selection criteria, resulted in 1025 data logarithmic reductions of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* in 30 types of vegetables, using 21 types of sanitizers. For thermal inactivation, the assembly of predictive models occurred as follows: (i) characteristics of the study (fruit, beverage type, presence of bacteriocin and clarification) were extracted and incorporated into a meta-analytic linear mixed effects model with based on the basic equation of Bigelow, describing the thermal resistance parameters of *A. acidoterrestris*. For evaluating the effectiveness of sanitizers (ii) data were to build three separate meta-analytic models to assess variability in logarithmic reduction of pathogens studied as a function of the type of plant and sanitizers. The results of the meta-analysis of thermal inactivation parameters of *A. acidoterrestris* show that the

highest coefficient of variance was observed in studies that evaluated: pH, soluble solids and acid content. The obtained $D_{95°C}$ (pasteurization temperature used for juices) values (1.5 to 5.7 min) were influenced by pH and concentration of soluble solids. Z values ranged from 6,1-29,1ºC. The results of the meta-analysis of log reduction data indicated that the pathogens for most sanitizers, concentration, temperature and contact time have a direct effect on microbial log reduction. Overall, *L. monocytogenes* showed a lower intercept, which means it can be tougher than *E. coli* O157: H7 and *Salmonella* to the sanitizers. Moreover, the slightly acidic electrolyzed water (SAEW) showed the highest bactericidal efficacy of sanitizers all evaluated. The use of meta-analysis approach provided the integration results of several studies and a large amount of data and allowed predictive models were created in order to consider many variables, and thus find most widespread application in aspects related to safety and microbiological quality of food.

**Key-words:** pasteurization, fruit juice, *Alicyclobacillus acidoterrestris*, MPV, *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, sanitizers, washing

## RESUMO GERAL

As frutas e legumes têm o seu consumo amplamente recomendado em virtude da sua importância na prevenção de doenças. Os sucos de frutas e os vegetais minimamente processados (VMP) constituem duas alternativas de grande aceitação pela população para o consumo de derivados das frutas e legumes. Neste sentido, a garantia a estabilidade microbiológica e da inocuidade destes produtos torna-se primordial. No caso dos sucos de frutas pasteurizados e estáveis a temperatura ambiente, por conta de seu pH ácido e temperatura de pasteurização, micro-organismos esporulados, acidófilos e deteriorantes como *Alicyclobacillus acidoterrestris* são a causa de grande preocupação e muitas vezes, os limitantes de sua vida útil. No caso dos VMP's, a grande preocupação está na ocorrência cada vez mais frequente de surtos de doenças causados por micro-organismos patogênicos (*Salmonella* spp., *Escherichia coli* 0157:H7*, Listeria monocytogenes*) e a lavagem com sanitizantes é a única etapa durante o processamento destes produtos, capaz de inativar micro-organismos patogênicos potencialmente presentes. Tanto a pasteurização dos sucos de frutas, quanto a lavagem e desinfecção das frutas e vegetais, tem sua eficiência afetada por diversos parâmetros, e desta forma, a inativação dos micro-organismos alvo poderá resultar em uma grande variabilidade. Na literatura existem diversos estudos sobre a inativação térmica de *A. acidoterrestris* em sucos de frutas e sobre o uso de diferentes sanitizantes durante a lavagem de vegetais e frutas visando a inativação de patógenos bacterianos. Se observados individualmente, estes estudos podem não fornecer resultados consistentes sobre os problemas estudados. No entanto, se avaliados em conjunto, podem possibilitar a obtenção de informações novas e mais consistentes acerca dos fenômenos estudados e, podem inclusive, resultar na indicação de novos temas a serem pesquisados ou que mereçam maior aprofundamento. A meta-análise constitui-se na aplicação de métodos estatísticos que integra os resultados de diferentes estudos disponíveis na literatura sobre uma mesma questão. No presente estudo, técnicas de meta-análise foram utilizadas objetivando-se: (*i*) integrar diferentes valores *D* (tempo a uma determinada temperatura necessário para provocar a redução de 1 ciclo-log numa população microbiana) e valor z (variação da temperatura necessária para resultar na diminuição de 1 ciclo-log no valor *D*) de *A. acidoterrestris* em sucos de frutas e (*ii*) compilar os resultados de reduções logarítmicas (log UFC $mL^{-1}$) em *Salmonella* spp, *E. coli* O157:H7 e *L. monocytogenes* causada por sanitizantes aplicados durante a lavagem de vegetais e frutas. No primeiro estudo, um total de 55 trabalhos foram obtidos na literatura científica. Baseando-se em critérios específicos, 11 estudos foram selecionados, resultando em 142 valores *D* obtidos em amplas faixas de temperatura (70-105ºC) e pH (2,28-4,00). No segundo estudo, foram selecionados 55 estudos, sendo que 40 trabalhos que atenderam aos critérios de seleção resultaram em 1025 dados de reduções logarítmicas de *Salmonella* spp, *E. coli* O157:H7 e *L. monocytogenes* em 30 tipos de vegetais, utilizando-se 21 tipos de sanitizantes. Para a inativação térmica, a montagem dos modelos preditivos ocorreu do seguinte modo: (*i*) as características do estudo (fruta, tipo de bebida, presença de bacteriocina e clarificação)

foram extraídas e incorporadas a um modelo linear meta-analítico de efeitos mistos com base na equação básica de Bigelow, descrevendo os parâmetros de resistência térmica de *A. acidoterrestris*. Já para a avaliação da eficiência dos sanitizantes (*ii*), os dados foram separados para construir três modelos meta-analíticos para avaliar a variabilidade na redução logarítimica em função dos patógenos estudados, do tipo de vegetal e dos sanitizantes. Os resultados da meta-análise dos parâmetros de inativação térmica de *A. acidoterrestris* demonstram que o maior coeficiente de variâncias foi observado nos estudos em que foram avaliados: pH, sólidos solúveis e teor de ácido. Os valores $D_{95^{\circ}C}$ (temperatura usual de pasteurização dos sucos) obtidos (1,5-5,7 min) foram influenciados pelo pH e concentração de sólidos solúveis. Valores-z variaram entre 6,1-29,1ºC. Os resultados da meta-análise dos dados de redução logarítmica dos patógenos indicaram que para a maioria dos sanitizantes, concentração, temperatura e tempo de contato têm um efeito direto na redução logarítmica microbiana. Em geral, *L. monocytogenes* apresentou um intercepto menor, o que significa que pode ser mais resistente do que *E. coli* O157:H7 e *Salmonella* aos sanitizantes. Além disso, a água eletrolizada ligeiramente ácida (SAEW) apresentou a maior eficácia bactericida entre todos os sanitizantes avaliados. O uso da abordagem de meta-análise proporcionou a integração de resultados de diversos estudos e de uma grande quantidade de dados, e permitiu que modelos preditivos fossem criados de maneira a considerar diversas variáveis, e desta forma, encontrar aplicações mais generalizadas em aspectos relacionados à segurança e qualidade microbiológica dos alimentos.

***Palavras-chave:*** pasteurização, suco de fruta, *Alicyclobacillus acidoterrestris,* VMP, *Salmonella, E. coli* O157:H7*, L. monocytogenes*, sanitizantes, lavagem

**Sumário**

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# Introdução Geral

As frutas e legumes, alimentos ricos em nutrientes essenciais ao organismo (vitaminas, aminoácidos, sais minerais e antioxidantes), tem o seu consumo amplamente difundido na sociedade em virtude da sua importância na prevenção de doenças (WHO/ FAO, 2003).

Grande parte da produção de frutas e legumes é perdida durante as etapas da cadeia produtiva, sendo que na pós-colheita os prejuízos são maiores devido, principalmente, ao manuseio, transporte e técnicas de conservação inadequadas. Desta maneira, o processamento adequado dos alimentos é uma alternativa inteligente de reduzir perdas e agregar valor aos produtos.

Dentre as frutas e legumes existem dois segmentos de importância na indústria de processamento de alimentos que são os sucos de frutas (não-pasteurizados, pasteurizados seguidos de refrigeração e estéreis comercialmente) e os vegetais minimamente processados (VMP).

Durante muito tempo a deterioração de suco de frutas foi, principalmente, atribuída a leveduras e bactérias láticas. Por conta deste fato, apenas a aplicação de condições brandas de pasteurização, já era considerada suficiente para a garantia da estabilidade destes produtos (Blocher e Busta, 1983).

No caso dos sucos estéreis comercialmente, a presença de bactérias patogênicas não é causa de preocupação devido às temperaturas de tratamento térmico (95ºC) e ao pH ácido ($\leq$ 3,8), que causam sua inativação e/ou inibem sua multiplicação. Nestes produtos, a grande preocupação está nos micro-organismos termorresistentes, como por exemplo, *Alicyclobacillus acidoterrestris,* bolores termorresistentes e alguns bacilos ácido tolerantes, únicos grupos capazes de crescer nas condições do produto.

*A. acidoterrestris* é uma bactéria ácido-termofílica formadora de esporos e motivo de grande preocupação para a indústria de sucos devido a sua resistência térmica e química, bem como o seu potencial de deterioração (produção de guaiacol em suco de laranja e maçã principalmente). Além disso, bolores termorresistentes (*Byssochlamys* spp.*, Neosartory* spp.*, Eupenicillium* spp.*, Talaromyces* spp.), que desenvolvem estruturas de resistência térmica (ascósporos) têm capacidade de deteriorarem produtos como os

sucos de frutas pasteurizados, pois suas características (pH ácido, baixa tensão de oxigênio, etc) favorecem o desenvolvimento destes tipos de micro-organismos (Tournas, 1994).

Falhas no processamento dos alimentos têm acarretado grandes perdas econômicas à indústria como é o caso da deterioração de suco de frutas por *A. acidoterrestris* (Chang e Kang, 2004). Neste sentido, visando a inativação térmica de *A. acidoterrestris* muitos estudos que reportam o valor *D* (tempo a uma determinada temperatura necessário para provocar a redução de 1 ciclo-log na população de uma bactéria alvo) e valor-z (variação da temperatura necessária para resultar na diminuição de 1 ciclo-log no valor *D*) estão disponíveis na literatura (Splittstoesser *et al*.,1994; Komitopoulou *et al*.,1999; Bahçeci e Acar, 2007; Walls, 1997; Silva *et al*., 1999; Maldonado *et al*., 2008; de Carvalho *et al*., 2008; Lopez *et al*., 2011; Alberice *et al*., 2012, Peña e Massager, 2006; McKnight *et al*., 2010). Sabendo que estes parâmetros (valor *D* e valor-*z*) são influenciados por diferentes parâmetros, como temperatura, pH, ºBrix, cepas, e métodos de inativação, etc., é previsível que exista heterogeneidade entre os estudos com relação a resistência térmica de *A. acidoterrestris*.

No caso dos VMP o problema está na presença cada vez maior de micro-organismos patogênicos como *Salmonella* spp., *Escherichia coli* O157:H7 e *Listeria monocytogenes* associados a surtos (Ackers *et al*., 1998; Rezende *et al*., 2009; Sant'Ana *et al*., 2012). Por conta disso, a indústria de alimentos tem aplicado e desenvolvido tecnologias para desinfecção destes produtos, já que estes são consumidos sem nenhum preparo adicional.

A lavagem de VMP com sanitizantes tem uma grande importância para reduzir a contaminação microbiana que possa ter impacto sobre a segurança e a vida de prateleira do produto final. Neste sentido, vários estudos quantificam as populações dos micro-organismos patogênicos em frutas e vegetais antes e após a lavagem com diferentes agentes sanitizantes (Behrsing *et al*., 2000; Allende *et al*., 2009; Ijabadeniyi e Ngcobo, 2013; Al-Nabusi *et al*., 2014). No entanto, os resultados de reduções de patógenos alcançados pelos sanitizantes são afetadas por condições específicas de cada estudo (protocolos, tipo de frutas e vegetais inteiros ou produtos frescos cortados, tipo de sanitizante e concentração, tempo de lavagem e temperatura, cepas patogénicas, ensaios microbiológicos, etc.) contribuindo para que exista uma variabilidade nos resultados,

mesmo que entre os estudos seja avaliado o impacto de um mesmo alimento e sanitizante.

Para este propósito, a meta-análise, que por definição é: *"análise estatística de um conjunto de resultados analíticos com a finalidade de integração dos resultados a partir de uma grande quantidade de estudos primários"* (DerSimonian e Laird, 1986), permite (i) a explicação das divergências nos resultados do estudo por parte da codificação das características de estudo (ou seja, moderando variáveis relacionadas ao desenho experimental, características de projeto, procedimentos de coleta de dados, o tipo de amostra, etc.) com o objetivo de reduzir a heterogeneidade ou variabilidade entre os estudos (Gonzales-Barron *et al*., 2013); e (ii) com o aumento do poder estatístico, mensurar um resultado global mais significativo (Sutton *et al*., 2001). Apesar das capacidades da meta-análise, já há muito tempo reconhecidas em estudos de medicina e clínicos, a aplicação desta técnica estatística em questões de segurança e de microbiologia de alimentos é recente (Gonzales-Barron *et al*, 2008;. Gonzales-Barron e Butler, 2011; Den Besten e Zwietering, 2012; Gonzales-Barron *et al*, 2013).

## Objetivos Gerais

O primeiro objetivo deste trabalho foi integrar diferentes valores *D* e valor-z de *A. acidoterrestris* em sucos de frutas e em segundo lugar compilar os resultados de reduções logarítmicas (log UFC $mL^{-1}$) nas populações de *Salmonella* spp, *E. coli* O157:H7 e *L. monocytogenes* causadas por sanitizantes aplicados durante a lavagem de vegetais e frutas.

# Capítulo I: Revisão de Literatura

## *1.* Deterioração de suco de frutas*: Alicyclobacillus acidoterrestris*

### 1.1. Histórico

*Alicyclobacillus spp.* foi primeiramente isolada em ambientes ácidos e termófilos, denominada a princípio como *Bacillus acidocaldarius*. Foram também descobertas espécies em solo neutro de *Bacillus acidocaldarius*, fontes não térmicas e nem ácidas, aumentando assim a abrangência da espécie (Hippchen *et al*., 1981). Em seguida, foi identificada a segunda espécie, *Bacillus acidoterrestris*, que foi isolada de suco de maçã pasteurizado deteriorados (Deinhard *et al*.,1987). A partir de análises realizadas dos primeiros isolados foi detectada a presença de ácidos graxos ω-aliciclícos e haponóides na membrana celular, e então, a partir dessa identificação e com o auxílio da técnica de sequeciamento 16S-RNA foi proposto a criação de uma nova espécie que hoje é conhecida como *Alicyclobacillus spp*. (Wisotzkey *et al*., 1992).

Outras espécies *Alicyclobacillus* spp. foram identificadas a partir de solos vulcânicos, como por exmplo *A. hesperidum* (Albuquerque *et al*., 2000). Além disso, existe uma espécie isolada de chá de hibisco, identificada como *A. herbarius*, e outra espécie de uma bebida ácida que ficou conhecida como *A. acidiphilus* (Goto *et al*., 2002; Matsubara *et al*., 2002).

### 1.2. Características Gerais

As espécies de *Alicyclobacillus* spp. se caracterizam, em geral, por se tratarem de bactérias termoacidófilas, em forma de bastonete e formadoras de esporos. Além disso, as espécies identificadas são classificadas como Gram-positivas, com exceção de uma espécie Gram-negativa, *A. sendaiensis* (Tsuruoka *et al*., 2003). A temperatura de crescimento, em geral, varia entre 20-70ºC, com exceção de espécies como *A. disulfidooxidans*, *A. tolerans* (Karavaiko *et al*., 2005) e *A. ferrooxydans* que são capazes de se desenvolverem em temperaturas abaixo de 20ºC. Assim, a temperatura ótima se encontra em torno de 42-60ºC para a maioria das espécies. Além disso, o pH de crescimento varia ao redor de 2,5-6,5 (Wisotzkey *et al*., 1992, Simbahan et al., 2004;

Jiang et al., 2008), podendo ter espécies como *A. disulfidooxidans*, *A. tolerans* (Karavaiko *et al*., 2005) que apresentaram crescimento em pH abaixo de 1,5.

Em estudos realizados com suco de uva foi observado que a espécie *A. acidoterrestris* é capaz de crescer em concentrações de SS de 5,40 a 16,20ºBrix, no entanto, a concentração de SS de 21,60º Brix foi suficiente para inibir o desenvolvimente da espécie (Splittstoesser *et al*., 1994). Desta maneira, o desenvolvimento deste micro-organismo deteriorante em sucos concentrados fica inviável, no entanto, por se tratarem de espécies formadoras de esporos todo o cuidado deve ser mantido, pois uma vez que este suco for diluido os esporos presentes podem se desenvolver e crescer até níveis de contaminação passíveis de deterioração do produto (Smit *et al*., 2011).

Devido a essas características trata-se de um micro-organismo resistente ao tratamento térmico (pasteurização) e causador de alterações no sabor dos sucos de frutas e assim gerando prejuízos econômicos para a indústria de suco de frutas (Chang e Kang, 2004).

### 1.3. Ácido graxo ω-alicíclico

A presença do ácido graxo ω-alicíclico na membrana plasmática das espécies de *Alicyclobacillus* spp. se trata da característica mais peculiar e que deu origem ao nome desta espécie bacteriana (Wisotzkey *et al*., 1992). Neste sentido, criou-se uma espectativa ao redor dessa característica em relação a sua influência na resistência térmica e à sua natureza acidófila. Assim, pesquisadores investigaram as propriedades do ácido graxo ω-ciclohexano em uma simulação de membrana celular e concluíram que a presença deste ácido graxo na membrana celular pode fornecer uma proteção extra ao núcleo bacteriano diminuindo a fluidez da membrana, estabilizando a estrutura da membrana e reduzindo a sua permeabilidade (Kannenberg *et al*., 1984).

### 1.4. Resistência Térmica

Tratando-se de uma espécie termorresistente, *Alicyclobacillus* spp. tem sido exaustivamente estudada com relação a sua resistência térmica sob condições diferentes e em uma variedade de sucos de frutas distintos, como por exemplo relatado em suco de maçã onde foi encontrado um valor *D* de 11 min e 0,7 min à 90ºC e 100ºC respectivamente e valor-z de 8,5ºC (Bahçeci e Acar, 2007).

Dentre os fatores, como temperatura, pH, ºBrix, entre outros que possam influenciar significativamente à resistência térmica da bactéria *Alicyclobacillus* spp. a temperatura trata-se do principal fator vinculado à resistência deste micro-organismo, por exemplo, um aumento de temperatura de 2ºC (95-97ºC) provocou a diminuição do valor *D* de 2,82 minutos para 0,57 minutos (Silva *et al*., 1999).

### 1.5. Deterioração

O interesse no micro-organismo *A. acidoterrestris* se deve a sua ação deteriorante desencadeada após a divulgação de um trabalho em 1984 indicando *A. acidoterrestris* como o micro-organismo causador de uma deterioração em grande escala na Alemanha envolvendo suco de maçã (Cerny *et al*., 1984). Subsequentemente, os incidentes de deterioração em todo o mundo atribuída às espécies *Alicyclobacillus* spp. foram relatados em diferentes produtos derivados de frutas, apresentados na tabela 1.

Sendo o suco de frutas (suco de laranja e maçã, principalmente) o principal produto, naturalmente ácido, deteriorado pelo crescimento da bactéria termoacidófila formadora de esporos, *Alicyclobacillus* spp., tem sido destaque em pesquisas cientifícas (Smit *et al*., 2011), que visam o compreendimento cada vez mais profundo deste tema, afinal, o suco de frutas é considerado um dos principais derivados de frutas e movimenta a econômia da indústria de alimentos.

Os compostos deteriorantes produzidos por *Alicyclobacillus* spp. já identificados são compostos fenólicos, e são eles: 2-methoxyphenol (guaiacol), 2,6-dibromophenol, 2,6-dichlorophenol e 2-methyltetrahydrothiophene-3-one, sendo o guaiacol o principal deles (Siegmund e Pöllinger-Zierler, 2006, Lottici *et al*., 2006, Siegmund e Pöllinger-Zierler, 2007, Concina *et al*., 2010). A principal característica que determina a deterioração do produto alimentício está relacionada a um sabor e odor característico, muitas vezes apontado como “cheiro e gosto de xarope”, embora se tenha relatos de que a deterioração pode causar turvação ou não e produção de sedimentos na embalagem (Duong e Jensen, 2000).

Tabela 1. Produtos, espécies deteriorantes e locais de ocorrência relacionados com deterioração de derivados de frutas.

| Produto | Espécie | Local | Fonte |
|---|---|---|---|
| Suco de frutas | *A. acidoterrestris* | Alemanha | Cerny *et al*., 1984 |
| Suco de frutas | *Bacillus* spp. | E.U.A | Splittstoesser *et al*., 1994 |
| Suco de frutas | *A. acidoterrestris* | Japão | Yamazaki *et al*., 1996 |
| Bebida gaseificada | *A. acidoterrestris* | Reino Unido | Pettipher *et al*., 1997 |
| Conserva de tomate | *A. acidoterrestris* | E.U.A | Walls e Chuyate, 2000 |
| Suco de frutas | *Alicyclobacillus* spp. | Austrália | Jensen, 2000 |
| Chá gelado | *Alicyclobacillus* spp. | Austrália | Duong e Jensen, 2000 |
| Suco de frutas | *A. acidiphilus* | Japão | Matsubara *et al*, 2002 |
| Misturas de suco de frutas | *A. acidoterrestris* | Austrália | Jensen e Whitfield, 2003 |
| Misturas de suco de frutas | *A. pomarum* | Japão | Goto *et al*, 2003 |
| Suco de manga | *A. acidocaldarius* | África do Sul | Gouws *et al*, 2005 |

### 1.5.1. Guaiacol (2-methoxyphenol)

O guaiacol (2-methoxyphenol) trata-se do principal composto deteriorante mais encontrado em amostras de produtos derivados de frutas contaminadas com a espécie *A. acidoterrestris*, embora tenha relatos de outras espécies envolvidas na deterioração de derivados de frutas como, por exemplo, *A. acidiphilus* (Matsubara *et al*., 2002; Goto *et al*., 2008), *A. pomorum* (Goto *et al*., 2003), *A. hesperidum*, *A. herbarius* (Goto *et al*., 2008), *A. cycloheptanicus* (Gocmen *et al*., 2005) e *A. acidocaldarius* (Gouws *et al*., 2005). Além de se tratar de um composto deteriorante, o guaiacol também está presente no odor defumado caracterísco da espécie de café mais conhecida no mundo, a *Coffea arabica* (Mayer *et al*., 1999).

A rota metabólica de produção guaiacol mais utilizada é a do ácido ferúlico (Crawford e Olson, 1978; Álvarez-Rodríquez *et al*., 2003) representada pela figura 1, podendo ser facilmente encontrado na natureza, em frutas, vegetais, grãos, folhas, sementes, nozes, ervas e flores (Rosazza *et al*., 1995).

Na maioria dos micro-organismos o primeiro passo do metabolismo do ácido ferúlico é a sua descarboxilação, formando o composto 4-vinilguaiacol (Rahouti *et al*., 1989; Mathew *et al*., 2007), embora possa também ser diretamente transformado em vanilina (Peleg *et al*., 1992) ou ácido vanílico (Huang *et al*., 1993), sem a produção de 4-vinilguaiacol.

*A. acidoterrestris* é capaz de produzir guaiacol a partir de vanilina (Bahçeci *et al*., 2005; Bahçeci e Acar, 2007) e ácido vanílico (Niwa e Kuriyama, 2003). A conversão de ácido vanílico em guaiacol é mais rápida do que a de vanilina, o que pode ser explicado pelo fato do ácido vanílico ser o precursor imediato de guaiacol durante o seu metabolismo. Outros precursores têm sido investigados, como é o caso do aminoácido tirosina que foi sugerido por Jensen (2000) como um provável composto envolvido no metabolismo de produção do guaiacol.

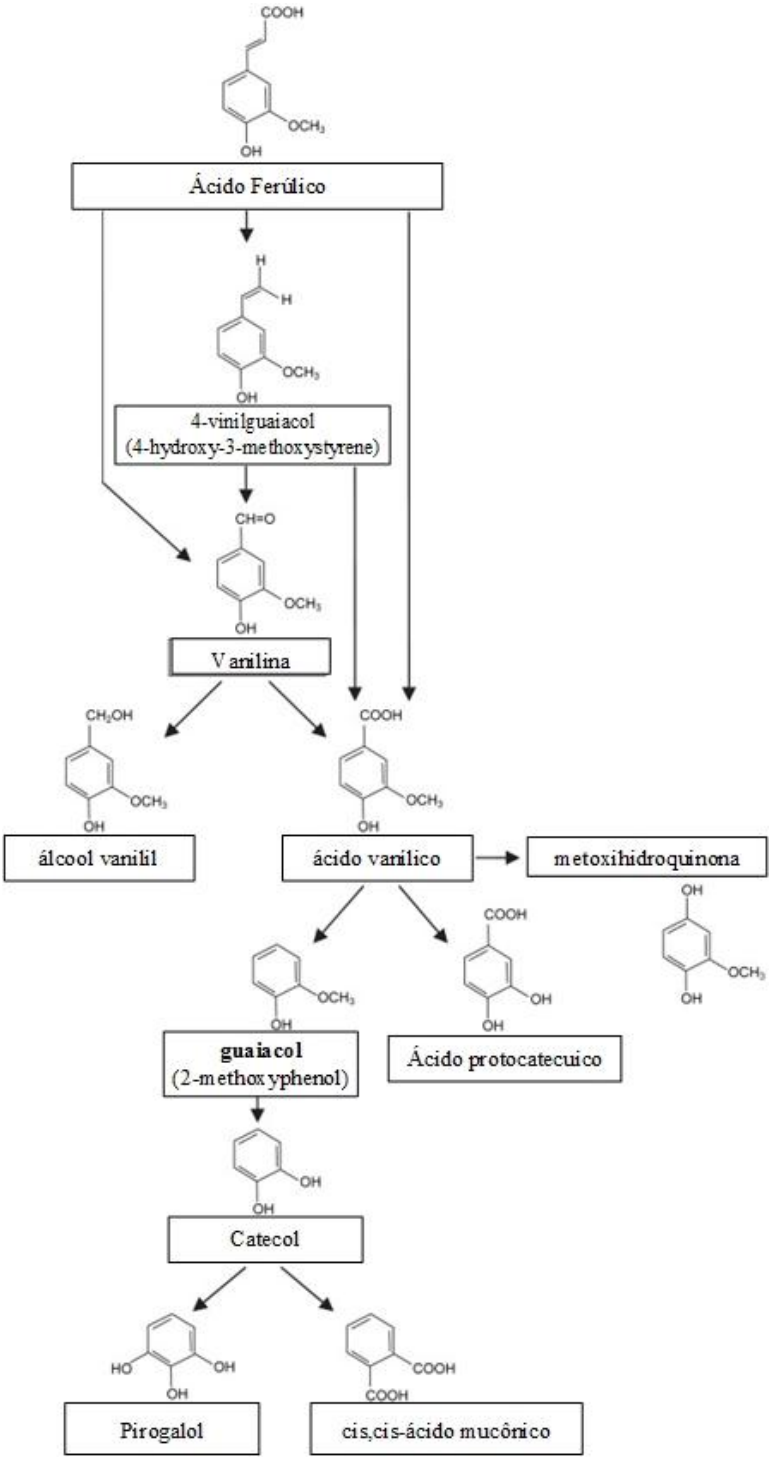


Figura 1. Vias de produção microbiana de guaiacol e outros produtos através do metabolismo do ácido ferúlico. (Adaptado de Smit *et al*., 2011).

## 2. Segurança Microbiológica de Vegetais Minimamente Processados (VMP)

Vegetais minimamente processados (VMP) são por definição qualquer tipo de hortaliça que venha a ter seu estado físico inicial alterado, mas que preserva seu estado *in natura* oferecendo frescor e qualidade ao consumidor (Cantwell, 1992). Consumidor este que está cada vez mais exigente e tem buscado praticidade aliada ao consumo de alimentos com alto valor nutritivo e funcional.

Assim, o processamento mínimo dos vegetais consiste na utilização de operações de limpeza, seleção e corte, sendo posteriormente embalados e distribuídos no varejo oferecendo aos consumidores um produto fresco, rico em nutriente e prático. De modo geral, o processamento mínimo de vegetais inclui operações unitárias de pós-colheita, seleção, lavagem, descascamento, corte, sanitização, enxágue, drenagem, seleção final, embalagem e armazenamento, conforme mostrado na figura 2 de maneira inespecífica, através de um fluxograma.

A lavagem representa um ponto crítico de controle do processo, visto que o uso de água potável (remoção física) para enxágue e aplicação de sanitizantes pode reduzir a carga microbiana superficial e evitar a infecção da parte comestível no momento do corte (Silva *et al.*, 2011).

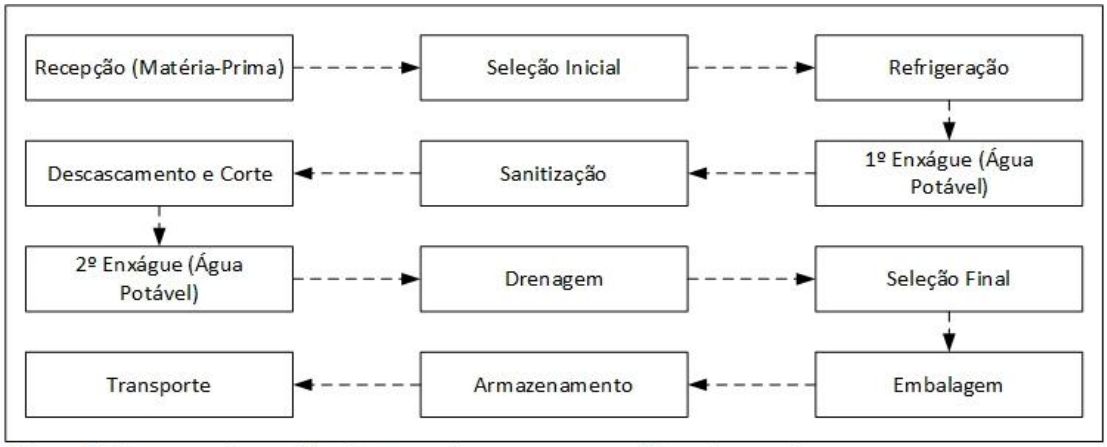

Figura 2. Fluxograma inespecifico das etapas do processamento minimo de vegetais.

Sabendo que os micro-organismos podem estar presentes na superfície dos vegetais (Abadias et al., 2008; Doyle e Erickson, 2008), tais como *Salmonella* sp, *L. monocytogenes* e *E. coli* O157:H7 (Brackett, 1999) torna o VMP susceptível à

contaminação no momento do corte. Visando a segurança microbiológica dos VMPs, a lavagem tem sido feita por meio de diversos tipos de sanitizantes como uma forma de reduzir a carga microbiana inicial dos vegetais (Alexandre *et al*., 2013; Allende *et al*., 2009; Al-Nabulsi *et al*., 2014; Behrsing *et al*., 2000; Chang *et al*., 2011; Chun *et al*., 2013; Forghani e Oh, 2013; Ge *et al*., 2013; Huang and Chen, 2011; Ijabadeniyi and Ngcobo, 2013; Lee *et al*., 2004; Li e Wu, 2013; Keeratipibul *et al*., 2011; Kenney e Beuchat, 2002 a, b; Keskinen e Annous, 2011; Kwon *et al*., 2011; Mahmoud *et al*., 2007; Rahman *et al*., 2010; Sengun *et al*., 2005; Su e D'Souza, 2012; Tian *et al*., 2013; Trinetta *et al*., 2010; Ukuku, 2004; Yuk *et al*., 2005).

Os sanitizantes mais utilizados pelas indústrias de processamento mínimo e recomendados por orgãos governamentais, em sua grande maioria, são à base de cloro (Silva *et al*., 2011). Nos Estados Unidos da América, a *Food and Drug Administration* (FDA, 2014) regulamenta o uso de produtos químicos utilizados na lavagem superficial de frutas e legumes através da normativa 21CFR173.315. Neste regulamento está permitido o uso de sanitizantes como hipoclorito de sódio, brometo de potássio, pirofosfato tetrapotássico, dicloreto de etileno, peróxido de hidrogênio, ácido peracético, entre outros para frutas e vegetais. No Brasil, no que se diz respeito à regulamentação de sanitizantes para indústria de alimentos está contida na RDC nº 220, de 29 de julho de 2005 e RDC nº 2, de 08 de janeiro de 2004, que permitem o uso de substâncias liberadoras de cloro ativo e ácido peracético como coadjuvante tecnológico, respectivamente.

De acordo com a *International Fresh-Cut Produce Association* (IFPA, 2001) recomenda-se que seja aplicado 200 mg $L^{-1}$ de cloro ativo à superfície do vegetal e o tempo seja definido pelo processador de acordo com a características individuais de cada vegetal e a sua respectiva carga microbiana inicial. De acordo com Su e D'Souza (2012) foi possível reduzir a carga microbiana inicial (7 log UFC $ml^{-1}$) de *S.* Typhimurium em alface em até 5,23 e 5,57 log UFC $ml^{-1}$ aplicando hipoclorito de sódio (200 mg $L^{-1}$) durante 15 s e 30 s respectivamente. Assim, a lavagem com sanitizante pode ser efetiva no controle microbiológico de VMP.

## 3. Micro-organismos patogênicos

De origem fecal, *Salmonella* spp. é um micro-organismo patogênico associado a diversos surtos de doenças transmitidas por alimentos (DTA) no mundo há muito tempo (Crump *et al*., 2004). Pertencente à família Enterobacteriaceae, são bastonetes Gram negativos, anaeróbia facultativa, não esporulada, e sua temperatura ótima de crescimento varia entre 35-43ºC e pH ótimo entre 7,0-7,5 (Silva *et al*., 2007).

A classificação do gênero *Salmonella* é complexa e divide opiniões na comunidade científica . De acordo com a literatura o gênero é dividido em três espécies: *S. enterica, S. bongori* e *S. subterranea* (Brenner *et al*., 2000; Shelobolina *et al.,* 2004) Entretanto, a classificação e nomenclatura mais usada para este gênero é o esquema de Kauffmann-White que leva em consideração os tipos sorológicos em relação aos três principais antígenos: somático (O), flagelar (H) e capsular (K). Além disso, um antígeno de virulência (Vi) foi recentemente incluído como um sub-tipo dentro do antígeno K e inclui três sorotipos: *S.* Typhi, *S.* Paratyphi C e *S.* Dublin (Eng *et al*., 2015). Considerando este conceito de classificação, atualmente estão identificados aproximadamente 2610 sorotipos do gênero *Salmonella* (Guibourdenche *et al*., 2010).

