



**MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRICULTURA E BIODIVERSIDADE**

**EXTENSÃO DA VIDA ÚTIL DE MORANGOS INTEIROS E
MINIMAMENTE PROCESSADOS TRATADOS COM
INFUSÃO A VÁCUO DE PECTINAMETILESTERASE E
LACTATO DE CÁLCIO**

ALYSSON CAETANO SOARES



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Tese apresentada à Universidade Federal de Sergipe, como parte das exigências do Curso de Doutorado em Agricultura e Biodiversidade, área de concentração em Agricultura e Biodiversidade, para obtenção do título de “Doutor em Ciências”.

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SÃO CRISTÓVÃO
SERGIPE – BRASIL

"À minha família, a quem dedico todas as minhas vitórias. Sem o amor, o carinho e o suporte de cada um de vocês, esta jornada teria sido impossível. Minha eterna gratidão por serem a minha maior fonte de inspiração."

Dedico

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LISTA DE ABREVIATURAS, SÍMBOLOS E SIGLAS

ATT	Acidez Total Titulável
ACP	Análise de Componentes Principais
Ca ²⁺	Íon Cálcio
CaCl ₂	Cloreto de Cálcio
CDTA	trans-1,2-ciclohexano diamina tetraacético
CIE L*a*b*	Sistema de Cores (CIE L-star a-star b-star)
CSP	Pectina Solúvel em Na ₂ CO ₃
CWM	Material da Parede Celular (Cell Wall Material)
C*	Croma
DIC	Delineamento Inteiramente Casualizado
h°	Ângulo Hue
ISP	Pectina Solúvel em CDTA
IV	Infusão a Vácuo
L*	Luminosidade
LCa	Lactato de Cálcio
LCa+PME	Lactato de Cálcio e Pectinametilesterase (Combinados)
N	Newton
Na ₂ CO ₃	Carbonato de Sódio
PCA	Principal Component Analysis (ACP em inglês)
PG	Poligalacturonase
PGN	Pelargonidina-3-Glicosídeo
PME	Pectinametilesterase
POD	Peroxidase
PPO	Polifenoloxidase
PVPP	Polivinilpirrolidona
Ratio	Relação Sólidos Solúveis/Acidez Total
SI	Sem Infusão
SST	Sólidos Solúveis Totais
W	Água
WSP	Pectina Solúvel em Água
PME	Pectinametilesterase
g	Grama
L	Litro
mg	Miligrama
g.L-1	Grama por litro
mm	Milímetro
cm	Centímetro
mL	Mililitro
atm	Atmosfera
°C	Grau Celsius
CO ₂	Dióxido de Carbono
PCA	Análise de Componentes Principais
CV	Coefficiente de Variação
PVP	Polivinilpirrolidona
NaCl	Cloreto de Sódio
SE	Sergipe

RESUMO

SOARES, Alysson Caetano. **Extensão da vida útil de morangos inteiros e minimamente processados tratados com infusão a vácuo de pectinametilesterase e lactato de cálcio.** São Cristóvão: UFS, 2026. 59p. (Tese – Doutorado em Agricultura e Biodiversidade).*

A demanda por alimentos saudáveis levou a um aumento no consumo de frutas frescas, como morangos. No entanto, o morango é um fruto altamente perecível, com vida útil limitada devido ao rápido amolecimento e deterioração, processos intensificados no produto minimamente processado (MP). O amolecimento e a perda da firmeza representam parâmetros cruciais para a aceitação do consumidor. O objetivo deste trabalho foi avaliar a eficácia do uso combinado de infusão a vácuo (IV), lactato de cálcio (LCa) e pectinametilesterase (PME) na manutenção da firmeza, integridade celular e qualidade pós-colheita de morangos inteiros (I) e minimamente processados (MP). Morangos (I e MP) foram submetidos à infusão a vácuo com água (W), PME (10 UI), LCa (1%) e LCa+PME. Morangos sem infusão foram utilizados como controle (C). Foram avaliados a firmeza, perda de massa fresca, antocianina, cor, teor de sólidos solúveis totais (SST), acidez total (AT), relação SST/AT, pH, deterioração e danos visíveis, qualidade visual, teor de ácido galacturônico, grau de metilação, efluxo de eletrólitos, atividade da PME e respiração, que foram medidos inicialmente e a cada 3 dias durante 9 dias de armazenamento a 5 °C para frutos MP e a cada 3 dias por 12 dias para frutos inteiros (I). Os resultados demonstram que o tratamento com LCa+PME foi eficaz em ambas as formas de apresentação. Para morangos minimamente processados (MP), a infiltração a vácuo de PME com lactato de cálcio aumentou a firmeza de morangos MP em 90,7% para LCa+PME e 119,8% para LCa no terceiro dia, retardou a perda de peso para menos de 0,8% em todos os tratamentos, exceto no tratamento com PME, manteve o frescor e o teor de antocianinas, reduziu o efluxo de eletrólitos em 38% em comparação com o controle e diminuiu as taxas respiratórias para 134,92 e 135,62 mg.kg⁻¹.h⁻¹ CO₂ para LCa+PME e LCa, respectivamente, enquanto o controle atingiu 163,36 mg.kg⁻¹.h⁻¹ CO₂. Além disso, minimizou os danos aos frutos. Para frutos inteiros (I), o LCa + PME aumentou a firmeza dos frutos em 48% após 3 dias de armazenamento quando comparado com frutos do tratamento controle (W), mantendo-se até 12 dias de armazenamento. Esta estabilidade foi atribuída à sinergia do tratamento entre LCa e PME exógena, o qual aumenta os grupos carboxílicos livres, otimizando a formação de ligações cruzadas de Ca-pectato, resultando na estabilização do CSP (pectina solúvel em carbonato de sódio). Adicionalmente, os tratamentos nos quais se utilizou o cálcio reduziram o extravazamento de eletrólitos em 22% e limitaram a perda de massa em cerca de 2,7% para LCa + PME, resultando em melhor frescor e menor incidência de danos e contaminação. O LCa + PME preservou significativamente os parâmetros de cor (L*, C*) e estendeu o tempo de prateleira em, no mínimo, 3 dias. A infusão a vácuo de lactato de cálcio e PME é uma tecnologia promissora capaz de estender significativamente a qualidade pós-colheita e a vida útil de morangos inteiros e minimamente processados.

Palavras-chave: Atividade enzimática, Firmeza, *Fragaria x ananassa*, Processamento-mínimo, Pós-colheita.

* Comitê Orientador: Marcelo Augusto Gutierrez Carnellosi – UFS (Orientador), Luiz Fernando Ganassali de Oliveira Junior – UFS (Coorientador).

ABSTRACT

SOARES, Alysso Caetano. **Shelf-life extension of whole and minimally-processed strawberries treated with vacuum infusion, pectin methylesterase and calcium lactate.** São Cristóvão: UFS, 2026. 59p. (Dissertation - Doctor of Science in Agriculture and Biodiversity).

