PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

GABRIELA DA SILVA PINHO

DESENVOLVIMENTO DE FILME BIODEGRADÁVEL CONTENDO SOBRENADANTE DE *PEDIOCOCCUS ACIDILACTICI* CE51 LIVRE DE CÉLULAS COMO EMBALAGEM PROTETORA DE CARNE DE FRANGO E BOVINA

Presidente Prudente - SP
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Orientadora:
Prof[a]. Dr[a]. Lizziane Kretli Winkelströter Eller

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DEDICATÓRIA

Dedico este trabalho aos meus pais por sempre lutarem para que eu tivesse acesso à educação de qualidade.

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RESUMO

Desenvolvimento de filme biodegradável contendo sobrenadante de *Pediococcus acidilactici* CE51 livre de células como embalagem protetora de carne de frango e bovina

Este estudo teve como objetivo avaliar o efeito bioconservador de um filme biodegradável com sobrenadante livre de células (SLC) de *P. acidilactici* CE51 em carne de frango e bovina contaminadas com *L. monocytogenes* e *E. faecium.* Filmes à base de amido e carboximetilcelulose foram produzidos com 0%, 50% e 100% de SLC. Foram avaliadas propriedades físicas e ação antimicrobiana. A solubilidade e opacidade dos filmes foram maiores naqueles que continham 100% de SLC. Houve um aumento na resistência aos testes de tensão à tração e aumento da ação antimicrobiana conforme a concentração de SLC aumentava. Em carne de frango, filmes com ≥ 50% de SLC reduziram *E. faecium* após 4 dias, alcançando 0,8 Log UFC/ml com 100% de SLC em 8 dias a 4ºC. Para *L. monocytogenes*, a maior redução (0,8 Log UFC/ml) ocorreu com 100% de SLC após 8 dias. Em carne bovina, filmes com 100% de SLC reduziram *E. faecium* (0,88 UFC/ml) após 8 dias, enquanto filmes com 50 e 100% de SLC reduziram *L. monocytogenes* a partir de 4 dias, atingindo 1,9 UFC/ml em 8 dias. Os filmes contendo SLC de *P. acidilactici* CE51 mostraram-se promissores como bioconservadores em alimentos.

Palavras-chave: Embalagem ativa; Bacteriocina; Bioconservação; *Listeria monocytogenes*; *Enterococcus faecium.*

ABSTRACT

Development of a biodegradable film containing cell-free supernatant of P*ediococcus acidilactici* CE51 as a protective packaging for chicken and beef meat

This study aimed to evaluate the bioconservative effect of a biodegradable film with cell-free supernatant (CFS) of *P. acidilactici* CE51 on chicken and beef meat contaminated with L. monocytogenes and E. faecium. Starch and carboxymethyl cellulose-based films were produced with 0%, 50% and 100% CFS. Physical properties and antimicrobial action were evaluated. The solubility and opacity of the films were higher in those containing 100% CFS. There was an increase in tensile strength and an increase in antimicrobial action as the CFS concentration increased. In chicken meat, films with ≥ 50% CFS reduced *E. faecium* after 4 days, reaching 0.8 Log CFU/ml with 100% CFS in 8 days at 4ºC. For *L. monocytogenes*, the greatest reduction (0.8 Log CFU/ml) occurred with 100% CFS after 8 days. In beef, films with 100% CFS reduced *E. faecium* (0.88 CFU/ml) after 8 days, while films with 50 and 100% CFS reduced *L. monocytogenes* from 4 days onwards, reaching 1.9 CFU/ml at 8 days. Films containing SLC from *P. acidilactici* CE51 showed promise as biopreservatives in food.

Keywords: Active packaging; Bacteriocins; Biopreservative; *Listeria monocytogenes; Enterococcus faecium*.

LISTA DE SIGLAS

ANOVA	Análise de variância
ASTM	American Society for Testing and Materials
BAL	Bactérias ácido láticas
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CMC	Carboximetilcelulose
E	Alongamento de ruptura
pH	Potencial hidrogeniônico
SLC	Sobrenadante livre de células
TS	Tensão (ou resistência) à tração
UFC	Unidade formadora de colônia
UNOESTE	Universidade do Oeste Paulista
UV	Ultravioleta

SUMÁRIO

1 ARTIGO CIENTÍFICO

Desenvolvimento de filme biodegradável contendo sobrenadante de *Pediococcus acidilactici* CE51 livre de células como embalagem protetora de carne de frango e bovina

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O trabalho está apresentado sob a forma de artigo, sob as normas do periódico o qual será submetido: Journal of Food Science and Technology; Fator de Impacto 2.6; Qualis A3.

Destaques

- Foram avaliadas propriedades físicas e ação antimicrobiana de filmes biodegradáveis contendo diferentes concentrações de sobrenadante livre de células (SLC) de *P. acidilactici* CE51.
- O efeito bioconservador dos filmes foi analisado em carne de frango e bovina contaminadas com dois microrganismos indicadores.
- Os filmes, quando em concentração máxima de SLC, reduziram significativamente a presença de *L. monocytogenes* e *E. faecium*.
- A incorporação de SLC de *P. acidilactici* CE51 em embalagens de alimentos biodegradáveis é promissora.

1. Introdução

As embalagens de alimentos são peças-chave para a garantia da qualidade dos alimentos, pois fornecem vários benefícios, como maior tempo útil do alimento embalado, melhor manuseio e proteção contra danos físico-químicos durante o armazenamento e transporte. Em especial, as embalagens são capazes de fornecer proteção química, biológica e física, prevenindo tanto a contaminação secundária dos alimentos, quanto os danos mecânicos durante o armazenamento e transporte, além de proteger contra a exposição à luz, oxigênio, radiação UV e umidade. Contudo, a embalagem também desempenha uma função informativa (nome do produto, lista de ingredientes, componentes alérgenos, prazo de validade e modo de preparo) e promocional (informações sobre o fabricante). Com avanços nos últimos anos, diversos sistemas de embalagem inovadores, incluindo materiais de embalagem ativos e inteligentes, vêm sendo amplamente explorados na indústria alimentícia (Suvarna et al. 2022).

Sistemas de embalagens inteligentes incluem ferramentas para monitorar os alimentos embalados ou o ambiente que os circundam. Dessa forma, fornecem informações em tempo

real sobre a qualidade e segurança dos alimentos, contribuindo não apenas para a prevenção de doenças de origem alimentar, mas também para a redução do desperdício de alimentos decorrente de seu deterioramento (Yousefi et al. 2019). Por outro lado, as embalagens ativas são aquelas que possuem efeitos antimicrobianos contra microrganismos patogênicos e/ou deteriorantes. Embalagens que contenham removedores de oxigênio, absorventes de umidade ou emissores de dióxido de carbono são exemplos que se enquadram na categoria de sistemas ativos (Yildirim and Röcker 2018). Em alguns casos, as embalagens ativas podem reduzir a necessidade de adição de conservantes aos alimentos, além de prolongarem a vida útil dos alimentos e preservar suas propriedades organolépticas (aspecto, aroma, consistência, textura e sabor), mantendo a qualidade e segurança do alimento.

A fim de eliminar os microrganismos indesejáveis presentes nos alimentos, é possível incluir antimicrobianos voláteis ou não voláteis nos polímeros, utilizar um revestimento ou agente antimicrobiano adsorvente nas superfícies desses materiais (Appendini and Hotchkiss 2002). As embalagens antimicrobianas são feitas, basicamente, de duas formas. Na primeira delas, a superfície antimicrobiana não entra em contato com o alimento conservado e os agentes ativos neles contidos podem migrar para o alimento. Essa embalagem é utilizada para alimentos embrulhados em papel alumínio ou à vácuo. Na outra forma, coloca-se o agente antimicrobiano dentro da embalagem, mas não em contato direto com o alimento, como por exemplo em embalagem com atmosfera modificada (Motelica et al. 2020).

Metabólitos de bactérias lácticas, como bacteriocinas, podem ser usados como substâncias antimicrobianas em embalagens ativas, impedindo o desenvolvimento indesejável de microrganismos em produtos alimentícios. As bacteriocinas são peptídeos ou proteínas extracelulares de baixo peso molecular sintetizados pelos ribossomos. Possuem atividade bactericida ou bacteriostática, em particular contra patógenos de origem alimentar. Com a crescente preocupação com o uso de conservantes químicos (como nitritos) que são

prejudiciais à saúde humana, o uso bacteriocinas na indústria de alimentos tem aumentado significativamente. As bacteriocinas são consideradas seguras e podem ser usadas como aditivos alimentares ou conservantes naturais (Gumienna and Górna 2021).

Dentre os patógenos de origem alimentar, *Listeria monocytogenes* possui destaque por levar a graves implicações econômicas e de saúde pública, sendo classificada entre os patógenos bacterianos de origem alimentar mais impactante em todo mundo (Noordhout et al. 2014). Este patógeno pode crescer em alta salinidade (10%), baixa atividade de água (<0,9), baixa temperatura (4°C) e em uma faixa de pH de 4,1 a 9,6, sendo capaz de sobreviver em equipamentos de processamento, materiais de embalagem e superfícies de contato com alimentos. A contaminação por *L. monocytogenes*, em sua maioria, se dá durante o processamento de alimentos. As cepas de *L. monocytogenes* são capazes de aderir em superfícies de contato com alimentos, podendo formar biofilme, uma colônia arquitetônica de microrganismos dentro de uma matriz de substâncias poliméricas extracelulares (EPS), o que permite sua persistência a longo prazo (El-sawy et al. 2024). Além disso, é um importante contaminante de alimentos prontos para consumo (ou "*ready-to-eat*"). Em relação às carnes, tanto de frango quanto bovina, a contaminação pode ocorrer por meio de matéria-prima contaminada ou de contaminação cruzada durante o processo de preparo, cozimento e serviço dos alimentos.