A patogenicidade de *Salmonella* está relacionada ao desencadeamento de doenças como septicemia e febre tifóide (*S.* Tiphy), febre paratifóide (*S.* Paratiphy A, B e C) e enterocolites (*S.* Enteritidis) (Gordon *et al*., 2008, Eng *et al*., 2015). A gravidade das doenças desencadeadas está intimamente relacionada ao sorotipo da *Salmonella* envolvida na infecção e à fragilidade do sistema imunológico do hospedeiro (Eng *et al*., 2015).

Também de origem fecal, *Escherichia coli* (Família Enterobacteriaceae), é um bastonete Gram negativo, não esporulado e anaeróbio facultativo que normalmente vive no intestino de pessoas e animais. Em sua maioria não são patogênicas e, integram uma parte importante do trato intestinal humano. No entanto, algumas *E. coli* patogênicas, podem causar distúrbios gastroentestinais e até mesmo doenças fora do trato gastrointestinal.

A sorotipagem de *E. coli*, assim como a *Salmonella*, basea-se nas diferenças entre os antígenos capsulares (antígenos K), somáticos (antígenos O) e flagelares (antígenos H).

Por exemplo, *E. coli* O157:H7 reporta a presença do antígeno somático O157, antígeno flagelar H7 e a ausência do antígeno capsular (Ernandez e Hofer, 1987).

As cepas patogênicas de *E. coli*, são classificadas em seis patotipos: - STEC (*E. coli* produtora de toxina Shiga, podendo também ser referida como produtora de verotoxina (VTEC), *E. coli* entero-hemorrágica (EHEC); - ETEC (*E. coli* enterotoxigênica); - EPEC (*E. coli* enteropatogênica); - EAEC (*E. coli* enteroagregativa); - EIEC (*E. coli* enteroinvasiva); - DAEC (*E. coli* de aderência difusa) (CDC, 2015). No entanto, recentemente foi identificado um sorotipo patogênico, *E. coli* O104:H4 que possui material genético que confere características dos grupos STEC e EAEC, sendo considerada um grande desafio na microbiologia de alimentos por se tratar de uma espécie extremamente versátil (Muniesa *et al*., 2012).

Dentre os sorotipos de *E. coli* produtora de toxina Shiga (STEC), a mais conhecida e frequentemente relacionada à surtos de DTA's é a *E. coli* O157:H7. Dentro da classe STEC existem outros sorotipos de *E. coli*, incluindo *E. coli* O145, às vezes são chamados de "STEC não-O157". O sorotipo O157:H7 é frequentemente encontrado nos E.U.A, Canadá, Reino Unido e Japão, e os sorotipos não-O157 são mais frequentes na América do Sul, Austrália e Europa Ocidental (Silva *et al*., 2007). Diarréias brandas e colite hemorrágica são os sintomas mais comuns dentro do espectro de doenças causadas pela classe STEC. Doenças mais graves como a síndrome hemolítico-urêmica (HUS) e a púrpura trobocitopênica trobótica (PTT) também podem ocorrer em consequência da infecção por estes micro-organismos (Eduardo *et al*., 2002). Além disso, foi registrado em 2011 na Alemanha um surto de grandes proporções envolvendo um sortotipo raro de STEC, *E. coli* O104:H4 (Frank *et al*., 2011).

De origem ubíqua, ou seja, amplamente distribuída no ambiente, *Listeria monocytogenes* pode ser encontrada em diversos locais (solo, vegetação, água, fezes, esgostos, etc). É uma bactéria patogênica que pode ser fatal para o homem e os animais, sendo a encefalite, septicemia e aborto as mais graves de suas complicações. As espécies do gênero *Listeria* apresentam-se em forma de bastonetes curtos Gram-positivos, não esporogênicos, móveis à 25ºC (flagelos perítriquios) e imóveis à 35º. Psicrotolerantes, crescem em uma ampla faixa de temperatura (1-45ºC) sendo de 30-37ºC sua faixa ótima de crescimento e pH que varia de 4,4-9,6 (Silva *et al*., 2007)

Atualmente vinte e três espécies e sub-espécies fazem parte do gênero *Listeria*: *L. monocytogenes, L. seeligeri, L. ivannovii, L. ivannovii subsp. ivanovii, L. ivannovii subsp. londoniensis, L. innocua, L. grayi, L. welshimeri*, *L. aquatica, L. booriae, L. cornellensis, L. denitrificans, L. fleischmannii, L. fleischmannii subsp. coloradonensis, L. fleischmannii subsp. fleischmannii, L. floridensis, L. grandensis, L. marthii, L. murrayi, L. newyorkensis, L. riparia, L. rocourtiae, L. weihenstephanensis* (DSMZ, 2015).

Dentre as espécies citadas, *L. monocytogenes* trata-se da espécie do gênero *Listeria* que é indiscutivelmente patogênica ao homem. *L. monocytogenes* é um micro-organismo oportunista, ou seja, acomete preferencialmente gestantes, indíviduos imunodeprimidos, idosos e recém-nascidos. Diversos sintomas associados à infecção de *L. monocytogenes* são caracterizados de forma geral como “Listeriose”. Dentre as enfermidades decorrentes da infecção por este micro-organismo, podemos incluir: septicemia, meningite, encefalite, endocardite, nascimento prematuro e aborto, doenças gastrontestinais (náusea, vômito e diarréia). A dose capaz de desencadear tais enfermidades varia de acordo com a virulência da cepa e a susceptibilidade do hospedeiro (Slutsker e Schuchat, 1999)

Tratando-se de micro-organismos patogênicos *Salmonella* spp., *L. monocytogeneges* e *E. coli* tem a sua incidência como um indicativo da qualidade sanitária de uma determinada região, estado ou país. No Brasil, ainda não se tem total controle da incidência destes micro-organimos relacionadas a surtos de doenças. No entanto, existe um trabalho que foi realizado no Estado de São Paulo que de um total de 1024 surtos de diarréia, que envolveu 27499 casos, 459 tiveram identificação da etiologia, sendo que 325 (70,8%) a causa infecção foi bactérias, dentre os surtos por bactéria, 140 (43,1%) foram identificadas como endo do gênero *Salmonella* (Eduardo *et al*., 2003). Nos Estados Unidos da América (E.U.A), onde existe um controle maior dos casos de surtos de doenças envolvendo a presença de micro-organimos de interesse em alimentos, foram reportados 43 surtos de doenças que envolve a presença do gênero *Salmonella* no período de 2006-2015, 22 surtos envolvendo *E. coli* e 8 surtos resultantes da presença de *L. monocytogenes* (CDC, 2015). Através de um levantamento (Tabela 2), com base nos dados disponibilizados pelo Centro de Controle e Prevenção e Doenças dos E.U.A (CDC), é possível verificar a incidência e a sua implicação (número de casos e mortes) resultante da contaminação por *Salmonella* spp., *E.coli* patogênica e *L. monocytogenes* envolvendo vegetais.

Tabela 2. Surtos de origem alimentar associados com vegetais nos Estados Unidos da América, entre o período de 2006-2015, notificados pelo Centro de Controle e Prevenção de Doenças (CDC).

| Gênero | Espécie / Sorotipo | Vegetal | Ano | Nº de Casos / Mortes |
|---|---|---|---|---|
| ***Salmonella* spp.** | *S.* Typhimurium | Tomate | 2006 | 183 / 0 |
| | *S.* Tennesse | Manteiga de Amendoim | 2007 | 425 / 0 |
| | *S.* Litchfield | Melão | 2008 | 60 / 0 |
| | *S.* Saintpaul | Pimenta | 2008 | 1442 / 2 |
| | *S.* Typhimurium | Manteiga de Amendoim | 2008-2009 | 714 / 9 |
| | *S.* Montevideo / *S.* Newport / *S.* Senftenberg | Pistache | 2009 | ND |
| | *S.* Saintpaul | Broto de Alfafa | 2009 | 235 / 0 |
| | *S.* Montevideo | Pimenta | 2010 | 272 / 0 |
| | *S.* Newport | Broto de Alfafa | 2010 | 44 / 0 |
| | *S.* Typhi | Polpa de Fruta | 2010 | 9 / 0 |
| | *Salmonella* spp. | Broto de Alfafa | 2010-2011 | 140 / 0 |
| | *S.* Panama | Melão | 2011 | 20 / 0 |
| | *S.* Enteritidis | Brotos (Alfafa e Pimenta) | 2011 | 25 / 0 |
| | *S.* Agona | Mamão | 2011 | 106 / 0 |
| | *S.* Enteritidis | Castanha | 2011 | 43 / 0 |
| | *S.* Typhimurium / *S.* Newport | Melão | 2012 | 261 / 3 |
| | *S.* Braenderup | Manga | 2012 | 127 / 0 |
| | *S.* Bredeney | Manteiga de Amendoim | 2012 | 42 / 0 |
| | *S.* Saintpaul | Pepino | 2013 | 84 / 0 |
| | *S.* Montevideo / *S.* Mbandaka | Pasta de Gergelim | 2013 | 16 / 1 |
| | *S.* Hartford / *S.* Newport / *S.* Oranienburg | Chia | 2014 | 31 / 0 |
| | *S.* Braenderup | Manteiga de Amendoim | 2014 | 6 / 0 |
| | *S.* Enteritidis | Broto de Feijão | 2015 | 115 / 0 |
| | *S.* Newport | Pepino | 2015 | 275 / 1 |
| ***E. coli*** | *E. coli* O157:H7 | Espinafre | 2006 | 199 / 3 |
| | *E. coli* O145 | Alface | 2010 | 26 / 0 |
| | *E. coli* O157:H7 | Avelã | 2011 | 8 / 0 |
| | *E. coli* O157:H7 | Alface | 2011 | 60 / 0 |
| | *E. coli* O26 | Broto de Alfafa | 2012 | 29 / 0 |
| | *E. coli* O157:H7 | Espinafre | 2012 | 33 / 0 |
| | *E. coli* O157:H7 | Salada Pronta | 2013 | 33 / 0 |
| | *E. coli* O121 | Broto de Alfafa | 2014 | 19 / 0 |
| ***Listeria*** | *L. monocytogenes* | Melão | 2011 | 147 / 33 |
| | *L. monocytogenes* | Broto de Soja | 2014 | 5 / 2 |
| | *L. monocytogenes* | Maçã | 2014 | 35 / 3 |

## 4. Meta-análise

Primeiramente, para se definir o termo meta-análise é preciso apresentar o conceito de "*revisão sistemática"*. De acordo com Dickson, Cherry e Boland (2014), revisão sistemática trata-se de uma revisão de literatura que visa encontrar, avaliar e sintetizar as melhores evidências de uma determinada pesquisa científica a fim de responder claramente a uma questão, previamente formulada. Para a elaboração de uma estratégia de busca eficiente é preciso que a "*questão de pesquisa"* seja factível, interessante, nova, ética e relevante (Hulley *et al*., 2011). Desta maneira, a questão de pesquisa deve estabelecer critérios de inclusão e exclusão de estudos durante a busca e a manutenção de um registro de exclusões facilita a administração da revisão sistemática. A elegibilidade dos estudos depende muito da elaboração da questão de pesquisa, ou seja, uma questão de pesquisa bem elaborada poderá facilitar no momento de seleção dos estudos a serem meta-analisados. Na figura 3 é possível visualizar os passos fundamentais no processo de revisão sistemática.

Deste modo, a meta-análise consiste em uma eficiente ferramenta estatística que auxilia na sistematização dos dados coletados, ou seja, integrar os resultados de estudos primários previamente selecionados e avaliá-los estatisticamente (Glass, 1976; Der

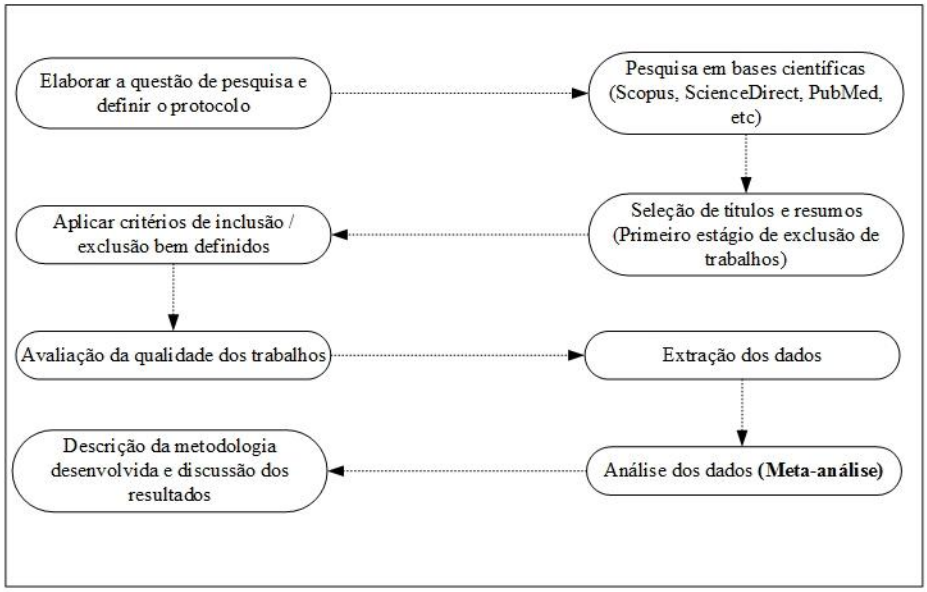


Figura 3. Fluxograma dos passos fundamentais para realização de uma revisão sistemática (Adaptado de Dickson, Cherry e Boland, 2014).

Simonian e Laird, 1986). Assim, o aumento do número de amostragem do estudo faz com que o poder estatístico das estimativas geradas pela meta-análise seja elevada já que com uma maior amostragem a tendência é que a variabilidade dos estudos observados seja reduzida (Dickson, Cherry e Boland, 2014).

Para que a meta-análise seja eficiente é necessário que sua condução obedeça a alguns critérios, como por exemplo, planejamento da coleta dos estudos e extração dos dados. Neste sentido, é necessário que os dados sejam suficientemente similares, ou seja, que exista coerência entre os dados extraídos, para que eles possam ser combinados entre si, no entanto, é aceitável que exista heterogeneidade entre os estudos.

Em meta-análise essa heterogeneidade entre os estudos primários observados é a medida da variabilidade entre os estudos. Podendo se tratar de uma heterogeneidade metodológica, que pode ser desde a variabilidade do desenho experimental e sua qualidade, ou até mesmo a uma heterogeneidade estatística, onde o acaso promove a variabilidade dos resultados do estudo (Dickson, Cherry e Boland (2014).

A heterogeneidade de uma meta-análise pode ser mensurada pela porcentagem (0-100%) de inconsistência ($I^2$) estatística entre os estudos, onde:

$I^2$ = 0%, não existe heterogeneidade entre os estudos;

$I^2$ = 25%, heterogeneidade baixa;

$I^2$ = 50%, heterogeneidade moderada; e

$I^2$ = 75%, heterogeneidade alta.

Na presença de heterogeneidade o condutor da meta-análise pode: ignorar, incorporar ou explicar (Figura 4). O pesquisador decidindo ignorar a heterogeneidade de seus resultados, deve estar, necessariamente, trabalhando com um modelo de efeitos fixos que permita-lhe assumir que a fonte da heterogeneidade de seu trabalho se deve a um fator irrelevante, ou ao acaso. Incorporando a heterogeneidade, o pesquisador pode não agrupar os dados que estão causando a heterogeneidade dos resultados e normalmente ocorre em modelos de efeito randômico. Quando se explica a heterogeneidade detectada no estudo, o pesquisador possui duas formas de avaliar a heterogeneidade, (i) análise de sub-grupos, onde é avaliado se os efeitos de diferentes

tratamentos são observados em diferentes sub-grupos; (ii) meta-regressão, que se trata de uma segunda análise estatística que associa o tamanho dos efeitos às características do estudo (Dickson, Cherry e Boland (2014).

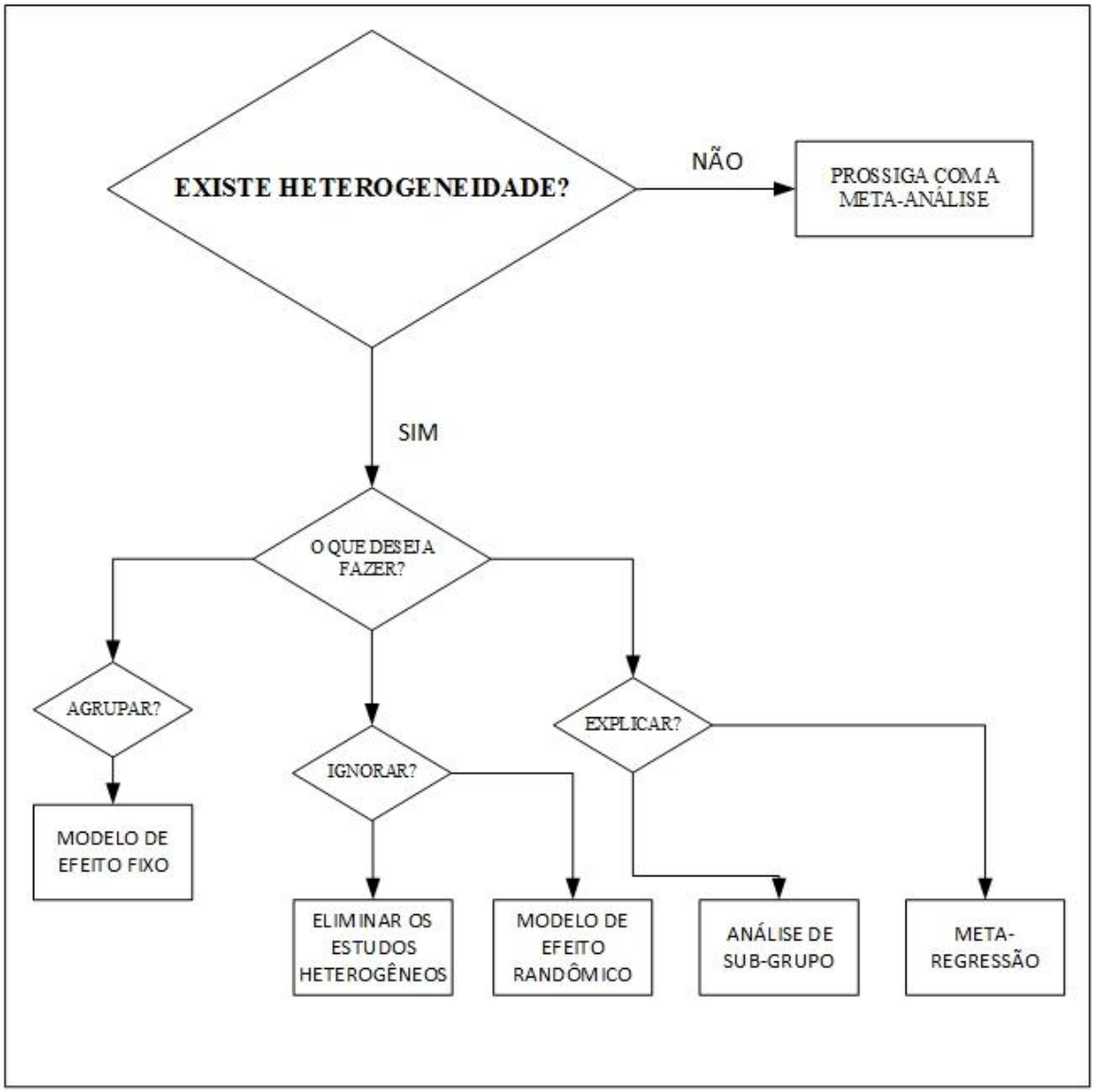


Figura 4. Fluxograma do efeito da heterogeneidade.

O uso da meta-análise em microbiologia e segurança dos alimentos, como uma ferramenta de coleta e análise de dados, a fim de reduzir a heterogeneidade dos resultados primários pode ser considerado recente (Gonzales-Barron *et al*, 2008;. Gonzales-Barron e Butler, 2011; Den Besten e Zwietering, 2012;. Gonzales-Barron *et al*, 2013; Rigaux *et al.*, 2013; Silva, *et al.*, 2015).

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## Capítulo II: Modeling the effects of temperature and pH on the resistance of *Alicyclobacillus acidoterrestris* in conventional heat-treated fruit beverages through a meta-analysis approach

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**Abstract**

In this work all publicly-accessible published findings on *Alicyclobacillus acidoterrestris* heat resistance in fruit beverages as affected by temperature and pH were compiled. Then, study characteristics (protocols, fruit and variety, ºBrix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) were extracted from the primary studies, and some of them incorporated to a meta-analysis mixed-effects linear model based on the basic Bigelow equation describing the heat resistance parameters of this bacterium. The model estimated mean $D^*$ (time needed for one log reduction) values of *Alicyclobacillus* in beverages of different fruits, two different concentration types, with and without bacteriocins, and with and without clarification). The $z_T$ (temperature changes needed to cause one log reduction in *D*-values) estimated by the meta-analysis model were contrasted to those ('observed' $z_T$ values) reported in the primary studies, and in all cases they were within the confidence interval of the model. The model was capable of predicting the heat resistance parameters of *Alicyclobacillus* in fruit beverage types beyond the combinations available in the meta-analytical data. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes.

**Keywords:** Bigelow, secondary model, mixed linear model, *D*-value, z-value, juice, pasteurization.

## 1. Introduction

The microbiological stability of shelf-stable fruit juices is based on the combination of their low pH values (usually ≤3.8) with heat treatments designed to inactivate the most heat resistant microorganisms found. Throughout the decades, several microorganisms have been used as targets of fruit juice pasteurization processes, including yeasts, lactic acid bacteria, heat resistant moulds and sporeforming bacteria (Tribst et al., 2009). However, since early 80's, fruit juice processors have been challenged by a bacterium showing remarkably heat and chemical resistances, ability to grow under acidic conditions and, consequently, to spoil shelf-stable fruit juices (Silva and Gibbs, 2001; Friedrich et al., 2009; Spinelli et al., 2009, 2010). This bacterium was characterized by the presence of ω-alicyclic fatty acids as major lipid components on the cellular membrane, which together with 16S rRNA sequencing analyses led to the proposal for creation of a new genus, *Alicyclobacillus* (Wisotzkey et al., 1992). Currently, it is known that members of the *Alicyclobacillus* genus are surprisingly diverse and not all species have been described as containing these characteristic fatty acids (Glaeser et al., 2013). Presently, more than 20 species have been reported to belong to *Alicyclobacillus* genus (Smit et al., 2011, Glaeser et al., 2013), while spoilage potential of fruit juices has been restricted to few species such as *A. acidoterrestris, A. acidiphillus, A. pomorum, A. herbarius, A. hesperidum, A. acidocaldarius* and *A. cycloheptanicus* (Cerny et al. 1984, Matsubara et al. 2002, Goto et al. 2003, AIJN, 2007, Smit et al. 2011). The spoilage potential of *Alicyclobacillus* species relies on their ability to produce off-flavor compounds such as 2-methoxyphenol (guaiacol), 2,6-dibromophenol, 2,6-dichlorophenol and 2-methyltetrahydrothiophene-3-one (Siegmund and Pöllinger-Zierler, 2006; Lottici et al., 2006; Siegmund and Pöllinger-Zierler, 2007; Concina et al., 2010).

Because of its spoilage potential, several reports are found on the incidence of *Alicyclobacillus* in fruit and vegetable beverages (Siegmund and Pollinger-Zierler, 2006, Durak et al., 2010, Steyn et al., 2011, Walls and Chuyate, 2000**,** Groenewald et al., 2009, McKnight et al., 2010, Danyluk et al., 2011, Oteiza et al., 2011). Also, as a major target for fruit juice pasteurization (Tribst et al., 2009), numerous studies are found that report thermal inactivation parameters of *Alicyclobacillus*, *i.e*., the *D* value (time at a determined temperature required to cause one-log cycle decrease in the population of a target bacterium) and the *z* value (temperature increase required to result in one-log cycle decrease of *D*-value) (Splittstoesser et al. (1994), Komitopoulou et al. (1999), Bahceci and Acar (2007), Walls (1997), Silva et al. (1999), Maldonado et al. (2008), de Carvalho et al. (2008), López et al. (2011), Alberice et al. (2012), Peña et al. (2009), McKnight et al. (2010). As known, *D*- and *z*-values of *Alicyclobacillus* are affected by the particular conditions or *study characteristics* (protocols, fruit and variety, ºBrix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) under which they were obtained. Therefore, variability in *D*- and *z*- values among primary studies is expected to occur, even among studies investigating the same type of fruit beverage. Nonetheless, by means of *a posteriori* analysis and identification – from each of the primary studies – of the sources of variability impacting on the thermal inactivation parameters of *Alicyclobacillus*, it may be possible to explain, to some extent, the differences found among the study outcomes.

To this respect, meta-analysis, defined as a *"statistical analysis of a collection of analytic results for the purpose of integrating the findings from a large amount of primary studies"* (DerSimonian & Laird, 1986), allows (i) the explanation of the divergences in the study outcomes by the codification of study characteristics (i.e., moderating variables related to research design features, data collection procedures, type of samples, etc.) aiming to reduce the between-study heterogeneity or variability (Gonzales-Barron et al.,

2013); and (ii) the accurate estimation of the overall outcome measure, with increased statistical power, using only a single study (Sutton et al., 2001). Despite the capabilities of meta-analysis, already long recognized in medicine and clinical studies, the application of this body of compiling statistical techniques in food safety and microbiology issues is recent (Gonzales-Barron et al., 2008; Gonzales-Barron and Butler, 2011; Den Besten and Zwietering, 2012; Gonzales-Barron et al., 2013). Thus, the first objective of this study is to compile all publicly-accessible published findings on the heat resistance of *Alicyclobacillus acidoterrestris* in fruit beverages as affected by temperature and pH, and quantitatively summarize these outcomes by means of a meta-analytical model based on a Bigelow-type secondary predictive model. A second objective is to attempt to explain a proportion of the total between-study heterogeneity in the heat resistance parameters by incorporating available study characteristics to the basic model. The resulting meta-analysis model (i.e., a mixed-effects linear model based on the Bigelow equation) should be effective in estimating the thermal inactivation parameters, *D*- and *z*- values, for the various types of beverage considered.

## 2. Methodology

### 2.1. Data collection

Literature identification was conducted using electronic search through Google with key terms, both in English and in Portuguese, including: "*Alicyclobacillus*", "ATSB", "Acidothermophilic sporeforming bacteria", "heat resistance", "*D*-value", "thermal resistance", "inactivation", "fruit juice", "juice", "beverages". Also, literature for inclusion in the study was identified from bibliographic databases such as Pubmed, Science Direct and Scopus, using the same key-words. Data included considered studies available in

scientific journals and electronically from 1980 to 2014. A total of 55 studies reporting on inactivation of *Alicyclobacillus* spores in fruit beverages were retrieved, however, these included also reports using high pressure processing, ultrasound, pulsed electric field and pulsed light. Nonetheless, for inclusion in the meta-analysis, only conventional heat-related studies were considered, which originated from peer-reviewed scientific papers. A second criterion used in the screening was the need for the primary study to model first-order reaction kinetics; said otherwise, studies reporting on inactivation of *Alicyclobacillus* in fruit beverages with no *D*-values were excluded from the meta-analysis. Additionally, for a primary study to be included in the meta-analysis, it had to report more than two *D*-values, measured either at different inactivation temperatures or at different beverage pH. The statistical reason for this was that, for the meta-analytical mixed-effects linear model explained in Section 2.2, the standard error about the $z_T$ or $z_{pH}$ value (inverse of the slope between log *D* and temperature or pH, respectively) of a particular experiment could be only measured with more than two points along a fitted straight line. This restriction caused the results from four primary studies to be omitted for the analysis: Yamazaki et al. (1997) who reported two *D*-values for orange juice; Baumgart (1999) with only one *D*-value for orange juice; Vieira et al. (2002) reporting one D-value for cupuaçu concentrate; and Baysal and Icier (2010) who reported only two *D*-values for orange juice. Thus, 11 primary studies were selected and considered appropriate for the meta-analysis model, providing a total of 142 *D*-values obtained at different inactivation temperatures and pH values (*Table 1*).

### 2.2. Description of the data set

Apart from the *D*-values, the corresponding beverage pH and the temperatures at which the isothermal experiments were conducted, additional information was also extracted from the primary studies. It is known that the content of soluble solids or ºBrix of

the beverage is an important physicochemical parameter affecting the heat resistance of *Alicyclobacillus* (Splittstoesser et al., 1998). However, as such information was not available for every primary study, a categorical variable "type of beverage" was created to assign fruit beverages either to a single strength juice or to a concentrate class. It was defined that *D*-values obtained from beverages of either Brix above 18º, or *concentrates* and *nectars* (stated as such in the primary studies yet with no indication of the level of soluble solids) were assigned to the "concentrates" category. Single strength juices presented an average concentration of soluble solids of 10.2% (ranging from 5.3 to 13.0%) while fruit concentrates an average concentration of 48.0% (ranging from 18.0 to 68.0%).

Another evident study characteristic to codify (or to disaggregate) was the fruit. *D*-values were assigned to ten different fruit classes: apple, berry, cupuaçu, grape, grapefruit, lemon, mango, orange, passion fruit and tangerine. A special class named as "model" (*Table 1*) was created within the moderating variable fruit to encompass the results from López et al. (2011) and Bahçeci and Acar (2007), who employed citrate phosphate McIlvaine buffer to estimate the heat resistance of *Alicyclobacillus* at different pH values. This buffer is an acidic model beverage that has been proposed in order to perform thermal processes and heat transfer studies in fruit products.

The third moderator variable was "clarification" to indicate whether or not fruit beverages underwent the normal clarification process followed by filtration to separate the particles in suspension in the beverage. This was a coded variable taking the value of 0 for non-clarified beverages and the value of 1 for clarified beverages. For the special case of the *model* category within the fruit moderating variable, the "clarified" class was assigned because of the low viscosity and the absence of particles in suspension in a buffer (*Table 1*). On the other hand, the study of de Carvalho et al. (2008), which focused on mango concentrate, did not specify whether the concentrate was clarified or not. However, as the main objective of such a study was to assess the effect of bovicin on the resistance of

*Alicyclobacilus* in mango pulp, a logical conclusion was that the mango pulp, which was two-fold diluted for their experiments (i.e., concentrate), was not clarified.

The fourth study characteristic was “presence of bacteriocins”, which was conceived because two of the primary studies investigated the effect of nisin (Komitopoulou et al., 1999; Peña et al., 2009) on the thermal resistance of *Alicyclobacillus*; and one study the effect of bovicin HC5 – a bacteriocin from *Streptococcus bovis* (de Carvalho et al., 2008). Thus, this categorical variable was coded to take up the value of 0 for absence of bacteriocins and the value of 1 for added bacteriocins. While de Carvalho et al. (2008) employed a concentration of bovicin HC5 of 80 IU/ml in mango concentrate, Komitopoulou et al. (1999) and Peña et al. (2009) assessed both a concentration of 50 IU/ml in apple and orange juice, respectively.

A summary of the input data for the meta-analysis study is presented in *Table 1*. It should be noticed that such meta-analytical data is highly sparse, meaning that for some fruits less data are available. For instance, for apple, lemon and orange, data for both types of beverages – juice and concentrate – were found, and additionally for clarified and non-clarified beverages, while for other fruits such as grape and passion fruit, data were limited to clarified juices only. This has some implications in the design of the meta-analysis mixed-effects model, as explained in Section 2.3.

### 2.3. Meta-analytical model

To describe the combined effect of temperature and pH on the heat resistance of *Alicyclobacillus* in fruit beverages, the Bigelow-type linear model was selected (Mafart and Leguerinel, 1998):

$$logD = logD^* - \left(\frac{1}{z_T}\right)(T - T^*) - \left(\frac{1}{z_{pH}}\right)(pH - pH^*) \quad \textit{Equation 1}$$

where *D* is time at a constant temperature *T* and at the pH of the food matrix required to cause one-log cycle decrease in the population of a target bacterium; *T** is the reference temperature (set at 95ºC, which is a common temperature for fruit juice pasteurization); pH* is the reference pH (chosen to be 3.5 to correspond to a common pH of fruit beverages); $z_T$ is the conventional thermal z-value; $z_{pH}$ is the distance of pH from pH* which leads to a ten-fold reduction of the decimal reduction time; and *D** is the decimal reduction time at *T** and pH*.

The Bigelow secondary predictive model was used to interpret the combined results of the primary studies. As the meta-analytical data obtained also contain a number of moderating variables or coded study characteristics (for example, fruit, type of beverage, addition of bacteriocin and application of clarification), the Bigelow model was transformed into a linear mixed-effects model in order to assess whether each of the moderating variables has any effect on *D* and/or $z_T$ and $z_{pH}$. Hence, the three parameters of *Equation 1* were modeled as,

$$logD^*_{ijlm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} = logD^*_{mean\ ij} + u_{lm} \quad \textit{Equation 2}$$

$$\frac{1}{z_{T_{ilm}}} = (\gamma_1 + \gamma_{2i} + v_{lm}) \quad \textit{Equation 3}$$

$$\frac{1}{z_{pH_k}} = (\delta_1 + \delta_{2k}) \quad \textit{Equation 4}$$

Where: $\beta_0$ is an intercept, $\beta_1$ is the fixed effect of the type of beverage *i* (coded as 0 for single strength juice and 1 for concentrates), $\beta_2$ is the fixed effect of the clarification stage *j* (coded as 0 for no clarification and 1 for regular clarification). The value of $D^*_{mean\ ij}$ represents the average decimal reduction time at the reference *T** and pH* applicable to the entire population of fruits, yet it is an intercept allowed to take up different independent values due to the variability in the fruit/primary study combination (viz. interaction). Because of the sparse nature of the data structure, whereby in most cases one primary study reported results for only one fruit (*Table 1*), for the analysis it was not feasible either

to separate the between-fruit variability from the between-study variability or to build a nested covariance of primary studies within fruit or fruits within primary study. To overcome this problem and still be able to account for the evident variability due to the different fruits (*l*) and primary studies (*m*), both variables had to be merged into an interaction variable (*lm*) providing sixteen levels to be used as the subject of variation of the random effects placed in *Equation 2*. These intercept random effects $u_{lm}$ are assumed to have a normal distribution with mean zero and variance $s^2_u$.

The coefficient $\gamma_1$ is the mean effect of a 1ºC-increment in temperature ($T$-$T^*$) for the entire population of fruit beverages; yet, the coefficient for the temperature difference slope is affected by the type of beverage *i* and by the specific combination of fruit (*l*) and primary study (*m*). Thus, $\gamma_2$ is the fixed effect of the interaction term between the type of beverage *i* and the temperature slope. Since preliminary analysis of the meta-analytical data had shown that the temperature slopes for single strength juice tended to be steeper than those for concentrates, this variability was accounted for. As done for the intercept random effects, the interaction between fruit and primary study (*lm*) was assumed to be the subject of variation of the random effects $v_{il}$. The random effects $v_{il}$ added to the slope $\gamma_1$+ $\gamma_2$ model the shifts in the temperature effect for each of the primary study×fruit existing in the data set. These slope random effects are assumed to follow a normal distribution with mean zero and variance $s^2_v$. Placing a fixed effect on the type of beverage and random effects for fruits (interacting with the primary studies) in *Equation 3* enables the model to compute the $z_T$ values for all the combinations of fruit and type of beverage, even beyond the combinations existing in the original meta-analytical data.