*

The demand for healthy foods has led to an increase in the consumption of fresh fruits, such as strawberries. However, strawberries are a highly perishable fruit with a limited shelf-life due to rapid softening and deterioration, which are intensified in minimally processed (MP) products. Softening and loss of firmness are crucial parameters for consumer acceptance. The aim of this study was to evaluate the effectiveness of the combined use of vacuum infusion (IV), calcium lactate (LCa), and pectin methylesterase (PME) in maintaining firmness, cell integrity, and post-harvest quality of whole (I) and minimally processed (MP) strawberries. The strawberries (I and MP) were subjected to vacuum infusion with water (W), PME (10 IU), LCa (1%), and LCa+PME, with a control group (C) without infusion. Firmness, fresh mass loss, anthocyanin, color, soluble solids content (SSC), total acidity (TA), SSC/TA ratio, pH, visible deterioration and damage, visual quality, galacturonic acid content, degree of methylation, electrolyte efflux, PME activity, and respiration were measured initially and every 3 days for 9 days of storage at 5°C for MP fruits and every 3 days for 12 days for whole fruits (I). The results demonstrate that the treatment with LCa+PME was effective in both presentation forms. In minimally processed strawberries (MP), vacuum infiltration of PME with calcium lactate increased firmness by 90.7% for LCa+PME and 119.8% for LCa on the third day, delayed weight loss to less than 0.8% in all treatments except the PME treatment, maintained freshness and anthocyanin content, reduced electrolyte efflux by 38% compared to the control, and decreased respiration rates to 134.92 and 135.62 mg.kg⁻¹.h⁻¹ CO₂ for LCa+PME and LCa, respectively, while the control reached 163.36 mg.kg⁻¹.h⁻¹ CO₂. Furthermore, it minimized fruit damage. For whole fruits (I), LCa + PME increased fruit firmness by 48% after 3 days of storage compared to fruits from the control treatment (W), maintaining it for up to 12 days of storage. This stability was attributed to the synergy of the treatment between LCa and exogenous PME, which increases free carboxylic groups, optimizing the formation of Ca-pectate cross-links, resulting in the stabilization of CSP (sodium carbonate soluble pectin). Additionally, treatments using calcium reduced electrolyte leakage by 22% and limited mass loss by approximately 2.7% for LCa + PME, resulting in better freshness and lower incidence of damage and contamination. LCa + PME significantly preserved color parameters (L*, C*) and extended shelf-life by at least 3 days. Thus, vacuum infusion of calcium lactate and PME is a promising technology capable of significantly extending the post-harvest quality and shelf-life of whole, minimally processed strawberries.

Keywords: Enzyme activity, Firming, *Fragaria x ananassa*, Fresh-Cut, Postharvest.

* Guidance Committee: Marcelo Augusto Gutierrez Canelossi (Advisor), Luiz Fernando Ganassali De Oliveira Junior – (Adjunct advisor).

1. INTRODUÇÃO GERAL

A busca por alimentos saudáveis, seguros e práticos que se adequem a qualquer estilo de vida tem levado a população a consumir mais frutas e hortaliças frescas, pois são altamente nutritivos, ajudam a manter uma dieta equilibrada e podem reduzir o risco de desenvolver doenças como a diabetes e o câncer (Kliemann *et al.*, 2022; Srouf *et al.*, 2020; Tracey *et al.*, 2023). O morango (*Fragaria x ananassa*), pertencente à família das rosáceas, é um exemplo de alimento amplamente consumido na forma fresca devido às suas características sensoriais agradáveis, como aroma, cor e sabor. Possui excelentes propriedades nutricionais, sendo rico em vitaminas e minerais e contendo grandes quantidades de compostos bioativos, como compostos fenólicos e vitamina C (Giampieri *et al.*, 2015; Méndez-Galarraga *et al.*, 2022). Contudo, por ser um fruto altamente perecível, a manutenção da qualidade nutricional e da segurança pós-colheita representa um grande desafio para a cadeia produtiva de alimentos (Tappi *et al.*, 2022).

O morango é um fruto não climatérico com tempo de prateleira reduzido, geralmente limitado de 5 a 7 dias sob refrigeração (Nour *et al.*, 2021). Essa perecibilidade é causada pela sua alta taxa respiratória, suscetibilidade a danos mecânicos, perda de água e rápida deterioração microbiológica (Luo *et al.*, 2020; Nour *et al.*, 2021). Devido a esses fatores, diversas técnicas vêm sendo exploradas para melhorar a vida útil e a qualidade dos frutos (Panou *et al.*, 2021; Giacalone & Chiabrando, 2019; Giannoglou *et al.*, 2021).

Em resposta à crescente demanda no consumo de produtos frescos e por conveniência, além do consumo de produtos *in natura*, a aplicação do processamento mínimo vem sendo estudada para uma grande variedade de frutas e hortaliças, como maçã (Perinban *et al.*, 2022), pitaia (Li *et al.*, 2022), kiwi (Hu *et al.*, 2022), cenoura (Li *et al.*, 2021), inhame (Teoh *et al.*, 2016), manga (Kirtil *et al.*, 2014) e morango (Méndez-Galarraga *et al.*, 2022). Segundo a International Fresh-cut Produce Association (IFPA), produto minimamente processado é qualquer fruta ou hortaliça, ou qualquer combinação delas, cortada ou descascada, 100% aproveitável e que é embalada para oferecer aos consumidores alta nutrição, conveniência e sabor, mantendo o frescor (Oliveira *et al.*, 2015). No entanto, frutas e hortaliças minimamente processadas são geralmente mais perecíveis, limitando a vida útil do produto (Hu *et al.*, 2022; Teoh *et al.*, 2016).

A qualidade e a vida útil dos frutos, sejam intactos ou minimamente processados, estão diretamente relacionadas à taxa de deterioração causada por operações físicas, como manipulação, lavagem, corte e descasque (Hu *et al.*, 2022; Teoh *et al.*, 2016). Estas operações podem afetar significativamente atributos sensoriais como cor, sabor, aroma, valor nutricional e, principalmente, a firmeza, que é um dos fatores críticos de qualidade, pois determina a aceitabilidade por parte do consumidor (Ma *et al.*, 2017). A diminuição da firmeza após a colheita e após o processamento mínimo de frutas tem sido atribuída às modificações e à degradação dos componentes da parede celular, tais como celulose, hemiceluloses e pectinas (Alandes *et al.*, 2009).

Nesse contexto, as enzimas pectolíticas, como a pectinametilesterase (PME) e a poligalacturonase (PG), são cruciais, pois são enzimas que agem na parede celular promovendo a solubilização e despolimerização da pectina (Alandes *et al.*, 2009). A PME catalisa a hidrólise dos grupos metila da pectina, formando pectina de baixa metilação. Este substrato desesterificado é, então, disponibilizado para a ação das PGs, acelerando o amolecimento (Alandes *et al.*, 2009; Quiles *et al.*, 2007).