Enterococcus faecium é um coco gram-positivo, facultativo, com arranjo em forma de cadeia e não formador de endósporos (Jiang et al. 2021). O patógeno está presente na microbiota de mamíferos, aves, insetos e répteis, sendo comumente encontrados no solo, nas plantas e na água. Por possuírem alta capacidade de adaptação às tensões ambientais, são particularmente difíceis de eliminar do ambiente. Por isso, é possível encontrar variantes de enterococos resistentes aos antimicrobianos recuperadas de carnes, produtos lácteos e alimentos prontos para consumo (Hayes et al. 2003). Além disso, os enterococos de origem

alimentar podem transferir genes de resistência a patógenos como *Campylobacter* spp., *Listeria* spp. e *E. coli* (Jurado-Rabadán et al. 2014).

Desta forma, filmes contendo bioconservantes (substâncias antimicrobianas extraídas de fontes naturais e ecologicamente corretas) são promissores porque ajudam a prevenir a deterioração dos alimentos, melhorando sua qualidade e segurança (Gumienna and Górna 2021). Além disso, é importante destacar que o uso de filmes plásticos convencionais pode causar sérios problemas ambientais. Por isso, o desenvolvimento de filmes biodegradáveis surge como uma alternativa viável às embalagens tradicionais. Neste contexto, o objetivo deste estudo foi avaliar o efeito bioconservador de um filme biodegradável com sobrenadante livre de células (SLC) de *P. acidilactici* CE51 em carne de frango e carne bovina, utilizando dois microrganismos indicadores: *L. monocytogenes* e *E. faecium*.

2. Material e Métodos

2.1 Produção de sobrenadante livre de células (SLC) de *P. acidilactici* CE51

Uma cultura de *P. acidilactici* CE51 (isolada previamente de presunto) (Vieira et al. 2020) foi centrifugada a 10.000 rpm por 15 min a 4°C e o SLC foi coletado. O sobrenadante foi neutralizado a pH 6,0 e em seguida filtrado em membrana de 0,22 μm. O espectro de atividade do sobrenadante da cultura láctica CE51 foi quantificada por meio do método de diluição crítica demonstrando espectro antimicrobiano de 3.200 UA/mL para *L. monocytogenes* e 1.600 UA/mL para *E. faecium* (Vieira et al., 2020). A atividade antibacteriana foi então testada contra *L. monocytogenes* ATCC 19015 (cepa indicadora) e *E. faecium* ATCC 6569 (cepa indicadora).

2.2 Preparo do filme biodegradável contendo SLC de *P. acidilactici* CE51

Para a preparação do filme, utilizou-se o método descrito por Ghanbarzadeh et al. (2010). Primeiramente, dissolveram-se 2g de amido em 100mL de água destilada por 5

minutos, juntamente com glicerol (1mL) e ácido cítrico (0,13g). Essas suspensões foram homogeneizadas em agitador magnético a 500 rpm por 30 minutos em banho-maria a 90°C. A carboximetilcelulose (CMC) (0,5g) e a solução de amido foram misturadas e agitadas a 75°C durante 10 minutos.

Alterações na fase líquida dessa etapa foram feitas para incluir diferentes concentrações de sobrenadante contendo bacteriocina de *P. acidilactici* CE51, substituindo a água destilada. As amostras foram preparadas com 0%, 50% e 100% de SLC.

As dispersões foram resfriadas a 40°C e misturadas suavemente por 20 minutos para eliminar todas as bolhas de ar. Em seguida, alíquotas de aproximadamente 30g das soluções filmogênicas foram distribuídas em placas de Petri de 150mm de diâmetro e secadas em estufa de 45-50ºC por 24 horas.

2.3 Caracterização do filme biodegradável contendo SLC de *P. acidilactici* CE51

2.3.1 Transparência

A transparência dos filmes foi avaliada de acordo com o proposto por Yang et al. (2023). Os filmes foram cortados e fixados de forma perpendicular ao feixe de luz, utilizando uma cubeta vazia como referência. Em seguida, a faixa de comprimento de onda da luz visível foi varrida, começando em 500 nm para cada filme.

A opacidade foi então calculada utilizando a fórmula: Opacidade = Absorbância em 500nm / Espessura do filme (mm).

Essa medida foi escolhida devido à sua relação direta com a intensidade da luz absorvida pelo filme em um determinado comprimento de onda e espessura do filme.

2.3.2 Espessura

A espessura média dos filmes (μm) foi obtida com o auxílio de um micrômetro digital (datamed). Foram tomadas medidas em dez pontos diferentes em cada filme. A espessura

final foi determinada pela média das dez leituras, sendo realizadas três repetições (Rodrigues et al. 2021).

2.3.3 Solubilidade em água

Amostras do filme (de forma circular de 20 mm de diâmetro) foram secadas, pesadas e mergulhadas em béquer contendo 50 ml de água destilada. O sistema foi mantido sob lenta agitação em banho-maria a 25ºC, por 24 h. Após as 24 h, as amostras foram removidas da água e secadas em estufa a 105 ºC por 24 h para se determinar o peso seco final do material que não foi solubilizado. As amostras foram preparadas em triplicata. A solubilidade foi expressa pela porcentagem de material seco solubilizado (Rodrigues et al. 2021).

2.3.4 Propriedades mecânicas

Para análise da resistência à tração (TS) e alongamento de ruptura (E), os filmes foram medidos com uma máquina de tração Universal seguindo o Método Padrão D 882-88 da American Society for Testing and Materials (ASTM), com algumas modificações. A separação inicial das garras foi ajustada em 50 mm e a velocidade da travessa foi ajustada em 50 mm/min. Os testes mecânicos foram repetidos cinco vezes para cada tipo de filme (Chaudhary et al. 2022).

2.3.5 Avaliação da ação antimicrobiana

A atividade antibacteriana das diferentes concentrações dos filmes contra *L. monocytogenes* e *E. faecium* foi avaliada utilizando uma versão modificada do método de difusão em ágar descritos por Vasconcelos et al. (2021). O inóculo padrão da cepa indicadora (0,5 na escala de McFarland) foi semeado na superfície de placas de Petri contendo meio Mueller Hinton. Em seguida, poços com 5.0 mm de diâmetro foram preenchidos com 100 µL de cada solução filmogênica com diferentes concentrações de sobrenadante de *P. acidilactici* CE51. Como controle positivo, foi utilizado sobrenadante de *P. acidilactici* CE51. As placas

inoculadas foram incubadas a 37 °C por 24 horas. O diâmetro da zona de inibição do crescimento ao redor do poço (medido em triplicata) foi o parâmetro utilizado para avaliar a atividade antibacteriana.

2.3.6 Avaliação do efeito bioconservador de filme biodegradável contendo SLC de *P. acidilactici* CE51 em carne de frango e bovina

Experimentos foram realizados conforme descrito por Maresca and Mauriello (2022). Foram utilizadas amostras de peito de frango e carne bovina obtidas no mercado local e imediatamente transportadas para o laboratório sob condições de refrigeração. As amostras foram picadas assepticamente e, em seguida, tratadas com luz ultravioleta em uma cabine de segurança biológica por 30 minutos (15 minutos para cada lado, com uma intensidade de luz UV de 36.000 μJ/cm²) para eliminar a microbiota nativa (Kuan et al. 2019).

Foi pesado 50g de carne de frango (peito de frango) e 50g de carne bovina (acém) e, em seguida, realizada a contaminação artificial com 500 μL de uma suspensão de *L. monocytogenes* ATCC 19015 (0,5 McFarland que corresponde 8 x 10^8 UFC/ml). O mesmo foi realizado para amostras que utilizaram *E. faecium* ATCC 6569 como microrganismo teste. Após a homogeneização, alíquotas de 10g de carne foi acondicionada no centro dos filmes biodegradáveis (10cm x 5cm) de variadas concentrações, e em seguida embrulhadas e armazenadas a 4°C por até 8 dias (Feng et al. 2021). O tempo de armazenamento foi baseado no tempo de vida útil de carnes de frango.

Foi realizado análise para a quantificação dos microrganismos indicadores nos dias 0, 2, 4, 6 e 8. Em cada dia, a embalagem foi aberta em sistema asséptico, e uma diluição decimal seriada contendo 10g de carne em 90ml água peptonada estéril foi realizada com agitação em vórtex. Cada diluição foi plaqueada em ágar PALCAM e ágar Bile Esculina, seguido de incubação a 37°C por 24-48 horas, respectivamente, para amostras contaminadas com *L.*

monocytogenes e E. faecium. Os experimentos foram realizados em triplicata, e os resultados foram expressos em Log UFC/g de produto.

3. Análise estatística

Todos os dados analisados foram submetidos ao teste de normalidade usando o teste Kolmogorov-Smirnov. A comparação entre dois grupos foi realizada através do teste t de Student para dados não pareados ou pelo teste de Mann-Whitney. Quando três ou mais grupos foram comparados, foi usado a análise de variância (ANOVA), seguido do pós-teste de Bonferroni para verificar a diferença entre os grupos. Os resultados foram considerados significativos para $p < 0,05$. O programa de estatística usado foi o GraphPadPrism 3.0.

4. Resultados

Foi possível observar uma redução significativa da solubilidade da membrana quando utilizado a concentração 100% do sobrenadante de *P. acidilactici* CE51 no filme ($p<0,05$) conforme demonstrado na figura 1.