The coefficient $\delta_1$ represents the effect of the increment in the pH difference (pH-pH*), and $\delta_2$ the coefficient of the interaction term between addition/non-addition of a bacteriocin (*k*) and the pH slope. This interaction allows for a change in the pH difference slope when a bacteriocin is added to the beverage. Random variations in the pH slope due

to beverage type and fruit were not modelled in *Equation 3* as they turned out to be non-significant. The variances of the random effects placed on the intercept and temperature slope, $s^2_u$ and $s^2_v$, were assumed to be correlated with a covariance $s^2_{uv}$. As all those variance and covariance terms can be thought of as realisations of a *primary study*, the presence of heterogeneity among primary studies can be assessed by the Wald's test of significance of each of the variance, $s^2_u$ and $s^2_v$, and covariance $s^2_{uv}$ parameters. Hence, if those terms were statistically significant, the between-study variability $\tau^2$ can be approximated by $s^2_u + s^2_u + s^2_{uv}$, and the $I^2$ statistics or intra-class correlation, estimating the proportion of between-study variability from the total variance, can be approached as $(s^2_u + s^2_u + s^2_{uv}) / (s^2_u + s^2_v + s^2_{uv} + s^2)$, where $s^2$ is the variance of the normally-distributed residual random errors $\varepsilon_{ijklm}$.

Thus, putting together *Equations 2, 3* and *4*, the linear mixed-effects model adjusted to the meta-analytical data was,

$$logD_{ijklm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} - (\gamma_1 + \gamma_{2i} + v_{lm})(T - T^*) - (\delta_1 + \delta_{2k})(pH - pH^*) + \varepsilon_{ijklm} \quad (5)$$

Notice that the values of *log D**, $z_T$ and $z_{pH}$ can be estimated from the model's fitted parameters using *Equations 2, 3* and *4*, respectively In building the meta-analysis mixed model, all the interaction terms between the categorical moderating variables, and with pH and temperature were evaluated. Because of data sparseness, only interactions of two terms were considered. However, only two interaction terms were found to be statistically significant (i.e., slope of temperature difference with type of beverage and slope of pH with presence of bacteriocins), which were retained in the model. Similarly, a series of combinations of random effects attempting to extract the variability between fruits and the variability between primary studies, both separately and as interactions, were placed in *Equations 2, 3* and *4*, and their results compared one-to-one by a log-likelihood ratio test

and the Bayesian Information Criterion (BIC). The model presented in *Equation 5* was the most parsimonious (i.e., least parameters with the best goodness-of-fit), and yet, with a fully interpretable arrangement. Since primary studies are expected to differ from each other in the reliability of estimating the true heat resistance parameters of *A. acidoterrestris* in fruit beverages*,* for instance, due to differences in study sizes, a *weighted* linear mixed model was preferred, with weights representing the precision in estimating the population lethality parameters. Because not all primary studies reported the standard error of the *D*-value, the precision was defined as some measure proportional to the sample size N used in the bacterial kinetics experiments to calculate a single *D*-value. Hence, the weight – level of confidence on each *D* measure – was given by the sample size. *Table 1* also compiles the sample size used to determine each of the *D*-values, which was calculated as the number of sample units analysed multiplied by the number of points in time where samples were taken to measure the concentration of *Alicyclobacillus*. Once the model was fitted, the normality of residuals was assessed and the studentised residuals examined for identifying spurious data points lower than -3.0 and higher than 3.0. The weighted mixed-effects linear model was fitted in R version 2.14.2 (R Development Core Team) using the 'nlme' package (Pinheiro et al., 2013).

## 3. Results and Discussion

The management of microbial spoilage of fruit beverages requires the ability to predict the thermal resistance of the spores of *Alicyclobacillus acidoterrestris*. During this systematic review, it was realized that there are in the literature numerous studies reporting useful data on the thermal death kinetics of this spoilage microorganism, which, in principle, could be applied for the determination and optimisation of the process variables for heat treatment. However, the large number and variety of data, and

principally, the different estimates of the thermal inactivation parameters among studies, make further developments difficult. For instance, the study of Komitopoulou et al. (1999) reported a $z_T$-value of 12.9 for orange juice at a pH of 3.9, while Yamazaki et al. (1997) found a lower $z_T$-value of 9.5 for orange juice at a similar pH of 3.7. Similarly, for apple juice at a pH of 3.5, Komitopoulou et al. (1999) and Splittstoesser et al. (1994) found dissimilar $z_T$-values of 12.2 and 7.7, respectively. The degree of discrepancies in the relationship between *D*-value and temperature observed in the input data set can be visually assessed in *Figure 1*. In such a *Figure*, the same markers depict a sub-group of observations from a given set of heat inactivation isothermal experiments conducted to determine a $z_T$ value at fixed conditions; said otherwise, a sub-group is formed by the paired observations (*D*-value, temperature) extracted for a given fruit, type of beverage, clarification, bacteriocin and pH value. From *Table 1*, it can be deduced that there were 37 sub-groups. *Figure 1* shows that the *D*-values from the 37 sub-groups were all consistent as they decrease with increasing temperatures, yet it also hinted that, in designing a meta-analytical linear model, some allowance had to be made in relation to the variability of the intercepts and slopes (inverse of $z_T$) by incorporating random effects. In a multilevel meta-analysis, as is the case here, one usually starts assessing the null random-effects model. In our case, the null random-effects model is the simple Bigelow model (*Equation 1*) with random effects placed on the intercept and the temperature difference slope. Such a model produced a value of heterogeneity $\tau^2$ of 0.072 while the variance of the residuals was 0.094 (results not shown). Thus, the intra-class correlation can be estimated ($I^2$=0.072/(0.072+0.094)=0.44) at 44%. This value, being higher than the rule of thumb of 25% (Hunter and Schmidt, 1990), underscored the presence of significant heterogeneity; and, consequently, confirmed that some study characteristics had to be coded in an attempt to explain, understand and reduce such variability.

When the null random-effect model (basic Bigelow) was extended to a multilevel model (mixed-effects linear model comprising study characteristics or moderating variables; *Equation 5*), the variance of the residuals reduced to 0.038, and the heterogeneity $\tau^2$ reduced to 0.044 (*Table 2*). This indicated that approximately 40% ((0.073-0.044)/0.073 = 0.397) of the total amount of heterogeneity due to primary studies and fruits could be explained by the categorical variables type of beverage, clarification and presence of bacteriocins. Because the residual heterogeneity $\tau^2$ of 0.044 is still significant (*Table 2*), it can be concluded that there may be other study characteristics, not coded in the present meta-analysis, that are likely to be also noteworthy. As Hox and De Leeuw (2003) pointed out, it is highly unlikely that the available study-level variables could cover all the artefacts causing variation between study outcomes. This occurs because the information given in research reports and articles is not enough to cover all the study characteristics; and in fact this was attested during the conduction of the present meta-analysis. For instance, not all primary studies specified the content of soluble solids of the fruit beverage.

As expected, the inactivation temperature affected ($p<.0001$) the resistance of *Alicyclobacillus* (*Table 2*). In comparison with the predominant effect of temperature (F-value=100.7), the influence of pH on the heat resistance of *Alicyclobacillus* was weaker (F-value=32.5), as suggested by the more disperse scatterplot between log *D* and beverage pH (not shown). Nonetheless, the meta-analysis model was still able to detect the significance of this physicochemical property (*Table 2*). In an earlier study, Pontius et al. (1998) detected as well a significant effect of pH, although they showed that it becomes more notorious only at lower inactivation temperatures. In this work, as the summarised data comprised a narrow range of pH from 2.8 to 4.0, it is natural that the effect of higher temperatures (from 80ºC) surpasses the effect of the matrix acidity. Although the

mechanisms of resistance to pasteurisation of *Alicyclobacillus* are still unclear, the thermal resistance of other bacterial spores is influenced by several environmental factors such as pH, water activity and menstruum composition (Baysal and Icier, 2010). However, the most significant parameter in the inactivation of microorganisms is the thermal effect itself, regardless of the type of thermal treatment.

The heat sensitivity of *Alicyclobacillus* was shown to be significantly different between single strength juices and concentrates (i.e., see variable type in *Table 2*). Independently of the kind of fruit, the concentrates had on average log $D^*$ values higher than juices in 0.115 units. This finding was in agreement with Alberice et al. (2012), who found that the *D*-values in all temperatures assayed were slightly higher in concentrated juice than in reconstituted juice. In our meta-analysis study, the type of beverage is not only responsible for causing a shift in the intercept (log $D^*$) of the relationship between log *D* and temperature but also causes a shift in the slope. Notice that the interaction term temperature×type is significant ($p<0.05$; *Table 2*), therefore bringing about differences in $z_T$ values for juices and concentrates (*Table 4*). The estimate of 0.014 for temperature×type (*Table 2*) indicates that, in single strength juices, the slope between log *D* and temperature is higher (steeper) than in concentrates by 0.014 units. In other words, the same increase in the pasteurisation temperature for concentrates will have a lower effect on the heat resistance of *Alicyclobacillus* than for juices. This is, as a consequence, reflected in the $z_T$ values estimated by the meta-analysis model (*Table 4*), which in all cases are higher in concentrates than in single strength juices. An explanation of the fact that the inactivation rate of *Alicyclobacillus* is higher in single strength juices than in concentrates can be found in Gombas (1983), who sustained that an apparent increase in spore heat resistance is achieved when it is balanced in low water activity or dissolved in a solution of high osmotic potential. High sugar concentrations like sucrose exert a similar osmotic pressure that

exists in the spore cortex. Thus, protoplast dehydration is induced mechanically and osmotically by pressure, and this dehydration mechanism present in the spores is probably responsible for heat resistance.

It was also demonstrated that *Alicyclobacillus* possesses less thermal resistance in clarified beverages than in non-clarified beverages. In the meta-analysis model, the variable clarification had an effect ($p<.0001$) on the *D*-values as a single term but not in interactions either with temperature or with pH (*Table 2*). Hence, clarification only affects the estimation of log *D**, meaning that, in the relationships between log *D* and temperature or log *D* and pH, the process of clarification will only cause a downward shift in the straight line, and will not affect either the temperature slope or the pH slope; hence, will not affect the $z_T$ or $z_{pH}$ values. On average, the model estimated that a non-clarified beverage will exhibit an increase in the intercept or log *D** value of 0.26 units (*Table 2*). It may be hypothesised that the greater particles in suspension in a non-clarified juice slows down the heat transfer rate, retarding also the thermal inactivation of *Alicyclobacillus*.

The meta-analysis also demonstrated that there is a significant effect of the addition of bacteriocins prior to heating on the thermal resistance of *Alicyclobacillus*, increasing the lethality of pasteurisation. Although the variable bacteriocin was not statistically significant when it entered the model as a single term (i.e., as a predictor of log *D**), it was highly significant as an interaction term with pH (*Table 2*). The negative estimate of pH×bacteriocin suggests that for a constant value of beverage pH, the addition of bacteriocins (either nisin or bovicin in the doses studied in their respective primary studies) will increase the thermal sensitivity of *Alicyclobacillus* (i.e., lower log *D*). On the other hand, the fact that there is an interaction between pH and the presence of bacteriocins implies that the effect of a bacteriocin on the thermal sensitivity of *Alicyclobacillus* becomes more evident at higher pH. This is a greater bactericide effect is revealed when a

bacteriocin is added to a less acidic beverage in comparison to a highly acidic beverage, because in a highly acidic matrix the effect of the low pH itself on *Alicyclobacillus* lethality may mask the effect of the bacteriocin, and hence, the effect of the latter becomes less evident. As a consequence, the value of $z_{pH}$ estimated for beverages with bacteriocins (0.586) was significantly lower than the one for beverages without bacteriocins (5.750) (*Table 4*). The bacteriocins in doses between 50-80 IU/ml reduced by a factor of ten the $z_{pH}$ value of *Alicyclobacillus*. In this meta-analysis study, the addition of bacteriocins did not play a role on the reduction of $z_T$ as the interaction temperature×bacteriocin turned out to be non-significant. Yet, our model still confirmed that the bacteriocins, nisin and bovicin, were bactericidal against *Alicyclobacillus*, as the *D*-values – hence, the viable cell numbers – decreased in their presence. Although there is evidence that higher doses of bacteriocins have greater effect on increasing the lethality of *Alicyclobacillus* spores (Peña et al., 2009; Komitopoulou et al., 1999), this was not assessed in this meta-analysis study.

The variances $s^2_u$ and $s^2_v$ of the random effects placed on the model's intercept (log *D**) and temperature slope, respectively, were both significant (*Table 2*), confirming statistically the presence of heterogeneity that was initially observed in *Figure 1*. As the subject of variation of the random effects was the interaction study×fruit, it can be conceived (i) that there is an infinite population (past, present and future) of primary studies reporting lethality data of *Alicyclobacillus* for a fruit beverage (ii) that there is an infinite population of fruits that can be subject of study; and (iii) that each of the studies associated to a fruit introduces inherent heterogeneity in the reported outcomes because of the differences in the methods for assessing microbial thermal resistance, in the composition of the beverage, in the bacteria strains inoculated, in the microbiological essay to quantify *Alicyclobacilus*, etc.. As explained before, the fixed effects or coded study characteristics could explain 40% of such heterogeneity. Yet, there is a residual

heterogeneity ($\tau^2$~0.044; *Table 2*), which is still significant. The purpose of the random effects is therefore to absorb this unexplained heterogeneity.

Because “primary study” and “fruit” could not enter the meta-analysis model as separate subjects of random effects – since in the input data, in most cases, one primary study was associated to one fruit (*Table 1*) – consequently, the estimate of variability cannot be separated into that due to differences among primary studies and that due to differences among fruits. By entering primary study in interaction with fruit, both subjects of variability are acknowledged although they cannot be disaggregated. At most, it could be hypothesised that a primary study involves many more sources of variability in the estimates of bacterial heat resistance than the kind of fruit does; and therefore, that the between-study heterogeneity is much greater than the between-fruit heterogeneity. Based on this assumption, the between-study heterogeneity $\tau^2$ was approximated by using the variances $s^2_u$, $s^2_v$ and the covariance $s^2_{uv}$ (*Table 2*).

Nevertheless, using such a model design, it is possible to provide estimates of log $D^*$ and $z_T$ for beverages (single strength juices or concentrates) of any of the ten fruits considered. This is possible by computing the random effects $u_{lm}$ and $v_{lm}$ (*Equations 2 and 3*, respectively) for a given fruit, and average them over the primary studies associated with such a fruit – in case that more than one primary study was in interaction with that fruit. In this way, the log $D^*$ and $z_T$-values were estimated for single strength juices and concentrates made of different fruits (*Tables 3* and *4*). A test of contrasts showed that there are statistical differences in the log $D^*$ and $z_T$-values among the kinds of beverage. For instance, in terms of the $D$-value at 95ºC and at matrix pH of 3.5, *Alicyclobacillus* in berry juice presented a low heat resistance of 1.8 min (log $D^*$=0.252 in *Table 3*), while in orange juice exhibited a higher thermal resistance with a $D$-value of 4.9 min (log $D^*$=0.695 in *Table 3*). The growth and inactivation of *Alicyclobacillus* spores in commercial beverages depends, among other factors, on the compositional properties of food. For

instance, in Splittstoesser et al. (1994), apple and tomato juice consistently supported growth, whereas grape juice at both pH 2.9 and 3.3 did not permit it. Different components present in fruits might increase the heat resistance of *Alicyclobacillus* spores, and this was clear for apple juice and apple nectar in Bahçeci and Acar (2007). Similar levels of heat resistance of *Alicyclobacillus* were found for tangerine juice (López et al., 2011) and orange juice (Conesa et al., 2009). Our meta-analysis study produced also relatively high log *D** values for tangerine and orange juice (*Table 3*). Nonetheless, because of the structure of our meta-analysis model, we cannot conclude that such significant differences in log *D** between, for instance, berry and orange juice (*Table 3*), can be entirely assigned to the composition of the fruits since it may as well be due to the heterogeneity among the primary studies that determined the *D*-values of *Alicyclobacillus* in berry and orange juice. Remember that the random effects had as subject the interaction primary study and fruit. Hence, some care should be taken in the interpretation of the statistical differences in the *D*-values and *z*-values estimates of the beverages across fruits listed in *Tables 3* and *4*. It is more prudent instead to interpret each of these estimates as *mean effect size* or overall average from all the meta-analysed literature sources. In fact, such summarisation of the research outcomes (i.e., available knowledge) increases the statistical confidence of the individual studies alone, and it is what constitutes one of the strengths of meta-analysis.

The mixed-effects linear model estimated a mean *D** value of 3.8 min with a 95% CI: 3.0 - 4.9 min (log *D** = 0.584; 95% CI: 0.474 - 0.694 in *Table 3*) to decrease one-log population of *Alicyclobacillus* in fruit beverages, on average (single strength juices or concentrates, clarified or non-clarified), at a temperature of 95ºC and a pH of 3.5. As this value is an estimate from a random-effects model, it can be generalised to the entire population of fruits and primary studies. More specifically, the mean *D** value estimate for single strength juices, whether clarified or not (3.3 min; 95% CI: 2.6 – 4.3 min), was lower ($p<0.05$) than for the concentrates (4.4 min; 95% CI: 3.3 – 5.9 min). The mean *D** value for

clarified juices (2.5 min; 95% CI: 1.9 – 3.2 min) was significantly lower than for non-clarified single strength juices (4.5 min; 95% CI: 3.4 – 6.0 min), and the same can be said for the clarified concentrates (3.2 min; 95% CI: 2.4 – 4.4 min) and the non-clarified concentrates (5.9 min; 95% CI: 4.4 – 8.0 min). The significant effects of the type of beverage and the clarification have been explained earlier in this section. As expected, the mean log $D^*$ values for the concentrates of each fruit were higher than their respective single strength juices (*Table 3*).

Because of the model design, it was possible to compute for the beverages of each fruit (whether single strength juice or concentrate), the log $D^*$ estimates in case they were clarified or not clarified. In *Table 3*, three examples are presented for apple, mango and orange. Notice that the meta-analytical model allows us to estimate *Alicyclobacillus* thermal lethality parameters beyond those originally available in the input data set; and this represents the main capability of this model. For instance, no *D*-values were available for mango single strength juice, but only for non-clarified mango concentrate (*Table 1*). However, the meta-analysis model can predict *D*-values for clarified mango single strength juice, non-clarified single strength mango juice and clarified mango concentrate at different inactivation temperature and matrix pH. The confidence about these extrapolated estimates remains to be tested by other thermal inactivation laboratory experiments; these are, experiments for which *D*-values were not available in the literature, namely, for mango juice, cupuaçu concentrate, berry concentrate, grape concentrate, tangerine concentrate and passion fruit concentrate.

Using *Equation 3*, the mean temperature shift required for the thermal destruction curve to move one-log cycle ($z_T$-value) was summarised for single strength juices (11.23; 95% CI: 9.03 – 13.42) and concentrates (13.35; 95% CI: 9.89 – 16.80), which are values that can be generalised to all the population of fruits and primary studies. As explained

before, because the interaction temperature×type (*Table 2*) was significant – hence, the slope of the relationship between log *D* and temperature lower for concentrates – for all fruits, the estimates of $z_T$ values were higher for concentrates than for single strength juices (*Table 4*). Once again, notice that, as occurred with the log *D** estimates, predictions of $z_T$ could be produced for *Alicyclobacillus* in types of beverages whose lethality kinetics were not investigated in the primary studies. Nonetheless, such extrapolated $z_T$ estimates were subject to greater uncertainty, reason as to why their confidence intervals were slightly broader. For example, for cupuaçu single strength juice (present in the meta-analytical data), the 95% confidence interval of $z_T$ was 6.96 – 11.56, while for (the non-investigated) concentrate of cupuaçu, it was 7.33 – 14.00 (*Table 4*).

The $z_T$ values of the fruit beverages estimated by the meta-analysis model were contrasted to those ('observed' $z_T$ values) reported in the primary studies, and in all cases they were within the confidence interval of the model. For instance, the $z_T$ value of *Alicyclobacillus* reported for mango concentrate in the corresponding primary study, (de Carvalho et al., 2008) was 21.27, while the mean estimate of the meta-analysis model was 23.07 with a 95% CI of 13.06 – 33.08 (*Table 4*). For grapefruit juice, Komitopoulou et al. (1999) found $z_T$ values of 11.60, 11.53 and 10.49 at a pH of 3.42, 3.0 and 4.0, respectively, whereas the mean $z_T$ value estimated by the meta-analysis model for grapefruit juice was in agreement at 11.17 with a 95% CI of 9.38 – 12.96. For the model, the lowest mean $z_T$ values belonged to berry juice (8.02; 95% CI: 5.34 – 10.70) and grape juice (7.95; 95% CI: 5.86 – 10.06), and these were not statistically different one from the other. Both model's estimates were very close to the observed $z_T$ values for berry and grape juice, both of 7.2, found in Walls et al. (1997) and Splittstoesser et al. (1994), respectively.

To further illustrate the model's accuracy, *Figure 2* shows a comparison of log *D*, as affected by temperature, between the observed values (directly extracted from the primary studies) and the values predicted by the meta-analytical model for different types of beverages at a fixed pH. In all cases, the lines predicted by the model lay close to the observations. This set of examples also demonstrates the flexibility of the model to describe the same or different slopes and intercepts. For clarified apple juice (*Figure 2*; top left), the use of a bacteriocin causes a downward shift in the intercept (diminishes the heat resistance) while the random effects realizations from the two primary studies (apple juice with bacteriocin and without bacteriocin) explain the different slopes. For lemon concentrate (*Figure 2*; top right) and tangerine juice (*Figure 2*; bottom right), the clarification process causes the downward shift in the intercept whereas there is no change in the slope because the variable 'clarification' did not enter the model in significant interaction with temperature. Notice that the model predictions for clarified tangerine juice (*Figure 2*; bottom right) could not be validated given the absence of thermal resistance data in the literature for this subgroup. For the clarified beverages made of orange (*Figure 2*; bottom left), the intercept belonging to the single strength juice is lower than that of the concentrate, and its slope is also affected because of the significant interaction between type of beverage (single strength juice or concentrate) and temperature. Notice that the slope for the single strength juice is steeper than for the concentrate.

In assessing the fitting quality of the meta-analytical model, it was found that the studentised residuals fell between -2.5 and 2.5, and according to the Shapiro-Wilk test, their distribution could be approximated to a normal distribution (not shown). Furthermore, the residuals versus the fitted values (i.e., log *D*) did not exhibit any singular pattern (*Figure 3*), as they were randomly spread with a coefficient of correlation of 0.047. In

addition, there was good agreement between the fitted and the observed log *D* (*Figure 4*) with a high coefficient of correlation of 0.972.

## 4. Conclusions

Typically, fruit juices will be pasteurized at temperatures around 95ºC for c. 20 s to 2 min (Komitopoulou et al., 1999; Silva and Gibbs, 2001). While the heat treatment alone applied in acidic fruit products can decrease concentrations of *Alicyclobacillus*, if starting concentrations are high enough, it may not be able to inactivate spores completely. Moreover, as Gouws et al. (2005) pointed out, the heat treatment may even act as a stimulus to germination, which follows outgrowth of the microorganism. The meta-analysis results indicated that the harsh conditions may be insufficient to inactivate the spores of this spoilage microorganism. For instance, the meta-analysis estimated a mean *D*-value of 4.9 min for orange juice at 95ºC and pH 3.5 (log $D^*$=0.695; *Table 3*), suggesting that spores could survive the processing conditions generally used in the fruit beverage industry. Thus, the use of other barriers along with heat treatment to undermine the resistance of *Alicyclobacillus*, such as the addition of bacteriocins prior to pasteurization, may be contemplated. It is known that, even at low levels of 50 IU/ml, the residual nisin would prevent the outgrowth of any surviving spores (Komitopoulou et al., 1999).

Statistical techniques, such as meta-analysis, are very useful to perform a synthesis of a set of distinct but similar experiments. This particular work exemplifies how a common microbiology predictive model such as the Bigelow secondary model can be the basic equation on which a meta-analytical model (i.e., a weighted mixed-effects linear model) is built upon. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes.

Nevertheless, although the results of this work should in principle provide a summary of the state-of-the-art of *Alicyclobacillus* thermal resistance in fruit beverages, further experimental studies should still be conducted in order to validate the log $D^*$ and $z^*$ values predicted for some types of beverages, such as mango juice, passion fruit concentrate or grapefruit concentrate, for which there were not available information in the literature

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**Figure Captions and Tables**

***Figure 1:*** Scatter plot of the available meta-analytical data of log *D* and temperature for the 37 sub-groups of isothermal experiments to determine a *z*-value.

***Figure 2:*** Relationship between temperature (ºC; x-axis) and log *D* (y-axis), as predicted (lines) by the meta-analysis linear mixed model for different subgroups of types of beverages, in comparison with observed data (markers) when available.

***Figure 3:*** Relationship between residual values and log *D* fitted by the meta-analytical mixed-effects linear model.

***Figure 4:*** Relationship between the observed log *D* extracted from the primary studies and the log *D* fitted by the meta-analytical mixed-effects linear model.

***Figure 1:***

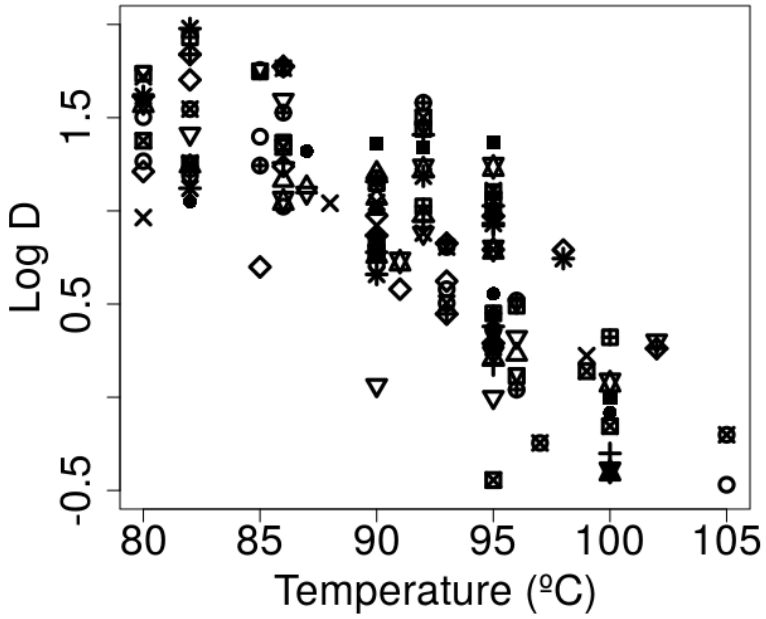

***Figure 2:***

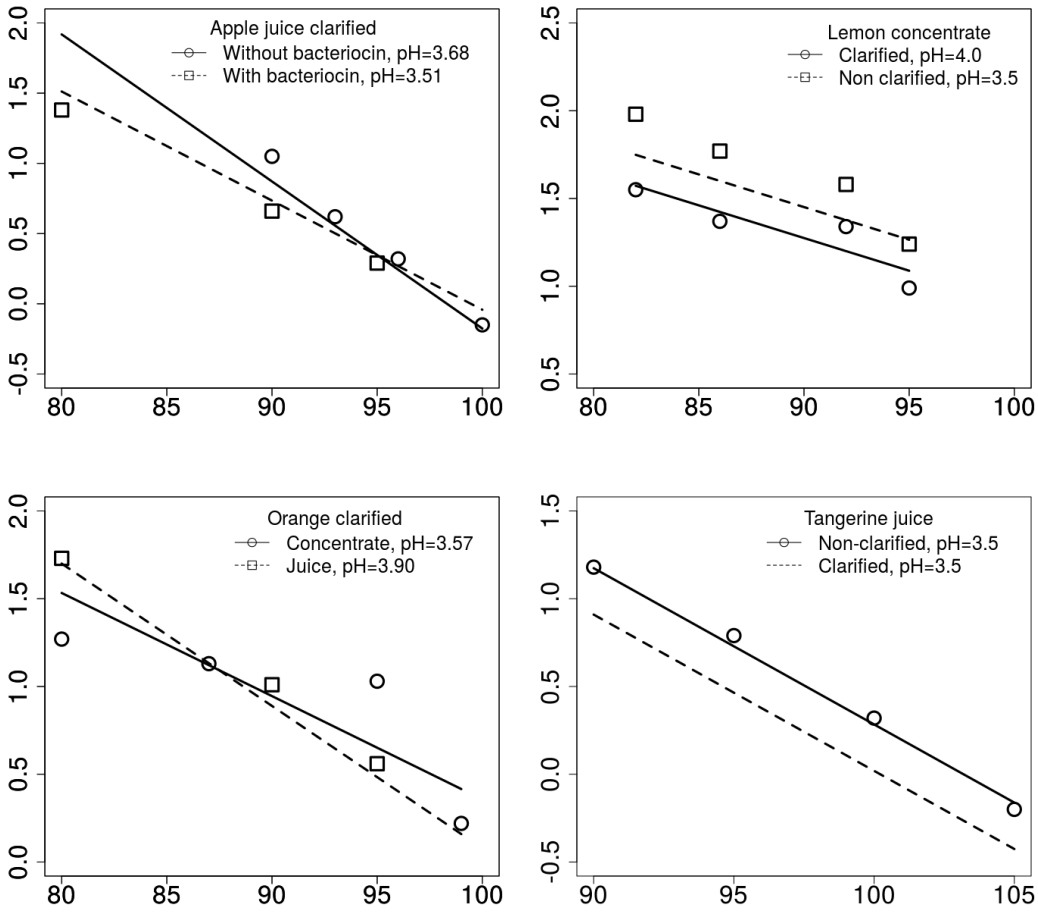
Apple juice clarified
Without bacteriocin, pH=3.68
With bacteriocin, pH=3.51
Lemon concentrate
Clarified, pH=4.0
Non clarified, pH=3.5
Orange clarified
Concentrate, pH=3.57
Juice, pH=3.90
Tangerine juice
Non-clarified, pH=3.5
Clarified, pH=3.5

***Figure 3:***

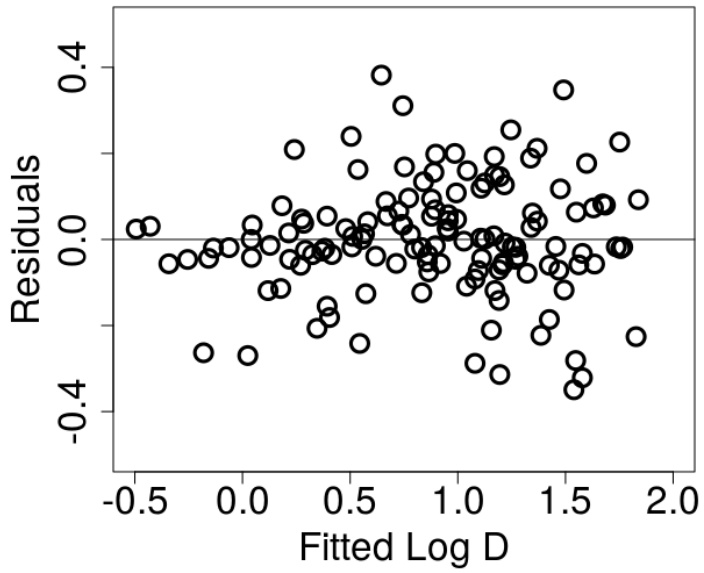

***Figure 4:***

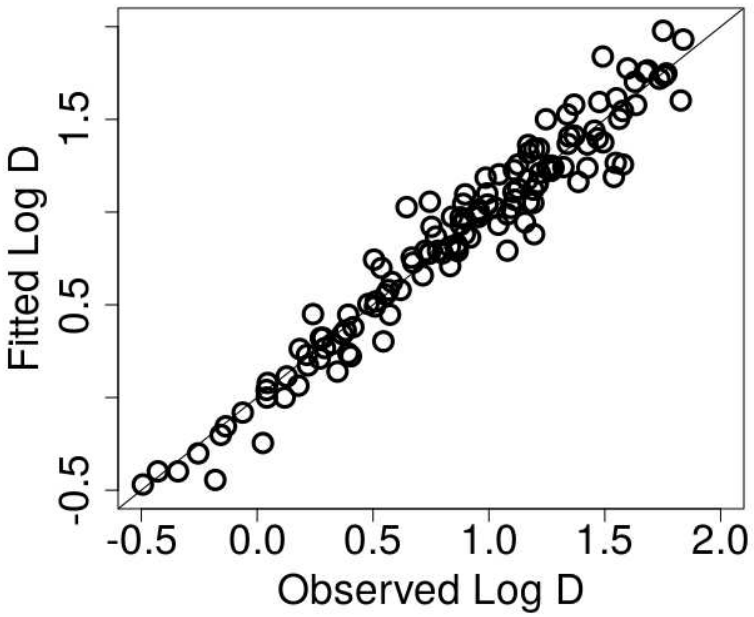

***Table 1:*** Meta-analytical data of *D*-values of *Alicyclobacillus acidoterrestris* in beverages at different temperature and pH, with extracted study characteristics of fruit, type (single strength or concentrate), clarification (0=no, 1=yes), bacteriocins (0=no, 1=yes) and sample size *N* used to estimate a single *D*-value.