Para combater esse amolecimento, o cálcio (Ca^{2+}) desempenha um papel fundamental. O íon cálcio liga-se inter e intramolecularmente aos grupos de ácido carboxílico das substâncias pectínicas, anteriormente desesterificadas pela PME, estabilizando o complexo pectina-proteína da lamela média. Esse processo, conhecido como formação cruzada de Ca-pectato, modelo “caixa de ovo”, aumenta a estabilidade da parede celular e limita a vulnerabilidade ao ataque das enzimas pectolíticas endógenas (Kirtil *et al.*, 2014; Quiles *et al.*, 2007). Dessa forma, a

aplicação de cálcio tem o poder de diminuir o amaciamento, aumentar a vida de prateleira e, consequentemente, a aceitação dos consumidores (Sirijariyawat *et al.*, 2012).

Estudos têm demonstrado a eficácia de abordagens combinadas envolvendo cálcio e pectinametilesterase (PME) na manutenção da firmeza de frutos. Em frutos intactos, Huang *et al.* (2023) observaram que a injeção exógena de PME associada a Ca^{2+} em frutos de nêspera resultou em aumento significativo da firmeza, associado à maior presença de homogalacturonanos com baixo grau de metilesterificação e à formação de estruturas do tipo “caixa de ovos”, indicando maior reforço estrutural da parede celular. De forma semelhante, Sun *et al.* (2023) relataram que frutos de tomate cereja submetidos ao tratamento com cálcio e ultrassom apresentaram manutenção elevada da firmeza ao longo de todo o período de armazenamento. Resultados convergentes foram descritos por Yan *et al.* (2021), que verificaram que o tratamento com CaCl_2 combinado à PME promoveu a manutenção da firmeza e reduziu a perda de massa em frutos de framboesa.

Para frutos minimamente processados, evidências semelhantes têm sido reportadas. Suriati *et al.* (2021), ao avaliarem as características físico-químicas de manga, rambutan e mangostão minimamente processados submetidos à aplicação de cálcio, constataram aumento da vida útil, maior firmeza, maiores teores de vitamina C e melhor aparência dos frutos em comparação ao controle. Yan *et al.* (2021), ao investigarem o efeito da aplicação de CaCl_2 e pectinametilesterase no amaciamento de framboesas durante o armazenamento, observaram manutenção da firmeza, indicando o potencial do tratamento como estratégia para retardar o amolecimento dos frutos. De modo complementar, Guillemín *et al.* (2008) avaliaram a infusão a vácuo de PME e verificaram aumento da rigidez das pectinas e, consequentemente, da rigidez mecânica do tecido da maçã.

Desta forma, a hipótese deste trabalho foi que a aplicação combinada de cálcio e PME, utilizando a técnica de infusão a vácuo, auxilia na manutenção da firmeza e prolonga a qualidade e vida útil de morangos inteiros e minimamente processados. O objetivo deste trabalho foi avaliar o efeito da infusão a vácuo de cálcio e de pectinametilesterase (PME) na firmeza, qualidade e vida útil dos morangos inteiros e minimamente processados.

2. REVISÃO DE LITERATURA

2.1 Morango

O morango possui grande importância econômica, pois é utilizado como matéria-prima para diversos produtos como sucos, vinhos, geleias, compotas, entre outros (Zhang *et al.*, 2022). Na forma *in natura*, é uma das frutas mais consumidas do mundo devido às excelentes propriedades sensoriais, como aroma e sabor agradável (Zhao *et al.*, 2022). Além disso, o morango possui excelentes propriedades nutricionais, visto que é rico em vitamina C (81,14 mg/100g), elementos minerais como potássio (8,4g/kg), zinco (16,37 mg/kg), cálcio (1,8g/kg) e ferro (62,02 mg/kg), além de compostos antioxidantes como antocianinas e flavonoides que podem auxiliar na prevenção de doenças, como, por exemplo, o câncer (Li *et al.*, 2021; Rocha *et al.*, 2008; Zhao *et al.*, 2022).

A produção mundial de morangos vem crescendo nos últimos anos, passando de 7.879.108 toneladas em 2013 para 12.106.585 toneladas em 2019. No Brasil, a produção em 2021 foi, em média, 165.000 toneladas, correspondendo à produtividade de 38,5 t/ha, com destaque para as regiões sul e sudeste, colocando o país na 17ª posição entre os maiores produtores de morango (EMBRAPA, 2021). Apesar dos crescentes números, o Brasil não se encontra entre os principais exportadores de morango, pois diversos fatores afetam a sua produção e comercialização, como o clima, apresentando grandes dificuldades na adaptação da cultura, o que requer, em muitas circunstâncias, o uso de tecnologias de melhoramento, e outro fator está relacionado à sua suscetibilidade à senescência, dificultando a comercialização dos frutos. A gestão de temperatura e o manuseio físico são os fatores mais críticos na cadeia de suprimentos transfronteiriça e doméstica. Mesmo com pré-resfriamento adequado, os hematomas (bruising) ocorridos durante o transporte de longa distância são os principais gatilhos para o aumento da taxa respiratória e perda de massa fresca, superando inclusive o mofo como fator limitante inicial da qualidade comercial (Person *et al.*, 2025). Em cadeias logísticas longas, a exposição a temperaturas acima de 10 °C e as vibrações mecânicas aceleram o amolecimento dos tecidos, criando feridas que servem como porta de entrada para patógenos (Person *et al.*, 2025). Esse cenário é agravado pela suscetibilidade do fruto à senescência e pelas dificuldades de adaptação da cultura ao clima em certas regiões (Liu *et al.*, 2018; Zeist & Resende, 2019).

O morango é um fruto não climatérico, altamente perecível, com tempo de prateleira reduzido, pois apresenta alta taxa respiratória, alta suscetibilidade a danos mecânicos, à deterioração microbiológica, à perda de água, dentre outros fatores, que reduzem consideravelmente a qualidade dos frutos, dificultando a sua comercialização (Luo *et al.*, 2020; Nour *et al.*, 2021; Zhang *et al.*, 2022). Nesse contexto, a vida útil do morango dura aproximadamente de 5 a 7 dias sob temperatura de 0 a 4 °C, necessitando de técnicas complementares que auxiliem na manutenção da qualidade do mesmo (Nour *et al.*, 2021). Atualmente, diversos estudos vêm sendo desenvolvidos a fim de melhorar a vida de prateleira e a qualidade dos frutos de morango, aplicando técnicas como o tratamento térmico e ozônio (Panou *et al.*, 2021), irradiação gama (Panou *et al.*, 2019), embalagem ativa (Giacalone & Chiabrando, 2019) e campos eletromagnéticos (Giannoglou *et al.*, 2021), bem como o uso do processamento mínimo (Huang *et al.*, 2022; Méndez-Galarraga *et al.*, 2022). Este último vem ganhando espaço devido à busca por uma melhor qualidade de vida, o que vem movimentando o mercado de frutas e hortaliças na busca por técnicas que minimizem perdas na qualidade, associadas ou não ao processamento, a fim de oferecer alimentos frescos e de boa qualidade nutricional (Huang *et al.*, 2022; Méndez-Galarraga *et al.*, 2022).