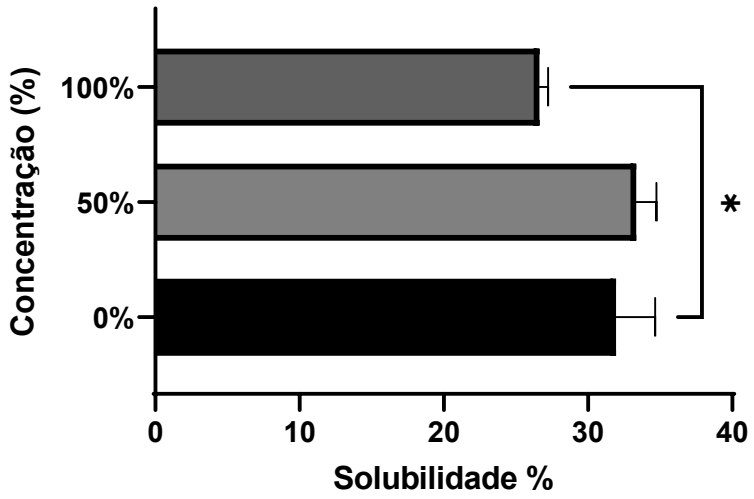

Figura 1. Representação gráfica dos valores de médias e desvios padrão encontrados para solubilidade em água de filmes com diferentes concentrações de SLC de *P. acidilactici* CE51 (0, 50 e 100%).

Para avaliar a opacidade dos filmes, foi fundamental mensurar a espessura desses materiais. A opacidade foi determinada como uma medida da capacidade do filme em impedir a passagem da luz. Foi possível observar uma correlação direta entre a concentração do sobrenadante de *P. acidilactici* CE51 e a opacidade dos filmes, conforme evidenciado na Tabela 1. Foi notado um aumento progressivo na opacidade em função da concentração do sobrenadante utilizado. O filme com ausência de sobrenadante (0%) apresentou uma opacidade de 1,06 ± 0,05, enquanto o filme com 100% de sobrenadante registrou uma opacidade de 3,01 ± 0,25, destacando-se como o mais opaco dentre as diferentes concentrações ($p<0,05$).

Tabela 1. Opacidade determinada pela razão entre absorbância e espessura em diferentes concentrações dos filmes (* $p<0,05$ quando comparado com 0%).

Filmes	Absorbância 500λ	Espessura (mm)	Opacidade
0%	0,137 ± 0,001	0,129 ± 0,004	1,06 ± 0,05
50%	0,356 ± 0,033	0,156 ± 0,007	2,27 ± 0,17*
100%	0,554 ± 0,019	0,185 ± 0,013	3,01 ± 0,25*

O protocolo para avaliação das propriedades mecânicas do filme incluiu testes de tensão à tração (Figura 2) e deslocamento até a ruptura (Figura 3). Durante o teste de tensão à tração, observou-se que o filme com 0% de concentração apresentou a menor resistência, com uma média de 2,48 ± 1,2 N. Em contraste, o filme de 100% de concentração demonstrou a maior resistência, com uma média de 5,8 ± 0,7 N, conforme mostrado na Figura 2.

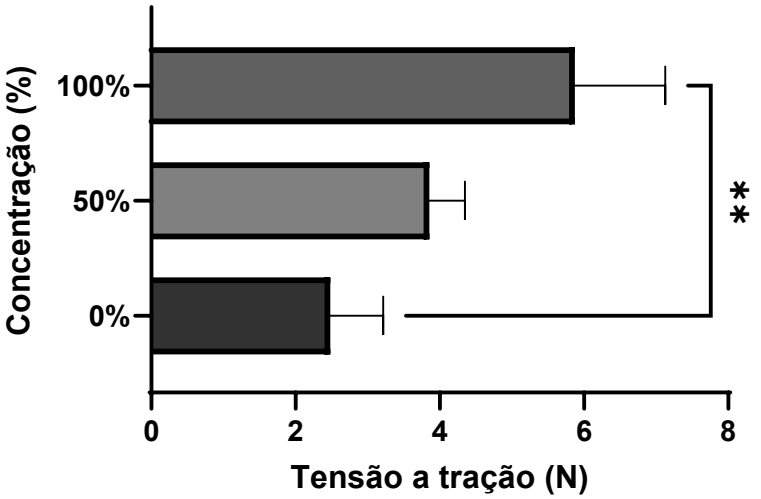

Figura 2. Média da tensão à tração (N) de filmes com diferentes concentrações de SLC de *P. acidilactici* CE51 (0, 50 e 100%).

Em relação ao deslocamento até a ruptura, o filme com concentração de 50 e 100% exibiram maior capacidade de alongamento se comparado com filme sem sobrenadante ($p<0,05$). O filme com 100% de sobrenadante apresentou o maior aumento, aproximadamente um aumento de 69,9 ± 9,3mm, conforme ilustrado na Figura 3.

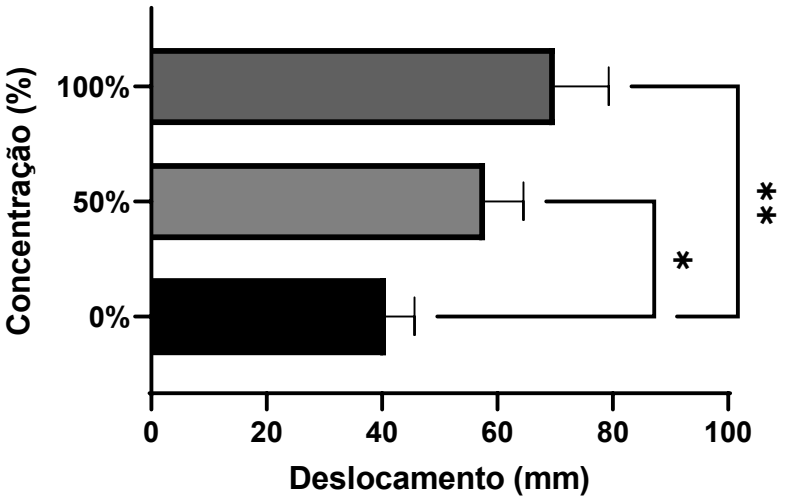

Figura 3. Perfil de alongamento à ruptura de filmes com diferentes concentrações de SLC de *P. acidilactici* CE51 (0, 50 e 100%).

O potencial inibitório dos filmes foi avaliado por meio da medição de halo de inibição do crescimento de *L. monocytogenes* e *E. faecium*. Os resultados indicaram que o maior halo de inibição para ambos os patógenos foram observados no filme com a concentração de 100% de sobrenadante, com halos de 17 mm e 16 mm.

O efeito bioconservador de filme biodegradável contendo SLC de *P. acidilactici* CE51 foi avaliado em carne de frango e bovina com dois microrganismos indicadores (*L. monocytogenes* e *E. faecium).* Foi possível observar uma redução significativa na contagem de *E. faecium* quando a carne de frango foi embalada com filmes de concentração a partir 50% e com 4 dias de incubação, sendo obtido uma redução de até 0,8 Log UFC/g em filme contendo 100% de sobrenadante e armazenado por 8 dias a 4ºC. Entretanto, foi notado aumento na contagem de *E. faecium* em algumas condições quando utilizado filme 0% ($p<0,05$) (Figura 4).

Para amostras de carne de frango contaminadas com *L. monocytogenes* foi possível observar reduções a partir da concentração de 50% e 2 dias de incubação, sendo obtido uma redução máxima de 0,8 Log UFC/mg em filme contendo 100% de sobrenadante e armazenado por 8 dias a 4ºC ($p<0,05$) (Figura 4). Nesse grupo experimental, também foi notado uma redução nas contagens de *L. monocytogenes* quando utilizados filme 0% ($p<0,05$), possivelmente devido interação com componentes para síntese do filme.

Na carne bovina, foi notado uma redução da presença de *E. faecium* a partir de filme contendo 50% de sobrenadante, entretanto, o maior decréscimo (0,88 UFC/g) foi observado em filme contendo 100% de sobrenadante e armazenado por 8 dias a 4ºC. Em relação a *L. monocytogenes* foi possível observar que filmes contendo 50 e 100% de sobrenadante apresentaram reduções significativas das contagens a partir de 4 dias de incubação ($p<0,05$), entretanto, a redução mais pronunciada (1,9 UFC/g) foi notada quando incubado por 8 dias a 4ºC em filme contendo 100% de sobrenadante (Figura 5). Nesta mesma condição (carne

bovina) foi também notado aumento das contagens de *L. monocytogenes* quanto utilizados filmes 0% após 6 e 8 dias de incubação ($p<0,05$).