| Fruit | Type | Clarification | Bacteriocins | pH | T (°C) | *D* (min) | N | Source |
|---|---|---|---|---|---|---|---|---|
| Apple | Single strength | 0 | 0 | 3.50 | 85 | 56.0 | 20 | Splittstoesser et al. (1994) |
| | | 0 | 0 | 3.50 | 90 | 23.0 | 20 | |
| | | 0 | 0 | 3.50 | 95 | 2.80 | 20 | |
| | | 1 | 0 | 3.51 | 80 | 41.2 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.51 | 90 | 7.38 | 22 | |
| | | 1 | 0 | 3.51 | 95 | 2.30 | 22 | |
| | | 1 | 0 | 3.68 | 90 | 11.1 | 25 | Bahceci and Acar (2007) |
| | | 1 | 0 | 3.68 | 93 | 4.20 | 25 | |
| | | 1 | 0 | 3.68 | 96 | 2.10 | 25 | |
| | | 1 | 0 | 3.68 | 100 | 0.70 | 25 | |
| | | 1 | 1 | 3.51 | 80 | 23.8 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 1 | 3.51 | 90 | 4.56 | 22 | |
| | | 1 | 1 | 3.51 | 95 | 1.95 | 22 | |
| | concentrate | 1 | 0 | 2.97 | 90 | 14.4 | 25 | Bahceci and Acar (2007) |
| | | 1 | 0 | 2.97 | 93 | 6.70 | 25 | |
| | | 1 | 0 | 2.97 | 96 | 3.30 | 25 | |
| | | 1 | 0 | 2.97 | 100 | 1.20 | 25 | |
| | | 1 | 0 | 2.95 | 90 | 14.1 | 25 | Bahceci and Acar (2007) |
| | | 1 | 0 | 2.95 | 93 | 6.40 | 25 | |
| | | 1 | 0 | 2.95 | 96 | 3.10 | 25 | |
| | | 1 | 0 | 2.95 | 100 | 1.00 | 25 | |
| Berry | single strength | 1 | 0 | 3.50 | 88 | 11.0 | 20 | Walls (1997) |
| | | 1 | 0 | 3.50 | 91 | 3.80 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 1.00 | 20 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cupuaçu | single strength | 1 | 0 | 3.60 | 85 | 17.5 | 20 | Silva et al. (1999) |
| | | 1 | 0 | 3.60 | 91 | 5.35 | 20 | |
| | | 1 | 0 | 3.60 | 95 | 2.82 | 20 | |
| | | 1 | 0 | 3.60 | 97 | 0.57 | 20 | |
| Grape | single strength | 1 | 0 | 3.30 | 85 | 57.0 | 20 | Splittstoesser et al. (1994) |
| | | 1 | 0 | 3.30 | 90 | 16.0 | 20 | |
| | | 1 | 0 | 3.30 | 95 | 2.40 | 20 | |
| Grapefruit | single strength | 1 | 0 | 3.42 | 80 | 37.8 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.42 | 90 | 5.95 | 22 | |
| | | 1 | 0 | 3.42 | 95 | 1.85 | 22 | |
| | | 1 | 0 | 3.00 | 80 | 31.85 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.00 | 90 | 5.69 | 22 | |
| | | 1 | 0 | 3.00 | 95 | 1.49 | 22 | |
| | | 1 | 0 | 4.00 | 80 | 52.35 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 4.00 | 90 | 9.44 | 22 | |
| | | 1 | 0 | 4.00 | 95 | 1.73 | 22 | |
| Lemon | single strength | 0 | 0 | 2.45 | 82 | 16.72 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 2.45 | 86 | 11.32 | 20 | |
| | | 0 | 0 | 2.45 | 95 | 9.98 | 20 | |
| | | 0 | 0 | 2.45 | 82 | 17.82 | 20 | |
| | | 0 | 0 | 2.45 | 95 | 9.44 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 11.23 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 3.50 | 86 | 10.54 | 20 | |
| | | 1 | 0 | 3.50 | 92 | 9.47 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 8.55 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 13.21 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 9.38 | 20 | |
| | concentrate | 0 | 0 | 2.28 | 82 | 15.50 | 20 | Maldonado et |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 2.28 | 86 | 14.54 | 20 | al. (2008) |
| 0 | 0 | 2.28 | 92 | 8.81 | 20 | |
| 0 | 0 | 2.28 | 95 | 8.55 | 20 | |
| 0 | 0 | 2.80 | 82 | 50.50 | 20 | Maldonado et al. (2008) |
| 0 | 0 | 2.80 | 86 | 39.30 | 20 | |
| 0 | 0 | 2.80 | 92 | 31.67 | 20 | |
| 0 | 0 | 2.80 | 95 | 22.03 | 20 | |
| 0 | 0 | 3.50 | 82 | 95.15 | 20 | Maldonado et al. (2008) |
| 0 | 0 | 3.50 | 86 | 59.50 | 20 | |
| 0 | 0 | 3.50 | 92 | 38.00 | 20 | |
| 0 | 0 | 3.50 | 95 | 17.22 | 20 | |
| 0 | 0 | 4.00 | 82 | 85.29 | 20 | Maldonado et al. (2008) |
| 0 | 0 | 4.00 | 86 | 58.15 | 20 | |
| 0 | 0 | 4.00 | 92 | 27.48 | 20 | |
| 0 | 0 | 4.00 | 95 | 23.33 | 20 | |
| 0 | 0 | 2.45 | 82 | 15.50 | 20 | Maldonado et al. (2008) |
| 0 | 0 | 2.45 | 86 | 14.54 | 20 | |
| 0 | 0 | 2.45 | 92 | 8.81 | 20 | |
| 0 | 0 | 2.45 | 95 | 8.56 | 20 | |
| 1 | 0 | 2.28 | 82 | 17.36 | 20 | Maldonado et al. (2008) |
| 1 | 0 | 2.28 | 86 | 18.06 | 20 | |
| 1 | 0 | 2.28 | 92 | 7.60 | 20 | |
| 1 | 0 | 2.28 | 95 | 6.20 | 20 | |
| 1 | 0 | 2.80 | 82 | 25.81 | 20 | Maldonado et al. (2008) |
| 1 | 0 | 2.80 | 86 | 22.01 | 20 | |
| 1 | 0 | 2.80 | 92 | 15.35 | 20 | |
| 1 | 0 | 2.80 | 95 | 11.3 | 20 | |
| 1 | 0 | 3.50 | 82 | 68.9 | 20 | Maldonado et |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 3.50 | 86 | 33.7 | 20 | al. (2008) |
| | | 1 | 0 | 3.50 | 92 | 16.8 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 12.6 | 20 | |
| | | 1 | 0 | 4.00 | 82 | 35.2 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 4.00 | 86 | 23.2 | 20 | |
| | | 1 | 0 | 4.00 | 92 | 21.9 | 20 | |
| | | 1 | 0 | 4.00 | 95 | 9.72 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 18.1 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 3.50 | 86 | 17.4 | 20 | |
| | | 1 | 0 | 3.50 | 92 | 7.60 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 6.20 | 20 | |
| Mango | concentrate | 0 | 0 | 4.00 | 80 | 40.0 | 15 | de Carvalho et al. (2008) |
| | | 0 | 0 | 4.00 | 85 | 25.0 | 15 | |
| | | 0 | 0 | 4.00 | 90 | 11.7 | 15 | |
| | | 0 | 0 | 4.00 | 95 | 8.33 | 15 | |
| | | 0 | 1 | 4.00 | 80 | 9.20 | 15 | de Carvalho et al. (2008) |
| | | 0 | 1 | 4.00 | 85 | 5.00 | 15 | |
| | | 0 | 1 | 4.00 | 90 | 1.16 | 15 | |
| | | 0 | 1 | 4.00 | 95 | 0.36 | 15 | |
| Model | single strength | 1 | 0 | 3.00 | 90 | 6.00 | 25 | Bahceci and Acar (2007) |
| | | 1 | 0 | 3.00 | 93 | 2.80 | 25 | |
| | | 1 | 0 | 3.00 | 96 | 1.10 | 25 | |
| | | 1 | 0 | 3.00 | 100 | 0.40 | 25 | |
| | | 1 | 0 | 3.50 | 90 | 6.50 | 25 | Bahceci and Acar (2007) |
| | | 1 | 0 | 3.50 | 93 | 3.20 | 25 | |
| | | 1 | 0 | 3.50 | 96 | 1.30 | 25 | |
| | | 1 | 0 | 3.50 | 100 | 0.40 | 25 | |
| | | 1 | 0 | 4.00 | 90 | 7.30 | 25 | Bahceci and |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 0 | 4.00 | 93 | 3.80 | 25 | Acar (2007) |
| | | 1 | 0 | 4.00 | 96 | 1.70 | 25 | |
| | | 1 | 0 | 4.00 | 100 | 0.50 | 25 | |
| | | 1 | 0 | 3.50 | 90 | 6.00 | 18 | López et al. (2011) |
| | | 1 | 0 | 3.50 | 95 | 2.20 | 18 | |
| | | 1 | 0 | 3.50 | 100 | 0.83 | 18 | |
| | | 1 | 0 | 3.50 | 105 | 0.34 | 18 | |
| Orange | single strength | 1 | 0 | 3.90 | 80 | 54.3 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.90 | 90 | 10.3 | 22 | |
| | | 1 | 0 | 3.90 | 95 | 3.59 | 22 | |
| | | 1 | 0 | 3.57 | 80 | 16.3 | 15 | Alberice et al. (2012) |
| | | 1 | 0 | 3.57 | 87 | 12.5 | 15 | |
| | | 1 | 0 | 3.57 | 95 | 10.8 | 12 | |
| | | 1 | 0 | 3.57 | 99 | 1.38 | 12 | |
| | concentrate | 0 | 0 | 3.68 | 92 | 25.6 | 10 | Peña et al. (2009) |
| | | 0 | 0 | 3.68 | 95 | 12.9 | 10 | |
| | | 0 | 0 | 3.68 | 98 | 6.16 | 10 | |
| | | 0 | 0 | 3.68 | 102 | 2.01 | 10 | |
| | | 0 | 1 | 3.68 | 95 | 11.4 | 10 | Peña et al. (2009) |
| | | 0 | 1 | 3.68 | 98 | 5.55 | 10 | |
| | | 0 | 1 | 3.68 | 102 | 1.83 | 10 | |
| | concentrate | 1 | 0 | 2.95 | 80 | 18.4 | 15 | Alberice et al. (2012) |
| | | 1 | 0 | 2.95 | 87 | 13.4 | 15 | |
| | | 1 | 0 | 2.95 | 95 | 10.6 | 12 | |
| | | 1 | 0 | 2.95 | 99 | 1.67 | 12 | |
| Passion | single strength | 1 | 0 | 3.50 | 87 | 20.9 | 12 | McKnight et al. (2010) |
| fruit | | 1 | 0 | 3.50 | 90 | 5.12 | 12 | |
| | | 1 | 0 | 3.50 | 95 | 1.62 | 12 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tangerine | single strength | 0 | 0 | 3.50 | 90 | 15.0 | 18 | López et al. (2011) |
| | | 0 | 0 | 3.50 | 95 | 6.20 | 18 | |
| | | 0 | 0 | 3.50 | 100 | 2.10 | 18 | |
| | | 0 | 0 | 3.50 | 105 | 0.63 | 18 | |

***Table 2:*** Parameter estimates of the Bigelow-type meta-analysis mixed-effects linear model predicting the *D*-value of *Alicyclobacillus acidoterrestris* in fruit beverages as a function of temperature, pH and moderating variables.

| Parameters | Mean | Standard error | Pr > \|t\|, Z | AIC / BIC |
|---|---|---|---|---|
| Predictors of log $D^*$ | | | | |
| $\beta_0$ (intercept) | 0.396 | 0.056 | <.0001 | -80.0 / -50.0 |
| $\beta_1$ (type) | -0.115 | 0.048 | 0.018 | |
| $\beta_2$ (clarification) | -0.261 | 0.037 | <.0001 | |
| Predictors of $(1/z_T)$ | | | | |
| $\gamma_1$ (temperature) | -0.089 | 0.008 | <.0001 | |
| $\gamma_2$ (temperature×type) | 0.014 | 0.006 | 0.025 | |
| Predictors of $(1/z_{pH})$ | | | | |
| $\delta_1$ (pH) | 1.707 | 0.207 | <.0001 | |
| $\delta_2$ (pH×bacteriocin) | -1.881 | 0.206 | <.0001 | |
| Variances | | | | |
| $s^2_u$ (log $D^*_{mean}$) | 0.0389 | 0.0162 | 0.008 | $\tau^2$~0.044 |
| $s^2_v$ (temperature) | 0.0010 | 0.0004 | 0.012 | $I^2$~53.9% |
| $s^2_{uv}$ (covariance) | 0.0045 | 0.0023 | 0.050 | |
| $s^2$ (residual) | 0.0380 | 0.0053 | <.0001 | |

***Table 3:*** Estimates of log *D** (log *D*-value at 95ºC and pH 3.5) for different combinations of fruits and the moderating variables, type of beverage and with/without clarification process.

| Parameter | Mean | Standard error | 95% CI |
|---|---|---|---|
| Overall mean | 0.584 | 0.055 | [0.474 – 0.694] |
| Single strength juice | 0.526 | 0.056 | [0.414 – 0.638] |
| Clarified | 0.396 | 0.056 | [0.285 – 0.507] |
| Non-clarified | 0.656 | 0.063 | [0.532 – 0.781] |
| Concentrate | 0.642 | 0.064 | [0.514 – 0.769] |
| Clarified | 0.511 | 0.068 | [0.376 – 0.645] |
| Non-clarified | 0.772 | 0.066 | [0.641 – 0.903] |
| Apple single strength juice | 0.470[c] | 0.047 | [0.377 – 0.565] |
| Berry single strength juice | 0.252[a] | 0.108 | [0.036 – 0.467] |
| Cupuaçu single strength juice | 0.355[b] | 0.078 | [0.200 – 0.510] |
| Grape single strength juice | 0.577[d] | 0.109 | [0.361 – 0.793] |
| Grapefruit single strength juice | 0.437[bc] | 0.064 | [0.310 – 0.564] |
| Lemon single strength juice | 1.007[f] | 0.048 | [0.912 – 1.103] |
| Mango single strength juice | 0.425[b] | 0.102 | [0.223 – 0.628] |
| Orange single strength juice | 0.695[e] | 0.054 | [0.587 – 0.803] |
| Passion fruit single strength juice | 0.401[b] | 0.130 | [0.142 – 0.660] |
| Tangerine single strength juice | 0.594[d] | 0.067 | [0.462 – 0.727] |
| Apple concentrate | 0.586[c] | 0.053 | [0.470 – 0.702] |
| Berry concentrate | 0.367[a] | 0.115 | [0.138 – 0.596] |
| Cupuaçu concentrate | 0.470[b] | 0.088 | [0.295 – 0.646] |
| Grape concentrate | 0.693[d] | 0.115 | [0.463 – 0.922] |
| Grapefruit concentrate | 0.552[bc] | 0.076 | [0.401 – 0.703] |
| Lemon concentrate | 1.122[f] | 0.036 | [1.050 – 1.195] |
| Mango concentrate | 0.541[b] | 0.096 | [0.348 – 0.732] |

| | | | |
|---|---|---|---|
| Orange concentrate | 0.810[e] | 0.053 | [0.705 – 0.915] |
| Passion fruit concentrate | 0.517[b] | 0.136 | [0.247 – 0.787] |
| Tangerine concentrate | 0.709[d] | 0.084 | [0.544 – 0.876] |
| Apple single strength juice | | | |
| Clarified | 0.340 | 0.049 | [0.243 – 0.438] |
| Non-clarified | 0.601 | 0.052 | [0.497 – 0.705] |
| Apple concentrate | | | |
| Clarified | 0.456 | 0.064 | [0.328 – 0.583] |
| Non-clarified | 0.716 | 0.058 | [0.601 – 0.832] |
| Mango single strength juice | | | |
| Clarified | 0.295 | 0.104 | [0.088 – 0.502] |
| Non-clarified | 0.555 | 0.103 | [0.350 – 0.761] |
| Mango concentrate | | | |
| Clarified | 0.410 | 0.101 | [0.209 – 0.612] |
| Non-clarified | 0.671 | 0.095 | [0.482 – 0.860] |
| Orange single strength juice | | | |
| Clarified | 0.565 | 0.053 | [0.458 – 0.670] |
| Non-clarified | 0.825 | 0.062 | [0.703 – 0.947] |
| Orange concentrate | | | |
| Clarified | 0.680 | 0.057 | [0.567 – 0.793] |
| Non-clarified | 0.940 | 0.055 | [0.830 – 1.051] |

(*) Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

**Table 4:** Estimates of $z_T$ and $z_{pH}$ obtained by the meta-analytical secondary predictive model.

| **Parameter** $z_T$ | **Mean** | **Standard error** | **95% CI** |
|---|---|---|---|
| Single strength juices – all fruits | 11.23 | 1.107 | [9.034 – 13.42] |
| Concentrates – all fruits | 13.35 | 1.744 | [9.893 – 16.80] |
| | | | |
| Apple single strength juice | 10.34[c] | 0.728 | [8.898 – 11.78] |
| Berry single strength juice | 8.019[ab] | 1.354 | [5.339 – 10.70] |
| Cupuaçu single strength juice | 9.261[b] | 1.163 | [6.958 – 11.56] |
| Grape single strength juice | 7.957[a] | 1.059 | [5.860 – 10.06] |
| Grapefruit single strength juice | 11.17[c] | 0.907 | [9.378 – 12.96] |
| Lemon single strength juice | 15.86[e] | 1.467 | [12.95 – 18.76] |
| Mango single strength juice | 17.39[e] | 3.375 | [10.71 – 24.08] |
| Orange single strength juice | 12.48[d] | 1.162 | [10.17 – 14.78] |
| Passion fruit single strength juice | 8.907[b] | 1.789 | [5.365 – 12.45] |
| Tangerine single strength juice | 11.35[c] | 1.382 | [8.611 – 14.08] |
| | | | |
| Apple concentrate | 12.19[c] | 1.164 | [9.886 – 14.50] |
| Berry concentrate | 9.045[ab] | 1.780 | [5.520 – 12.57] |
| Cupuaçu concentrate | 10.65[b] | 1.682 | [7.326 – 14.00] |
| Grape concentrate | 8.967[a] | 1.424 | [6.147 – 11.78] |
| Grapefruit concentrate | 13.27[c] | 1.679 | [9.945 – 16.59] |
| Lemon concentrate | 20.43[e] | 1.827 | [16.81 – 24.05] |
| Mango concentrate | 23.07[e] | 5.054 | [13.06 – 33.08] |
| Orange concentrate | 15.15[d] | 1.752 | [11.68 – 18.62] |
| Passion fruit concentrate | 10.19[b] | 2.408 | [5.422 – 14.96] |
| Tangerine concentrate | 13.52[c] | 2.269 | [9.023 – 18.01] |
| $z_{pH}$ | | | |
| Overall mean | 1.305 | 0.180 | [0.948 – 1.661] |

| | | | |
|---|---|---|---|
| With bacteriocin | 0.586 | 0.071 | [0.445 – 0.726] |
| Without bacteriocin | 5.750 | 0.950 | [3.869 – 7.631] |

(*) Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

**Capítulo III: Meta-analysis of the effects of sanitizing treatments for reducing *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* concentrations in fresh produce**

**Running Title:** Meta-analysis of sanitization in produce processing

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**Abstract**

The aim of this study was to perform a meta-analysis of the effects of sanitizing treatments of fresh produce on *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*. From 55 primary studies found to report on such effects, 40 were selected based on specific criteria. Data were partitioned to build three meta-analytical models that could allow the assessment of differences in log reductions among pathogens, fresh produce and sanitizers. Moderating variables assessed in the meta-analytical models included type of fresh produce, type of sanitizer, concentration, treatment time and temperature. Further, a proposal is done to classify the sanitizers according to their bactericidal efficacy by means of a meta-analytical dendogram. Herein, we were able to assess more than 1000 data on log reduction of the three main bacterial pathogens impairing the safety of fresh produce. The results reported seem to be an important achievement for advancing the global understanding of the effectiveness of sanitizers for microbial safety of fresh produce. The resulting meta-analysis models have the capability to provide mean log-reduction estimates for a particular pathogen when using a given sanitizer at known concentration, time/temperature of application. Altogether, the outcomes of the present study can serve as scientific information for decision-making (risk-benefit analysis). Regulations can be further harmonized and developed taking into account the integrated findings reported herein.

**Keywords:** Fruits, vegetables, pathogens, sanitizers, washing, disinfection.

## 1. Introduction

The consumption of fresh fruits and vegetables comprises an essential element of a healthy diet and a protective factor against several chronic diseases (1,2). Even though, the ingestion of these products is highly recommended by health authorities, guaranteeing fresh, safe and high quality fruits and vegetables remains an enormous challenge for fresh produce industries.

In order to deliver the health benefits (2), fruits and vegetables must be safe. The main concern related to the safety of these products is their recurrent and increased association with disease outbreaks (3-6). Epidemiological investigations indicate that *Salmonella,* pathogenic *Escherichia coli* and *Listeria monocytogenes* stand out as the most important bacterial agents linked to fresh produce disease outbreaks (3, 5, 6, 7). Recent studies have reported the occurrence and high diversity of these microorganisms in the environment or in close areas of produce farming areas (8-13).

Given the above, fresh produce industries have been implementing measures at pre- and post-harvest steps to reduce/avoid the contamination of these products and, consequently to diminish the burden of disease outbreaks. At post-harvest steps, disinfection is the critical step for reduction of microbial contamination (14, 15). During disinfection, fresh produce are allowed to stay in contact with sanitizers added to the washing tanks aiming to reduce their microbial load. Finally yet importantly, during disinfection, washing water should not become a point of cross-contamination (15, 16). The phenomenon of cross-contamination during produce washing has been indicated as the potential cause of the Spinach and *E. coli* 0157:H7 outbreak that resulted in 205 illnesses and three deaths in the fall of 2006 in the USA (17, 18). As disinfection of fresh produce constitutes a critical control point, several reports are found that quantify the

pathogens' concentrations in these foods before and after disinfection with different sanitizers (19-26). However, because the log-reductions of pathogens attained by the sanitizers are affected by the particular conditions or *study characteristics* (protocols for washing, type of fruit and vegetable, whole or cut fresh produce, type of sanitizer and concentration, washing time and temperature, pathogenic strains, microbiological essays, etc.) under which the measurements were obtained, variability in the effect size reported in the primary studies is expected to occur. This variability can be observed even among studies investigating the same type of fresh produce and sanitizer. Nonetheless, by means of *a posteriori* analysis and identification of the sources of variability likely to affect the log-reduction of the pathogenic flora due to disinfection, it may be possible to explain, at least to some extent, the differences found among the study outcomes. Most importantly, it may be realistic to build a model that can be generalized to different types of fresh produce.

Meta-analysis has been defined as a *statistical analysis of a collection of analytic results for the purpose of integrating the findings from a large amount of primary studies* (27). Meta-analysis allows the explanation of the divergences in the study outcomes by the codification of moderating variables representing study characteristics related to research design features, data collection procedures, type of samples, etc., aiming to reduce the between-study heterogeneity or variability (28). Through meta-analysis, it may also be possible to accurately estimate the overall outcome measure, with increased statistical power, than is possible using only a single study (29).

Considering the capabilities of the meta-analysis and the significance of *Salmonella,* pathogenic *E. coli* and *L. monocytogenes* for the microbial safety of fresh produce and for the public health (3, 5, 6, 7), the objective of this study is four-fold: (i) firstly, to compile all publicly-accessible published findings on the effects of sanitizers on the mean reduction in the log-counts of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* on fresh produce, and quantitatively summarise the outcomes by means of meta-analytical models

based on mixed-effects linear regressions; (ii) secondly, to explain a proportion of the total between-study heterogeneity in the reduction of pathogens' populations by incorporating available study characteristics to the basic meta-analysis model, such as type of fresh produce, type of sanitizer, concentration, treatment time and temperature; (iii) thirdly, to assess possible differences in the susceptibility of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* to selected sanitizers, as well as differences in the overall microbial log-reduction among fresh produce for a given disinfectant treatment; and (iv) finally, to evaluate the effectiveness of the sanitizers to reduce each of the pathogen's populations using a common disinfectant treatment, and to propose a classification of sanitizers according to their bactericidal efficacy by means of a meta-analytical dendogram. The resulting meta-analysis models have the capability to provide overall log-reduction estimates for a particular pathogen when using a given sanitizer and sanitizing treatment.

## 2. Materials and methods

### 2.1. Data collection and effect size parameterization

Before commencing any meta-analysis study, the research problem must be stated and three important facets should be defined: population, intervention or treatment and measured outcome. In this meta-analysis, the *problem statement* was the estimation of the overall effect of disinfecting fresh and minimally processed fruits and vegetables with aqueous and gaseous chemicals on the final microbial concentration (number of log-reductions) of three pathogens (i.e., *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7). The *population* was specified as fresh produce, fruits and vegetables, prior to the sanitizing treatment, while the *intervention* or *treatment* was represented by the

disinfection unit operation using aqueous or gaseous sanitizers. The *measured outcome* is derived from the pathogen's concentration on the fruits/vegetables before and after treatment, giving the number of log-reductions.

The next step of literature identification was conducted using electronic search through Google with key terms, both in English and in Portuguese, encompassing: "*Salmonella*", "*Escherichia coli* O157:H7", "*Listeria monocytogenes*", "pathogens", "sanitizers", "chemicals", "solutions", "organic acids", "detergents", "washing", "inactivation", "antibacterial effect", "reduction", "fruits", "vegetables" and "produce". Also, literature for inclusion in the study was identified from bibliographic databases such as Pubmed, Science Direct and Scopus using the same key-words. Data included considered studies available in scientific journals and electronically from 1990 to 2014. A total of 55 studies were found to report on the effect of sanitizers on the concentration of pathogens in fresh produce. However, these encompassed also reports using ultrasound and irradiation treatments, which were disregarded as only conventional washing with sanitizers was considered for inclusion in the meta-analysis. A second criterion used in the screening was the need for the primary study to report the concentration of either of the pathogens (*Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes*) in fresh produce both before (i.e., control) and after the disinfection treatment; or, alternatively, the microbial log-reduction attained by the disinfection treatment. As a third criterion for inclusion, the primary study had to clearly specify the type of sanitizer and its concentration, washing/exposure time and temperature, as well as sample size and/or standard deviations. As a fourth criterion, an approved microbiological method for pathogen enumeration had to be employed. Considering all of those requirements, forty primary studies were regarded as appropriate for inclusion in the present meta-analysis study (19-26, 30-62).

As a next step, a parameterization of the intervention's effect size should be determined. The effect size ($\theta$) refers to the degree to which the hypothetical phenomenon (i.e., reduction in the concentration of pathogens on fresh produce due to disinfection treatment) is present in the population. For the studies' outcomes to be compatible and meaningful for analysis, such effect size should be converted to a common scale that permits direct comparison and summation of the primary studies. Because the data generated by the primary studies are the means of a continuous variable (i.e., microbial concentration in/on fresh produce), the possible parameters to measure effect size or treatment difference are raw (unstandardized) mean difference, standardized mean difference and response ratios (63). The most suitable parameter to measure effect size was the *raw mean difference* between control and treatment means, because all the primary studies reported in the same log CFU scale and it is an intuitively meaningful parameter. This is, referring in terms of, say, 2- or 3-log microbial reduction is of widespread use among food microbiologists.

Consider a primary study *j* that reports means for two groups (*control* or before disinfection treatment, and *treated* or after disinfection treatment). Let $\bar{x}_C$ and $\bar{x}_T$ be the sample means of the two independent groups; hence, the effect size estimate $\hat{\theta}$, which, in our case, is the difference in sample means or *mean log-reduction R*, is defined as,

$$\hat{\theta}_j = R_j = \bar{x}_{Cj} - \bar{x}_{Tj} \quad (1)$$

Now, let $s_C$ and $s_T$ be the sample standard deviations of the two groups, and $n_C$ and $n_T$ be the samples sizes in the two groups, control and treated, respectively. If we assume that the two population standard deviations are different, then the standard error *SE* of the mean log-reduction *R* can be estimated as,

$$SE(R_j) = \sqrt{\frac{s_C^2}{n_C} + \frac{s_T^2}{n_T}} \quad (2)$$

Mean log-reductions (*R*) and their standard errors (*SE*) for the three pathogens were estimated from the primary studies whose results were reported separately for the control and treated groups. Nonetheless, in some primary studies, mean log-reductions and their standard errors were provided as such, so none of the above formulas needed to be applied, and their values were extracted directly from tables or charts.

### 2.2. Description of meta-analytical data set

The microbial log-reduction values for the three pathogens, whether estimated using Equations (1-2) or directly extracted from the primary studies, were the outcomes of experiments carried out with a specific fresh produce under a certain disinfection treatment. Hence, all this additional information was also annotated from the primary studies in the form of *study characteristics* or *moderating variables*. The study characteristics considered were: bacteria (a categorical variable), type of sanitizer (a categorical variable), sanitizer concentration (a continuous variable), type of produce (a categorical variable), treatment or washing time (a continuous variable) and treatment or washing temperature (a continuous variable). As explained in the previous sub-section, sample sizes ($n_C$, $n_T$) and standard deviations ($s_C$, $s_T$) of the control (pre-disinfection) and treated (post-disinfection) groups were also extracted. Depending upon the sanitizer, a specific concentration unit was used in a primary study. For instance, for gaseous chlorine dioxide, the concentration was often expressed in ppm, while for sodium chlorite, it was in g/L. In order to facilitate comparisons among sanitizers, all concentrations were converted to g/100 ml. For the 27 sanitizers recovered (namely, acetic acid [AA], acidified sodium chlorite [ASC], benzalkonium chloride [Bzc], citric acid [CA], calcined oyster shell [Ca-Oy], calcined Sakhalin surf clam [Ca-SS], calcium hypochlorite [CH], Citrox™, chlorine dioxide gas [CD], dodecyl-benzenesulfonic acid [DA], sodium 2-ethylhexyl-sulfate [EHS], hydrogen peroxide [HP], lactic acid [LA], malic acid [MA], nisin, ozonized water [OW], ozone gas

[Oz], pediocin, peroxyacetic acid [PAA], phytic acid [Phy], slightly acidic electrolysed water [SAEW], sodium chlorite [SC], sodium-dodecyl sulphate [SDS], sodium hypochlorite [SH], tartaric acid [TA], trisodium phosphate [TSP], Tsunami-100™), the concentrations ranged between 0.0001 to 4.8 g/100 mL, although it is important to bear in mind that every sanitizer is associated to a specific concentration range. For instance, in the primary studies, fresh fruits and vegetables are treated with chlorine dioxide gas in concentrations from 0.00015 to 0.00030 g/100 mL, while they are washed with lactic acid in higher concentrations from 0.003 to 2.0 g/100 mL. In addition to the 27 sanitizers identified, primary studies also provided microbial log-reductions from washing using only water (i.e., a blank treatment). The water types were tap water (W), distilled water (DisW) and deionised water (DioW), which were categorised within sanitizers, although a solute concentration of 0 g/100 mL was assigned to all water types. Washing times were in the range between 0.15 to 180 min, yet the longer times belonged to chlorine dioxide gas treatments. Temperatures for washing were mostly ambient, although overall they were in the interval from 4º to 55ºC.

To get some insight into the spread of the microbial log-reduction data among the categorical study characteristics, *Table 1* compiles the number of log-reduction observations partitioned by sanitizer, pathogen and fresh produce. It should be noticed that the meta-analytical data is highly sparse, meaning that for some sanitizers, less data are available. For instance, for SAEW, microbial log-reduction observations were reported for the three pathogens, while for ozone gas, data were limited to *E. coli* O17:H7 only. Moreover, the heterogeneity in the distribution of fresh produce across pathogens and sanitizers caused further sparseness. Said otherwise, for a given sanitizer, the types of produce studied did not coincide for all pathogens. From the 27 sanitizers, whose microbial log-reduction information was available in the literature, nine were excluded from the meta-analyses (i.e., Bzc, Ca-Oy, Ca-SS, DA, EHS, Nisin, Pediocin, Phy and SC) for

presenting too few observations (threshold was set as equal to or less than four observations per sanitizer; *Table 1*).

Thus, for the 18 sanitizers remaining for the meta-analyses plus the three water types (i.e., 21 sanitizers), a total of 1025 microbial log-reduction values were brought together. In the primary studies, those values were obtained by measuring the effects of disinfectant treatment in 30 types of fresh produces: apple, baby spinach, blueberry, broccoli, buckwheat, cabbage, cantaloupe, carrot, cherry tomato, Chinese cabbage, Chinese celery, cilantro, cucumber, daikon, green onion, honeydew, lettuce, mung bean, mung bean sprouts, onion, peach, pepper, rocket leaves, romaine lettuce, sesame leaf, spinach, spring onion, strawberry, tatsoi and tomato.

The sparseness of the data has some implications in the choice and the design of the meta-analysis mixed-effect models. Because of the considerable dispersion in the number of microbial log-reduction observations among the sanitizer – pathogen combinations (*Table 1*), a general meta-analysis model encompassing all data could not be adjusted. Hence, separate meta-analysis studies were conducted, first on data partitioned by sanitizer – in order to make comparisons among pathogens and fresh produce – and, subsequently, on data partitioned by pathogen – in order to make comparisons among the bactericidal efficacy of sanitizers. This is explained in detail in the following subsections.

### 2.3. Meta-analysis models by sanitizer

When conducting separate meta-analysis models by sanitizer, it is possible to assess both, whether there are differences in the resistance to the sanitizer agent among the three pathogens, and whether there are differences among produce in the microbial log-reduction attained by a disinfection treatment.

### 2.4. Assessing differences among pathogens

To assess the bactericidal efficacy of fresh produce disinfection among pathogens, nine sanitizing agents with the least sparseness in the number of observations across pathogens were selected. These were: acetic acid (AA), acidified sodium chlorite (ASC), chlorine dioxide gas (CD), citric acid (CA), hydrogen peroxide (HP), malic acid (MA), peroxyacetic acid (PAA), slightly acidic electrolysed water (SAEW) and sodium hypochlorite (SH). Tap water (W) was also selected for comparison, as it can be regarded as a blank treatment for washing (i.e., washing without sanitizer).

A meta-analysis model can be considered a special case of a multilevel analysis using hierarchical linear models, with subjects between studies at the first level and studies at the second level. In a multilevel meta-analysis, one usually starts from the random-effects model, and if the between-study variance is shown to be noteworthy, study characteristic can be added to the model to account for at least part of the heterogeneity in the true effect size $\theta$ (in our case, the log-reduction $R$). Thus, for each of the ten selected sanitizers (including tap water), the microbial log-reduction $R$ was modelled as,

$$R_{ijk} = \beta_{0i} + \beta_1 logC + \beta_2 T + \beta_3 t + u_{jk} + \varepsilon_{ijk} \quad (3)$$

where: $\beta_0$ is the fixed effect of the pathogen $i$, $\beta_1$ the mean effect of the increment in the logarithm of the sanitizer concentration $C$, $\beta_2$ the mean effect of a 1ºC- increment in disinfection temperature $T$, and $\beta_3$ the mean effect of a 1 min-increment in disinfection time $t$. Because of the sparse nature of the data structure, whereby in many cases one primary study reported results for only one or two fresh produce, for this meta-analysis it was not feasible either to separate the between-produce variability from the between-study variability, or to build a nested covariance of primary studies within a fresh produce. To

overcome this problem, and still be able to account for the evident variability due to the different primary studies *j*, and the different fresh produce *k*, both variables were merged into an interaction variable (*jk*). Such interaction was assumed to be the subject of variation of the intercept random effects $u_{jk}$ placed in Equation (3). The random effects $u_{jk}$ are assumed to be normally-distributed with mean zero and variance $s^2_u$. The errors or residuals $\varepsilon_{ijk}$ are also assumed to follow a normal distribution with mean zero and variance $s^2$. Using this model design, the estimated value of $R_{ijk}$ represents therefore the *overall* mean microbial log-reduction for the pathogen *i* attained by a particular sanitizing treatment (*C*, *T* and *t*), applicable to the entire population of fresh produce and primary studies. Nonetheless, if we wished to estimate the mean microbial log-reduction for a particular fresh produce, it can still be done extracting its corresponding random effect $u_{jk}$ and replacing in Equation (3).