2.2 Processamento mínimo

O processamento mínimo (PM) é a técnica que resulta na alteração física de uma fruta ou hortaliça, consistindo em operações como seleção, lavagem, classificação, descascamento, corte, sanitização, enxágue, drenagem, embalagem e refrigeração, com a finalidade de produzir

um produto fresco e conveniente que possa ser consumido sem a necessidade de qualquer preparo (Soliva-Fortuny & Martín-Belloso, 2003; Tappi *et al.*, 2016).

Na última década, o mercado de frutas e produtos hortícolas preparados na hora cresceu mais de 30%, tornando-se popular em todo o mundo (Pan *et al.*, 2020; Qiao *et al.*, 2021). Uma das principais causas desse crescimento foi o aumento da demanda por alimentos saudáveis que ofereçam qualidade e credibilidade aos consumidores. Além disso, organizações como a Organização Mundial da Saúde (OMS) e a Food and Agriculture Organization (FAO) recomendam o aumento do consumo de frutas e hortaliças frescas para promover a redução de doenças como câncer e fortalecer o sistema imunológico contra a COVID-19 (FAO, 2020; Oliveira *et al.*, 2015).

As frutas e hortaliças minimamente processadas, quando comparadas com frutas e hortaliças *in natura*, são produtos com maior valor agregado que fornecem ao consumidor novas variedades de produtos com 100% de aproveitamento (Oliveira *et al.*, 2015). Além da proposta de reduzir o tempo gasto no preparo de refeições, onde a ênfase está em adquirir um produto fresco, oferecem também extrema praticidade para a cadeia das indústrias, restaurantes e instituições diversas, juntamente com a economia do espaço físico e mão de obra durante o seu preparo, consistência na qualidade e segurança e melhor controle de custo operacional (Ayala-Zavala *et al.*, 2008; Bahram-Parvar & Lim, 2018).

Além desses benefícios, outra vantagem na aplicação do processamento mínimo é reduzir as perdas pós-colheita, minimizando também os desperdícios de alimentos ao longo da cadeia produtiva, que são geralmente associados ao manuseio, armazenamento e embalagens inadequadas, bem como à contaminação microbiológica (Ayala-Zavala *et al.*, 2008; Maringgal *et al.*, 2020). Estimativas apontam que cerca de 40% das frutas e legumes produzidos nos países em desenvolvimento são perdidos ou desperdiçados, desde o abastecimento até o seu consumidor final (Ayala-Zavala *et al.*, 2008; FAO, 2020).

Os grandes desafios para a aplicação do processamento mínimo (PM), desde que foram disponibilizados pela primeira vez em 1940, são preservar e estender a vida de prateleira dos produtos após o processamento (Rico *et al.*, 2006). Embora o processamento mínimo teoricamente mantenha a qualidade dos produtos, ainda ocorrem processos de deterioração dos alimentos devido ao estresse causado, pois o produto continua vivo e com suas atividades metabólicas ativas, influenciando na qualidade do produto (Caleb *et al.*, 2012).

A qualidade de frutas é determinada por diversos fatores, como alterações fisiológicas e bioquímicas que vão definir a aceitação de um produto por parte do consumidor (Farcuh *et al.*, 2020; Shewfelt, 1999). Essas alterações estão relacionadas à minimização da taxa de deterioração e, consequentemente, à manutenção das características do produto, como textura (firmeza), cor, sabor e aroma, de forma a mantê-los atraentes ao consumidor pelo maior tempo possível (Farcuh *et al.*, 2020).

2.3 Firmeza e parede celular

A firmeza é considerada um parâmetro crítico na avaliação do frescor e na qualidade de frutas, visto que é o atributo mais importante associado à sua estrutura, pois determina a taxa de amolecimento e é um dos principais fatores associados à deterioração pós-colheita dos frutos, desempenhando um papel de fundamental importância na vida útil dos produtos vegetais (Chen & Opara, 2013; Farcuh *et al.*, 2020). Além disso, a textura do produto tem grande impacto na aceitação do mesmo, podendo ser um aspecto limitante de aceitação quando o alimento se encontra muito diferente daquilo que o consumidor espera (Asghar *et al.*, 2022; Chen & Opara, 2013).

A mudança na textura (firmeza) dos frutos, incluindo os minimamente processados, está associada à senescência, amadurecimento ou perda de líquidos que o produto sofre durante o período de armazenamento (Asghar *et al.*, 2022). Essas mudanças são provocadas por alterações nos polissacarídeos que compõem a parede celular, como celulose, hemicelulose e pectina, que sofrem ação de algumas enzimas endógenas do fruto (Priya Sethu *et al.*, 1996;

Zhang *et al.*, 2019). Entre os polissacarídeos, as pectinas são as principais responsáveis pela firmeza dos frutos, são constituintes da parede celular primária e lamelas médias, são ricas em ácido anidrogalaacturônico, ligadas covalentemente e podem representar um terço de todas as macromoléculas da parede celular primária (Willats *et al.*, 2001).

A diminuição da firmeza da polpa durante o amadurecimento é atribuída à ação coordenada ou interdependente de uma série de enzimas endógenas, como, por exemplo, a poligalacturonase (PG; EC 3.2.1.15) e a pectinametilesterase (PME; EC 3.1.1.11), que são as principais enzimas envolvidas na desmontagem das substâncias pécticas (Zhang *et al.*, 2019). Essa desmontagem da parede celular ocorre em uma ação conjunta em que a pectinametilesterase catalisa a desmetilação da pectina, danificando os elos horizontais da cadeia polissacarídica ácida, levando à separação celular, resultando em um maior número de grupos de ácido carboxílicos disponibilizados como substrato adequado para a atuação da poligalacturonase. Por sua vez, a PG atua hidrolisando o ácido da pectina, causando a degradação da pectina, a dissolução da parede celular e o amaciamento do fruto (Brummell *et al.*, 2004; Wakabayashi *et al.*, 2000).

2.3 Cálcio e pectina

A aplicação de cálcio é uma alternativa para a redução do amolecimento em frutas inteiras e minimamente processadas, pois quando disponível, o cálcio suprime o declínio da qualidade, preserva a integridade e reduz a permeabilidade da membrana celular durante o armazenamento, melhorando a firmeza dos frutos (Aguayo *et al.*, 2015; Pinheiro & Almeida, 2008). Isso ocorre porque, após a desmetilação da pectina provocada pela ação da PME, o íon de cálcio (Ca^{+2}) se liga covalentemente às cargas negativas dos ácidos galaacturônicos desesterificados, formando uma rede, mantendo a estabilidade da pectina (**Erro! Fonte de referência não encontrada.**) e a estrutura da parede celular durante o armazenamento (Anthon & Barrett, 2006; Beirão-da-Costa *et al.*, 2014).