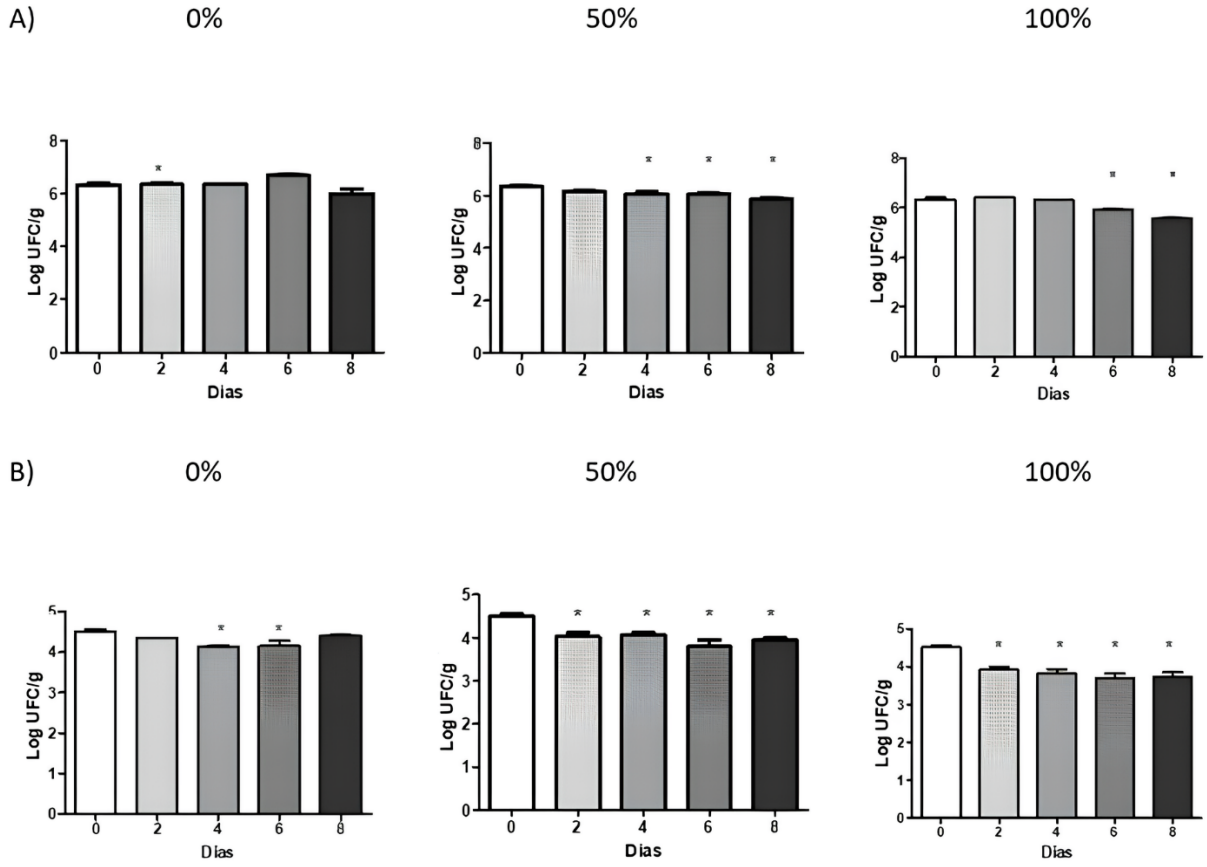

Figura 4. Resultados de contagem em placa de *E. faecium* (A) e *L. monocytogenes (B)* de carne de frango contaminadas propositalmente e embaladas com filme biodegradável acrescidos com 0, 50 e 100% de cultura livres de células de *P. acidilactici* CE51 e incubadas a 37°C por 0, 2, 4, 6, 8 dias.

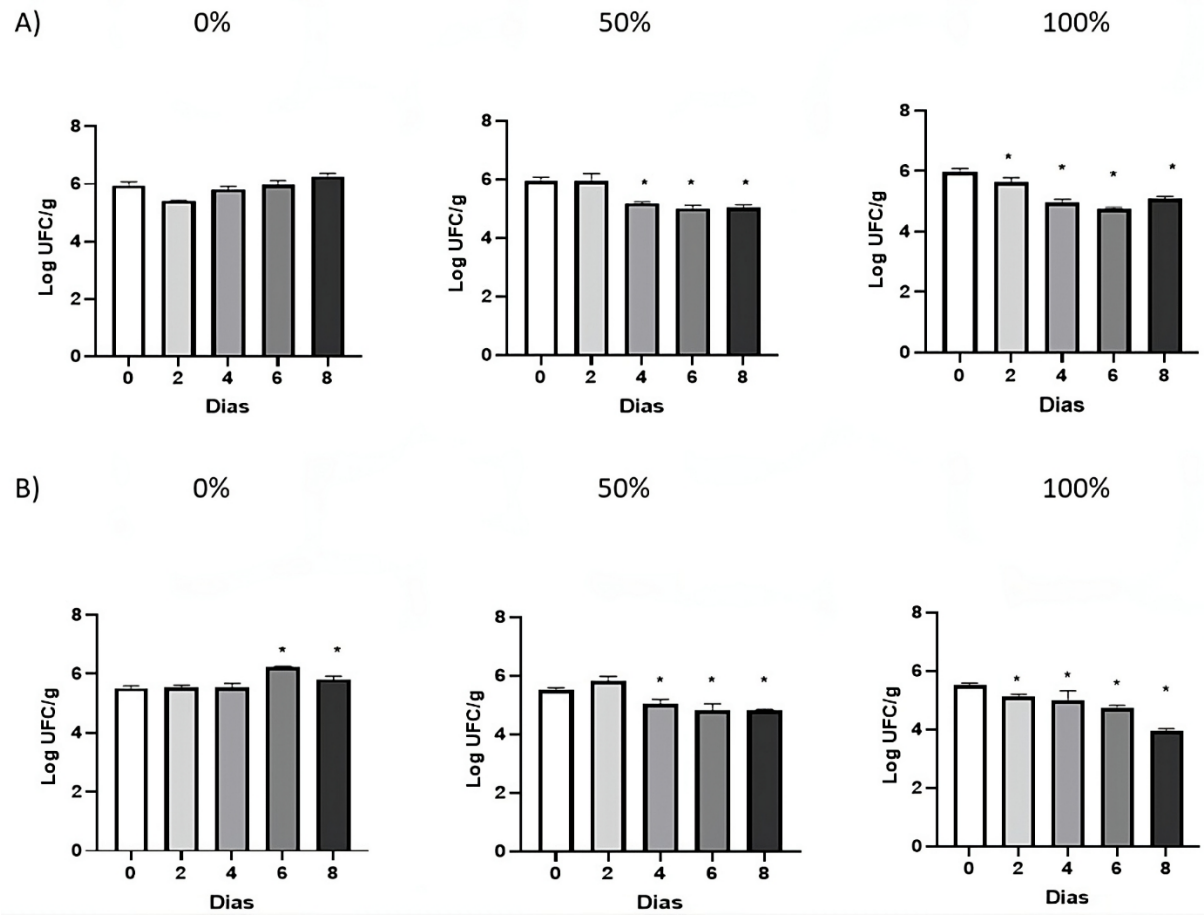

Figura 5. Resultados de contagem em placa de *E. faecium* (A) e *L. monocytogenes* (B) de carne bovina contaminadas propositalmente e embaladas com filme biodegradável acrescidos com 0, 50 e 100% de cultura livres de células de *P. acidulactici* e incubadas a 37°C por 0, 2, 4, 6, 8 dias.

5. Discussão

Alimentos de origem animal, como a carne de frango e bovina, são altamente suscetíveis à deterioração bacteriana devido à alta atividade de água, pH favorável e alto teor de nutrientes. Por isso, tecnologias emergentes de embalagens ativas que buscam aumentar o tempo de vida de alimentos são promissoras.

Para que uma embalagem atinja seu objetivo (proteção e conservação do alimento, além da função de informação), o material utilizado necessita passar por diversos testes de propriedade física. No presente trabalho, o primeiro deles foi a solubilidade, onde foi observado que quanto maior a quantidade de sobrenadante presente no filme (100%), menor a

solubilidade em água. Esses dados contrastam com os obtidos por Contessa et al. (2021) que, apesar de utilizarem uma bacteriocina diferente da utilizada no trabalho, perceberam aumento da solubilidade em água ao adicionarem extratos de bacteriocina de *Lactobacillus sakei* em filmes de quitosana/ágar devido, possivelmente, às características hidrofílicas do extrato de *L. sakei*. Dessa forma, mais estudos são necessários para entender os mecanismos que levaram à diminuição da solubilidade nos filmes do presente estudo, especialmente quanto à hidrofobicidade do SLC de *P. acidilactici* CE51.

A solubilidade é uma característica significativa para determinar a resistência à água dos filmes, especialmente nos filmes à base de amido. A insolubilidade é desejável em embalagens de alimentos, pois melhora a integridade do produto, as propriedades de barreira à umidade e o prazo de validade (Tongdeesoontorn et al. 2011).

A opacidade dos filmes também aumentou conforme a quantidade de SLC de *P. acidilactici* CE51 aumentava. Esse resultado foi semelhante aos obtidos por Contessa et al. (2021) que, além do aumento da opacidade, os filmes apresentaram coloração amarelada com a adição do extrato purificado de bacteriocina de *L. sakei* em filmes de quitosana/ágar. Em contrapartida, Dicastillo et al. (2021) não observaram alteração na opacidade em três filmes testados (caseinato de sódio, alginato de sódio com gelatina e álcool polivinílico) ao adicionarem bacteriófago Listex P100.

A transparência dos filmes é um fator que influencia a aparência geral e a qualidade dos materiais para aplicações em embalagens de alimentos, permitindo um aumento na aceitação geral pelos consumidores (Dicastillo et al. 2021). Por outro lado, a alta opacidade atua como barreira, prevenindo a deterioração oxidativa induzida pela luz, evitando perdas de nutrientes, descoloração e sabores estranhos. Quando aplicados em materiais usados para embalar alimentos perecíveis, como carnes e laticínios, possuem bons resultados para evitar a perda de nutrientes por foto-oxidação e prolongar a vida útil do produto (Wai et al. 2022).