Since primary studies are expected to differ from each other in the reliability of estimating the true effect of disinfection on the pathogens' numbers on fresh produce, for instance, due to differences in study sizes, analytical methods or experimental designs, a *weighted* linear mixed model was preferred, with weights representing the *precision* in estimating the true microbial log-reduction. In meta-analysis, it is common practice to use the standard error of the effect size as a measure of precision to assign weights to each of the primary studies. However, in the present meta-analysis, it was not possible to obtain the standard errors of the log-reductions *R* for all primary studies; and consequently, the precision was instead re-defined as some measure proportional to the sample size *N* used in every primary study. Hence, the weight – level of confidence on each of the measured log-reductions *R* – was given by the sample size. A weighted mixed-effects linear model (Equation 3) was adjusted to each of the ten selected sanitizers.

### 2.5. Assessing differences among fresh produce

By partitioning data by sanitizer, it is also possible to appraise whether the same disinfection treatment would achieve variable effects depending upon the type of fresh produce. However, to carry out this assessment, we need to choose sanitizers that have been tested in a wide range of fresh produce, and that these types of fresh produce are roughly the same at least across two pathogens. For instance, observing the data dispersion shown in *Table 1*, using the data from the gaseous chlorine dioxide (CD) is a good option because it was tested on cabbage, cantaloupe, lettuce and strawberry, for the three pathogens, and tested on spinach for *E. coli* O157:H7 and *Salmonella.* Following this reasoning, the sanitizers ASC, CD, SAEW and SH were considered suitable for this analysis, and the following meta-analysis model was adjusted to each of the four data sets,

$$R_{ijk} = \beta_{0k} + \beta_1 logC + \beta_2 T + \beta_3 t + u_{i(j)} + \varepsilon_{ijk} (4)$$

Now, $\beta_0$ is the fixed effect of the type of fresh produce *k*, and $u_{i(j)}$ are the intercept random effects with subject of variation pathogen *i* nested in the primary study *j*. The nested random effects $u_{i(j)}$ are assumed to be normally-distributed with means zero and variances $s^2_i$ and $s^2_j$. With such a model design, the variability due to pathogens is extracted, and the response variable $R_{ijk}$ can be thought of the overall mean log-reduction in the entire population of pathogens, from treating a fresh produce *k* by a particular sanitizing treatment (*C*, *T* and *t*). In a similar fashion, a weighted regression was opted for, in order to account for the differences in precision among primary studies. The sample size *N* was used as the weight of each log-reduction observation.

### 2.6. Meta-analysis models by pathogen

The microbial data were also partitioned by pathogen, producing three data sets for *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. Separate meta-analyses were then performed by pathogen, so as to compare the bactericidal efficacy of sanitizers for a common treatment (*C*, *T*, *t*). For each of the pathogens' data sets, a mixed-effects linear model of the type

$$R_{jkl} = \beta_{0l} + \beta_1 logC + \beta_2 T + \beta_3 t + u_{jk} + \varepsilon_{jkl} \quad (5)$$

was fitted; where $\beta_0$ now represents the fixed effect of the type of sanitizer *l*, and $u_{jk}$ are intercept random effects, whose subject of variation is the interaction study×fresh produce, which account for the variability due to both the different primary studies *j*, and the different fresh produce *k*. The regression models were fitted using the sample size N as the weight of each of the observations. For each of the models explained in Sub-sections 2.2-2.4, the normality of residuals was assessed and the studentised residuals examined for identifying spurious data points lower than -3.0 and higher than 3.0. In those cases, outliers were removed from the data, and regression models re-fitted. The mixed-effects linear models were also used to construct meta-analytical forest plots (63), in order to allow a better visualization of the difference in the effect of a given sanitizer and disinfection treatment among fresh produce (from Equation (4)), and the difference among sanitizers for a given disinfection treatment (from Equation (5)). The weighted mixed-effects linear models were fitted in R version 2.14.2 (R Development Core Team) using the 'lme' function from the 'nlme' package (64). Forest plots were built using the 'metafor' package (65).

## 2.7. Cluster analysis of sanitizers

In order to examine similarities and dissimilarities among sanitizers in their bactericidal effect, so that clusters of sanitizers could be identified and separated from others, a hierarchical cluster analysis was performed on the microbial data from selected sanitizers. Since cluster analysis is a multivariate data analysis technique – hence, it requires continuous variables as inputs, it is necessary to obtain, firstly, some measurements of the characteristics of each sanitizer in the form of a continuous variable. In a regression analysis of the type,

$$R_{ijk} = \beta_0 + \beta_1 logC + \beta_2 T + \beta_3 t + u_{jk} + \varepsilon_{ijk} \quad (6)$$

adjusted to the whole data from a given sanitizer, the parameter estimates $\beta_0$, $\beta_1$, $\beta_2$, and $\beta_3$ can be thought of the *continuous* variables characterizing the disinfectant capacity of the sanitizer. This is because, for a sanitizer, the higher the intercept $\beta_0$ (representing the mean log reduction *R* at the mean log-concentration *C*, the mean temperature *T* and the mean time *t*), the higher the microbial mean log-reduction *R*. Similarly, a sanitizer with higher slopes $\beta_1$, $\beta_2$, and $\beta_3$, will produce a greater mean log-reduction *R* for a given log-concentration *C*, temperature *T* and time *t*, respectively. Thus, for the cluster analysis, the sanitizers selected needed to be those presenting microbial log-reduction observations measured over a wide range of temperature, concentration and time, so that precise slope estimates could be computed. Suitable sanitizers for analysis were AA, ASC, CA, CD, CH, HP, LA, MA, PAA, SAEW and SH. The water types, W, DisW and DioW, were also included in the list of sanitizers as a mechanism for testing the performance of the clustering algorithm to build meaningful groups (said otherwise, because it is known a

priori that the water types are not sanitizers and their bactericidal effect is the lowest of all, they should be grouped together by the clustering method chosen).

Equation (6) was then fitted to each of the sanitizers $l$, and the parameter estimates $\beta_{0l}$, $\beta_{1l}$, $\beta_{2l}$, and $\beta_{3l}$ for $l$ = {1, 2, …14} were organized in an 14 x 4 matrix, where the rows corresponded to the sanitizers and the columns to the four parameter estimates. As a next step, the Euclidean distance between each pair of sanitizers was computed, and arranged in a distance matrix. The clustering was performed using a hierarchical algorithm, whereby the partition with $k$ = 1 cluster (all sanitizers are together in the same cluster) is part of the output, and also the situation with $k$ = j (each sanitizer forms a separate cluster with only a single element). In between, all values of $k$ = 2, 3, … $j$-1 are covered in a kind of gradual transition: The only difference between $k$=$r$ and $k$=$r$+1 is that one of the $r$ clusters splits up in order to obtain $r$+1 clusters (i.e., two of the $r$+1 clusters combine to yield $r$ clusters). The clustering method chosen was that of Ward's that is a minimum variance method aiming at finding compact and spherical clusters (For further information on hierarchical clustering and clustering methods, refer to (66)). The distance matrix was computed using the 'dist' function; and the agglomerative hierarchical cluster analysis producing the dendogram using the 'hclus' function, both from the R 'stats' package.

# 3. Results

## 3.1. Meta-analysis models by sanitizer

*Table 2* shows the results from fitting *Equation 3* to nine sanitizers plus water (n=10) studied. These are the overall mean log–reductions for the specific pathogens caused by each sanitizer treatment applied to fruits and vegetables. From *Table 2*, it can

be seen that for most sanitizers, concentration, temperature and time have a direct effect on the microbial log-reduction, even though for water and ASC a quadratic effect of temperature on mean log–reduction was also identified. Covariate was not included for temperature when the treatment was SAEW as data was available only for ambient temperature. The concentration covariate was neither included when treatment was water as it had no meaning for this treatment.

Through the meta-analytical model, it was possible to find that the pathogens studied may differ in terms of their resistance depending on the sanitizers. *L. monocytogenes* presented the lower intercept, meaning that it may be more resistant to CA, PA and ASC treatments. On the other hand, pathogenic *E. coli* seems to be more resistant to MA, CD, AA and HP treatments (*Table 2*). *Salmonella* presented the lowest intercepts (higher resistance) only when the treatment was done with water, while *L. monocytogenes* and pathogenic *E. coli* presented similar resistance to this treatment (*Table 2*). SH had a similar impact on *L. monocytogenes* and pathogenic *E. coli* inactivation, whereas *L. monocytogenes* and *Salmonella* were equally resistant to CD. SAEW was the only sanitizer for which no differences in inactivation resistance were found for the three pathogens studied (*Table 2*). It should be highlighted that for some sanitizers, such as AA and HP, no data on *L. monocytogenes* inactivation was available. Therefore, for these sanitizers, only *Salmonella* and pathogenic *E. coli* were considered with the latter being more resistant than the former (*Table 2*).

The $I^2$ intra-class correlation values obtained were generally >48%, except for the treatment with MA (12%) (*Table 2*), which suggest that, for most sanitizers, there may be other moderating variables explaining the remaining between-study variability that were not codified in the present meta-analysis study. Despite the above, for each of the sanitizers, a reasonable agreement was shown between the observed mean log-reduction

values extracted from the primary studies, and those fitted by the models from *Table 2* (Figure 1).

Taking into account that the inactivation of *L. monocytogenes*, *Salmonella* and pathogenic *E. coli* by four sanitizers (ASC, CD, SAEW and SH) was assessed in at least four different types of produce (*Table 1*), *Equation 4* has been used to assess whether the inactivation effectiveness of the same washing treatment would be affected by the type of fresh produce. Forest plots were constructed for each of the four sanitizing treatments using realistic sanitizer concentration and washing time as shown in *Figures 2, 3, 4*, and *5*. Also, it should be highlighted that these forest plots were constructed based on the meta-analytical model (*Equation 4*) fitted to ASC, CD, SAEW and SH. For example, data on *L. monocytogenes*, *Salmonella* and pathogenic *E. coli* inactivation by ASC was only available for six types of fresh produce, namely cucumber, cherry tomato, tatsoi, cilantro, tomato and carrots (*Figure 2*). Based on the model represented by *Equation 4*, it can be seen that different mean log reductions were achieved according to the type of fresh produce studied. When ASC was the sanitizer used, mean log reductions varied from 1.68 for cucumber to 5.38 for carrots (*Figure 2*), while log reductions varied between 0.68-3.61, 2.04-3.68 and 0.91-3.38 for CD, SAEW and SH treatments, respectively (*Figures 3-5*). Data obtained suggest, in general, that sanitizing treatments seemed to be less effective (achieve lower log reductions of pathogens) when applied in leafy vegetables in comparison to other fresh produce (*Figures 2, 3, 4*, and *5*).

### 3.2. Meta-analysis models by pathogen

A further approach taken was to build separate meta-analytical inactivation models for each of the pathogens studied, i.e., *L. monocytogenes*, *Salmonella* and pathogenic *E. coli*. This was done aiming at comparing the bactericidal effects of sanitizers for a common treatment (*C, T, t*). *Tables 3, 4* and *5* show the parameter estimates obtained using

*Equation* 5 fitted to each of the pathogens predicting their log reductions. The $I^2$ values were >60% for the three models predicting the inactivation of *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella*, suggesting significant remaining heterogeneity in the outcomes from the primary studies. (*Tables 3, 4* and *5*). Considering that the meta-analysis models were fitted by pathogen, it is now possible to see the differences among sanitizers and rank them from the lowest to the highest effects on pathogens inactivation (log reduction) (*Tables 3, 4* and *5*). It should be underlined that the sanitizers listed in these tables are not the same for the three pathogens because of the data sparseness. A total of 15, 12 and 8 different sanitizers were used for *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella*, respectively. Despite this, it was possible to find some similarity regarding the log reductions caused by the sanitizers over the three pathogens studied. Comparing the sanitizers' intercept values, SAEW, ASC and CD appeared as the most effective sanitizers, against *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella* (*Tables 3, 4* and *5*). On the other hand, Oz, HP and AA, and CA, SH and LA caused lower log reductions in *E. coli* O157:H7 and *Salmonella* (*Tables 3* and *5*), while, AA, LA and SDS caused lower log reductions in *L. monocytogenes* (*Table 4*). The fitted intercepts presented in *Tables 3, 4* and *5*, also suggest that *E. coli* 0157:H7 seems to be more resistant to the most effective sanitizers. While SAEW, ASC and CD caused 3.4, 5.1 and 3.6 mean log reductions in *E. coli* O157:H7 (*Table 3*), the number of log reductions caused in *L. monocytogenes* and *Salmonella* were 6.9, 8.0 and 7.0 and 5.1, 5.4 and 7.0, respectively (*Tables 4* and *5*). Among these three sanitizers, ASC and CD were more effective against *L. monocytogenes* and *Salmonella*, while SAEW was the less effective against *E. coli* 0157:H7 (*Tables 3, 4* and *5*).

A way to visualise the effect of type of sanitizer on microbial log reduction is through the construction of forest plots. To this end, the fitted meta-analysis models from *Tables 3, 4* and *5* were solved for a hypothetical treatment with sanitizer concentration of 0.001

g/100mL and exposure time of 3 minutes at ambient temperature in order to predict the mean log reductions of *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella.* These predicted values are illustrated as forest plots in *Figures 6, 7* and *8*. Under the hypothetical treatment conditions, it was found that Oz and ASC resulted in the lowest (0.14 [-0.50, 0.78]) and highest (3.86 [3.24, 4.49]) log reductions of *E. coli* 0157:H7, respectively (*Figure 6*). For *L. monocytogenes*, the lowest and highest log reduction would be obtained with the use of CA (0.37 [-0.77, 1.50]) and ASC (2.47 [1.41, 3.53]) as sanitizers (*Figure 7*), while for *Salmonella*, AA and ASC led to the lowest (0.49 [-0.58, 1.57]) and highest (4.40 [3.40, 5.40)] log reductions, respectively (*Figure 8*).

### 3.3. Cluster analysis of sanitizers

Through a hierarchical clustering analysis, the sanitizers were grouped in four clusters according to their bactericidal efficacy. The meta-analytical dendogram of sanitizers is shown in *Figure 9*. Through this approach it was possible to find four groups: waters (blanks), “low bactericidal efficacy”, “medium bactericidal efficacy” and finally, a group with the “highest bactericidal efficacy”.

## 4. Discussion

Microbial safety is a major concern for fresh produce industry because of the recurrent implication of fruits and vegetables in foodborne disease outbreaks (3, 5, 6). The use of sanitizers during washing step comprises the main measure aiming to safeguard the safety of fruits and vegetables at post-harvest steps. It is recognized that the effectiveness of washing procedures applied during processing of ready-to-eat fruits and vegetables is affected by several factors such as washing conditions (temperature, time, water circulation and etc.), type of produce (whole, pieces, leafy and etc.) and sanitizers

(chemical principle, concentration and etc.) (15). In view of this, models predicting the global effectiveness of sanitizers used in washing treatments of minimally processed vegetables are not available in the literature. In order to contribute to the field, in this study was applied a meta-analysis approach to assess *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella* inactivation by sanitizers during fresh produce washing. We were able to collect data on the microbial log-reduction achieved by the sanitizers used during washing treatment of minimally processed fruits and vegetables from 55 primary studies. This firstly resulted in data on inactivation of *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella* by 27 sanitizers. These records were further refined leading to data of 18 sanitizers, 30 types of fruits and vegetables and 1025 microbial log-reduction values (*Table 1*).

### 4.1. Meta-analysis models by sanitizer

Our first approach in this study was to construct a meta-analysis model by sanitizer, which allowed us to compare the effectiveness of treatments among pathogens and fruits/vegetables (*Table 2*). From *Table 2* it can be seen that log reduction for pathogens was affected by the sanitizer concentration, washing water temperature and increase in time. This is particularly true for treatments with AA, CA, water, ASC and HP, but the opposite was found for treatments with SH and CD (*Table 2*). The higher sensibility of chlorine-based solutions to increase of washing water temperature is well known and this is deemed as one of the major limitations for a wider application of these compounds during fresh produce sanitation (14, 15, 67).

A major finding of the meta-analytical model by sanitizer (*Equation 3*) is that we found the susceptibility of the three pathogens studied to the treatments (*Table 2*). The pathogens tend to be more resistant when lower intercepts are obtained in a specific treatment, while when higher intercepts are attained the pathogen tend to be less resistant . The fact that *E. coli* 0157:H7 and *L. monocytogenes* presented the lower intercepts for

treatments such AA, CA, MA, SAEW, PAA, SH, CD, ASC and HP, indicate that these pathogens are the most resistant to sanitizing treatments applied during washing of fruits and vegetables (*Table 2*). The higher global resistance of *E. coli* 0157:H7 to sanitizers widely used by the fresh produce industry such as SH and CD, can provide further insights on the reasons why this pathogen is commonly involved in fresh produce disease outbreaks (68). Despite the fact that *L. monocytogenes* presented similar resistance to almost all the same sanitizers deemed also ineffective against *E. coli* 0157:H7 (*Table 2*), it is known that the former pathogen is more susceptible to inhibition in vegetables (69, 70). *Salmonella* was found to be more resistant than *E. coli* 0157:H7 and *L. monocytogenes* only when water was assessed as a washing treatment (*Table 2*). Nonetheless, as we used water [tap water (W), distilled water (DisW) and deionised water (DioW)] as blanks (concentration of 0 g/100 mL), the inactivation effect, even low, is due to factors such as temperature and time. If one considers the application of water at increasing time and temperature (*Table 1*), the inactivation of *Salmonella* and other pathogens will be higher. SAEW seemed to be the most effective sanitizer for the inactivation of three pathogens studied (*Table 2*). The fact that temperature and concentration covariates were not included in the meta-analysis model for some sanitizing treatments indicate the need for these data to be generated. This will further allow the improvement of meta-analysis predictions (*Equation 3, Table 2*).

Despite the fact that data was gathered from different primary studies (*Table 1*), scatter plots show that there is still a reasonable agreement between observed and predicted values for each of the sanitizers (*Figure 1*). The fitted versus observed plots highlight how good the meta-analysis models represented the data. This is a great accomplishment of this study, as we were able to combine data from 40 primary studies (*Table 1*).

Further, when the impact of a realistic sanitizing treatment (equal sanitizer concentration and washing time) was assessed, we found that the same sanitizing treatment would achieve different log reduction, which is dependent upon the type of produce (*Figures 2-5*). The fact that sanitizing treatments applied to leafy greens seemed to result in lower log reductions when compared to other types of produce (for instance, carrot, tomato, cantaloupe and etc.), might be related to physicochemical nature of leafy green surfaces (71). Besides, when leafy vegetables are diced, chopped or shredded plant tissue damage takes place, which may result in their increased attachment (72, 73) and even growth in vegetable tissues (74). Another possible reason for the differences obtained between the inactivation of pathogens during washing of leafy greens and other produce, such as carrot, tomato, cantaloupe, could be related to the different mechanics during washing (brushing can be applied) or even the physicochemical nature of plant surfaces (71, 75, 76).

### 4.2. Meta-analysis models by pathogen

Data partitioned by pathogen was used to make comparisons among the bactericidal efficacy of sanitizers (*Tables 3-5*). It should be highlighted that sanitizers listed in these tables are not the same for the three pathogens considering these data was not available. Thus, because of data sparseness we fitted the model to data available. The fact that more sanitizing treatment data (n=15) on the inactivation of *E. coli* 0157:H7 during fresh produce washing was available, followed by their effects on *Salmonella* (n=12) and *L. monocytogenes* (n=8) can reflect the relative concern of commodity-pathogen combinations. It is noteworthy that *E. coli* O157:H7 and *Salmonella* are the most frequent pathogens associated to foodborne disease outbreaks linked to fresh produce (3, 5, 6, 68). As such, it would expected to find more sanitizer options to be applied during washing aiming to ensure effective fresh produce disinfection and safeguard public health.

From *Tables 3-5*, it can be seen that weak organic acids such as CA, AA, and LA presented the lowest effect on microbial log-reduction. The antimicrobial efficiency of weak organic acids is highly dependent on the pH of the final solution applied for fresh produce disinfection as pH affects the concentration of undissociated acid formed (77, 78). Moreover, it is known that the antimicrobial activity of organic acids is highly dependent on the type of acid (77, 79). This limitation, summed to the fact that depending on the organic acid, there might be impact on food taste and flavour and that high BOD and COD values may be found in wastewater, which will certainly limit their application in washing water of fresh produce industry (78).

SH was another class of sanitizers that appeared among those compounds with lowest effects on microbial log reduction (*Tables 4-5*). SH is a highly used chemical principle for sanitization of fresh produce, given its high cost-benefit (14, 15), despite the fact that SH solution are highly affected by organic matter concentration and pH of the washing water (14, 15, 78). Another weakness of SH application as sanitizer for fresh produce is the concern with the formation of compounds with potentially carcinogenic or mutagenic effects, such as chloramines and trihalomethanes (14, 80, 81). Because of these risks, the use of SH for fresh produce sanitation has been prohibited in some parts of the world, such as Europe (15, 77, 78). From the data presented in *Tables 3-5*, it becomes clear that SH presented a higher effect on microbial log reductions only for *E. coli* 0157:H7 (*Table 3*). Nonetheless, this should be carefully interpreted as the mean effects of all sanitizers tested against *E. coli* 0157:H7 were lower when compared to those found for *Salmonella* and *L. monocytogenes* (*Tables 4-5*). This may reinforce the hypothesis that *E. coli* 0157:H7 presents an intrinsic higher resistance to sanitizing agents commonly used for fresh produce sanitation.

In contrast to SH and organic acid sanitizers, SAEW, ASC and PAA were found to be the highest efficient chemicals in reducing microbial contamination during fresh produce

washing (*Tables 3-5*). PAA is a chemical successfully used in sanitation of equipment used in food industry (82, 83). PAA can be applied in a wide range of temperature, water physico-chemical parameters (including pH and calcium and magnesium contents), presence of organic matter (15, 77, 78). On the other hand, SAEW is deemed as a highly effective sanitizing, less inexpensive, easy of application and of handling (84). Nonetheless, SAEW has some limitations concerning equipment corrosion and low stability of the antimicrobial solution (15, 77, 78, 85, 86). ASC has been approved for application in fresh produce sanitation 15 years ago (87). It has been proved to be a highly efficient antimicrobial treatment when applied in the range of 0.5–1.2 g $L^{-1}$ (50, 88). Nonetheless, ASC has been found to cause physiological damages in fresh produce even when used in concentration to 1.2 g $L^{-1}$ allowed by the FDA (89, 31).

Although data presented in *Tables 3-5* already suggest the range of efficiency of the assessed sanitizing treatments over the three pathogens studied, we further established a hypothetical treatment (0.001 g/100mL, washing time/temperature of 3 min/25°C) to be able to visualise, through forest plots, the log-reductions caused by each sanitizer for each pathogen. As seen in *Figures 6-8*, Oz, CA and AA would cause the lowest log reductions on *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella*, respectively. Oz effects on the microbial log reductions for *E. coli* 0157:H7 were found to be lower in comparison with other sanitizers (*Table 3*). The antimicrobial efficacy of Oz is known to be highly influenced by the level of $O_3$ soluble in the washing water, contact time, water agitation, water pH and organic matter content (78, 90-92). Although Oz has been reported as a highly antimicrobial agent for fresh produce washing applications (15), its application in high concentration (>1ppm) is not feasible because of likely damages prone to be caused in fresh produce as well as corrosion potential of equipment (77, 78). This can reinforce that the antimicrobial effectiveness of these compounds seems to be highly dependent upon factors, such as time and temperature (78).

On the other hand, ASC was consistently the most effective sanitizer for the three pathogens studied (*Figures 6-8*). Nonetheless, these findings should be interpreted with care because the rankings given in these figures were created for a constant sanitizer concentration (0.001 g/100mL, washing time/temperature of 3 min/25°C), when in fact each sanitizer operates at a recommended and proper concentration. These rankings are useful to illustrate the power of sanitizers, but in practice, the use of some of these sanitizers may require specific time/temperature conditions and specific concentrations. For example, it is know that chlorine-based sanitizers have a range of increased antimicrobial activity and that above a certain pH; the increase in chlorine concentration will not result in any further gain from the antimicrobial point of view (14).

### 4.3. Cluster analysis of sanitizers

A further assessment of the meta-analysis models was the use of cluster analysis to group the sanitizers according to bactericidal efficacy by means of hierarchical clustering analysis (*Figure 9*). This is a better approach than the previous forest plots (*Figures 6, 7* and *8*), because the clustering method instead takes into account the slope of the sanitizer concentration (*Equation 6*), and implicitly it considers the specific range of concentration at which each sanitizer operates (viz. sanitizer concentration is not assumed to be constant for all the chemicals). Moreover, the clustering method combines the log reduction data for all three pathogens. The four clusters seen in *Figure 9* clearly show that sanitizers could be grouped based on their antimicrobial activity. For example, all the waters (DioW, DisW and Water) have been grouped together, indicating that their bactericidal power is the lowest of all. A second group with slightly higher bactericidal efficacy is that formed by HP, AA and CH (for the concentrations recommended for fresh produce washing), and we can label the group as “low bactericidal efficacy”. A second category of “medium bactericidal efficacy” is given by the organic acids CA, LA, MA, PAA,

and the inorganic SH. Although SH apparently should have a stronger bactericidal effect, it is grouped with the organic acids because for the low concentrations allowed for produce washing, its effect is comparable to the organic acids. The fourth group can be labelled as "high bactericidal efficacy" and is given by SAEW, ASC and the gaseous CD. SAEW has the highest bactericidal effect of all (*Figure 9*).

## 5. Conclusions

Through a meta-analysis approach, we were able to assess more than 1000 data on log reduction of the three main bacterial pathogens impairing the safety of fresh produce. We were able to build predictive models by sanitizer and by pathogen. The study is the first to gather data from a great number of papers (n=40) and packed in such way that the outputs could be compared. This has been cited as one of the major limitations of the works in this field (15). Furthermore, through the hierarchical clustering analysis performed, we were able to classify sanitizers by their bactericidal efficacy.

The findings of this study can be seen as an achievement of very practical relevance as it can serve regulators to rank sanitizers based on their antimicrobial efficiency. For example, depending on pathogen of greatest concern in a specific produce item, a sanitizer with the highest bactericidal power could be suggested as preferential for use. Altogether, the outcomes of the present study can serve as scientific information for decision-making (risk-benefit analysis). Regulations can be further harmonized and developed taking into account the findings reported herein.

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## Figure Legends and Tables

**Figure 1:** Scatter plots of mean microbial log-reduction values (y-axis) fitted by the independent meta-analysis linear mixed models by sanitizer (from Table 2) in comparison with the observed data (x-axis).

**Figure 2:** Forest plot of the overall mean log-reduction of pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp.) on different fresh produce achieved by sanitizing washing with 0.04 g/100 ml acidified sodium chloride (ASC) at a time/temperature of 3 min/25ºC

**Figure 3:** Forest plot of the overall mean log-reduction of pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp.) on different fresh produce achieved by sanitizing treatment with 0.00033 g/100 ml gaseous chlorine dioxide (CD) at a time/temperature of 10 min/25ºC

**Figure 4:** Forest plot of the overall mean log-reduction of pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp.) on different fresh produce achieved by sanitizing washing with 0.005 g/100 ml slightly acidic electrolysed water (SAEW) at a time/temperature of 3 min/25ºC

**Figure 5:** Forest plot of the overall mean log-reduction of pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp.) on different fresh produce achieved by sanitizing washing with 0.012 g/100 ml sodium hypochlorite (SH) at a time/temperature of 3 min/25ºC

**Figure 6:** Forest plot of the overall mean log-reduction of *E. coli* O157:H7 on a population of fresh fruits and vegetables that would be achieved by different sanitizers using a common hypothetical treatment of 0.001 g/100 ml concentration and a washing/exposure time/temperature of 3 min/25ºC

**Figure 7:** Forest plot of the overall mean log-reduction of *L. monocytogenes* on a population of fresh fruits and vegetables that would be achieved by different sanitizers using a common hypothetical treatment of 0.001 g/100 ml concentration and a washing/exposure time/temperature of 3 min/21.0ºC

**Figure 8:** Forest plot of the overall mean log-reduction of *Salmonella* spp. on a population of fresh fruits and vegetables that would be achieved by different sanitizers using a common hypothetical treatment of 0.001 g/100 ml concentration and a washing/exposure time/temperature of 3 min/22.5ºC

**Figure 9:** Dendogram of sanitizers clustered hierarchically showing four main groups according to bactericidal efficacy

***Figure 1:***

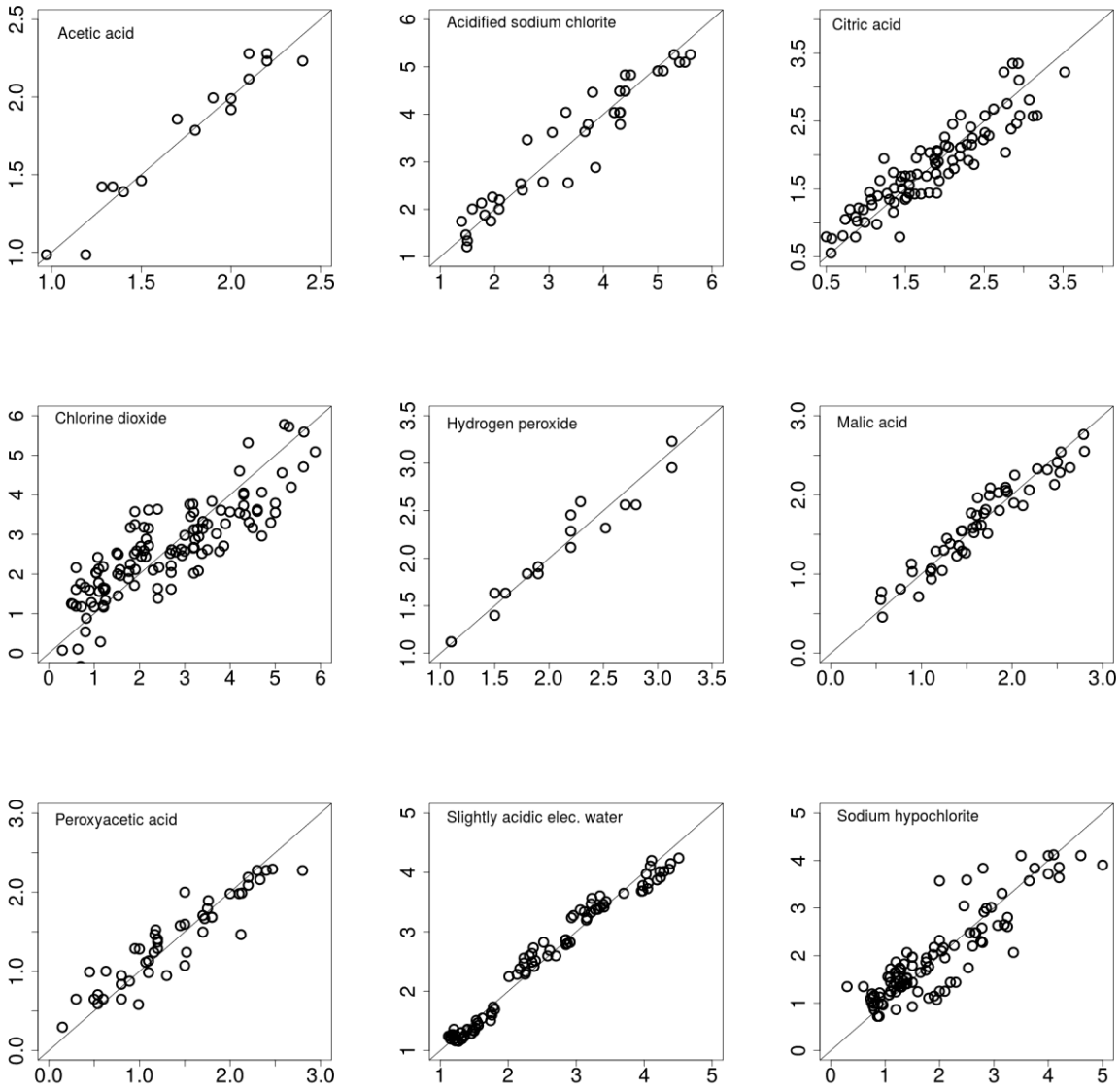
Acetic acid
Acidified sodium chlorite
Citric acid
Chlorine dioxide
Hydrogen peroxide
Malic acid
Peroxyacetic acid
Slightly acidic elec. water
Sodium hypochlorite

***Figure 2:***

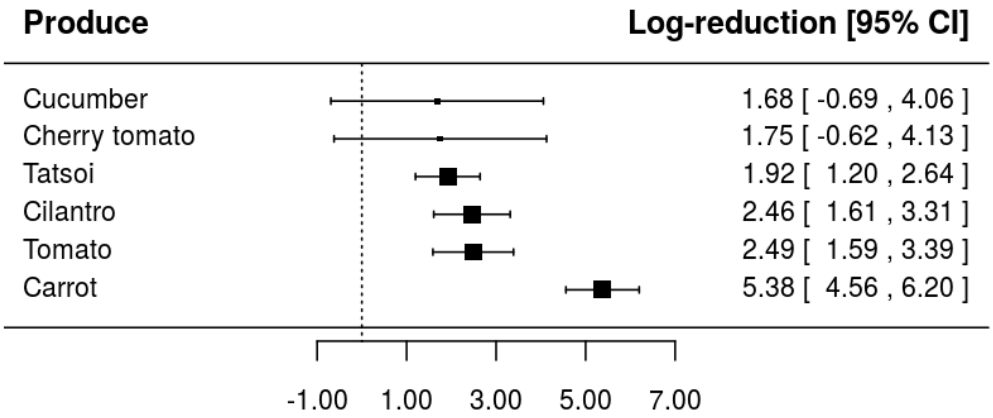
Produce
Log-reduction [95% CI]
Cucumber
Cherry tomato
Tatsoi
Cilantro
Tomato
Carrot
1.68 [ -0.69 , 4.06 ]
1.75 [ -0.62 , 4.13 ]
1.92 [ 1.20 , 2.64 ]
2.46 [ 1.61 , 3.31 ]
2.49 [ 1.59 , 3.39 ]
5.38 [ 4.56 , 6.20 ]
-1.00
1.00
3.00
5.00
7.00