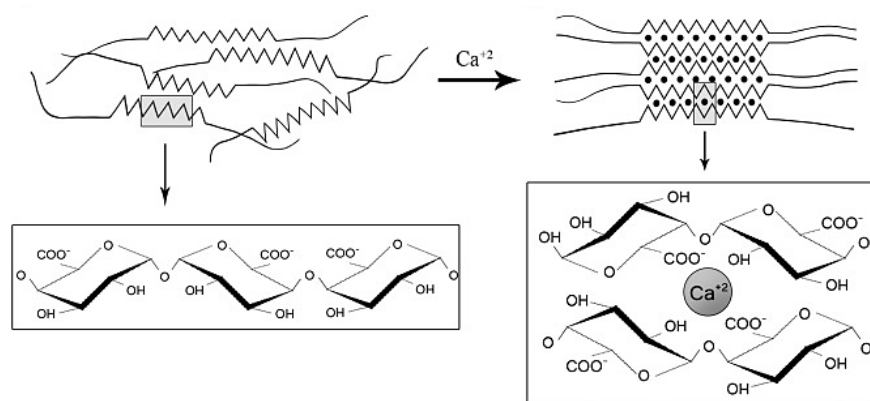


Figura 1. Estrutura e ligação da pectina com o cálcio. Fonte: (Kirtil *et al.*, 2014).

Atualmente, diversas fontes de cálcio são utilizadas para reduzir os distúrbios fisiológicos de frutas, como o fosfato de cálcio, propionato de cálcio, gluconato de cálcio e cloreto de cálcio, este último sendo o mais utilizado (Prajapati *et al.*, 2021). Apesar de ser o mais utilizado, o cloreto de cálcio pode conferir atributos indesejáveis, como sabor amargo e salgado (Hanson *et al.*, 1993; Luna-Guzmán & Barrett, 2000). Para contornar esses problemas, o lactato de cálcio (CA-L) é um sal orgânico que pode servir como fonte alternativa de cálcio, visto que, além de poder manter melhores atributos de textura dos frutos, evita os efeitos indesejáveis que o cloreto de cálcio confere ao produto (Hu *et al.*, 2022; Rico *et al.*, 2006).

Estudos têm sido conduzidos para avaliar o efeito da aplicação do cálcio e/ou da PME exógena na firmeza em diferentes matrizes vegetais, incluindo mamão (Yang *et al.*, 2017), mangas (Kirtil *et al.*, 2014), morangos (Carnelossi *et al.*, 2018), damascos (Liu *et al.*, 2017), goiabas (Werner *et al.*, 2009) e framboesas (Hanson *et al.*, 1993). A técnica que tem sido

utilizada para essa aplicação é a impregnação a vácuo, que consiste na troca de gás e líquidos nativos com uma solução de impregnação sob a ação do mecanismo hidrodinâmico (Derossi *et al.*, 2013).

A eficácia dessa abordagem combinada é amplamente demonstrada na literatura. Por exemplo, a impregnação a vácuo de PME e lactato de cálcio foi mais eficaz em melhorar a firmeza dos frutos de mamão do que a infusão apenas de cálcio, melhorando a firmeza do fruto quando armazenado por 8 dias (Yang *et al.*, 2017). Efeito semelhante foi verificado, que a infusão a vácuo de PME + Ca^{+2} em morangos minimamente processados resultou em aumento da firmeza (Carnelossi *et al.*, 2018).

A aplicação de cálcio em produtos minimamente processados também tem mostrado ser eficaz na extensão da vida útil e na melhoria das características físico-químicas. As características físico-químicas da manga, rambutan e mangostão minimamente processados com aplicação de cálcio aumentaram a vida útil dos frutos, com alta firmeza, vitamina C e aparência dos frutos (Suriati *et al.*, 2021).

Adicionalmente, a aplicação de CaCl_2 e pectinametilesterase em framboesa durante o armazenamento retardou o amolecimento do fruto, uma vez que houve a manutenção da firmeza, destacando o método como promissor para inibir o amolecimento (Yan *et al.*, 2022). De forma complementar, Guillemín *et al.* (2008) avaliaram a ação da PME impregnada a vácuo e observaram o aumento da rigidez das pectinas e, finalmente, a rigidez mecânica do tecido da maçã.

2.4 Infusão a Vácuo (IV)

A infusão a vácuo (IV) é uma tecnologia de processamento não térmico que utiliza diferenciais de pressão para introduzir rapidamente solutos na matriz porosa de frutas e hortaliças (Zhao & Xie, 2004). O processo é regido pela interação entre o mecanismo hidrodinâmico (HDM) e os fenômenos de deformação-relaxamento (FDR) (Durán-Castañeda *et al.*, 2024). Durante a aplicação de vácuo, os gases e líquidos presentes nos espaços intercelulares do tecido vegetal são removidos; subsequentemente, ao restaurar a pressão atmosférica, a solução externa é forçada para dentro desses espaços, ocupando os capilares anteriormente desgaseificados (Radziejewska-Kubzdela *et al.*, 2014; Durán-Castañeda *et al.*, 2024).

Diferente da impregnação osmótica convencional à pressão atmosférica, a IV reduz significativamente o tempo de processamento e aumenta a eficiência da transferência de massa sem comprometer a integridade estrutural da matriz (Méndez-Galarraga *et al.*, 2022). Em morangos, essa técnica é particularmente vantajosa devido à alta porosidade e à natureza esponjosa do parênquima do fruto. Diferente de matrizes compactas, o morango possui uma estrutura tecidual com grandes espaços intercelulares (porosidade efetiva), o que facilita a penetração profunda dos agentes de conservação através do mecanismo hidrodinâmico (Durán-Castañeda *et al.*, 2024). Essa característica do material biológico permite que a infusão atue de forma volumétrica e não apenas superficial, garantindo a manutenção da firmeza em toda a polpa. Segundo Durán-Castañeda *et al.* (2024), a solução impregnante atua como um preenchedor dos espaços intercelulares. Quando combinada com agentes de conservação, como o lactato de cálcio, essa técnica permite uma distribuição homogênea de íons que reforçam a parede celular, minimizando a deformação estrutural e mitigando o estresse metabólico causado pelo processamento mínimo (Huang *et al.*, 2022).

A eficácia da infusão a vácuo na preservação de estruturas vegetais tem sido amplamente demonstrada em estudos recentes. Méndez-Galarraga *et al.* (2022) observaram que a aplicação de IV em morangos permite uma incorporação mais homogênea de solutos quando comparada à imersão simples, resultando em uma melhor manutenção das propriedades físico-químicas após o processamento mínimo. Complementarmente, Huang *et al.* (2022) destacam que a tecnologia de vácuo, ao facilitar a entrada de agentes firmadores e antioxidantes nos espaços intercelulares, minimiza o estresse metabólico causado pelo corte, sendo uma estratégia

indispensável para prolongar a vida útil e garantir a segurança microbiológica de frutas frescas e processadas. Assim, os resultados da literatura sugerem que a aplicação de infusão a vácuo de cálcio e PME é uma estratégia eficaz e pode auxiliar na manutenção da firmeza e na qualidade de frutas, especialmente em produtos minimamente processados (Guillemin *et al.*, 2008; Kirtil *et al.*, 2014). O objetivo deste trabalho foi avaliar o efeito da infusão a vácuo de cálcio e de pectinametilesterase (PME) na firmeza, qualidade e vida útil dos morangos inteiros e minimamente processados.