A resistência ou tensão à tração mostra a tensão máxima que os filmes podem suportar quando esticados. Nesse estudo, o filme com 0% de concentração apresentou a menor resistência e o filme de 100% de concentração demonstrou a maior resistência. O que pode ser considerado um resultado desejável, visto que a resistência à tração dos materiais de embalagem é importante na proteção e resistência à violação das embalagens de alimentos. Geralmente, uma maior resistência à tração é preferível em embalagens, pois garantem uma melhor vedação com estabilização segura da carga, enquanto contribuem para produtos mais seguros e de alta qualidade para o consumidor final.

Outros estudos mostraram que a adição de bacteriocina em filmes de diferentes formulações aumenta a tensão à tração (Feng et al. 2021), resultando em filmes fortes e com maior resistência mecânica, o que corrobora com nossos resultados.

O alongamento na ruptura indica o aumento do comprimento do filme desde o ponto inicial até o ponto de ruptura, medindo a extensibilidade do filme. O filme com concentração de 100% obteve a maior capacidade de alongamento, dado que corrobora com Gumienna and Górna (2021) quando afirmam que a adição de pediocina aumenta os valores de alongamento na ruptura. Quando se objetiva envolver e embrulhar alimentos, valores altos de alongamento são preferíveis.

Grande parte do efeito antimicrobiano das bactérias lácticas se deve à produção de bacteriocinas que podem ser usadas em embalagens para impedir o crescimento de microrganismos indesejáveis (Vieira et al. 2020; Gumienna and Górna 2021). Em nosso estudo, obteve-se o resultado esperado em relação à eficácia antimicrobiana do filme contendo SLC de *P. acidilactici* CE51.

Esperava-se que o filme fosse capaz de inibir o crescimento de *L. monocytogenes*, como já visto na literatura (Kho et al. 2024). No presente estudo, a ação antimicrobiana contra amostras de frango contaminadas por *L. monocytogenes* obteve redução máxima (0,8 Log

UFC/g) em filme contendo 100% de sobrenadante e armazenado por 8 dias a 4°C. Em carne bovina, ao utilizar o filme contendo 100% de SLC, com 8 dias de incubação, observou-se a maior redução entre todas as variáveis (1,9 UFC/g). Efeitos semelhantes foram observados por Nieto-Lozano et al. (2006) ao utilizarem bacteriocina de *P. acidilactici* na superfície de carnes cruas. Kho et al. (2024) também obtiveram o mesmo resultado, ao observaram atividade antilisterial do SLC de *P. acidilactici* incorporado em celulose bacteriana após 12 horas de incubação.

A maior sensibilidade de *L. monocytogenes* ao agente antimicrobiano em carne bovina em comparação à carne de frango pode estar associada a diferenças na matriz alimentícia dessas duas carnes, especialmente ao teor de gordura, que é maior no acém bovino do que no peito de frango, cortes utilizados no estudo. Um trabalho realizado por Degnan and Luchansky (1992) avaliou e monitorou a atividade antilisterial de pediocina AcH em sebo e músculo bovino. Após a adição em suspensões nas amostras, foram observadas perdas da atividade da pediocina. A recuperação da atividade da pediocina AcH foi maior em suspensões à base de sebo do que de músculo. Esses dados mostram a relevância do monitoramento da atividade residual de bacteriocinas em alimentos ao avaliar a eficácia de embalagens ativas. Estudos adicionais são necessários para avaliar o mecanismo de ação da pediocina utilizada neste estudo e a interação em diferentes matrizes alimentícias.

O tempo de incubação considerado ótimo (redução de 0,8 Log UFC/g) para atividade antimicrobiana contra *E. faecium* também foi de 8 dias, tanto em amostras de frango, quanto de carne bovina (0,88 Log UFC/g). Na carne de frango, com 4 dias de incubação e com concentração a partir de 50%, foi possível observar redução de *E. faecium.* Se comparado à redução *L. monocytogenes,* é necessário mais tempo e uma maior concentração do SLC para reduzir a população de *E. faecium*.

A atividade antimicrobiana da pediocina envolve a formação de poros na membrana alvo, provocando mudanças na integridade, o que resulta na perda de materiais celulares vitais e consequente morte celular. Para que isso ocorra, é necessária uma atração inicial entre a pediocina e a bactéria alvo. A atração se dá principalmente devido às interações eletrostáticas entre o domínio catiônico antiparalelo da folha β na região N-terminal com o ácido lipoteicóico, que é o principal componente presente na superfície de bactérias gram-positivas, em especial, a *L. monocytogenes*. Sabe-se que a composição lipídica da membrana da célula alvo é um fator importante de suscetibilidade à pediocina e outras bacteriocinas (Xu et al. 2022). Nesse contexto, o motivo de o efeito antibacteriano apresentar-se mais pronunciado contra *L. monocytogenes* do que contra *E. faecium* esteja, possivelmente, na morfologia das duas bactérias, dado o modo de ação da pediocina.

Na carne de frango contaminada com *L. monocytogenes* e embalada com filme contendo 0% de SLC, observou-se uma redução significativa nos dias 4 e 6 de incubação. A redução pode ter ocorrido devido ao ácido cítrico presente no filme. O ácido cítrico é ácido orgânico natural de origem biológica e possui propriedades antibacterianas, especialmente contra *L. monocytogenes*, sendo amplamente utilizado na como aditivo na indústria (Wu et al. 2017; Zhang et al. 2023).

Houve aumento na contagem de *E. faecium* (carne de frango) quando utilizado filme 0%, com 2 dias de incubação. Em carne bovina, foi notado aumento das contagens de *L. monocytogenes* quanto utilizados filmes 0% após 6 e 8 dias de incubação, o que mostra que, mesmo sob refrigeração (4^{o}C), os microrganismos foram capazes de se multiplicar.

A eficácia antimicrobiana foi comprovada contra os dois microrganismos indicadores em filmes que continham 100% de SLC, demonstrando um avanço significativo na preservação da qualidade dos alimentos. Além disso, essa mesma composição resultou em melhorias nas propriedades mecânicas, com aumento da tração e deslocamento, características

essenciais para a resistência e durabilidade das embalagens alimentícias. A incorporação de SLC (100%) conferiu maior robustez aos filmes, o que torna essa tecnologia especialmente vantajosa para a indústria de alimentos. Contudo, embora o aumento da opacidade seja benéfico em termos de funcionalidade, ele pode representar uma limitação para a estética das embalagens uma vez que a transparência óptica é crucial para os consumidores, pois possibilita a avaliação visual da aparência dos alimentos no ponto de venda. Assim, o aumento da opacidade pode constituir uma barreira comercial, embora os benefícios mecânicos e antimicrobianos ofereçam grande potencial para a evolução das embalagens alimentícias (Eldesouky et al., 2015).

Apesar de diversos estudos terem utilizado cepas de *P. acidilactici* contra *L. monocytogenes* (Kho et al. 2024), nenhum deles avaliou a eficácia contra *E. faecium* e/ou simulou as condições de temperatura (4^{o}C), tempo de incubação (8 dias), concentrações de SLC (0, 50 e 100%) avaliadas no presente trabalho, bem como os tipos de amostras testadas (carnes de frango e bovina). A análise do efeito dos filmes contendo SLC aplicado às carnes é capaz de simular a realidade das carnes comercializadas, (quando embaladas, refrigeradas e armazenadas em temperaturas baixas) fator que contribui para seu uso comercial.

Conclusão

Os dados do presente estudo sugerem que a aplicação do SLC em filmes *P. acidilactici* CE51 atingem sua eficácia antimicrobiana contra os dois microrganismos testados quando em 100% de concentração. A incorporação do SLC (100%) nos filmes levou à redução da solubilidade, aumento da opacidade, maiores resistência à tração e capacidade de alongamento. Sendo assim, o uso dos filmes como embalagem protetora de carne de frango e bovina é promissor, devido à redução significativa de *L. monocytogenes* e *E. faecium*.

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ANEXO A- NORMAS DE PUBLICAÇÃO DO PERIÓDICO JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

1.0 Aims & Scope

The Journal of Food Science and Technology (JFST) is an international peer reviewed scientific journal published monthly and is the official publication of the Association of Food Scientists and Technologists of India (AFSTI). The journal considers high-quality, original research representing complete studies and scientific advances dealing with the innovative application of fundamental and applied science to enhance the understanding of product attributes, processes, technologies and bioactive constituents of foods, including antioxidants, phytochemicals, antinutrients of food and their impact on health.

The Journal's basis is food science and technology with increasing emphasis on findings that enhance product quality and safety of foods., extend shelf life of fresh and processed food products and improve process efficiency.

Out of scope of the journal

Manuscripts (MS) that report studies, which are (i) not of international interest or do not have a substantial impact on either food science or food technology, (ii) submissions which comprise merely data collections and (iii) based on the use of routine analytical methods, shall be rejected without review as out of scope.

Further, authors should please note that any manuscripts dealing with bacteriological cultures or strains must include the culture deposition numbers as given by an authentic public culture collection (e.g., ATCC, MTCC, NCIM etc), failing which such MS will not be considered for the review process and rejected as out of scope.

2.0 Pre-submission tips to ensure your manuscript is handled promptly

Authors SHOULD NOT contact the Editor-in-Chief (EiC) or Editor(s) for seeking opinion on suitability of your manuscript (MS) for submission. This decision is best left to you (and your co-authors). EiC, Editors or the Editorial Office (EO) cannot pre-screen MS outside the electronic system as JFST uses the Editorial Manager® (EM) electronic submission system.

For smooth handling of MS by the Editorial Office (EO), authors may ensure that the:

• MS fits the *Aims & Scope* of the journal.

• Cover letter is prepared, introducing your article and explaining the novelty of the research, identifying important outcomes of the work.