***Figure 3:***

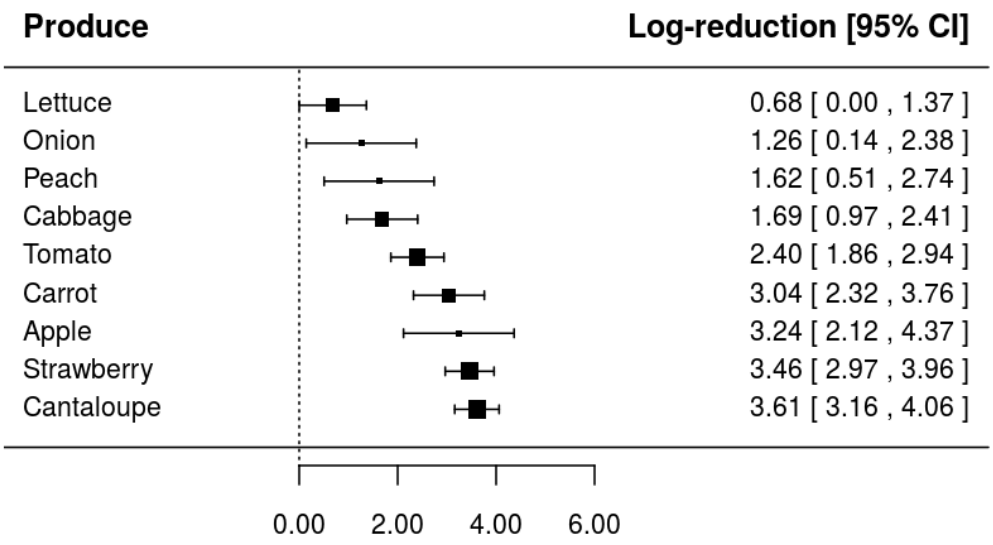
Produce
Log-reduction [95% CI]
Lettuce
0.68 [ 0.00 , 1.37 ]
Onion
1.26 [ 0.14 , 2.38 ]
Peach
1.62 [ 0.51 , 2.74 ]
Cabbage
1.69 [ 0.97 , 2.41 ]
Tomato
2.40 [ 1.86 , 2.94 ]
Carrot
3.04 [ 2.32 , 3.76 ]
Apple
3.24 [ 2.12 , 4.37 ]
Strawberry
3.46 [ 2.97 , 3.96 ]
Cantaloupe
3.61 [ 3.16 , 4.06 ]
0.00
2.00
4.00
6.00

***Figure 4:***

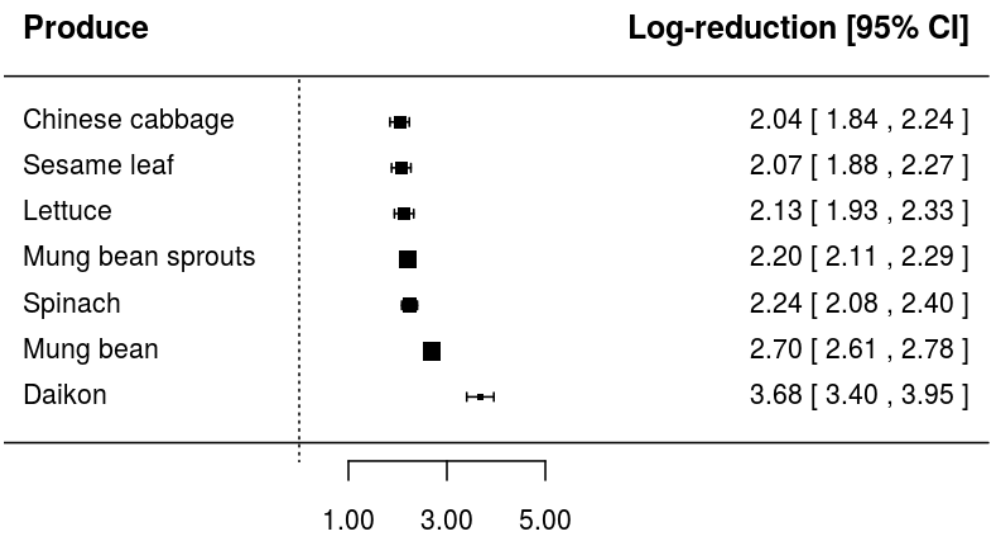
Produce
Log-reduction [95% CI]
Chinese cabbage
2.04 [ 1.84 , 2.24 ]
Sesame leaf
2.07 [ 1.88 , 2.27 ]
Lettuce
2.13 [ 1.93 , 2.33 ]
Mung bean sprouts
2.20 [ 2.11 , 2.29 ]
Spinach
2.24 [ 2.08 , 2.40 ]
Mung bean
2.70 [ 2.61 , 2.78 ]
Daikon
3.68 [ 3.40 , 3.95 ]
1.00
3.00
5.00

***Figure 5:***

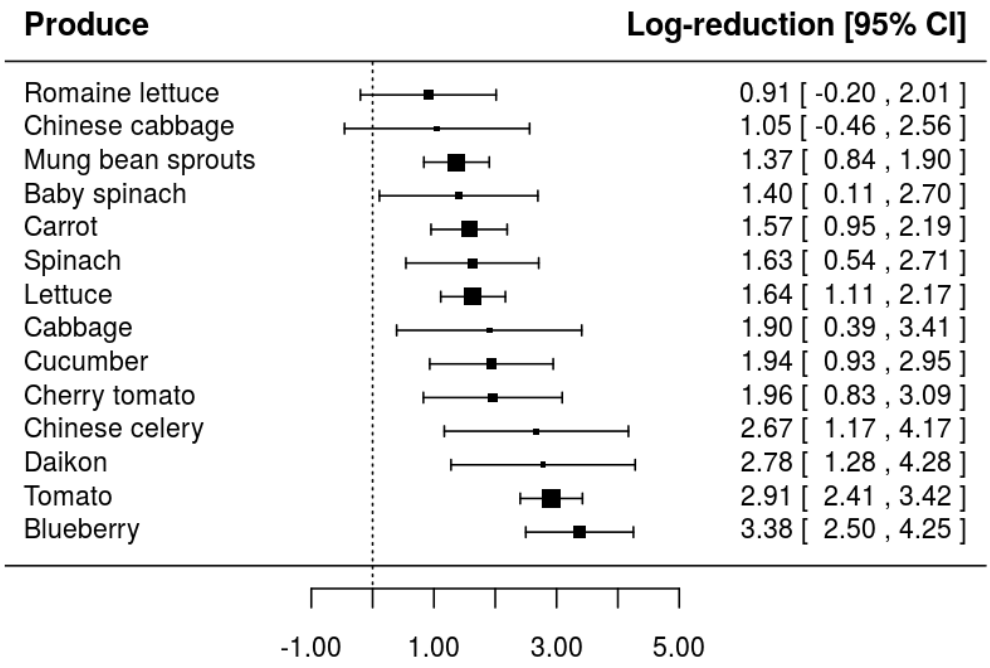
Produce
Log-reduction [95% CI]
Romaine lettuce
0.91 [ -0.20 , 2.01 ]
Chinese cabbage
1.05 [ -0.46 , 2.56 ]
Mung bean sprouts
1.37 [ 0.84 , 1.90 ]
Baby spinach
1.40 [ 0.11 , 2.70 ]
Carrot
1.57 [ 0.95 , 2.19 ]
Spinach
1.63 [ 0.54 , 2.71 ]
Lettuce
1.64 [ 1.11 , 2.17 ]
Cabbage
1.90 [ 0.39 , 3.41 ]
Cucumber
1.94 [ 0.93 , 2.95 ]
Cherry tomato
1.96 [ 0.83 , 3.09 ]
Chinese celery
2.67 [ 1.17 , 4.17 ]
Daikon
2.78 [ 1.28 , 4.28 ]
Tomato
2.91 [ 2.41 , 3.42 ]
Blueberry
3.38 [ 2.50 , 4.25 ]
-1.00
1.00
3.00
5.00

***Figure 6:***

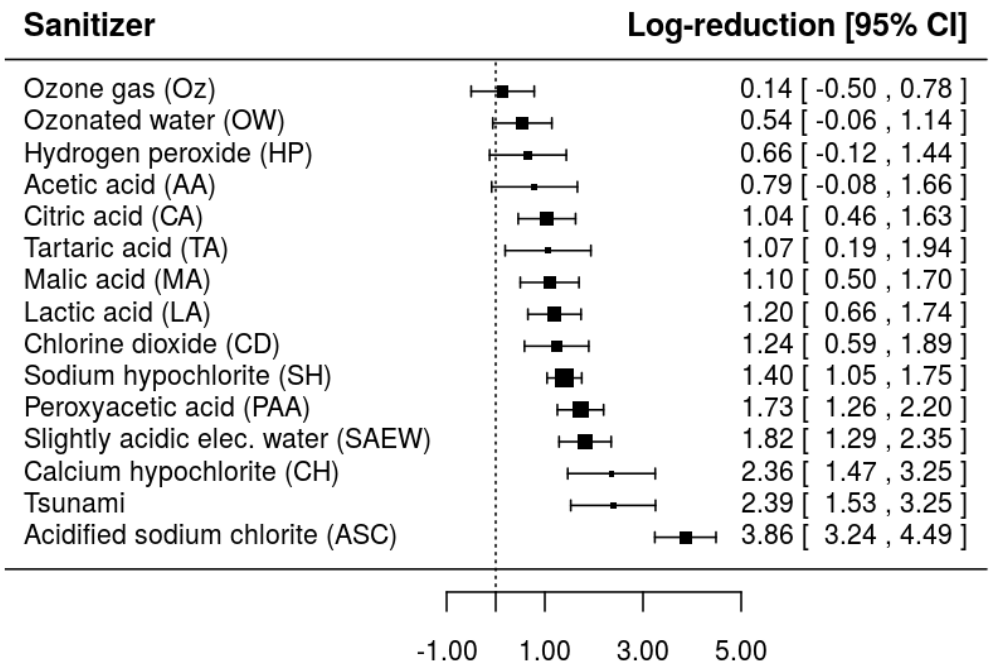
Sanitizer
Log-reduction [95% CI]
Ozone gas (Oz) 0.14 [ -0.50 , 0.78 ]
Ozonated water (OW) 0.54 [ -0.06 , 1.14 ]
Hydrogen peroxide (HP) 0.66 [ -0.12 , 1.44 ]
Acetic acid (AA) 0.79 [ -0.08 , 1.66 ]
Citric acid (CA) 1.04 [ 0.46 , 1.63 ]
Tartaric acid (TA) 1.07 [ 0.19 , 1.94 ]
Malic acid (MA) 1.10 [ 0.50 , 1.70 ]
Lactic acid (LA) 1.20 [ 0.66 , 1.74 ]
Chlorine dioxide (CD) 1.24 [ 0.59 , 1.89 ]
Sodium hypochlorite (SH) 1.40 [ 1.05 , 1.75 ]
Peroxyacetic acid (PAA) 1.73 [ 1.26 , 2.20 ]
Slightly acidic elec. water (SAEW) 1.82 [ 1.29 , 2.35 ]
Calcium hypochlorite (CH) 2.36 [ 1.47 , 3.25 ]
Tsunami 2.39 [ 1.53 , 3.25 ]
Acidified sodium chlorite (ASC) 3.86 [ 3.24 , 4.49 ]
-1.00
1.00
3.00
5.00

***Figure 7:***

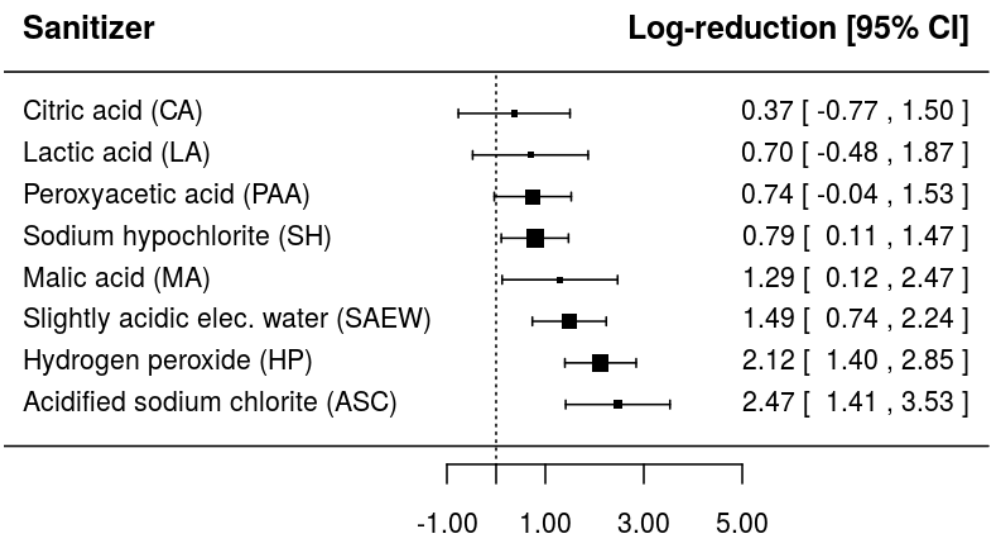
Sanitizer
Log-reduction [95% CI]
Citric acid (CA)
0.37 [ -0.77 , 1.50 ]
Lactic acid (LA)
0.70 [ -0.48 , 1.87 ]
Peroxyacetic acid (PAA)
0.74 [ -0.04 , 1.53 ]
Sodium hypochlorite (SH)
0.79 [ 0.11 , 1.47 ]
Malic acid (MA)
1.29 [ 0.12 , 2.47 ]
Slightly acidic elec. water (SAEW)
1.49 [ 0.74 , 2.24 ]
Hydrogen peroxide (HP)
2.12 [ 1.40 , 2.85 ]
Acidified sodium chlorite (ASC)
2.47 [ 1.41 , 3.53 ]
-1.00
1.00
3.00
5.00

***Figure 8:***

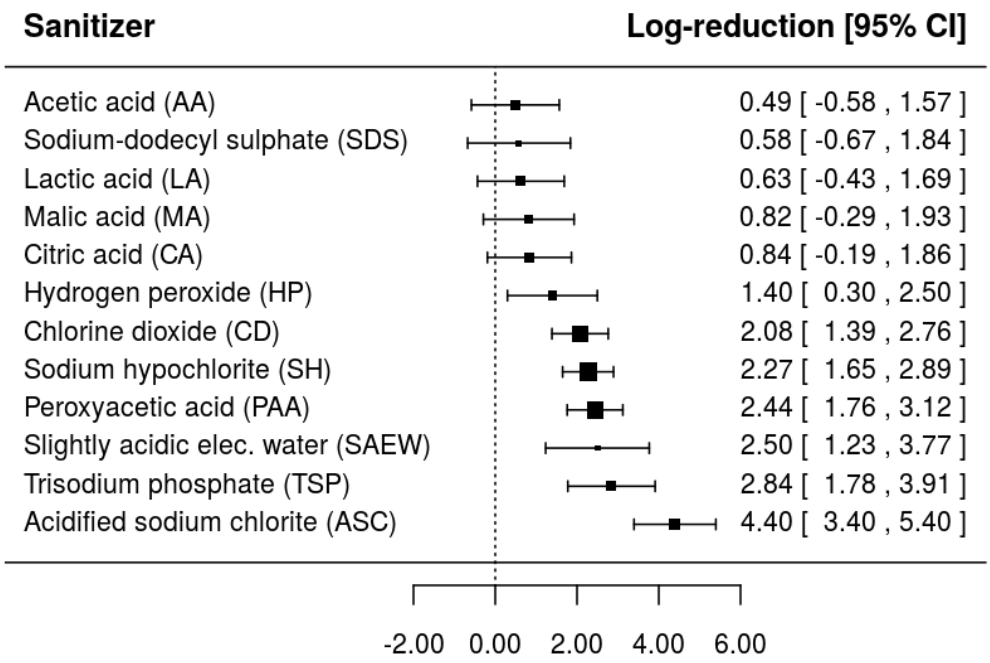
Sanitizer
Log-reduction [95% CI]
Acetic acid (AA)
0.49 [ -0.58 , 1.57 ]
Sodium-dodecyl sulphate (SDS)
0.58 [ -0.67 , 1.84 ]
Lactic acid (LA)
0.63 [ -0.43 , 1.69 ]
Malic acid (MA)
0.82 [ -0.29 , 1.93 ]
Citric acid (CA)
0.84 [ -0.19 , 1.86 ]
Hydrogen peroxide (HP)
1.40 [ 0.30 , 2.50 ]
Chlorine dioxide (CD)
2.08 [ 1.39 , 2.76 ]
Sodium hypochlorite (SH)
2.27 [ 1.65 , 2.89 ]
Peroxyacetic acid (PAA)
2.44 [ 1.76 , 3.12 ]
Slightly acidic elec. water (SAEW)
2.50 [ 1.23 , 3.77 ]
Trisodium phosphate (TSP)
2.84 [ 1.78 , 3.91 ]
Acidified sodium chlorite (ASC)
4.40 [ 3.40 , 5.40 ]
-2.00
0.00
2.00
4.00
6.00

***Figure 9:***

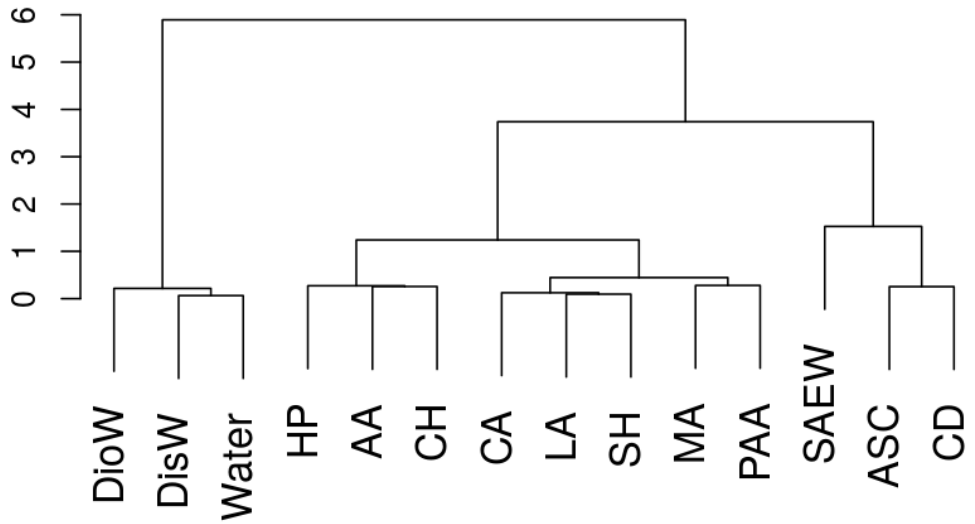

**Table 1.** Number of microbial log-reduction observations (*n*) found in the literature according to type of fresh produce, pathogen and sanitizer, extracted from published studies

| Sanitizer | *E. coli* O157:H7 | *L. monocytogenes* | *Salmonella* spp. |
|---|---|---|---|
| **AA** (acetic acid) | Baby spinach (6) | - | Blueberry (4), rocket leaves (8), spring onion (8) |
| **ASC** (acidified sodium chlorite) | Carrot (6), cilantro (4), tatsoi (9) | Carrot (7), cherry tomato (1), cucumber (1) | Carrot (3), cherry tomato (1), cucumber (1), tomato (6) |
| **Bzc** (benzalkonium chloride) | Lettuce (2), tomato (2) | - | - |
| **CA** (citric acid) | Baby spinach (9), cilantro (1), lettuce (20), spinach (1) | Lettuce (20), spinach (1) | Blueberry (2), lettuce (20), rocket leaves (8), spring onion (8) |
| **Ca-Oy** (calcined oyster shell) | Tomato (4) | - | - |
| **Ca-SS** (calcined Sakhalin surf clam) | Tomato (4) | - | - |
| **CH** (calcium hypochlorite) | Broccoli (11), lettuce (12), spinach (1) | - | Spinach (1) |
| **Citrox™** | Lettuce (9) | - | - |
| **CD** (chlorine dioxide) | Cabbage (3), cantaloupe (10), carrot (3), lettuce (6), spinach (2), strawberry (6) | cabbage (3), cantaloupe (10), carrot (3), lettuce (6), strawberry (5) | Apple (3), cabbage (3), carrot (3), cantaloupe (9), lettuce (6), onion (3), peach (3), spinach (2), strawberry (6), tomato (29) |
| **DA** (dodecyl-benzenesulfonic acid) | Romaine lettuce (2) | - | - |
| **DioW** (deionised water) | Baby spinach (6), broccoli (3), lettuce (6), mung bean (4), mung bean sprouts (4), romaine lettuce (2), spinach (1) | Lettuce (2), spinach (1) | Blueberry (2), lettuce (2), mung bean (4), mung bean sprouts (4), pepper (2) |
| **DisW** (distilled water) | Buckwheat (1), Chinese cabbage (3), lettuce (5), sesame (1), spinach (4), tomato (6), cabbage (2), apple (3), mung bean sprouts (2) | Broccoli (1), cabbage (1), mung bean sprouts (3), Chinese cabbage (1), lettuce (4), sesame leaf (1), spinach (1), cucumber (1) | Buckwheat (1), apple (3), blueberry (3), spinach (1), mung bean sprouts (2), lettuce (1), cucumber (3) |
| **EHS** (sodium 2-ethylhexyl-sulfate) | Romaine lettuce (2) | - | - |
| **HP** (hydrogen | Baby spinach (9) | Cucumber (1) | Blueberry (5), cantaloupe |

| | | | |
|---|---|---|---|
| peroxide) | | | (2), honeydew (2) |
| **LA** (lactic acid) | Apple (2), baby spinach (9), lettuce (33), spinach (1), tomato (4) | Lettuce (20) | Apple (2), blueberry (2), lettuce (20), spinach (1) |
| **MA** (malic acid) | Baby spinach (9), lettuce (20) | Lettuce (20) | Lettuce (20) |
| **Nisin** | - | Broccoli (1), cabbage (1), mung bean sprouts (1) | - |
| **OW** (ozonized water) | Cabbage (2), Chinese cabbage (2), lettuce (2), spinach (5) | Spinach (1) | Spinach (2) |
| **Oz** (ozone gas) | Cabbage (2), Chinese cabbage (2), lettuce (2), spinach (2) | - | - |
| **PAA** (peroxyacetic acid) | Carrot (4), lettuce (4), mung bean sprouts (7), rocket leaves (1), spinach (1), tomato (3) | Carrot (4), mung bean sprouts (7) | Carrot (4), green onion (4), lettuce (4), mung bean sprouts (7), spinach (1), tomato (8) |
| **Pediocin** | - | Broccoli (1), cabbage (1), mung bean sprouts (1) | - |
| **Phy** (phytic acid) | Tomato (4) | - | - |
| **SAEW** (slightly acidic electrolysed water) | Chinese cabbage (2), lettuce (2), daikon lettuce (2), mung bean (16), mung bean sprouts (16), sesame leaf (2), spinach (4) | Chinese cabagge (2), lettuce (2), sesame leaf (2), spinach (4) | Chinese celery (1), daikon lettuce (1), lettuce (1), mung bean (16), mung bean sprouts (16) |
| **SC** (sodium chlorite) | Cilantro (1) | - | - |
| **SDS** (sodium-dodecyl sulphate) | - | - | Blueberry (11) |
| **SH** (sodium hypochlorite) | Baby spinach (3), cabbage (2), carrot (4), Chinese cabbage (2), Chinese celery (1), cilantro (1), daikon lettuce (1), lettuce (12), mung bean sprouts (7), romaine lettuce (4), rocket leaves (1), spinach (3), tatsoi (1), tomato (9) | Carrot (6), cherry tomato (2), cucumber (3), lettuce (2), mung bean sprouts (7), spinach (1) | Blueberry (7), carrot (6), cherry tomato (2), Chinese celery (1), cucumber (2), daikon lettuce (1), green onion (4), lettuce (7), mung bean sprouts (7), pepper (2), tomato (20) |
| **TA** (tartaric acid) | Baby spinach (6) | - | - |
| **TSP** (trisodium | - | Lettuce (4) | Lettuce (4), pepper (4) |

| | | | |
|---|---|---|---|
| phosphate) | | | |
| **Tsunami-100 ™** | Lettuce (6) | - | - |
| **Water** | Carrot (4), daikon lettuce (1), lettuce (3), tatsoi (1), tomato (1) | Carrot (4), cucumber (1) | Cantaloupe (1), carrot (4), Chinese celery (1), daikon honeydew (1), lettuce (2), tomato (8) |

**Table 2.** Parameter estimates of the individual meta-analysis mixed-effects linear models by sanitizer, predicting the microbial log-reduction (R) in fresh produce as a function of microorganism, sanitizer concentration, and washing time and temperature

| Sanitizer | Parameters | Mean | Standard error | Pr > \|t\| | AIC / BIC |
|---|---|---|---|---|---|
| AA (acetic acid) | Predictors of R | | | | |
| | *E. coli* | 1.234[x] | 0.909 | 0.307 | 27/31 |
| | *Listeria*[a] | - | - | - | |
| | *Salmonella* | 1.325[y] | 0.565 | 0.143 | |
| | Concentration | 0.103 | 0.072 | 0.181 | |
| | Temperature | 0.022 | 0.004 | <.0001 | |
| | Time | 0.029 | 0.007 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.705 | | | $I^2$=99% |
| | $s^2$ (residual) | 0.004 | | | |
| CA (citric acid) | Predictors of R | | | | |
| | *E. coli* | 2.576[y] | 0.429 | <.0001 | 102/121 |
| | *Listeria* | 2.337[x] | 0.430 | <.0001 | |
| | *Salmonella* | 2.868[z] | 0.425 | <.0001 | |
| | Concentration | 0.500 | 0.057 | <.0001 | |
| | Temperature | 0.032 | 0.008 | <.0001 | |
| | Time | 0.043 | 0.003 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.436 | | | $I^2$=82% |
| | $s^2$ (residual) | 0.095 | | | |
| MA (malic acid) | Predictors of R | | | | |
| | *E. coli* | 4.223[x] | 0.207 | <.0001 | 5/18 |
| | *Listeria* | 4.538[z] | 0.212 | <.0001 | |
| | *Salmonella* | 4.444[y] | 0.207 | <.0001 | |
| | Concentration | 0.692 | 0.041 | <.0001 | |
| | Temperature[b] | - | - | - | |
| | Time | 0.051 | 0.003 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.0007 | | | $I^2$=12% |
| | $s^2$ (residual) | 0.0053 | | | |
| SAEW (slightly acidic electrolysed water) | Predictors of R | | | | |
| | *E. coli* | 11.34[x] | 0.340 | <.0001 | 32/49 |
| | *Listeria* | 11.45[x] | 0.359 | <.0001 | |
| | *Salmonella* | 11.31[x] | 0.341 | <.0001 | |
| | Concentration | 1.573 | 0.039 | <.0001 | |
| | Temperature[b] | - | - | - | |
| | Time | 0.019 | 0.005 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.591 | | | $I^2$=94% |
| | $s^2$ (residual) | 0.037 | | | |
| PAA (peroxi acetic acid) | Predictors of R | | | | |
| | *E. coli* | 3.843[z] | 0.655 | <.0001 | 62/74 |
| | *Listeria* | 3.295[x] | 0.658 | <.0001 | |
| | *Salmonella* | 3.535[y] | 0.653 | <.0001 | |
| | Concentration | 0.491 | 0.116 | <.0001 | |
| | Temperature | ns | | - | |
| | Time | 0.057 | 0.030 | 0.067 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.568 | | | $I^2$=89% |
| | $s^2$ (residual) | 0.067 | | | |

| | | | | | |
|---|---|---|---|---|---|
| SH (sodium hypo-chlorite) | Predictors of R | | | | |
| | *E. coli* | 3.143[x] | 0.712 | <.0001 | 289/307 |
| | *Listeria* | 3.233[x] | 0.719 | <.0001 | |
| | *Salmonella* | 3.861[y] | 0.719 | <.0001 | |
| | Concentration | 0.383 | 0.148 | 0.012 | |
| | Temperature | ns | | | |
| | Time | 0.097 | 0.047 | 0.043 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 1.040 | | | $I^2$=94% |
| | $s^2$ (residual) | 0.061 | | | |
| CD (chlorine dioxide gas) | Predictors of R | | | | |
| | *E. coli* | 8.450[x] | 1.085 | <.0001 | 359/378 |
| | *Listeria* | 8.914[y] | 1.084 | <.0001 | |
| | *Salmonella* | 8.855[y] | 1.066 | <.0001 | |
| | Concentration | 0.854 | 0.115 | <.0001 | |
| | Temperature | ns | | | |
| | Time | 0.054 | 0.010 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 1.413 | | | $I^2$=92% |
| | $s^2$ (residual) | 0.119 | | | |
| Water | Predictors of R | | | | |
| | *E. coli* | 1.765[y] | 0.318 | <.0001 | 27/40 |
| | *Listeria* | 1.754[y] | 0.331 | <.0001 | |
| | *Salmonella* | 1.653[x] | 0.322 | <.0001 | |
| | Concentration[c] | - | - | - | |
| | Temperature | -0.080 | 0.022 | 0.001 | |
| | Temperature$^2$ | 0.001 | 0.000 | <.0001 | |
| | Time | -0.003 | 0.005 | 0.600 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.181 | | | $I^2$=98% |
| | $s^2$ (residual) | 0.003 | | | |
| ASC (acidified sodium chlorite) | Predictors of R | | | | |
| | *E. coli* | 2.053[y] | 0.525 | <.0001 | 88/100 |
| | *Listeria* | 1.625[x] | 0.628 | 0.015 | |
| | *Salmonella* | 2.686[z] | 0.682 | <.0001 | |
| | Concentration | 0.493 | 0.119 | <.0001 | |
| | Temperature | 0.472 | 0.059 | 0.001 | |
| | Temperature$^2$ | -0.011 | 0.002 | 0.001 | |
| | Time | -0.254 | 0.046 | <.0001 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.052 | | | $I^2$=48% |
| | $s^2$ (residual) | 0.056 | | | |
| HP (hydrogen peroxide) | Predictors of R | | | | |
| | *E. coli* | 1.739[x] | 0.751 | 0.146 | 8/14 |
| | *Listeria*[a] | - | | | |
| | *Salmonella* | 2.906[y] | 0.601 | 0.040 | |
| | Concentration | 0.348 | 0.137 | 0.027 | |
| | Temperature | 0.031 | 0.007 | 0.001 | |
| | Time | 0.059 | 0.043 | 0.197 | |
| | Variances | | | | |
| | $s^2_u$ (intercept) | 0.052 | | | $I^2$=66% |
| | $s^2$ (residual) | 0.026 | | | |

[a] No data for *L. monocytogenes* was available

[b] As data was available only for ambient temperature, a temperature covariate could not be included

[c] The concentration covariate was not included as it has no meaning for water
[x,y,z] Different superscript letters denote significant differences in log-reduction among microorganisms

**Table 3.** Parameter estimates of the meta-analysis model predicting the log-reduction (R) of *E. coli* O157:H7 in fresh produce as a function of sanitizer, sanitizer concentration, and washing time and temperature

| Parameters | Mean | Standard error | Pr > \|t\| | AIC / BIC |
|---|---|---|---|---|
| Predictors of R | | | | |
| Sanitizer | | | | |
| AA | 2.094[b] | 0.535 | <.0001 | 742/816 |
| ASC | 5.103[g] | 0.419 | <.0001 | |
| CA | 2.362[c] | 0.440 | <.0001 | |
| CH | 3.192[e] | 0.526 | <.0001 | |
| CD | 3.555[f] | 0.615 | <.0001 | |
| HP | 1.987[b] | 0.507 | <.0001 | |
| LA | 2.537[d] | 0.425 | <.0001 | |
| MA | 2.429[cd] | 0.445 | <.0001 | |
| OW | 2.060[b] | 0.436 | <.0001 | |
| Oz | 1.628[a] | 0.490 | 0.001 | |
| PAA | 3.167[e] | 0.441 | <.0001 | |
| SAEW | 3.455[f] | 0.485 | <.0001 | |
| SH | 2.677[d] | 0.393 | <.0001 | |
| TA | 2.369[c] | 0.535 | <.0001 | |
| Tsunami | 2.976[e] | 0.472 | <.0001 | |
| log(Concentration) | 0.367 | 0.053 | <.0001 | |
| Temperature | 0.019 | 0.009 | 0.049 | |
| Time | 0.071 | 0.007 | <.0001 | |
| Variances | | | | |
| $s^2_u$ (intercept) | 0.689 | | | $I^2$=64% |
| $s^2$ (residual) | 0.392 | | | |

[a-g] Different superscript letters indicate that sanitizers have significantly-different effects

**Table 4.** Parameter estimates of the meta-analysis model predicting the log-reduction (R) of *L. monocytogenes* in fresh produce as a function of sanitizer, sanitizer concentration, and washing time and temperature

| Parameters | Mean | Standard error | Pr > \|t\| | AIC / BIC |
|---|---|---|---|---|
| Predictors of R | | | | |
| Sanitizer | | | | |
| ASC | 5.442[f] | 0.493 | <.0001 | 327/361 |
| CA | 3.282[a] | 0.696 | <.0001 | |
| CD | 7.058[g] | 0.857 | <.0001 | |
| LA | 3.620[b] | 0.711 | <.0001 | |
| MA | 4.218[d] | 0.711 | <.0001 | |
| PAA | 3.980[c] | 0.622 | <.0001 | |
| SAEW | 5.147[e] | 0.678 | <.0001 | |
| SH | 3.594[b] | 0.502 | <.0001 | |
| log(Concentration) | 0.617 | 0.091 | <.0001 | |
| Temperature | nd[x] | | | |
| Time | 0.081 | 0.008 | <.0001 | |
| Variances | | | | |
| $s^2_u$ (intercept) | 0.613 | | | $I^2$=60% |
| $s^2$ (residual) | 0.397 | | | |

[a-g] Different superscript letters indicate that sanitizers have significantly-different effects[x] Not determined. Temperature effect could not be estimated as log reduction values for *L. monocytogenes* were mostly obtained at ambient temperature (mean=21.0ºC)

**Table 5.** Parameter estimates of the meta-analysis model predicting the log-reduction (R) of *Salmonella* spp. in fresh produce as a function of sanitizer, sanitizer concentration, and washing time and temperature

| Parameters | Mean | Standard error | Pr > \|t\| | AIC / BIC |
|---|---|---|---|---|
| Predictors of R | | | | |
| Sanitizer | | | | |
| AA | 3.622[a] | 0.504 | <.0001 | 850/908 |
| ASC | 8.095[g] | 0.517 | <.0001 | |
| CA | 4.010[b] | 0.500 | <.0001 | |
| CD | 8.225[g] | 0.672 | <.0001 | |
| HP | 4.451[c] | 0.553 | <.0001 | |
| LA | 3.797[a] | 0.515 | <.0001 | |
| MA | 4.000[b] | 0.532 | <.0001 | |
| PAA | 6.675[ef] | 0.504 | <.0001 | |
| SAEW | 6.901[f] | 0.755 | <.0001 | |
| SDS | 3.779[a] | 0.582 | <.0001 | |
| SH | 5.980[d] | 0.436 | <.0001 | |
| TSP | 6.315[e] | 0.535 | <.0001 | |
| log(Concentration) | 0.771 | 0.070 | <.0001 | |
| Temperature | nd[x] | | | |
| Time | 0.049 | 0.007 | <.0001 | |
| Variances | | | | |
| $s^2_u$ (intercept) | 1.269 | | | $I^2$=80% |
| $s^2$ (residual) | 0.313 | | | |

[a-g] Different superscript letters indicate that sanitizers have significantly-different effects

[x] Not determined. Temperature effect could not be estimated as log reduction values for *Salmonella* spp. were mostly obtained at ambient temperature (mean=22.5ºC)

## Conclusão geral

O uso da meta-análise, como ferramenta estatística, foi bastante útil no estudo de problemas relacionados a qualidade e segurança microbiológica de alimentos. Neste sentido, aliar a abordagem meta-análitica com a modelagem preditiva, de maneira inovadora, permitiu compilar dados disponíveis na literatura científica até então não relacionados uns com os outros. Como esperado, a integralização dos dados compilados apresentou heterogeneidade entre os estudos. No entanto, se tratando de estudos similares a abordagem meta-analítica aliada à modelagem preditiva permitiu a elevação do poder estatístico e que os dados fossem relacionados entre si e novos resultados a partir dessa relação fossem extraídos.