3. REFERÊNCIAS BIBLIOGRÁFICAS

- AGUAYO, E.; REQUEJO-JACKMAN, C.; STANLEY, R.; WOOLF, A. Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality. *Postharvest Biology and Technology*, v. 110, p. 158–165, 1 dez. 2015.
- ALANDES, L.; PÉREZ-MUNUERA, I.; LLORCA, E.; QUILES, A.; HERNANDO, I. Use of calcium lactate to improve structure of “Flor de Invierno” fresh-cut pears. *Postharvest Biology and Technology*, v. 53, n. 3, p. 145–151, 1 set. 2009.
- ANTHON, G. E.; BARRETT, D. M. Characterization of the temperature activation of pectin methylesterase in green beans and tomatoes. *Journal of Agricultural and Food Chemistry*, v. 54, n. 1, p. 204–211, 11 jan. 2006.
- ASGHAR, A.; RASHID, M. H.; AHMED, W.; ROOBAB, U.; INAM-UR-RAHEEM, M.; SHAHID, A.; KAFEEL, S.; AKRAM, M. S.; ANWAR, R.; AADIL, R. M. An in-depth review of novel cold plasma technology for fresh-cut produce. *Journal of Food Processing and Preservation*, v. 46, n. 7, p. e16560, 1 jul. 2022.
- AYALA-ZAVALA, J. F.; DEL-TORO-SÁNCHEZ, L.; ALVAREZ-PARRILLA, E.; GONZÁLEZ-AGUILAR, G. A. High relative humidity in-package of fresh-cut fruits and vegetables: advantage or disadvantage considering microbiological problems and antimicrobial delivering systems? *Journal of Food Science*, v. 73, n. 4, maio 2008.
- BAHRAM-PARVAR, M.; LIM, L. T. Fresh-Cut Onion: A Review on Processing, Health Benefits, and Shelf-Life. *Comprehensive Reviews in Food Science and Food Safety*, v. 17, n. 2, p. 290–308, 1 mar. 2018.
- BEIRÃO-DA-COSTA, S.; EMPIS, J.; MOLDÃO-MARTINS, M. Fresh-Cut Kiwifruit Structure and Firmness as Affected by Heat Pre-treatments and Post-cut Calcium Dips. *Food and Bioprocess Technology*, v. 7, n. 4, p. 1128–1136, 20 abr. 2014.
- BRUMMELL, D. A.; DAL CIN, V.; CRISOSTO, C. H.; LABAVITCH, J. M. Cell wall metabolism during maturation, ripening and senescence of peach fruit. *Journal of Experimental Botany*, v. 55, n. 405, p. 2029–2039, 1 set. 2004.
- CALEB, O. J.; MAHAJAN, P. V.; AL-SAID, F. A. J.; OPARA, U. L. Modified Atmosphere Packaging Technology of Fresh and Fresh-cut Produce and the Microbial Consequences—A Review. *Food and Bioprocess Technology*, v. 6, n. 2, p. 303–329, 27 jul. 2012.
- CARNELOSSI; HUBER; D.; CARVALHO. Vacuum infusion of pectin methylesterase and calcium maintains firmness of the fresh-cut strawberry 2018
- CHEN, L.; OPARA, U. L. Approaches to analysis and modeling texture in fresh and processed foods – A review. *Journal of Food Engineering*, v. 119, n. 3, p. 497–507, 1 dez. 2013.
- DEROSSA, A.; PILLI, T. DE; SEVERINI, C. Application of Vacuum Impregnation Techniques to Improve the pH Reduction of Vegetables: Study on Carrots and Eggplants. *Food and Bioprocess Technology*, v. 6, n. 11, p. 3217–3226, 1 nov. 2013.

DURÁN-CASTAÑEDA, A. C.; GONZÁLEZ-MOYA, S.; SÁNCHEZ-BURGOS, J. A.; SÁYAGO-AYERDI, S. G.; ZAMORA-GASGA, V. M. Applications of vacuum impregnation as a technology to incorporate functional components in plant matrices. *Advances in Food Chemistry*, v. 4, p. 100579, 2024.

EMBRAPA. *Morango: produção aumenta ano a ano*. Campo e Negócio, 2021. Disponível em: Acesso em: <http://www.alice.cnptia.embrapa.br/alice/handle/doc/1131057>

FAO. *Fruit and vegetables: your dietary essentials*. Rome: FAO, 2020. Disponível em: Acesso em: <http://www.fao.org/faostat/en/#data/QC>

FARCUH, M.; COPES, B.; LE-NAVENEC, G.; MARROQUIN, J.; JAUNET, T.; CHI-HAM, C.; CANTU, D.; BRADFORD, K. J.; DEYNZE, A. VAN. Texture diversity in melon (*Cucumis melo* L.): sensory and physical assessments. *Postharvest Biology and Technology*, v. 159, p. 111024, 1 jan. 2020.

GIACALONE, G.; CHIABRANDO, V. Active packaging to improve the shelf-life and nutritional quality of strawberries. *Acta Horticulturae*, v. 1256, p. 379–385, 2019.

GIAMPIERI, F.; FORBES-HERNANDEZ, T. Y.; GASPARRINI, M.; ALVAREZ-SUAREZ, J. M.; AFRIN, S.; BOMPADRE, S.; QUILS, J. L.; MEZZETTI, B.; BATTINO, M. Strawberry as a health promoter: an evidence based review. *Food & Function*, v. 6, n. 5, p. 1386–1398, 14 maio 2015.

GIANNOGLOU, M.; XANTHOU, Z. M.; CHANIOTI, S.; STERGIOU, P.; CHRISTOPOULOS, M.; DIMITRAKELLIS, P.; EFTHIMIADOU, A.; GOGOLIDES, E.; KATSAROS, G. Effect of cold atmospheric plasma and pulsed electromagnetic fields on strawberry quality and shelf-life. *Innovative Food Science & Emerging Technologies*, v. 68, p. 102631, 1 mar. 2021.

GUILLEMIN, A.; GUILLON, F.; DEGRAEVE, P.; RONDEAU, C.; DEVAUX, M. F.; HUBER, F.; BADEL, E.; SAUREL, R.; LAHAYE, M. Firming of fruit tissues by vacuum-infusion of pectin methylesterase: visualisation of enzyme action. *Food Chemistry*, v. 109, n. 2, p. 368–378, 15 jul. 2008.

HANSON, E. J.; BEGGS, J. L.; BEAUDRY, R. M. Applying Calcium Chloride Postharvest to Improve Highbush Blueberry Firmness. *HortScience*, v. 28, n. 10, p. 1033–1034, 1 out. 1993.

HU, X.; CHEN, Y.; WU, X.; LIU, W.; JING, X.; LIU, Y.; YAN, J.; LIU, S.; QIN, W. Combination of calcium lactate impregnation with UV-C irradiation maintains quality and improves antioxidant capacity of fresh-cut kiwifruit slices. *Food Chemistry: X*, v. 14, p. 100329, jun. 2022.