• List of at least four potential reviewers with contact details (i.e., Full name, designation affiliations and official address, official e-mail and alternate email, if available). None of the reviewers should be from the author's own institution; and, at least 3 out of the 4 reviewers from countries than the one to which the authors belong.
• The text is written in good English.
• The MS must have, on a separate page, stand-alone highlights of the work (in such a way that one need not have to read the article to understand what authors mean). There has to be a minimum 3 highlights (and a maximum of 5), with each highlights not exceeding 100 characters including spaces.
• MS is in accordance with ARTICLE TYPES and strictly adheres to the limits prescribed for the number of words, references and of figures/tables is within the stipulated limits:
Research article (6000 words, 30 references, 6 tables & figures combined)
Review article (7000 words, 60 references, 8 tables & figures combined)
Short communication (3000 words, 25 references, 3 tables & figures combined)
Any additional tables and figures as supplementary material
• Text in the MS is clearly divided into sections as mentioned in the instructions; that the line and page numbers are continuous and the text is is double-spaced.
• Declarations section is included on the Title Page to facilitate double-blind peer review
• Any experiments involving humans/animals are accompanied by an ethical statement.
• Conflict-of-interest statement is included in your Declarations section
• Additional electronic material in support of your MS, if any.
• All relevant sources (i.e. peer-reviewed articles, websites, books, theses etc.) are included in the Reference list. Number of references do not exceed the prescribed limit. Any pre-print edition/online repository of the theses referred in the MS must be upfront declared in the cover letter and appropriately referenced in the MS.

3.0 Declaration on submission

Submission of an MS to JFST implies that – (i) the work described has not been published before (except in the form of an abstract, a published lecture or academic thesis), (ii) it is not under consideration for publication elsewhere, (iii) its submission to JFST publication has been approved by all authors as well as the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out, (iv) if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically

without the written consent of the copyright holder, and (v) JFST will not be held legally responsible should there be any claims for compensation or dispute on authorship.

4.0 Submissions

4.1 Cover letter

All manuscripts must be accompanied by a cover letter, addressed to the EiC, which should clearly present the descriptions about the significance of research work, including its originality, its contribution to new knowledge in the field, and its relevance to the journal's aims & scope especially in the context of core food science and technology.

If author(s) do not enclose a covering letter covering aspects as mentioned below, the MS would be returned by the EO.

The cover letter, from among other things, should specifically address the following aspects –

(i) the type of article being submitted (original research article / review / short communication).

(ii) the total word count of the MS (excluding tables and figure legends), number of references and number of tables and/or figures in the MS.

(iii) should clearly mention about the originality of work, its non-submission / consideration in another journal.

(iv) highlights/novelty of the work being submitted (minimum of 3 and a maximum of 5 as bullets). Each highlight has to be a separate statement or bullet; and, each highlight shall not exceed 100 characters including spaces.

(v) a statement on conflict of interest, if any or otherwise.

(vi) a statement to the effect that all authors have read and approved the MS; and, that all co-authors are aware of its submission to JFST including the concerned authorities.

(vii) the corresponding author must undertake in the covering letter that he/she shall review at least three manuscripts (in his/her own specialization) submitted to JFST.

(viii) If the manuscript is one of a series of companion manuscripts that will be published sequentially, please describe the planned series in the cover letter, mentioning previously published parts and giving an estimate of when subsequent parts will be submitted.

4.2 Online submissions

Authors should submit their manuscripts online only using the platform provided for submission to JFST. Electronic submission substantially reduces the editorial processing and reviewing times and shortens overall publication times. Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given

on the screen. Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

4.3 Online Submission of revised manuscript

Highlight all changes made in the revised MS for faster processing and provide a pointby-point reply to the reviewer(s) comments as a separate file and a list of changes. Insert continuous line numbers and page numbers throughout the text to facilitate the reviewing process.

4.4 Confidentiality

Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share information.

5.0 Permissions

Author(s) wishing to include figures, tables, or text passages from other copyrighted works must obtain written permission from the copyright owner(s) for both the print and online format and appropriately credit the source(s) in the article. Please be aware that some publishers do not grant electronic rights for free and that the journal/publisher will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used. Authors must submit evidence that such permission has been granted when submitting their manuscript. Any material received without such evidence will be assumed to originate from the authors.

6.0 Types of Manuscripts

The journal accepts Research Articles, Reviews and Short communications. In addition, starting July 2020, the journal also intends to introduce Rapid Communications. Limits set for each type of the article are separately detailed below.

6.1 Research Articles

Research articles are complete reports of original, scientifically sound research that have not been published previously, except in a preliminary form in symposia/conferences etc. The article must contribute new knowledge and original research that is expected to have a definable impact on the advancement of food science and technology. Originality comprises of novel experiments and results, interpretations of data, and absence of prior publications on the same/similar topics. Fragmentation of work into an incremental series (that amounts to

data slicing) of manuscripts is not acceptable. The research articles shall adhere to the following, in terms of its text attributes –

Abstract : ≤ 200 words

Word count : ≤ 6000 (incl. abstract & references; excl. tables & figure legends)

Total Figures & Tables : ≤ 06

Number of references : ≤ 30

7.0 Review Process

A double blind peer review system is used to ensure high quality of manuscripts accepted for publication. All contributions will be initially assessed for suitability. The EiC and Editors have the right to decline formal review of the manuscript when it is deemed that the manuscript is either/or -

(i) outside the scope of the Journal,

(ii) not within the priority subject of the journal,

(iii) makes no contribution to the advancement of food science and/or food technology,

(iv) lacks scientific and technical merit,

(v) not innovative, lacks novelty or any new information,

(vi) fragmentary and provides marginally incremental results,

(vii) closely duplicates research previously published by the author (e.g., just changing the source or species)

(viii) reports only routine work (lacks novelty)

(ix) poorly written or lacks clarity in English usage and grammar

Manuscripts that meet the journal's criteria for scope, relevance and scientific merit will be sent for peer review to at least two independent expert reviewers assigned by the Editor. The review will be conducted against established criteria to determine scientific and technical merit. Each Reviewer submits a recommendation regarding the merit of the manuscript, but the Editor provides the final decision on acceptance of the paper for publication. The EiC's and/or Editor's decision is final, and no communication would be entertained in this regard with the Editor or EiC.

7.1 Reviewer suggestions/exclusion

Authors, mandatorily, should suggest at least 4 suitable reviewers on the EM system; and/or request for the exclusion of certain experts as reviewers, if any, when they submit their manuscripts. Please note that the Journal may use some or none of the suggested reviewers. These suggestions are appreciated as it helps facilitate the peer review process. When

suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way nor related to the author.

Authors should note the following while making suggestions on the potential reviewers -

(i) Suggest a mix of reviewers from different countries and different institutions.

(ii) The recommended reviewers should be experts in the subject matter of the manuscript.

(iii) Should not be anyone who is or has been a former adviser/advisee/research collaborator/ and/or co-author of papers and patents or in any other way has a conflict of interest.

(iv) The reviewers suggested should not be a colleague in the same institution.

(v) They all cannot be from your own country (if suggesting from your own country, restrict it to only one of four or more reviewers suggested).

(vi) Provide an institutional email address for each suggested reviewer, or, if this is not possible to include, provide other means of verifying the identity (such as a link to a personal homepage, a link to the publication record or a researcher or author ID) in the submission letter.

8.0 General responsibilities of Author(s)

Author(s) who are submitting the manuscript should be aware of their responsibilities which include, but not limited to, the following –

(1) All authors are collectively responsible for the content of the work submitted for consideration of publication. It is also a collective responsibility of all authors to ensure to check the publication for correctness through all stages of publication to ensure that the methods, results and conclusions are reported accurately as intended.

(2) Author(s) should proof read and check all the calculations, formulae, data presentation/interpretations, typesetting and correctness of typescripts during submission/revision(s), reviewing and galley proofing (post-acceptance, if accepted).

(3) Use appropriate methods of data analysis and display is completely the responsibility of all authors; and, if any specialist advice is used, he/she should be appropriately acknowledged (either through an authorship or by acknowledging the person in the acknowledgement section) as deemed appropriate by the author(s).

(4) Images (e.g. micrographs, X-rays, pictures of electrophoresis gels) should not be modified in any misleading way; and, only the original images as produced during the investigation(s) shall only be used for the purpose of research publication.

(5) Author(s) should alert the EO or EiC promptly, if they discover an error in any submitted, accepted or published manuscript.

(6) Author(s) should cite only relevant references which they have read; and, must not quote any reference(s) from other publications if they have not read the cited work.

(7) Author(s) must not use acknowledgements misleadingly to imply a contribution or endorsement by individuals who have not, in fact, been involved with the work or given an endorsement.

(8) It is the responsibility of author(s), especially the Corresponding Author, that the authorships of the submitted manuscript accurately reflect an individual's contribution. Author(s) must refrain from the practices of guest, gift, and/or ghost authorship.

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(10) It is author(s) responsibility to ensure due and proper acknowledgement of any funding received both in the text of the MS as well at appropriate place during the online submission.

(11) Author(s) should duly obtain any institutional/organizational permission required before submitting the MS. Journal shall not be responsible in any way for any act of omission or commission in this regard.

(Most of the contents in this section are sourced and modified from the - *Responsible research publication: International standards for authors 'A position statement developed at the 2nd World Conference on Research Integrity', Singapore, July 22-24, 2010*)

8.1 Ethical Responsibilities

This journal is committed to upholding the integrity of the scientific record. Authors should refrain from misrepresenting research results, which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by adhering to the rules of good scientific practices, but not limited to, as mentioned below:

(1) The research being reported should have been conducted in an ethical and responsible manner.