Em geral, a variabilidade dos dados coletados no estudo da modelagem dos efeitos da temperatura e pH na resistência térmica de *A. acidoterrestris* se encontra no tipo de fruta, no tipo de suco (concentrado ou reconstituído), na presença de bacteriocinas e se ocorreu o processo de clarificação. Com base nisso, o modelo meta-analítico linear de efeitos mistos construído foi responsável por correlacionar todas as variáveis e reduzir a heterogeneidade de $\mathbf{t}^2$=0,072 para $\mathbf{t}^2$=0,044.

Dessa maneira, o modelo meta-analítico desenvolvido foi capaz de mostrar que o pH influenciou menos na resistência térmica de *A. acidoterrestris* do que a temperatura. Sucos concentrados tornam o esporo de *A. acidoterrestris* mais resistente às temperaturas de pasteurização. O fato de o suco ser clarificado não tem efeito sobre as variações de temperatura ($Z_T$) e pH ($Z_{pH}$), no entanto, a variável clarificação tem efeito significativo ($p < 0,0001$) quando tratado individualmente no valor *D*. De maneira oposta, a presença de bacteriocinas não interfere significativamente sobre o valor *D*, porém quando interagida com o pH elevou a sensibilidade térmica dos esporos.

Para o estudo dos sanitizantes a construção de três modelos meta-analíticos (modelo meta-analítico por sanitizante, por patógeno e análise de agrupamento) permitiu que, por meio do tratamento dos dados das mais de 1000 reduções logarítmicas dos três principais patógenos (*Salmonella* spp., *Escherichia coli* 0157:H7*, Listeria monocytogenes*), foi possível verificar a susceptibilidade dos três patógenos estudados em relação aos sanitizantes. Quanto menor for o intercepto os patógenos tendem a ser mais resistentes ao sanitizante. Por meio da análise de agrupamentos (*cluster*) os sanitizantes foram classificados de acordo a sua eficácia bactericida.

Os dados tratados por essa meta-análise revelam que a *L. monocytogenes* por apresentar o menor intercepto e a *Salmonella* o maior, são, respectivamente, mais e menos resistentes à ação dos sanitizantes. Dentre os 21 tipos de sanitizantes apurados, a água eletrolizada ligeiramente ácida (SAEW) apresentou a maior eficácia bactericida de todos os sanitizantes. Em relação aos 30 tipos de vegetais computados, os dados coletados sugerem que em vegetais folhosos a eficiência bactericida dos sanitizantes parece ser menos eficiente do que para os demais vegetais.

Os resultados obtidos neste trabalho podem servir de base para que outros estudos de meta-análise sejam planejados e conduzidos a fim de solucionar problemas da indústria de alimentos, no que se diz respeito à qualidade e segurança microbiológica dos alimentos.

# ANEXOS

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# Modeling the effects of temperature and pH on the resistance of *Alicyclobacillus acidoterrestris* in conventional heat-treated fruit beverages through a meta-analysis approach

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ABSTRACT

In this work, all publicly-accessible published findings on *Alicyclobacillus acidoterrestris* heat resistance in fruit beverages as affected by temperature and pH were compiled. Then, study characteristics (protocols, fruit and variety, °Brix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) were extracted from the primary studies, and some of them incorporated to a meta-analysis mixed-effects linear model based on the basic Bigelow equation describing the heat resistance parameters of this bacterium. The model estimated mean $D^*$ values (time needed for one log reduction at a temperature of 95 °C and a pH of 3.5) of *Alicyclobacillus* in beverages of different fruits, two different concentration types, with and without bacteriocins, and with and without clarification. The $z_T$ (temperature change needed to cause one log reduction in *D*-values) estimated by the meta-analysis model were compared to those ('observed' $z_T$ values) reported in the primary studies, and in all cases they were within the confidence intervals of the model. The model was capable of predicting the heat resistance parameters of *Alicyclobacillus* in fruit beverages beyond the types available in the meta-analytical data. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, will be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes.



## 1. Introduction

The microbiological stability of shelf-stable fruit juices is based on the combination of their low pH values (usually ≤3.8) with heat treatments designed to inactivate the most heat resistant microorganisms found. Throughout the decades, several microorganisms have been used as targets of fruit juice pasteurization processes, including yeasts, lactic acid bacteria, heat resistant molds and spore-forming bacteria (Tribst et al., 2009). However, since early 80's, fruit juice processors have been challenged by a bacterium showing remarkable heat and chemical resistances, ability to grow under acidic conditions and, consequently, to spoil shelf-stable fruit juices (Silva and Gibbs, 2001; Friedrich et al., 2009; Spinelli et al., 2009, 2010). This bacterium was characterized by the presence of ω-alicyclic fatty acids as major lipid components on the cellular membrane, which together with 16S rRNA sequencing analyses led to the proposal for creation of a new genus, *Alicyclobacillus* (Wisotzkey et al., 1992). Currently, it is known that members of the *Alicyclobacillus* genus are surprisingly diverse and not all species have been described as containing these characteristic fatty acids (Glaeser et al., 2013). Presently, more than 20 species have been reported to belong to *Alicyclobacillus* genus (Smit et al., 2011; Glaeser et al., 2013), while spoilage potential of fruit juices has been restricted to few species such as *Alicyclobacillus acidoterrestris*, *Alicyclobacillus acidiphillus*, *Alicyclobacillus pomorum*, *Alicyclobacillus herbarius*, *Alicyclobacillus hesperidum*, *Alicyclobacillus acidocaldarius* and *Alicyclobacillus cycloheptanicus* (Cerny et al., 1984; Matsubara et al., 2002; Goto et al., 2003; AIJN, 2007; Smit et al., 2011). The spoilage potential of *Alicyclobacillus* species relies on their ability to produce off-flavor compounds such as 2-methoxyphenol (guaiacol), 2,6-dibromophenol, 2,6-dichlorophenol and 2-methyltetrahydrothiophene-3-one (Siegmund and Pöllinger-Zierler, 2006; Lottici et al., 2006; Siegmund and Pöllinger-Zierler, 2007; Concina et al., 2010).

Because of its spoilage potential, several reports are found on the incidence of *Alicyclobacillus* in fruit and vegetable beverages

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(Siegmund and Pöllinger-Zierler, 2006; Durak et al., 2010; Steyn et al., 2011; Walls and Chuyate, 2000; Groenewald et al., 2009; McKnight et al., 2010; Danyluk et al., 2011; Oteiza et al., 2011). Also, as a major target for fruit juice pasteurization (Tribst et al., 2009), numerous studies are found that report thermal inactivation parameters of *Alicyclobacillus*, *i.e.*, the *D* value (time at a determined temperature required to cause one-log cycle decrease in the population of a target bacterium) and the *z* value (temperature increase required to result in one-log cycle decrease of *D*-value) (Splittstoesser et al., 1994; Komitopoulou et al., 1999; Bahçeci and Acar, 2007; Walls, 1997; Silva et al., 1999; Maldonado et al., 2008; de Carvalho et al., 2008; López et al., 2011; Alberice et al., 2012, Peña et al., 2009; McKnight et al., 2010). As known, *D*- and *z*-values of *Alicyclobacillus* are affected by the particular conditions or *study characteristics* (protocols, fruit and variety, °Brix, pH, temperature, heating medium, culture medium, inactivation method, strains, etc.) under which they were obtained. Therefore, variability in *D*- and *z*-values among primary studies is expected to occur, even among studies investigating the same type of fruit beverage. Nonetheless, by means of *a posteriori* analysis and identification – from each of the primary studies – of the sources of variability impacting on the thermal inactivation parameters of *Alicyclobacillus*, it may be possible to explain, to some extent, the differences found among the study outcomes.

To this respect, meta-analysis, defined as a *"statistical analysis of a collection of analytic results for the purpose of integrating the findings from a large amount of primary studies"* (DerSimonian and Laird, 1986), allows (i) the explanation of the divergences in the study outcomes by the codification of study characteristics (i.e., moderating variables related to research design features, data collection procedures, type of samples, etc.) aiming to reduce the between-study heterogeneity or variability (Gonzales-Barron et al., 2013); and (ii) the accurate estimation of the overall outcome measure, with increased statistical power than using only a single study (Sutton et al., 2001). Despite the capabilities of meta-analysis, already long recognized in medicine and clinical studies, the application of this body of compiling statistical techniques in food safety and microbiology issues is recent (Gonzales-Barron et al., 2008; Gonzales-Barron and Butler, 2011; Den Besten and Zwietering, 2012; Gonzales-Barron et al., 2013). Thus, the first objective of this study is to compile all publicly-accessible published findings on the heat resistance of *A. acidoterrestris* in fruit beverages as affected by temperature and pH, and quantitatively summarize these outcomes by means of a meta-analytical model based on a Bigelow-type secondary predictive model. A second objective is to attempt to explain a proportion of the total between-study heterogeneity in the heat resistance parameters by incorporating available study characteristics to the basic model. The resulting meta-analysis model (i.e., a mixed-effects linear model based on the Bigelow equation) should be effective in estimating the thermal inactivation parameters, *D*- and *z*-values, for the various types of beverage considered.

## 2. Methodology

### 2.1. Data collection

Literature identification was conducted using electronic search through Google with key terms, both in English and in Portuguese, including: "*Alicyclobacillus*", "ATSB", "Acidothermophilic sporeforming bacteria", "heat resistance", "*D*-value", "thermal resistance", "inactivation", "fruit juice", "juice", "beverages". Also, literature for inclusion in the study was identified from bibliographic databases such as Pubmed, Science Direct and Scopus, using the same keywords. Data included studies electronically available in scientific journals and electronically from 1980 to 2014. A total of 55 studies on inactivation of *Alicyclobacillus* spores in fruit beverages were retrieved, however, these included also reports using high pressure processing, ultrasound, pulsed electric field and pulsed light. Nonetheless, for inclusion in the meta-analysis, only conventional heat-related studies were considered, which originated from peer-reviewed scientific papers. A second criterion used in the screening was the need for the primary study to model first-order reaction kinetics; said otherwise, studies reporting on inactivation of *Alicyclobacillus* in fruit beverages with no *D*-values were excluded from the meta-analysis. Additionally, for a primary study to be included in the meta-analysis, it had to report more than two *D*-values, measured either at different inactivation temperatures or at different beverage pH. The statistical reason for this was that, for the meta-analytical mixed-effects linear model explained in Section 2.2, the standard error about the $z_T$ or $z_{pH}$ value (inverse of the slope between log *D* and temperature or pH, respectively) of a particular experiment could be only measured with more than two points along a fitted straight line. This restriction caused the results from four primary studies to be omitted for the analysis: Yamazaki et al. (1997) who reported two *D*-values for orange juice; Baumgart (1999) with only one *D*-value for orange juice; Vieira et al. (2002) reporting one D-value for cupuaçu concentrate; and Baysal and Icier (2010) who reported only two *D*-values for orange juice. Thus, 11 primary studies were selected and considered appropriate for the meta-analysis model, providing a total of 142 *D*-values obtained at different inactivation temperatures and pH values (Table 1).

### 2.2. Description of the data set

Apart from the *D*-values, the corresponding beverage pH and the temperatures at which the isothermal experiments were conducted, additional information was also extracted from the primary studies. It is known that the content of soluble solids or °Brix of the beverage is an important physicochemical parameter affecting the heat resistance of *Alicyclobacillus* (Splittstoesser et al., 1998). However, as such information was not available for every primary study, a categorical variable "type of beverage" was created to assign fruit beverages either to a single strength juice or to a concentrate class. It was defined that *D*-values obtained from beverages of either Brix above 18°, or *concentrates* and *nectars* (stated as such in the primary studies yet with no indication of the level of soluble solids) were assigned to the "concentrates" category. Single strength juices presented an average concentration of soluble solids of 10.2% (ranging from 5.3 to 13.0%) while fruit concentrates presented an average concentration of 48.0% (ranging from 18.0 to 68.0%).

Another study characteristic to codify (or to disaggregate) was the fruit. *D*-values were assigned to ten different fruit classes: apple, berry, cupuaçu, grape, grapefruit, lemon, mango, orange, passion fruit and tangerine. A special class named as "model" (Table 1) was created within the moderating variable fruit to encompass the results from López et al. (2011) and Bahçeci and Acar (2007), who employed citrate phosphate McIlvaine buffer to estimate the heat resistance of *Alicyclobacillus* at different pH values. This buffer is an acidic solution that has been proposed to model thermal process and heat transfer studies in fruit products.

The third moderator variable was "clarification" to indicate whether or not fruit beverages underwent the normal clarification process followed by filtration to separate the particles in suspension in the beverage. This was a coded variable taking the value of 0 for non-clarified beverages and the value of 1 for clarified beverages. For the special case of the *model* category within the fruit moderating variable, the "clarified" class was assigned because of the low viscosity and the absence of particles in suspension in a buffer (Table 1). On the other hand, the study of de Carvalho et al. (2008), which focused on mango concentrate, did not specify

**Table 1**
Meta-analytical data of *D*-values of *Alicyclobacillus acidoterrestris* in beverages at different temperature and pH, with extracted study characteristics of fruit, type (single strength or concentrate), clarification (0 = no, 1 = yes), bacteriocins (0 = no, 1 = yes) and sample size *N* used to estimate a single *D*-value.

| Fruit | Type | Clarification | Bacteriocins | pH | T (°C) | *D* (min) | *N* | Source |
|---|---|---|---|---|---|---|---|---|
| Apple | Single strength | 0 | 0 | 3.50 | 85 | 56.0 | 20 | Splittstoesser et al. (1994) |
| | | 0 | 0 | 3.50 | 90 | 23.0 | 20 | |
| | | 0 | 0 | 3.50 | 95 | 2.80 | 20 | |
| | | 1 | 0 | 3.51 | 80 | 41.2 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.51 | 90 | 7.38 | 22 | |
| | | 1 | 0 | 3.51 | 95 | 2.30 | 22 | |
| | | 1 | 0 | 3.68 | 90 | 11.1 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 3.68 | 93 | 4.20 | 25 | |
| | | 1 | 0 | 3.68 | 96 | 2.10 | 25 | |
| | | 1 | 0 | 3.68 | 100 | 0.70 | 25 | |
| | | 1 | 1 | 3.51 | 80 | 23.8 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 1 | 3.51 | 90 | 4.56 | 22 | |
| | | 1 | 1 | 3.51 | 95 | 1.95 | 22 | |
| | Concentrate | 1 | 0 | 2.97 | 90 | 14.4 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 2.97 | 93 | 6.70 | 25 | |
| | | 1 | 0 | 2.97 | 96 | 3.30 | 25 | |
| | | 1 | 0 | 2.97 | 100 | 1.20 | 25 | |
| | | 1 | 0 | 2.95 | 90 | 14.1 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 2.95 | 93 | 6.40 | 25 | |
| | | 1 | 0 | 2.95 | 96 | 3.10 | 25 | |
| | | 1 | 0 | 2.95 | 100 | 1.00 | 25 | |
| Berry | Single strength | 1 | 0 | 3.50 | 88 | 11.0 | 20 | Walls (1997) |
| | | 1 | 0 | 3.50 | 91 | 3.80 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 1.00 | 20 | |
| Cupuaçu | Single strength | 1 | 0 | 3.60 | 85 | 17.5 | 20 | Silva et al. (1999) |
| | | 1 | 0 | 3.60 | 91 | 5.35 | 20 | |
| | | 1 | 0 | 3.60 | 95 | 2.82 | 20 | |
| | | 1 | 0 | 3.60 | 97 | 0.57 | 20 | |
| Grape | Single strength | 1 | 0 | 3.30 | 85 | 57.0 | 20 | Splittstoesser et al. (1994) |
| | | 1 | 0 | 3.30 | 90 | 16.0 | 20 | |
| | | 1 | 0 | 3.30 | 95 | 2.40 | 20 | |
| Grapefruit | Single strength | 1 | 0 | 3.42 | 80 | 37.8 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.42 | 90 | 5.95 | 22 | |
| | | 1 | 0 | 3.42 | 95 | 1.85 | 22 | |
| | | 1 | 0 | 3.00 | 80 | 31.85 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.00 | 90 | 5.69 | 22 | |
| | | 1 | 0 | 3.00 | 95 | 1.49 | 22 | |
| | | 1 | 0 | 4.00 | 80 | 52.35 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 4.00 | 90 | 9.44 | 22 | |
| | | 1 | 0 | 4.00 | 95 | 1.73 | 22 | |
| Lemon | Single strength | 0 | 0 | 2.45 | 82 | 16.72 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 2.45 | 86 | 11.32 | 20 | |
| | | 0 | 0 | 2.45 | 92 | 10.58 | 20 | |
| | | 0 | 0 | 2.45 | 95 | 9.98 | 20 | |
| | | 0 | 0 | 2.45 | 82 | 17.82 | 20 | |
| | | 0 | 0 | 2.45 | 95 | 9.44 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 11.23 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 3.50 | 86 | 10.54 | 20 | |
| | | 1 | 0 | 3.50 | 92 | 9.47 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 8.55 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 13.21 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 9.38 | 20 | |
| | Concentrate | 0 | 0 | 2.28 | 82 | 15.50 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 2.28 | 86 | 14.54 | 20 | |
| | | 0 | 0 | 2.28 | 92 | 8.81 | 20 | |
| | | 0 | 0 | 2.28 | 95 | 8.55 | 20 | |
| | | 0 | 0 | 2.80 | 82 | 50.50 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 2.80 | 86 | 39.30 | 20 | |
| | | 0 | 0 | 2.80 | 92 | 31.67 | 20 | |
| | | 0 | 0 | 2.80 | 95 | 22.03 | 20 | |
| | | 0 | 0 | 3.50 | 82 | 95.15 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 3.50 | 86 | 59.50 | 20 | |
| | | 0 | 0 | 3.50 | 92 | 38.00 | 20 | |
| | | 0 | 0 | 3.50 | 95 | 17.22 | 20 | |
| | | 0 | 0 | 4.00 | 82 | 85.29 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 4.00 | 86 | 58.15 | 20 | |
| | | 0 | 0 | 4.00 | 92 | 27.48 | 20 | |
| | | 0 | 0 | 4.00 | 95 | 23.33 | 20 | |
| | | 0 | 0 | 2.45 | 82 | 15.50 | 20 | Maldonado et al. (2008) |
| | | 0 | 0 | 2.45 | 86 | 14.54 | 20 | |

(*continued on next page*)

**Table 1** (*continued*)

| Fruit | Type | Clarification | Bacteriocins | pH | T (°C) | *D* (min) | *N* | Source |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 0 | 2.45 | 92 | 8.81 | 20 | |
| | | 0 | 0 | 2.45 | 95 | 8.56 | 20 | |
| | | 1 | 0 | 2.28 | 82 | 17.36 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 2.28 | 86 | 18.06 | 20 | |
| | | 1 | 0 | 2.28 | 92 | 7.60 | 20 | |
| | | 1 | 0 | 2.28 | 95 | 6.20 | 20 | |
| | | 1 | 0 | 2.80 | 82 | 25.81 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 2.80 | 86 | 22.01 | 20 | |
| | | 1 | 0 | 2.80 | 92 | 15.35 | 20 | |
| | | 1 | 0 | 2.80 | 95 | 11.3 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 68.9 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 3.50 | 86 | 33.7 | 20 | |
| | | 1 | 0 | 3.50 | 92 | 16.8 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 12.6 | 20 | |
| | | 1 | 0 | 4.00 | 82 | 35.2 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 4.00 | 86 | 23.2 | 20 | |
| | | 1 | 0 | 4.00 | 92 | 21.9 | 20 | |
| | | 1 | 0 | 4.00 | 95 | 9.72 | 20 | |
| | | 1 | 0 | 3.50 | 82 | 18.1 | 20 | Maldonado et al. (2008) |
| | | 1 | 0 | 3.50 | 86 | 17.4 | 20 | |
| | | 1 | 0 | 3.50 | 92 | 7.60 | 20 | |
| | | 1 | 0 | 3.50 | 95 | 6.20 | 20 | |
| Mango | Concentrate | 0 | 0 | 4.00 | 80 | 40.0 | 15 | de Carvalho et al. (2008) |
| | | 0 | 0 | 4.00 | 85 | 25.0 | 15 | |
| | | 0 | 0 | 4.00 | 90 | 11.7 | 15 | |
| | | 0 | 0 | 4.00 | 95 | 8.33 | 15 | |
| | | 0 | 1 | 4.00 | 80 | 9.20 | 15 | de Carvalho et al. (2008) |
| | | 0 | 1 | 4.00 | 85 | 5.00 | 15 | |
| | | 0 | 1 | 4.00 | 90 | 1.16 | 15 | |
| | | 0 | 1 | 4.00 | 95 | 0.36 | 15 | |
| Model | Single strength | 1 | 0 | 3.00 | 90 | 6.00 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 3.00 | 93 | 2.80 | 25 | |
| | | 1 | 0 | 3.00 | 96 | 1.10 | 25 | |
| | | 1 | 0 | 3.00 | 100 | 0.40 | 25 | |
| | | 1 | 0 | 3.50 | 90 | 6.50 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 3.50 | 93 | 3.20 | 25 | |
| | | 1 | 0 | 3.50 | 96 | 1.30 | 25 | |
| | | 1 | 0 | 3.50 | 100 | 0.40 | 25 | |
| | | 1 | 0 | 4.00 | 90 | 7.30 | 25 | Bahçeci and Acar (2007) |
| | | 1 | 0 | 4.00 | 93 | 3.80 | 25 | |
| | | 1 | 0 | 4.00 | 96 | 1.70 | 25 | |
| | | 1 | 0 | 4.00 | 100 | 0.50 | 25 | |
| | | 1 | 0 | 3.50 | 90 | 6.00 | 18 | López et al. (2011) |
| | | 1 | 0 | 3.50 | 95 | 2.20 | 18 | |
| | | 1 | 0 | 3.50 | 100 | 0.83 | 18 | |
| | | 1 | 0 | 3.50 | 105 | 0.34 | 18 | |
| Orange | Single strength | 1 | 0 | 3.90 | 80 | 54.3 | 18 | Komitopoulou et al. (1999) |
| | | 1 | 0 | 3.90 | 90 | 10.3 | 22 | |
| | | 1 | 0 | 3.90 | 95 | 3.59 | 22 | |
| | | 1 | 0 | 3.57 | 80 | 16.3 | 15 | Alberice et al. (2012) |
| | | 1 | 0 | 3.57 | 87 | 12.5 | 15 | |
| | | 1 | 0 | 3.57 | 95 | 10.8 | 12 | |
| | | 1 | 0 | 3.57 | 99 | 1.38 | 12 | |
| | Concentrate | 0 | 0 | 3.68 | 92 | 25.6 | 10 | Peña et al. (2009) |
| | | 0 | 0 | 3.68 | 95 | 12.9 | 10 | |
| | | 0 | 0 | 3.68 | 98 | 6.16 | 10 | |
| | | 0 | 0 | 3.68 | 102 | 2.01 | 10 | |
| | | 0 | 1 | 3.68 | 95 | 11.4 | 10 | Peña et al. (2009) |
| | | 0 | 1 | 3.68 | 98 | 5.55 | 10 | |
| | | 0 | 1 | 3.68 | 102 | 1.83 | 10 | |
| | | 1 | 0 | 2.95 | 80 | 18.4 | 15 | Alberice et al. (2012) |
| | | 1 | 0 | 2.95 | 87 | 13.4 | 15 | |
| | | 1 | 0 | 2.95 | 95 | 10.6 | 12 | |
| | | 1 | 0 | 2.95 | 99 | 1.67 | 12 | |
| Passion fruit | Single strength | 1 | 0 | 3.50 | 87 | 20.9 | 12 | McKnight et al. (2010) |
| | | 1 | 0 | 3.50 | 90 | 5.12 | 12 | |
| | | 1 | 0 | 3.50 | 95 | 1.62 | 12 | |
| Tangerine | Single strength | 0 | 0 | 3.50 | 90 | 15.0 | 18 | López et al. (2011) |
| | | 0 | 0 | 3.50 | 95 | 6.20 | 18 | |
| | | 0 | 0 | 3.50 | 100 | 2.10 | 18 | |
| | | 0 | 0 | 3.50 | 105 | 0.63 | 18 | |

whether the concentrate was clarified or not. However, as the main objective of such a study was to assess the effect of bovicin on the resistance of *Alicyclobacilus* in mango pulp, a logical conclusion was that the mango pulp, which was two-fold diluted for their experiments (i.e., concentrate), was not clarified.

The fourth study characteristic was "presence of bacteriocins", which was conceived because two of the primary studies investigated the effect of nisin (Komitopoulou et al., 1999; Peña et al., 2009) on the thermal resistance of *Alicyclobacillus*; and one study the effect of bovicin HC5 — a bacteriocin from *Streptococcus bovis* (de Carvalho et al., 2008). Thus, this categorical variable was coded to take up the value of 0 for absence of bacteriocins and the value of 1 for added bacteriocins. While de Carvalho et al. (2008) employed a concentration of bovicin HC5 of 80 IU/ml in mango concentrate, Komitopoulou et al. (1999) and Peña et al. (2009) assessed both a concentration of 50 IU/ml in apple and orange juice, respectively.

A summary of the input data for the meta-analysis study is presented in Table 1. It should be noticed that such meta-analytical data is highly sparse, meaning that for some fruits less data are available. For instance, for apple, lemon and orange, data for both types of beverages — juice and concentrate — were found, and additionally for clarified and non-clarified beverages, while for other fruits such as grape and passion fruit, data were limited to clarified juices only. This has some implications in the design of the meta-analysis mixed-effects model, as explained in Section 2.3.

### 2.3. Meta-analytical model

To describe the combined effect of temperature and pH on the heat resistance of *Alicyclobacillus* in fruit beverages, the Bigelow-type linear model was selected (Mafart and Leguerinel, 1998):

$$\log D = \log D^* - \left(\frac{1}{z_T}\right)(T - T^*) - \left(\frac{1}{z_{pH}}\right)(\mathrm{pH} - \mathrm{pH}^*) \quad (1)$$

where $D$ is time at a constant temperature $T$ and at the pH of the food matrix required to cause one-log cycle decrease in the population of a target bacterium; $T^*$ is the reference temperature (set at 95 °C, which is a common temperature for fruit juice pasteurization); pH* is the reference pH (chosen to be 3.5 to correspond to a common pH of fruit beverages); $z_T$ is the conventional thermal $z$-value; $z_{\mathrm{pH}}$ is the distance of pH from pH* which leads to a ten-fold reduction of the decimal reduction time; and $D^*$ is the decimal reduction time at $T^*$ and pH*.

The Bigelow secondary predictive model was used to interpret the combined results of the primary studies. As the meta-analytical data obtained also contain a number of moderating variables or coded study characteristics (for example, fruit, type of beverage, addition of bacteriocin and application of clarification), the Bigelow model was transformed into a linear mixed-effects model in order to assess whether each of the moderating variables has any effect on $D^*$ and/or $z_T$ and $z_{\mathrm{pH}}$. Hence, the three parameters of Equation (1) were modelled as.

$$\log D^*_{ijlm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} = \log D^*_{\mathrm{mean}\ ij} + u_{lm} \quad (2)$$

$$\frac{1}{z_{T\,ilm}} = (\gamma_1 + \gamma_{2i} + v_{lm}) \quad (3)$$

$$\frac{1}{z_{\mathrm{pH}_k}} = (\delta_1 + \delta_{2k}) \quad (4)$$

Where: $\beta_0$ is an intercept, $\beta_1$ is the fixed effect of the type of beverage $i$ (coded as 0 for single strength juice and 1 for concentrates), $\beta_2$ is the fixed effect of the clarification stage $j$ (coded as 0 for no clarification and 1 for regular clarification). A fixed effect of the addition of bacteriocin on log $D^*$ was not considered as it turned out to be non-significant. The value of $D^*_{\mathrm{mean}\ ij}$ represents the average decimal reduction time at the reference $T^*$ and pH* applicable to the entire population of fruits, yet it is an intercept allowed to take up different independent values due to the variability in the fruit/primary study combination (viz. interaction). Because of the sparse nature of the data structure, whereby in most cases one primary study reported results for only one fruit (Table 1), for the analysis it was not feasible either to separate the between-fruit variability from the between-study variability or to build a nested covariance of primary studies within fruit or fruits within primary study. To overcome this problem and still be able to account for the evident variability due to the different fruits ($l$) and primary studies ($m$), both variables had to be merged into an interaction variable ($lm$) providing sixteen levels to be used as the subject of variation of the random effects placed in Equation (2). These intercept random effects $u_{lm}$ are assumed to have a normal distribution with mean zero and variance $s^2_u$.

The coefficient $\gamma_1$ is the mean effect of a 1°C-increment in temperature ($T$–$T^*$) for the entire population of fruit beverages; yet, the coefficient for the temperature difference slope is affected by the type of beverage $i$ and by the specific combination of fruit ($l$) and primary study ($m$). Neither clarification $j$ nor bacteriocin $k$ was included as a predictor of the temperature difference slope because they were not statistically significant. $\gamma_2$ is the fixed effect of the interaction term between the type of beverage $i$ and the temperature slope. Since preliminary analysis of the meta-analytical data had shown that the temperature slopes for single strength juice tended to be steeper than those for concentrates, this variability was accounted for. As done for the intercept random effects, the interaction between fruit and primary study ($lm$) was assumed to be the subject of variation of the random effects $v_{il}$. The random effects $v_{il}$ added to the slope $\gamma_1 + \gamma_2$ model the shifts in the temperature effect for each of the primary study × fruit existing in the data set. These slope random effects are assumed to follow a normal distribution with mean zero and variance $s^2_v$. Placing a fixed effect on the type of beverage and random effects for fruits (interacting with the primary studies) in Equation (3) enables the model to compute the $z_T$ values for all the combinations of fruit and type of beverage, even beyond the combinations existing in the original meta-analytical data.

The coefficient $\delta_1$ represents the effect of the increment in the pH difference (pH–pH*), and $\delta_2$ the coefficient of the interaction term between addition/non-addition of a bacteriocin ($k$) and the pH slope. This interaction allows for a change in the pH difference slope when a bacteriocin is added to the beverage. Fixed effects of the type of beverage $i$ and the application of clarification $j$ were not included in Equation (3) for being non-significant. Random variations in the pH slope due to beverage type and fruit were not modelled in Equation (3) as they turned out to be non-significant. The variances of the random effects placed on the intercept and temperature slope, $s^2_u$ and $s^2_v$, were assumed to be correlated with a covariance $s^2_{uv}$. As all those variance and covariance terms can be thought of as realisations of a *primary study*, the presence of heterogeneity among primary studies can be assessed by the Wald's test of significance of each of the variance, $s^2_u$ and $s^2_v$, and covariance $s^2_{uv}$ parameters. Hence, if those terms were statistically significant, the between-study variability $\tau^2$ can be approximated by $s^2_u + s^2_v + s^2_{uv}$, and the $I^2$ statistics or intra-class correlation, estimating the proportion of between-study variability from the total variance, can be approached as $(s^2_u + s^2_v + s^2_{uv})/(s^2_u + s^2_v + s^2_{uv} + s^2)$, where $s^2$ is the variance of the normally-distributed residual random errors $\varepsilon_{ijklm}$.

Thus, putting together Equations (2)–(4), the linear mixed-effects model adjusted to the meta-analytical data was.

$$\log D_{ijklm} = (\beta_0 + \beta_{1i} + \beta_{2j}) + u_{lm} - (\gamma_1 + \gamma_{2i} + v_{lm})(T - T^*) - (\delta_1 + \delta_{2k})(\mathrm{pH} - \mathrm{pH}^*) + \varepsilon_{ijklm} \quad (5)$$

Notice that the values of log $D^*$, $z_T$ and $z_{\mathrm{pH}}$ can be estimated from the model's fitted parameters using Equations (2)–(4), respectively. In building the meta-analysis mixed model, all the interaction terms between the categorical moderating variables, and with pH and temperature were evaluated. Because of data sparseness, only interactions of two terms were considered. However, only two interaction terms were found to be statistically significant (i.e., slope of temperature difference with type of beverage and slope of pH with presence of bacteriocins), which were retained in the model. Similarly, a series of combinations of random effects attempting to extract the variability between fruits and the variability between primary studies, both separately and as interactions, were placed in Equations (2)–(4), and their results compared one-to-one by a log-likelihood ratio test and the Bayesian Information Criterion (BIC). The model presented in Equation (5) was the most parsimonious (i.e., least parameters with the best goodness-of-fit), and yet, with a fully interpretable arrangement. Since primary studies are expected to differ from each other in the reliability of estimating the true heat resistance parameters of *A. acidoterrestris* in fruit beverages, for instance, due to differences in study sizes, a *weighted* linear mixed model was preferred, with weights representing the precision in estimating the population lethality parameters. Because not all primary studies reported the standard error of the *D*-value, the precision was defined as some measure proportional to the sample size N used in the bacterial kinetics experiments to calculate a single *D*-value. Hence, the weight – level of confidence on each *D* measure – was given by the sample size. Table 1 also compiles the sample size used to determine each of the *D*-values, which was calculated as the number of sample units analysed multiplied by the number of points in time where samples were taken to measure the concentration of *Alicyclobacillus*. Once the model was fitted, the normality of residuals was assessed and the studentised residuals examined for identifying spurious data points lower than −3.0 and higher than 3.0. The weighted mixed-effects linear model was fitted in R version 2.14.2 (R Development Core Team) using the 'nlme' package (Pinheiro et al., 2013).