HUANG, S.; YING LIM, S.; LAU, H.; NI, W.; FONG YAU LI, S. Effect of glycinebetaine on metabolite profiles of cold-stored strawberry revealed by ¹H NMR-based metabolomics. *Food Chemistry*, v. 393, p. 133452, 1 nov. 2022.

HUANG, W., SHI, Y., YAN, H., WANG, H., WU, D., GRIERSON, D., & CHEN, K. (2023). The calcium-mediated homogalacturonan pectin complexation in cell walls contributes the firmness increase in loquat fruit during postharvest storage. *Journal of Advanced Research*, 49, 47–62. <https://doi.org/10.1016/j.jare.2022.09.009>

KIRTIL, E.; OZTOP, M. H.; SIRIJARIYAWAT, A.; NGAMCHUACHIT, P.; BARRETT, D. M.; MCCARTHY, M. J. Effect of pectin methyl esterase (PME) and CaCl₂ infusion on the cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study. *Food Research International*, v. 66, p. 409–416, 1 dez. 2014.

KLIEMANN, N.; NAHAS, A. AL; VAMOS, E. P.; TOUVIER, M.; KESSE-GUYOT, E.; GUNTER, M. J.; MILLETT, C.; HUYBRECHTS, I. Ultra-processed foods and cancer risk: from global food systems to individual exposures and mechanisms. *British Journal of Cancer*, v. 127, n. 1, p. 14–20, 1 jul. 2022.

Li, L., Li, C., Sun, J., Xin, M., Yi, P., He, X., Sheng, J., Zhou, Z., Ling, D., Zheng, F., Li, J., Liu, G., Li, Z., Tang, Y., Yang, Y., Tang, J. Synergistic effects of ultraviolet light irradiation and high-oxygen modified atmosphere packaging on physiological quality, microbial growth and lignification metabolism of fresh-cut carrots. *Postharvest Biology and Technology*, v. 173, p. 111365, 1 mar. 2021.

LI, X.; LI, B.; LI, M.; FU, X.; ZHAO, X.; MIN, D.; LI, F.; ZHANG, X. Ethylene pretreatment induces phenolic biosynthesis of fresh-cut pitaya fruit by regulating ethylene signaling pathway. *Postharvest Biology and Technology*, v. 192, p. 112028, 1 out. 2022.

LI, Z.; WEI, Y.; CAO, Z.; JIANG, S.; CHEN, Y.; SHAO, X. The Jasmonic Acid Signaling Pathway is Associated with Terpinen-4-ol-Induced Disease Resistance against *Botrytis cinerea* Strawberry Fruit. *Journal of Agricultural and Food Chemistry*, v. 69, n. 36, p. 10678–10687, 15 set. 2021.

LIU, C.; ZHENG, H.; SHENG, K.; LIU, W.; ZHENG, L. Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology*, v. 139, p. 47–55, 1 maio 2018.

LIU, H.; CHEN, F.; LAI, S.; TAO, J.; YANG, H.; JIAO, Z. Effects of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*). *Food Chemistry*, v. 225, p. 87–97, 15 jun. 2017.

LUNA-GUZMÁN, I.; BARRETT, D. M. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, v. 19, n. 1, p. 61–72, 1 maio 2000.

LUO, M.; ZHOU, X.; SUN, H.; ZHOU, Q.; WEI, B.; CHENG, S.; JI, S. J. Glycine betaine treatment alleviates loss of aroma-related esters in cold-stored ‘Nanguo’ pears by regulating the lipoxygenase pathway. *Food Chemistry*, v. 316, p. 126335, 30 jun. 2020.

MA, L.; ZHANG, M.; BHANDARI, B.; GAO, Z. Recent developments in novel shelf life extension technologies of fresh-cut fruits and vegetables. *Trends in Food Science & Technology*, v. 64, p. 23–38, 1 jun. 2017.

MARINGGAL, B.; HASHIM, N.; TAWAKKAL, I. S. M. A.; MOHAMED, M. T. M. Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. *Trends in Food Science & Technology*, v. 96, p. 253–267, 1 fev. 2020.

MÉNDEZ-GALARRAGA, M. P.; VAN DE VELDE, F.; PIAGENTINI, A. M.; PIROVANI, M. É. Kinetic modeling of the changes in bioactive compounds and quality attributes of fresh-

cut strawberries stored in controlled atmospheres with high oxygen alone or with carbon dioxide. *Postharvest Biology and Technology*, v. 190, p. 111947, 1 ago. 2022.

NOUR, V.; PLESOIANU, A. M.; IONICA, M. E. Effect of dip wash treatments with organic acids and acidic electrolyzed water combined with ultraviolet irradiation on quality of strawberry fruit during storage. *Bragantia*, v. 80, 10 mar. 2021.

OLIVEIRA, M.; ABADIAS, M.; USALL, J.; TORRES, R.; TEIXIDÓ, N.; VIÑAS, I. Application of modified atmosphere packaging as a safety approach to fresh-cut fruits and vegetables – A review. *Trends in Food Science & Technology*, v. 46, n. 1, p. 13–26, 1 nov. 2015.

PAN, Y.; CHEN, L.; PANG, L.; CHEN, X.; JIA, X.; LI, X. Ultrasound treatment inhibits browning and improves antioxidant capacity of fresh-cut sweet potato during cold storage. *RSC Advances*, v. 10, n. 16, p. 9193–9202, 3 mar. 2020.

PANOU, A. A.; AKRIDA-DEMERTZI, K.; DEMERTZIS, P.; RIGANAKOS, K. A. Effect of Gaseous Ozone and Heat Treatment on Quality and Shelf Life of Fresh Strawberries during Cold Storage. *International Journal of Fruit Science*, v. 21, n. 1, p. 218–231, 2021.

PANOU, A. A.; KARABAGIAS, I. K.; RIGANAKOS, K. A. Effect of Gamma-Irradiation on Sensory Characteristics, Physicochemical Parameters, and Shelf Life of Strawberries Stored under Refrigeration. *International Journal of Fruit Science*, v. 20, n. 2, p. 191–206, 2 abr. 2019.

PERINBAN, S.; ORSAT, V.; RAGHAVAN, V. Influence of plasma activated water treatment on enzyme activity and quality of fresh-cut apples. *Food Chemistry*, v. 393, p. 133421, 1 nov. 2022.

Person, T., Tobler, E., Schouten, R., Lukasse, L., & Defraeye, T. Multimetric assessment of packaged strawberry quality under controlled supply chain temperatures. *Food Packaging and Shelf Life*, v. 52, p. 101679, dez. 2025.

PINHEIRO, S. C. F.; ALMEIDA, D. P. F. Modulation of tomato pericarp firmness through pH and calcium: Implications for the texture of fresh-cut fruit. *Postharvest Biology and Technology*, v. 47, n. 1, p. 119–125, 1 jan. 2008.

PRAJAPATI, U.; ASREY, R.; VARGHESE, E.; SHARMA, R. R. Effects of calcium lactate on postharvest quality of bitter gourd fruit during cold storage. *Physiology and Molecular Biology of Plants*, v. 27, n. 8, p. 1811–1821, 1 ago. 2021.