(2) The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work.

(3) The MS shall not be submitted to any other Journal for simultaneous consideration.

(4) A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time.

(5) Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image-based manipulation).

(6) No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted. Please note that JFST screens each MS through an anti-plagiarism software and excessive reproduction of previous works will result in rejection.

(7) Authors should make sure they have permissions for the use of material(s) mentioned in the MS, including reproduction(s) of images/figures/tables from published papers.

(8) Include only those authors who have contributed meaningfully and have made primary contributions (like design of work, interpretation of results, writing of the paper etc.) to the work. It is the responsibility of the corresponding author to ensure that every author has read and approved the manuscript before submission.

(9) Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged; and, will be treated as an unethical publishing behaviour.

(10) Upon request, authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

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- an erratum/correction may be placed with the article

- an expression of concern may be placed with the article
- or in severe cases retraction of the article may occur.
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Authors are strongly advised to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Either adding and/or deleting authors /rearrangement of author names during the revision stages is generally not permitted, but in some cases may be warranted and possible, only if approved by the EiC. To request such a change, the EiC must receive the following from the corresponding author –

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(b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement.

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For the purposes of transparency, the journal requires authors to submit a statement outlining the individual contributions of each author(s) to the manuscript, to be placed in the Declarations on the Title Page. The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors. Authorship statements should be formatted by including names in the form of initials (e.g., if Dr Marie Curie was responsible for conceiving the idea, carried out the work and wrote the MS, while Prof CV Raman supervised the work and corrected the manuscript; then it should be written as "MC conceived, carried out the experiments and wrote the MS; CVR supervised the work and edited the manuscript").

8.5 Acknowledgements

Collate acknowledgements in a separate section on the Title Page. List here all those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.)

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If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent for publication. See our editorial policies for more information on consent for publication. If your manuscript does not contain data from any individual person, please state "Not applicable" in this section of the Declarations.

8.7 Availability of Data and Materials

All manuscripts must include an 'Availability of data and materials' statement in the Declarations. Data availability statements should include information on where data supporting the results reported in the article can be found including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. By data we mean the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. We recognise it is not always possible to share research data publicly, for instance when individual privacy could be compromised, and in such instances data availability should still be stated in the manuscript along with any conditions for access. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

 The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

 The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

 All data generated or analysed during this study are included in this published article [and its supplementary information files].

 The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

 Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

 The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

 Not applicable. If your manuscript does not contain any data, please state 'Not applicable' in this section.

More examples of template data availability statements, which include examples of openly available and restricted access datasets, are available here. Authors should cite any publicly available data on which the conclusions of the paper rely in the manuscript. Data citations should include a persistent identifier (such as a DOI) and should ideally be included in the reference list. Citations of datasets, when they appear in the reference list, should include the minimum information recommended by DataCite and follow journal style. Dataset identifiers including DOIs should be expressed as full URLs.

For example:

Hao Z, AghaKouchak A, Nakhjiri N, Farahmand A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. figshare. 2014. http://dx.doi.org/10.6084/m9.figshare.853801

With the corresponding text in the Availability of data and materials statement:

The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]. [Reference number]

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Please use the authors initials to refer to each authors' competing interests in this section. If you do not have any competing interests, please state "The authors declare that they have no competing interests" in this section.

9.0 Article Structure

Follow this order when typing manuscripts: Title, Authors, Affiliations, Declarations, Abstract, Keywords, Main text (Introduction, Materials and Methods, Results, Discussion), Author contributions, Acknowledgements, References and Figure Captions. Tables, Figures and supplementary material that will be uploaded as separate files during online submission, will be placed after the figure captions by the system, as Tables, figures and supplementary files, in that order.

9.1 Title page (Page 1)

The title page should include an informative title, name of the author(s), author(s) affiliations, corresponding author & his contact details, acknowledgement(s), and Declarations.

Title of the MS*:** The MS should have ***a concise, un-ambiguous and informative title. Titles are often used in information-retrieval systems. Use abbreviations and formulae, only if they are very essential and cannot be done away with.

The name(s) of the author(s): Please clearly indicate the full given name(s) and family name(s) of each author and check that all names are accurately spelled. Please ensure that names are listed in the order first name/FAMILY NAME (e.g. Marie CURIE) – this will ensure they are listed correctly in indexing services.

***Author(s) affiliations*:** Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the email address of each author.

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Declarations: All manuscripts must contain the following sections under the heading 'Declarations', to be placed on the Title Page. JFST follows a double-blind review process, at the time of initial submission and revision(s) and hence any such information is not desirable

in the MS. If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate consent statements)

Consent for publication (appropriate statements regarding publishing an individual's data or image)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions

9.2 Research highlights (Page 2)

Place research highlights on a separate page preceding the abstract. The research highlights should consist of short collection of bullet points. Highlights should identify and capture novel outcomes of your work and must be stand-alone (i.e., they should not require someone to read the article to understand what is being conveyed). Provide a minimum of 3 and a maximum of 5 highlights. Each highlight, provided as a bullet point, shall have a maximum of 100 characters including spaces.

9.3 Abstract (Page 3)

The abstract should be a clear and concise (**not exceeding the word limits as prescribed in section 6.0**) one-paragraph factual summary which is informative rather than descriptive. It should include the purpose of the research, major results and conclusions. Do not use statements such as "Results are discussed". An abstract is often presented separately from the article, so it must be able to stand alone and be comprehensible without the rest of the paper. References should be avoided, but if essential, then cite the author(s) and year(s) title, journal name, volume and page numbers. Avoid non-standard or uncommon abbreviations, but if essential they must be defined at their first mention in the abstract itself.

9.4 Keywords (Page 3)

Keywords allow the article to be found easily by search engines and considerably increase article citations when they are comprehensive. Provide a minimum of 4 and maximum of 6 significant keywords to aid the reader in literature retrieval. Keywords should be in singular, full word form.

9.5 Abbreviations (Page 3)

Define abbreviations that are not standard in food science and technology. These will be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there. Ensure consistency of abbreviations used throughout the article.

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The article should be divided into clearly defined sections *viz.,* Introduction, Materials and Methods, Results, Discussion, Author contributions, Acknowledgements, Conflict of Interest (if any), and References. Each section heading should appear on its own in a separate line. Any subsection may be given a brief heading. If the MS uses abbreviations, they should be defined at first mention and used consistently thereafter.

Introduction

Provide a brief review of pertinent work citing key references (do not resort to selfcitation, unless it is very essential) outlining the issue that is being addressed and clearly state the objectives. Discuss relationships of the study to previously published work, but do not reiterate or attempt to provide a complete literature survey. The purpose or rationale for the research being reported, and its significance, originality, or contribution to new knowledge in the field, should be clearly and concisely stated. Do not summarize the current findings.

Material and methods

In this section author(s) should clearly provide details of the materials and methods they have employed in the study in such a way that it can be easily carried out by other researchers if they wish to. Author(s) must emphasize any unexpected, new, and/or significant hazards or risks associated with the experimental work. Specific and new experimental methods should be sufficiently detailed so the work can be repeated by fellow researchers interested in the area. Give references to globally established methods, provide references and brief descriptions of methods that have been published but are not well-known, describe substantially modified methods, including statistical methods, give reasons for using them, and evaluate their limitations. Fo special equipment, reagents, kits, etc., the source, city, state, and country should be specified in parentheses.

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Manuscripts dealing with bacteriological cultures or strains, the culture deposition numbers as given by public culture collection (e.g., ATCC, MTCC, NCIM etc) must be provided, without which such MS will not be considered for the review process.

Experiment with live animals or human subjects are used must include a statement that such experiments were performed in compliance with the appropriate laws and institutional guidelines and also name the institutional committee that approved the experiments (*for details see section 8.3*).

If variation within a treatment (coefficient of variation (CV), that is, the standard deviation divided by the mean) is less than 10% and the difference among treatment means is greater than 3 standard deviations, it is not necessary to conduct a statistical analysis. If the data do not meet these criteria, statistical analysis must be conducted. In case of theoretical papers / engineering calculations, the section should provide an extended (not repeating what is already dealt in introduction) foundation for the current and further work. In other words, the calculation section should represent its practicality in the context of the MS from a theoretical basis.

Results and Discussion

Results and discussion may be presented in separate sections or combined into a single section, whichever format conveys the results lucidly. To avoid repetition of results in the discussion, a combined section of results and discussion is often more appropriate. Results should be very concise and clear. Cite tables and figures consecutively in text with Arabic numerals. Do not intersperse tables and figures in text. While discussing findings, compare results with previous work and proposing explanations for the results observed. Extensive citations and discussion of published literature without importance to the experimental results should be avoided. Avoid speculation unsupported by the data obtained.

If author(s) choose to separate results and discussion sections, the results section should be very clear, comprehensive and concise. Repetition of results in the discussion section should be avoided. Discussion should clearly focus on the significance of the results of the work and the improvements over the already available knowledge.

Conclusions

The main conclusions of the study may be presented in a short conclusions section, which may stand alone or form a subsection of the Discussion or Results and Discussion section. The conclusions section, as far as possible, should avoid presenting the results repetitively and must focus on the key takeaway from the study with emphasis on future prospects or requirements.

10.0 Scientific style

Please always use internationally accepted signs and symbols.

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Always use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Use mg/Kg instead of ppm and µg/Kg instead of ppb. Temperatures should be given in degrees Celsius (□C). The unit 'billion' is ambiguous and should not be used.