## 3. Results and discussion

The management of microbial spoilage of fruit beverages requires the ability to predict the thermal resistance of the spores of *A. acidoterrestris*. During this systematic review, it was realized that there are in the literature numerous studies reporting useful data on the thermal death kinetics of this spoilage microorganism, which, in principle, could be applied for the determination and optimisation of the process variables for heat treatment. However, the large number and variety of data, and principally, the different estimates of the thermal inactivation parameters among studies, make further developments difficult. For instance, the study of Komitopoulou et al. (1999) reported a $z_T$-value of 12.9 °C for orange juice at a pH of 3.9, while Yamazaki et al. (1997) found a lower $z_T$-value of 9.5 °C for orange juice at a similar pH of 3.7. Similarly, for apple juice at a pH of 3.5, Komitopoulou et al. (1999) and Splittstoesser et al. (1994) found dissimilar $z_T$-values of 12.2 °C and 7.7 °C, respectively. The degree of discrepancies in the relationship between *D*-value and temperature observed in the input data set can be visually assessed in Fig. 1. In such a *Figure*, the same markers depict a sub-group of observations from a given set of heat inactivation isothermal experiments conducted to determine a $z_T$ value at fixed conditions; said otherwise, a sub-group is formed by the paired observations (*D*-value, temperature) extracted for a given fruit, type of beverage, clarification, bacteriocin and pH value. From Table 1, it can be deduced that there were 37 sub-groups. Fig. 1 shows that the *D*-values from the 37 sub-groups were all consistent as they decrease with increasing temperatures, yet it also hinted that, in designing a meta-analytical linear model, some allowance had to be made in relation to the variability of the intercepts and slopes (inverse of $z_T$) by incorporating random effects. In a multilevel meta-analysis, as is the case here, one usually starts assessing the null random-effects model. In our case, the null random-effects model is the simple Bigelow model (Equation (1)) with random effects placed on the intercept and the temperature difference slope. Such a model produced a value of heterogeneity $\tau^2$ of 0.072 while the variance of the residuals was 0.094 (results not shown). Thus, the intra-class correlation can be estimated ($I^2 = 0.072/(0.072 + 0.094) = 0.44$) at 44%. This value, being higher than the rule of thumb of 25% (Hunter and Schmidt, 1990), underscored the presence of significant heterogeneity; and, consequently, confirmed that some study characteristics had to be coded in an attempt to explain, understand and reduce such variability.

When the null random-effect model (basic Bigelow) was extended to a multilevel model (mixed-effects linear model comprising study characteristics or moderating variables; Equation (5)), the variance of the residuals reduced to 0.038, and the heterogeneity $\tau^2$ reduced to 0.044 (Table 2). This indicated that approximately 40% ((0.072−0.044)/0.072 = 0.389) of the total amount of heterogeneity due to primary studies and fruits could be explained by the categorical variables type of beverage, clarification and presence of bacteriocins. Because the residual heterogeneity $\tau^2$ of 0.044 is still significant (Table 2), it can be concluded that there may be other study characteristics, not coded in the present meta-analysis, that are likely to be also noteworthy. As Hox and De Leeuw (2003) pointed out, it is highly unlikely that the available study-level variables could cover all the artefacts causing variation between study outcomes. This occurs because the information given in research reports and articles is not enough to cover all the study characteristics; and in fact this was attested during the conduction of the present meta-analysis. For instance, while the specific strain inoculated in the essay and the method used to measure heat resistance may explain some of the between-study variability observed among the measured *D*-values, they could not be considered in the model since not all primary studies reported such information.

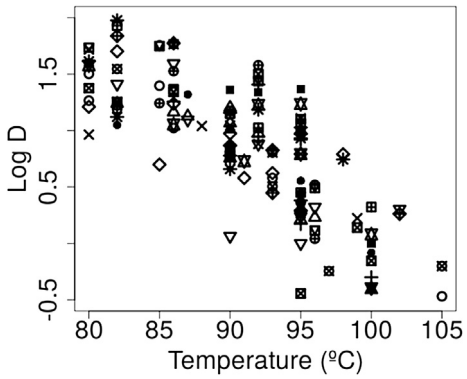


**Fig. 1.** Scatter plot of the available meta-analytical data of log *D* and temperature for the 37 sub-groups of isothermal experiments to determine a *z*-value.

**Table 2**
Parameter estimates of the Bigelow-type meta-analysis mixed-effects linear model predicting the log *D*-value of *Alicyclobacillus acidoterrestris* in fruit beverages as a function of temperature, pH and moderating variables.

| Parameters | Mean | Standard error | Pr > \|t\|, Z | AIC/BIC |
|---|---|---|---|---|
| **Predictors of log $D^*$** | | | | |
| $\beta_0$ (intercept) | 0.396 | 0.056 | <0.0001 | −80.0/−50.0 |
| $\beta_1$ (type) | −0.115 | 0.048 | 0.018 | |
| $\beta_2$ (clarification) | −0.261 | 0.037 | <0.0001 | |
| **Predictors of ($1/z_T$)** | | | | |
| $\gamma_1$ (temperature) | −0.089 | 0.008 | <0.0001 | |
| $\gamma_2$ (temperature × type) | 0.014 | 0.006 | 0.025 | |
| **Predictors of ($1/z_{pH}$)** | | | | |
| $\delta_1$ (pH) | 1.707 | 0.207 | <0.0001 | |
| $\delta_2$ (pH × bacteriocin) | −1.881 | 0.206 | <0.0001 | |
| **Variances** | | | | |
| $s^2_u$ (log $D^*_{mean}$) | 0.0389 | 0.0162 | 0.008 | $\tau^2$~0.044 |
| $s^2_v$ (temperature) | 0.0010 | 0.0004 | 0.012 | $I^2$~53.9% |
| $s^2_{uv}$ (covariance) | 0.0045 | 0.0023 | 0.050 | |
| $s^2$ (residual) | 0.0380 | 0.0053 | <0.0001 | |

**Table 3**
Estimates of log $D^*$ (log *D*-value at 95 °C and pH 3.5) for different combinations of fruits and the moderating variables, type of beverage and with/without clarification process.

| Parameter | Mean | Standard error | 95% CI |
|---|---|---|---|
| Overall mean | 0.584 | 0.055 | [0.474–0.694] |
| Single strength juice | 0.526 | 0.056 | [0.414–0.638] |
| Clarified | 0.396 | 0.056 | [0.285–0.507] |
| Non-clarified | 0.656 | 0.063 | [0.532–0.781] |
| Concentrate | 0.642 | 0.064 | [0.514–0.769] |
| Clarified | 0.511 | 0.068 | [0.376–0.645] |
| Non-clarified | 0.772 | 0.066 | [0.641–0.903] |
| Apple single strength juice | 0.470$^{c}$ | 0.047 | [0.377–0.565] |
| Berry single strength juice | 0.252$^{a}$ | 0.108 | [0.036–0.467] |
| Cupuaçu single strength juice | 0.355$^{b}$ | 0.078 | [0.200–0.510] |
| Grape single strength juice | 0.577$^{d}$ | 0.109 | [0.361–0.793] |
| Grapefruit single strength juice | 0.437$^{bc}$ | 0.064 | [0.310–0.564] |
| Lemon single strength juice | 1.007$^{f}$ | 0.048 | [0.912–1.103] |
| Mango single strength juice | 0.425$^{b}$ | 0.102 | [0.223–0.628] |
| Orange single strength juice | 0.695$^{e}$ | 0.054 | [0.587–0.803] |
| Passion fruit single strength juice | 0.401$^{b}$ | 0.130 | [0.142–0.660] |
| Tangerine single strength juice | 0.594$^{d}$ | 0.067 | [0.462–0.727] |
| Apple concentrate | 0.586$^{c}$ | 0.053 | [0.470–0.702] |
| Berry concentrate | 0.367$^{a}$ | 0.115 | [0.138–0.596] |
| Cupuaçu concentrate | 0.470$^{b}$ | 0.088 | [0.295–0.646] |
| Grape concentrate | 0.693$^{d}$ | 0.115 | [0.463–0.922] |
| Grapefruit concentrate | 0.552$^{bc}$ | 0.076 | [0.401–0.703] |
| Lemon concentrate | 1.122$^{f}$ | 0.036 | [1.050–1.195] |
| Mango concentrate | 0.541$^{b}$ | 0.096 | [0.348–0.732] |
| Orange concentrate | 0.810$^{e}$ | 0.053 | [0.705–0.915] |
| Passion fruit concentrate | 0.517$^{b}$ | 0.136 | [0.247–0.787] |
| Tangerine concentrate | 0.709$^{d}$ | 0.084 | [0.544–0.876] |
| Apple single strength juice | | | |
| Clarified | 0.340 | 0.049 | [0.243–0.438] |
| Non-clarified | 0.601 | 0.052 | [0.497–0.705] |
| Apple concentrate | | | |
| Clarified | 0.456 | 0.064 | [0.328–0.583] |
| Non-clarified | 0.716 | 0.058 | [0.601–0.832] |
| Mango single strength juice | | | |
| Clarified | 0.295 | 0.104 | [0.088–0.502] |
| Non-clarified | 0.555 | 0.103 | [0.350–0.761] |
| Mango concentrate | | | |
| Clarified | 0.410 | 0.101 | [0.209–0.612] |
| Non-clarified | 0.671 | 0.095 | [0.482–0.860] |
| Orange single strength juice | | | |
| Clarified | 0.565 | 0.053 | [0.458–0.670] |
| Non-clarified | 0.825 | 0.062 | [0.703–0.947] |
| Orange concentrate | | | |
| Clarified | 0.680 | 0.057 | [0.567–0.793] |
| Non-clarified | 0.940 | 0.055 | [0.830–1.051] |

*Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

As expected, the inactivation temperature affected ($p < 0.0001$) the resistance of *Alicyclobacillus* (Table 2). In comparison with the predominant effect of temperature (*F*-value = 100.7), the influence of pH on the heat resistance of *Alicyclobacillus* was weaker (*F*-value = 32.5), as suggested by the more disperse scatter plot between log *D* and beverage pH (not shown). Nonetheless, the meta-analysis model was still able to detect the significance of this physicochemical property (Table 2). In an earlier study, Pontius et al. (1998) detected as well a significant effect of pH, although they showed that it becomes more notorious only at lower inactivation temperatures. In this work, as the summarised data comprised a narrow range of pH from 2.8 to 4.0, it is natural that the effect of higher temperatures (from 80 °C) surpasses the effect of the matrix acidity. Although the mechanisms of resistance to pasteurisation of *Alicyclobacillus* are still unclear, the thermal resistance of other bacterial spores is influenced by several environmental factors such as pH, water activity and menstruum composition (Baysal and Icier, 2010). However, the most significant parameter in the inactivation of microorganisms is the thermal effect itself, regardless of the type of thermal treatment.

The heat sensitivity of *Alicyclobacillus* was shown to be significantly different between single strength juices and concentrates (i.e., see variable type in Table 2). Independently of the kind of fruit, the concentrates had on average log $D^*$ values higher than juices by 0.115 units. This finding was in agreement with Alberice et al. (2012), who found that the *D*-values in all temperatures assayed were slightly higher in concentrated juice than in reconstituted juice. An explanation of the fact that the inactivation rate of *Alicyclobacillus* is higher in single strength juices than in concentrates can be found in Gombas (1983), who sustained that an apparent increase in spore heat resistance is achieved when it is balanced in low water activity or dissolved in a solution of high osmotic potential. High sugar concentrations like sucrose exert a similar osmotic pressure that exists in the spore cortex. Thus, protoplast dehydration is induced mechanically and osmotically by pressure, and this dehydration mechanism present in the spores is probably responsible for heat resistance.

In our meta-analysis study, the type of beverage was not only responsible for causing a shift in the intercept (log $D^*$) of the relationship between log *D* and temperature but also for causing a shift in the slope. Notice that the interaction term temperature × type is significant ($p < 0.05$; Table 2), therefore bringing about differences in $z_T$ values for juices and concentrates (Table 4). The estimate of 0.014 for temperature × type (Table 2) indicates that, in single strength juices, the slope between log *D* and temperature is higher (steeper) than in concentrates by 0.014 units. In other words, the same increase in the pasteurisation temperature for concentrates will have a lower effect on the heat resistance of *Alicyclobacillus* than for juices. This is, as a consequence, reflected in the $z_T$ values estimated by the meta-analysis model (Table 4), which in all cases are higher in concentrates than in single strength juices.

It was also demonstrated that *Alicyclobacillus* possesses less thermal resistance in clarified beverages than in non-clarified beverages. In the meta-analysis model, the variable clarification had an effect ($p < 0.0001$) on the *D*-values as a single term (Table 2) but not in interactions either with temperature or with pH (results not shown). Hence, clarification only affects the estimation of log $D^*$, meaning that, in the relationships between log *D* and temperature or log *D* and pH, the process of clarification will only cause a downward shift in the straight line, and will not affect either the temperature slope or the pH slope; hence, will not affect the $z_T$ or $z_{pH}$ values. On average, the model estimated that a non-clarified

**Table 4**
Estimates of $z_T$ (°C) and $z_{pH}$ obtained by the meta-analytical secondary predictive model.

| Parameter | Mean | Standard error | 95% CI |
|---|---|---|---|
| $z_T$ | | | |
| Single strength juices – all fruits | 11.23 | 1.107 | [9.034–13.42] |
| Concentrates – all fruits | 13.35 | 1.744 | [9.893–16.80] |
| Apple single strength juice | 10.34[c] | 0.728 | [8.898–11.78] |
| Berry single strength juice | 8.019[ab] | 1.354 | [5.339–10.70] |
| Cupuaçu single strength juice | 9.261[b] | 1.163 | [6.958–11.56] |
| Grape single strength juice | 7.957[a] | 1.059 | [5.860–10.06] |
| Grapefruit single strength juice | 11.17[c] | 0.907 | [9.378–12.96] |
| Lemon single strength juice | 15.86[e] | 1.467 | [12.95–18.76] |
| Mango single strength juice | 17.39[e] | 3.375 | [10.71–24.08] |
| Orange single strength juice | 12.48[d] | 1.162 | [10.17–14.78] |
| Passion fruit single strength juice | 8.907[b] | 1.789 | [5.365–12.45] |
| Tangerine single strength juice | 11.35[c] | 1.382 | [8.611–14.08] |
| Apple concentrate | 12.19[c] | 1.164 | [9.886–14.50] |
| Berry concentrate | 9.045[ab] | 1.780 | [5.520–12.57] |
| Cupuaçu concentrate | 10.65[b] | 1.682 | [7.326–14.00] |
| Grape concentrate | 8.967[a] | 1.424 | [6.147–11.78] |
| Grapefruit concentrate | 13.27[c] | 1.679 | [9.945–16.59] |
| Lemon concentrate | 20.43[e] | 1.827 | [16.81–24.05] |
| Mango concentrate | 23.07[e] | 5.054 | [13.06–33.08] |
| Orange concentrate | 15.15[d] | 1.752 | [11.68–18.62] |
| Passion fruit concentrate | 10.19[b] | 2.408 | [5.422–14.96] |
| Tangerine concentrate | 13.52[c] | 2.269 | [9.023–18.01] |
| $z_{pH}$ | | | |
| Overall mean | 1.305 | 0.180 | [0.948–1.661] |
| With bacteriocin | 0.586 | 0.071 | [0.445–0.726] |
| Without bacteriocin | 5.750 | 0.950 | [3.869–7.631] |

*Different superscript letters denote statistical differences across fruits separately for single strength juices and for concentrates.

beverage will exhibit an increase in the intercept or log $D^*$ value by 0.26 units (Table 2). It may be hypothesised that the greater particles in suspension in a non-clarified juice slows down the heat transfer rate, retarding also the thermal inactivation of *Alicyclobacillus*. This is also affected by the method employed to assess microbial thermal resistance. For instance, the most common method of inoculating the microorganism in small closed vessels and immersing them in the heating medium, leads to the production of non-desirable heating lag times, which will accentuate the difference in D-values estimates between clarified and non-clarified beverages. On the contrary, methods whereby the inoculum is added to the sample only when it reaches the desired temperature will produce an insignificant thermal lag, leading to more accurate D-values, and probably smaller differences between clarified and non-clarified juices. The bias caused by the method used to determine microbial thermal resistance could not be assessed in the present meta-analysis as some primary studies failed to report the method in a clear way.

The meta-analysis also demonstrated that there is a significant effect of the addition of bacteriocins prior to heating on the thermal resistance of *Alicyclobacillus*, increasing the lethality of pasteurisation. Although the variable bacteriocin was not statistically significant when it entered the model as a single term (i.e., as a predictor of log $D^*$), it was highly significant as an interaction term with pH (Table 2). The negative estimate of pH × bacteriocin suggests that for a constant value of beverage pH, the addition of bacteriocins (either nisin or bovicin in the doses studied in their respective primary studies) will increase the thermal sensitivity of *Alicyclobacillus* (i.e., lower log $D$). On the other hand, the fact that there is an interaction between pH and the presence of bacteriocins implies that the effect of a bacteriocin on the thermal sensitivity of *Alicyclobacillus* becomes more evident at higher pH. This is, a greater bactericide effect is revealed when a bacteriocin is added to a less acidic beverage in comparison to a highly acidic beverage. This may stem from both of the following reasons: Firstly, in a highly acidic matrix, the effect of the low pH itself on *Alicyclobacillus* lethality may mask the effect of the bacteriocin, and hence, the effect of the latter becomes less evident. Secondly, there is a direct effect of pH on bacteriocin activity, which is higher at lower pH values (Davies et al., 1998; Houlihan et al., 2004). With this, the lower the pH of the matrix, the more active the bacteriocin becomes, and the more strongly *Alicyclobacillus* is inhibited, causing, at that lower pH, a greater increase in heat sensitivity in comparison to that when no bacteriocin was added.

As a consequence, the value of $z_{pH}$ estimated for beverages with bacteriocins (0.586) was significantly lower (i.e., the spore heat resistance is highly affected by changes in pH) than the one for beverages without bacteriocins (5.750) (Table 4). The bacteriocins in doses between 50 and 80 IU/ml reduced by a factor of ten the $z_{pH}$ value of *Alicyclobacillus*. In this meta-analysis study, the addition of bacteriocins did not play a role on the reduction of $z_T$ as the interaction temperature × bacteriocin turned out to be non-significant. Yet, our model still confirmed that the bacteriocins, nisin and bovicin, were bactericidal against *Alicyclobacillus*, as the $D$-values – hence, the viable cell numbers – decreased in their presence. Although there is evidence that higher doses of bacteriocins have greater effect on increasing the lethality of *Alicyclobacillus* spores (Peña et al., 2009; Komitopoulou et al., 1999), this was not assessed in this meta-analysis study.

The variances $s_u^2$ and $s_v^2$ of the random effects placed on the model's intercept (log $D^*$) and temperature slope, respectively, were both significant (Table 2), confirming statistically the presence of heterogeneity that was initially observed in Fig. 1. As the subject of variation of the random effects was the interaction study × fruit, it can be conceived (i) that there is an infinite population (past, present and future) of primary studies reporting lethality data of *Alicyclobacillus* for a fruit beverage (ii) that there is an infinite population of fruits that can be subject of study; and (iii) that each of the studies associated to a fruit introduces inherent heterogeneity in the reported outcomes because of the differences in the methods for assessing microbial thermal resistance, in the composition of the beverage, in the bacteria strains inoculated, in the microbiological essay to quantify *Alicyclobacilus*, etc. As explained before, the fixed effects or coded study characteristics could explain 40% of such heterogeneity. Yet, there is a residual heterogeneity ($\tau^2$~0.044; Table 2), which is still significant. The purpose of the random effects is therefore to absorb this unexplained heterogeneity.

Because “primary study” and “fruit” could not enter the meta-analysis model as separate subjects of random effects – since in the input data, in most cases, one primary study was associated to one fruit (Table 1) – consequently, the estimate of variability cannot be separated into that due to differences among primary studies and that due to differences among fruits. By entering primary study in interaction with fruit, both subjects of variability are acknowledged although they cannot be disaggregated. At most, it could be hypothesised that a primary study involves many more sources of variability in the estimates of bacterial heat resistance than the kind of fruit does; and therefore, that the between-study heterogeneity is much greater than the between-fruit heterogeneity. Based on this assumption, the between-study heterogeneity $\tau^2$ was approximated by using the variances $s^2_u$, $s^2_v$ and the covariance $s^2_{uv}$ (Table 2).

Nevertheless, using such a model design, it is possible to provide estimates of log $D^*$ and $z_T$ for beverages (single strength juices or concentrates) of any of the ten fruits considered. This is possible by computing the random effects $u_{lm}$ and $v_{lm}$ (Equations (2) and (3), respectively) for a given fruit, and average them over the primary studies associated with such a fruit – in case that more than one

primary study was in interaction with that fruit. In this way, the log $D^*$ and $z_T$-values were estimated for single strength juices and concentrates made of different fruits (Tables 3 and 4). A test of contrasts showed that there are statistical differences in the log $D^*$ and $z_T$-values among the kinds of beverage. For instance, in terms of the $D$-value at 95 °C and at matrix pH of 3.5, *Alicyclobacillus* in berry juice presented a low heat resistance of 1.8 min (log $D^* = 0.252$ in Table 3), while in orange juice exhibited a higher thermal resistance with a $D$-value of 4.9 min (log $D^* = 0.695$ in Table 3). The growth and inactivation of *Alicyclobacillus* spores in commercial beverages depends, among other factors, on the compositional properties of food. For instance, in Splittstoesser et al. (1994), apple and tomato juice consistently supported growth, whereas grape juice at both pH 2.9 and 3.3 did not permit it. Different components present in fruits might increase the heat resistance of *Alicyclobacillus* spores, and this was clear for apple juice and apple nectar in Bahçeci and Acar (2007). Similar levels of heat resistance of *Alicyclobacillus* were found for tangerine juice (López et al., 2011) and orange juice (Conesa et al., 2009). Our meta-analysis study produced also relatively high log $D^*$ values for tangerine and orange juice (Table 3). Nonetheless, because of the structure of our meta-analysis model, we cannot conclude that such significant differences in log $D^*$ between, for instance, berry and orange juice (Table 3), can be entirely assigned to the composition of the fruits since it may as well be due to the heterogeneity among the primary studies that determined the $D$-values of *Alicyclobacillus* in berry and orange juice. Remember that the random effects had as subject the interaction primary study and fruit. Hence, some care should be taken in the interpretation of the statistical differences in the $D$-values and $z$-values estimates of the beverages across fruits listed in Tables 3 and 4. It is more prudent instead to interpret each of these estimates as *mean effect size* or overall average from all the meta-analysed literature sources. In fact, such summarisation of the research outcomes (i.e., available knowledge) increases the statistical confidence of the individual studies alone, and it is what constitutes one of the strengths of meta-analysis.

The mixed-effects linear model estimated a mean $D^*$ value of 3.8 min with a 95% CI: 3.0–4.9 min (log $D^* = 0.584$; 95% CI: 0.474–0.694 in Table 3) to decrease one-log population of *Alicyclobacillus* in fruit beverages, on average (single strength juices or concentrates, clarified or non-clarified), at a temperature of 95 °C and a pH of 3.5. As this value is an estimate from a random-effects model, it can be generalised to the entire population of fruits and primary studies. More specifically, the mean $D^*$ value estimate for single strength juices, whether clarified or not (3.3 min; 95% CI: 2.6–4.3 min), was lower ($p < 0.05$) than for the concentrates (4.4 min; 95% CI: 3.3–5.9 min). The mean $D^*$ value for clarified juices (2.5 min; 95% CI: 1.9–3.2 min) was significantly lower than for non-clarified single strength juices (4.5 min; 95% CI: 3.4–6.0 min), and the same can be said for the clarified concentrates (3.2 min; 95% CI: 2.4–4.4 min) and the non-clarified concentrates (5.9 min; 95% CI: 4.4–8.0 min). The significant effects of the type of beverage and the clarification have been explained earlier in this section. As expected, the mean log $D^*$ values for the concentrates of each fruit were higher than their respective single strength juices (Table 3).

Because of the model design, it was possible to compute for the beverages of each fruit (whether single strength juice or concentrate), the log $D^*$ estimates in case they were clarified or not clarified. In Table 3, three examples are presented for apple, mango and orange. Notice that the meta-analytical model allows us to estimate *Alicyclobacillus* thermal lethality parameters beyond those originally available in the input data set; and this represents the main capability of this model. For instance, no $D$-values were available for mango single strength juice, but only for non-clarified mango concentrate (Table 1). However, the meta-analysis model can predict $D$-values for clarified mango single strength juice, non-clarified single strength mango juice and clarified mango concentrate at different inactivation temperature and matrix pH. The confidence about these extrapolated estimates remains to be tested by other thermal inactivation laboratory experiments; these are, experiments for which $D$-values were not available in the literature, namely, for mango juice, cupuaçu concentrate, berry concentrate, grape concentrate, tangerine concentrate and passion fruit concentrate.

Using Equation (3), the mean temperature shift required for the thermal destruction curve to move one-log cycle ($z_T$-value) was summarised for single strength juices (11.23 °C; 95% CI: 9.03–13.42 °C) and concentrates (13.35 °C; 95% CI: 9.89–16.80 °C), which are values that can be generalised to all the population of fruits and primary studies. As explained before, because the interaction temperature × type (Table 2) was significant – hence, the slope of the relationship between log $D$ and temperature lower for concentrates – for all fruits, the estimates of $z_T$ values were higher for concentrates than for single strength juices (Table 4). Once again, notice that, as occurred with the log $D^*$ estimates, predictions of $z_T$ could be produced for *Alicyclobacillus* in types of beverages whose lethality kinetics were not investigated in the primary studies. Nonetheless, such extrapolated $z_T$ estimates were subject to greater uncertainty, reason as to why their confidence intervals were slightly broader. For example, for cupuaçu single strength juice (present in the meta-analytical data), the 95% confidence interval of $z_T$ was 6.96–11.56 °C, while for (the non-investigated) concentrate of cupuaçu, it was 7.33–14.00 °C (Table 4).

The $z_T$ values of the fruit beverages estimated by the meta-analysis model were contrasted to those ('observed' $z_T$ values) reported in the primary studies, and in all cases they were within the confidence interval of the model. For instance, the $z_T$ value of *Alicyclobacillus* reported for mango concentrate in the corresponding primary study, (de Carvalho et al., 2008) was 21.27 °C, while the mean estimate of the meta-analysis model was 23.07 °C with a 95% CI of 13.06–33.08 °C (Table 4). For grapefruit juice, Komitopoulou et al. (1999) found $z_T$ values of 11.60, 11.53 and 10.49 °C at a pH of 3.42, 3.0 and 4.0, respectively, whereas the mean $z_T$ value estimated by the meta-analysis model for grapefruit juice was in agreement at 11.17 °C with a 95% CI of 9.38–12.96 °C. From the model, the lowest mean $z_T$ values belonged to berry juice (8.02 °C; 95% CI: 5.34–10.70 °C) and grape juice (7.95 °C; 95% CI: 5.86–10.06 °C), and these were not statistically different one from the other. Both model's estimates were very close to the observed $z_T$ values for berry and grape juice, both of 7.2 °C, found in Walls (1997) and Splittstoesser et al. (1994), respectively.

To further illustrate the model's accuracy, Fig. 2 shows a comparison of log $D$, as affected by temperature, between the observed values (directly extracted from the primary studies) and the values predicted by the meta-analytical model for different types of beverages at a fixed pH. In all cases, the lines predicted by the model lay close to the observations. This set of examples also demonstrates the flexibility of the model to describe the same or different slopes and intercepts. For clarified apple juice (Fig. 2; top left), the use of a bacteriocin causes a downward shift in the intercept (diminishes the heat resistance) while the random effects realizations from the two primary studies (apple juice with bacteriocin and without bacteriocin) explain the different slopes. For lemon concentrate (Fig. 2; top right) and tangerine juice (Fig. 2; bottom right), the clarification process causes the downward shift in the intercept whereas there is no change in the slope because the variable 'clarification' did not enter the model in significant interaction with temperature. Notice that the model predictions for clarified

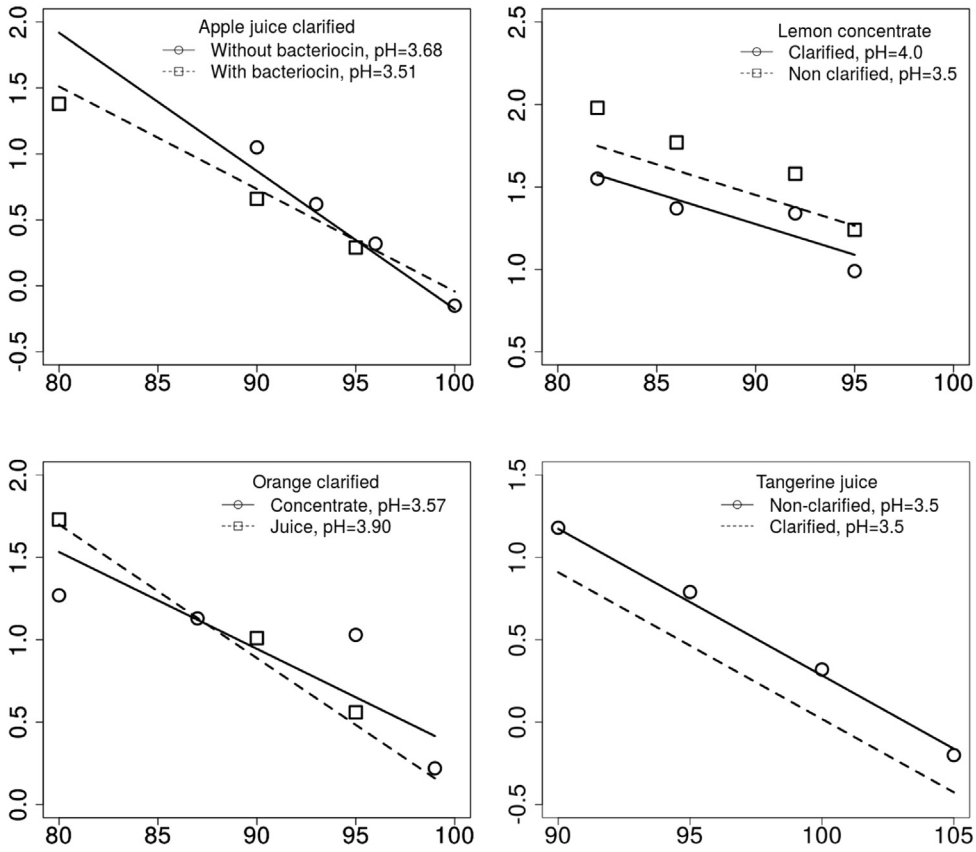


**Fig. 2.** Relationship between temperature (°C; *x*-axis) and log *D* (*y*-axis), as predicted (lines) by the meta-analysis linear mixed model for different subgroups of types of beverages, in comparison with observed data (markers) when available.

tangerine juice (Fig. 2; bottom right) could not be validated given the absence of thermal resistance data in the literature for this subgroup. For the clarified beverages made of orange (Fig. 2; bottom left), the intercept belonging to the single strength juice is lower than that of the concentrate, and its slope is also affected because of the significant interaction between type of beverage (single strength juice or concentrate) and temperature. Notice that the slope for the single strength juice is steeper than for the concentrate.

In assessing the fitting quality of the meta-analytical model, it was found that the studentised residuals fell between −2.5 and 2.5, and according to the Shapiro–Wilk test, their distribution could be approximated to a normal distribution (not shown). Furthermore, the residuals versus the fitted values (i.e., log *D*) did not exhibit any singular pattern (Fig. 3), as they were randomly spread with a coefficient of correlation of 0.047. In addition, there was good agreement between the fitted and the observed log *D* (Fig. 4) with a high coefficient of correlation of 0.972.

## 4. Conclusions

Typically, fruit juices will be pasteurized at temperatures around 95 °C for c. 20 s to 2 min (Komitopoulou et al., 1999; Silva and Gibbs, 2001). While the heat treatment alone applied in acidic fruit

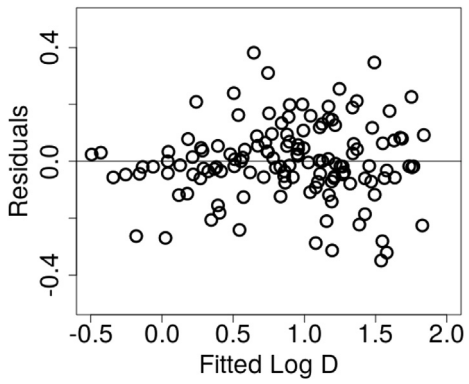


**Fig. 3.** Relationship between residual values and log *D* fitted by the meta-analytical mixed-effects linear model.

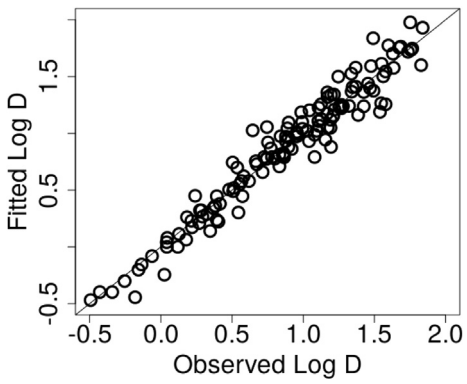


**Fig. 4.** Relationship between the observed log *D* extracted from the primary studies and the log *D* fitted by the meta-analytical mixed-effects linear model.

products can decrease concentrations of *Alicyclobacillus*, if starting concentrations are high enough, it may not be able to inactivate spores completely. Moreover, as Gouws et al. (2005) pointed out, the heat treatment may even act as a stimulus to germination, which follows outgrowth of the microorganism. The meta-analysis results indicated that the harsh conditions may be insufficient to inactivate the spores of this spoilage microorganism. For instance, the meta-analysis estimated a mean *D*-value of 4.9 min for orange juice at 95 °C and pH 3.5 ($\log D^* = 0.695$; Table 3), suggesting that spores could survive the processing conditions generally used in the fruit beverage industry. Thus, the use of other barriers along with heat treatment to undermine the resistance of *Alicyclobacillus*, such as the addition of bacteriocins prior to pasteurization, may be contemplated. It is known that, even at low levels of 50 IU/ml, the residual nisin would prevent the outgrowth of any surviving spores (Komitopoulou et al., 1999).

Statistical techniques, such as meta-analysis, are very useful to perform a synthesis of a set of distinct but similar experiments. This particular work exemplifies how a common microbiology predictive model such as the Bigelow secondary model can be the basic equation on which a meta-analytical model (i.e., a weighted mixed-effects linear model) is built upon. It is expected that the compilation of the thermal resistance of *Alicyclobacillus* in fruit beverages, carried out in this study, be of utility to food quality managers in the determination or validation of the lethality of their current heat treatment processes. Nevertheless, although the results of this work should in principle provide a summary of the state-of-the-art of *Alicyclobacillus* thermal resistance in fruit beverages, further experimental studies should still be conducted in order to validate the $\log D^*$ and $z^*$ values predicted for some types of beverages, such as mango juice, passion fruit concentrate or grapefruit concentrate, for which there were not available information in the literature.

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