Do not use a plural form for the symbols; for example, 5 kgs would be incorrect instead use 5 kg. Give a space between measurement and number (for example, 50 cm, 0.1 N) but no space between degree and sign (for example, 25 oC) and % sign (for example, 50%). A range is formatted as 0.5- 1.0 g.

Unit expression should not be in the form of exponent (e.g., L min-1 should be L/min). Centrifugation speed should be expressed as gravity (e.g., 10,000 □ g, not 10,000g or 10,000xg) and not rpm.

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Numerical data should be reported with the number of significant digits that corresponds to the magnitude of experimental uncertainty.

Compounds: The rules and recommendations of IUPAC should be used for abbreviation of chemical names, nomenclature of chemical compounds, isotopic compounds, optically active isomers, and spectroscopic data.

Enzymes: The trivial and systematic names of enzymes should be those recommended by the Nomenclature Committee of the IUBMB and not abbreviated except in terms of the substrates for which there are accepted abbreviations — e.g., ATPase and DNase.

Organisms: Genus and species names should be in italics.

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Please use the standard mathematical notation for formulae, symbols, etc.: Italic for single letters that denote mathematical constants, variables, and unknown quantities Roman/upright for numerals, operators, and punctuation, and commonly defined functions or abbreviations, e.g., cos, det, e or exp, lim, log, max, min, sin, tan, d (for derivative) Bold for vectors, tensors, and matrices**.**

10.4 Analytical methods

If analytical method or measurement results thereof are reported they should also be accompanied by the associated measurement uncertainty, precision, reproducibility, repeatability, trueness, selectivity, sensitivity, and where applicable, information on method validation and the traceability to Certified Reference Materials (CRM).

11.0 References

Responsibility for the accuracy of references cited lies entirely with the authors. References taken from a review or other secondary source should be checked for accuracy with the primary source. The manuscript should be carefully checked to ensure that the spelling of authors' names and dates are exactly the same in the text as in the reference list. Ensure that every reference cited in the text is also present in the reference list at the end of the manuscript (and vice versa).

11.1 Citation in running text

Cite references in the text by name and year in parentheses. Some examples: 'Negotiation research spans many disciplines (Thompson 1990). This result was later contradicted by Becker and Seligman (1996). This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1993)'.

All citations in the text should refer to -

(1) Single author: the author's name (without initials, unless there is ambiguity) and the year of publication. e.g., Thompson 1990

(2) Two authors: both authors' names and the year of publication. e.g., Becker and Seligman 1996

(3) Three or more authors: first author's name followed by et al. and the year of publication. e.g., Barakat et al. 1995

(4) Citations may be made directly or in parenthesis. e.g., "as demonstrated earlier (Thompson 1990). Further Barakat et al (1995) have recently shown..."

(5) Groups of references cited together should be listed first alphabetically, then chronologically. e.g., "as demonstrated (Becker 1996ab; Becker 1999; Becker and Seligman 1996; Thompson 1990). Note references are separated using semi colon.

(6) Any references cited in the abstract must be given in full in the abstract itself.

(7) Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text.

(8) Papers should not depend for their usefulness on unpublished material, and excessive reference to material "in press" is discouraged.

(9) Reference to the author(s) own unpublished work is permitted if the subject is of secondary importance to the manuscript in question, but any unpublished results of central importance must be described in sufficient detail within the manuscript.

(10) If pertinent references are "in press" or unpublished for any reason, furnish copies to enable reviewers to evaluate the manuscript. An electronic copy of these materials should be uploaded according to the directions.

(11) “In press” references should include the Digital Object Identifier (DOI) assigned by the potential publisher

(12) Web references: As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. e.g., American Chemical Society, Chemical Health and Safety Division: http://tungsten.acs.org/health.html http://chas.cehs.siu.edu/ Accessed on 9th May, 2020

11.2 Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished work should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

The cited references should be arranged first alphabetically according to the last name of the first author and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Journal article: Author(s). Year in parenthesis, Article title (in sentence case). Journal title (abbreviated). Volume number: inclusive pages.

Example

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Article by DOI: Author(s). Year in parenthesis, Article title. Journal title. doi

Example

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Reference to a book: Author(s) or editor(s). Year in parenthesis, Title. Edition or volume. Publisher name, Place of publication.

Example

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Reference to a chapter in an edited book: Author(s) of the chapter. Year in parentheses Chapter title. Volume (if relevant). In: Author(s) or editor(s) Title of the book. Edition number (if relevant) Publisher name, Place of publication**.** Inclusive pages of the chapter.

Example

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document Author(s) year in parenthesis, Title URL. Accessed date.

Example

Cartwright J (2007) Big stars have weather too. IOP Publishing Physics Web. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2019

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see www.issn.org/2-22661-LTWA-online.php

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list. EndNote style (zip, 3kB)

11.3 Text Formatting

MS should be submitted in Word.

• Use a normal, plain font (e.g., 12-point Times Roman) for text.

• Use italics for emphasis.

• Use the automatic page numbering function to number the pages.

• Do not use field functions.

• Use tab stops or other commands for indents, not the space bar.

• Use the table function, not spreadsheets, to make tables.

• Use the equation editor or Math Type for equations.

• Save your file in docx format (Word 2007 or higher) or doc format (older version). MS with mathematical content can also be submitted in LaTeX.

• LaTeX macro package (zip, 182 kB)

12.0 Tables

Tables should be used when the data cannot be presented clearly in the narrative, when many numbers must be presented, or when more meaningful inter-relationships can be conveyed by the tabular format.

• Tables should supplement, not duplicate, information presented in the text and figures.

• Tables should be simple and concise**.**

• All tables are to be numbered using Arabic numerals.

• Tables should always be cited in text in consecutive numerical order.

• Each table, should have an overall title explaining the components of the table and each column within the table must have a heading. The title should be understandable without reference to the text. Details should be put in footnotes, not in the title

• The table should contain sufficient experimental detail to be understood without reference to the text. Each table should be stand alone.

• Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

• Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

13.0 Figures

Figure should be in a high-resolution original form. It is preferable to place any key to symbols used in the artwork itself, not in the caption. Ensure that any symbols and abbreviations used in the text agree with those in the artwork.

13.1 Numbering

• All figures are to be numbered using Arabic numerals.

• Figures should always be cited in text in consecutive numerical order.

• Figure parts should be denoted by lowercase letters (a, b, c, etc. e.g. Figure 1a, 1b).

• If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures (e.g., A1, A2, A3, etc). Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

13.2 Figure Captions

• Each figure should have a concise caption describing accurately what the figure depicts. The caption should be understandable without reference to the text

• Include the captions in the text file of the manuscript after References as “Figure captions”. Do not include in the figure file.

• Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type (**Fig.1**)

• No punctuation (full stop) is to be included after the number, nor is any punctuation to be placed at the end of the caption (e.g. **Fig.1** Functional properties of bioactive peptides and hydrolysates).

• Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs. It is preferable to place any key to symbols used in the artwork itself, not in the caption. Ensure that all symbols and abbreviations used in the text agree with those in the artwork.

• Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

13.3 Figure Placement and Size

• When preparing your figures, size figures to fit in the column width.

• The figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.

• If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Indicate the permission granted and cite as suggested by copyright owner. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

13.4 Artwork and Illustrations Guidelines

For the best quality final product, it is highly recommended to submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

• Supply all figures electronically.

• Indicate what graphics program was used to create the artwork.

• For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.

• Vector graphics containing fonts must have the fonts embedded in the files.

• Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art

Definition: Black and white graphic with no shading.

• Do not use faint lines and/or lettering and check that all lines **a**nd lettering within the figures are legible at final size.

• All lines should be at least 0.1 mm (0.3 pt) wide.

• Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

• Vector graphics containing fonts must have the fonts embedded in the files.

Accessibility to Visually Impaired

• In order to give all abled and disable persons access to the content of your figures, ensure that

• All figures have descriptive captions (visually impaired users could then use a textto- speech software or a text-to-Braille hardware)

• Patterns are used instead of or in addition to colors for conveying information (colorvision impaired users would then be able to distinguish the visual elements)

• Any figure lettering has a contrast ratio of at least 4.5:1

14.0 Electronic Supplementary Material

JFST accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature adds dimension to the author's article, as certain information either cannot be printed or is more effective in electronic form.

14.1 Submission

Supply all supplementary material in standard file formats. Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations - Always use MPEG-1 (.mpg) format.

Text and Presentations - Submit your material in PDF format .doc or .ppt files are not suitable for long-term viability. A collection of figures may also be combined in a PDF file.

Spreadsheets - Spreadsheets should be converted to PDF if no interaction with the data is intended. If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats - Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files - It is possible to collect multiple files in a .zip or .gz file.

14.2 Numbering and Captions

• If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

• Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

• Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

• For each supplementary material, please supply a concise caption describing the content of the file.

14.3 Processing of supplementary files

• Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

14.4 Accessibility

In order to provide all abled and disabled access to the content of the supplementary files, please make sure that the MS contains a descriptive caption for each supplementary material. Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

15.0 English language editing support

For editors and peer reviewers to accurately assess the work presented in your MS you need to ensure the English language is of sufficient quality to be understood.

MS that are accepted for publication will be checked by our copy editors for spelling and formal style. This may not be sufficient if English is not your native language and substantial editing would be required.

If English is not your native language you may want to have your MS edited by a native speaker or use a professional language editing service, where editors will improve the English to ensure that your meaning is clear and identify problems that require your review.

Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. They provide for scientific articles in medicine, biomedical and life sciences, chemistry, physics, engineering, business/economics, and humanities.

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Nature Research Editing Service and American Journal Experts will charge authors directly for these language polishing services. Please contact the editing service directly to make arrangements for editing and payment.

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