PRIYA SETHU, K. M.; PRABHA, T. N.; THARANATHAN, R. N. Post-harvest biochemical changes associated with the softening phenomenon in *Capsicum annuum* fruits. *Phytochemistry*, v. 42, n. 4, p. 961–966, 1 jul. 1996.

QIAO, L.; GAO, M.; ZHENG, J.; ZHANG, J.; LU, L.; LIU, X. Novel browning alleviation technology for fresh-cut products: Preservation effect of the combination of *Sonchus oleraceus* L. extract and ultrasound in fresh-cut potatoes. *Food Chemistry*, v. 348, p. 129132, 30 jun. 2021.

QUILES, A.; HERNANDO, I.; PÉREZ-MUNUERA, I.; LLUCH, M. Á. Effect of calcium propionate on the microstructure and pectin methylesterase activity in the parenchyma of

fresh-cut Fuji apples. *Journal of the Science of Food and Agriculture*, v. 87, n. 3, p. 511–519, 1 fev. 2007.

RADZIEJEWSKA-KUBZDELA, E.; BIEGAŃSKA-MARECIK, R.; KIDÓN, M. Applicability of vacuum impregnation to modify physicochemical, sensory and nutritive characteristics of plant origin products—a review. *International Journal of Molecular Sciences*, v. 15, n. 9, p. 16577-16610, 2014.

RICO, D.; MARTÍN-DIANA, A. B.; FRÍAS, J. M.; HENEHAN, G. T.; BARRY-RYAN, C. Effect of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce. *Journal of the Science of Food and Agriculture*, v. 86, n. 13, p. 2179–2188, 1 out. 2006.

ROCHA, D. A.; PATTO DE ABREU, C. M.; CORRÊA, A. D.; SANTOS, C. D.; FONSECA, E. W. N. Comparative analysis of functional nutrients in strawberries of different cultivars in the region of Lavras-MG. *Revista Brasileira de Fruticultura*, v. 30, n. 4, p. 1124–1128, 2008.

SHEWFELT, R. L. What is quality? *Postharvest Biology and Technology*, v. 15, n. 3, p. 197–200, 1 mar. 1999.

SIRIJARIYAWAT, A.; CHAROENREIN, S.; BARRETT, D. M. Texture improvement of fresh and frozen mangoes with pectin methylesterase and calcium infusion. *Journal of the Science of Food and Agriculture*, v. 92, n. 13, p. 2581–2586, 17 out. 2012.

SOLIVA-FORTUNY, R. C.; MARTÍN-BELLOSO, O. New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science & Technology*, v. 14, n. 9, p. 341–353, 1 set. 2003.

SROUR, B. et al. Ultraprocessed Food Consumption and Risk of Type 2 Diabetes Among Participants of the NutriNet-Santé Prospective Cohort. *JAMA Internal Medicine*, v. 180, n. 2, p. 283–291, 1 fev. 2020.

SUN, X., WANG, P., SHEN, X., CHEN, F., & ZHANG, L. (2023). Changes of Ca forms and chelate-soluble pectin in cherry tomatoes treated with ultrasound and calcium lactate. *LWT*, 173, 114393. <https://doi.org/10.1016/J.LWT.2022.114393>.

SURIATI, L.; UTAMA, IM. S.; HARSOJUWONO, B. A.; GUNAM, I. B. W.; ADNYANA, IM. Differences in Physicochemical Characters of Fresh-Cut Mango, Mangosteen and Rambutan Due to Calcium Chloride Application. *Food Science and Nutrition*, v. 7, n. 3, p. 1–6, 9 jul. 2021.

TAPPI, S.; GOZZI, G.; VANNINI, L.; BERARDINELLI, A.; ROMANI, S.; RAGNI, L.; ROCCULI, P. Cold plasma treatment for fresh-cut melon stabilization. *Innovative Food Science & Emerging Technologies*, v. 33, p. 225–233, 1 fev. 2016.

TAPPI, S.; VELICKOVA, E.; MANNOZZI, C.; TYLEWICZ, U.; LAGHI, L.; ROCCULI, P. Multi-Analytical Approach to Study Fresh-Cut Apples Vacuum Impregnated with Different Solutions. *Foods*, v. 11, n. 3, p. 488, 8 fev. 2022.

TEOH, L. S.; LASEKAN, O.; ADZAHAN, N. M.; HASHIM, N. The effect of combinations of UV-C exposure with ascorbate and calcium chloride dips on the enzymatic activities and

total phenolic content of minimally processed yam slices. *Postharvest Biology and Technology*, v. 120, p. 138–144, 1 out. 2016.

TRACEY, C. T.; KRYUCHKOVA, A. V.; BHATT, T. K.; KRIVOSHAPKIN, P. V.; KRIVOSHAPKINA, E. F. Silk for post-harvest horticultural produce safety and quality control. *Journal of Food Engineering*, v. 337, p. 111231, 1 jan. 2023.

WAKABAYASHI, K.; CHUN, J.-P.; HUBER, D. J. Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. *Physiologia Plantarum*, v. 108, n. 4, p. 345-352, 2000.

WERNER, E. T.; OLIVEIRA JUNIOR, L. F. G. DE; BONA, A. P. DE; CAVATI, B.; GOMES, T. D. U. H. Efeito do cloreto de cálcio na pós-colheita de goiaba Cortibel. *Bragantia*, v. 68, n. 2, p. 511–518, 2009.

WILLATS, W. G. T.; MCCARTNEY, L.; MACKIE, W.; KNOX, J. P. Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology*, v. 47, n. 1, p. 9–27, 2001.

YAN, R.; HAN, C.; FU, M.; JIAO, W.; WANG, W. Inhibitory effects of CaCl_2 and pectin methylesterase on fruit softening of raspberry during cold storage. *Horticulturae*, v. 8, n. 1, 1 jan. 2021.

YANG, H.; WU, Q.; NG, L. Y.; WANG, S. Effects of Vacuum Impregnation with Calcium Lactate and Pectin Methylesterase on Quality Attributes and Chelate-Soluble Pectin Morphology of Fresh-Cut Papayas. *Food and Bioprocess Technology*, v. 10, n. 5, p. 901–913, 1 maio 2017.

ZEIST, A. R.; RESENDE, J. T. V. DE. Strawberry breeding in Brazil: current momentum and perspectives. *Horticultura Brasileira*, v. 37, n. 1, p. 7–16, 15 abr. 2019.

ZHANG, C.; XIONG, Z.; YANG, H.; WU, W. Changes in pericarp morphology, physiology and cell wall composition account for flesh firmness during the ripening of blackberry (*Rubus* spp.) fruit. *Scientia Horticulturae*, v. 250, p. 59–68, 10 maio 2019.

ZHANG, Y., LI, S., DENG, M., GUI, R., LIU, Y., CHEN, X., LIN, Y., LI, M., WANG, Y., HE, W., CHEN, Q., ZHANG, Y., LUO, Y., WANG, X., TANG, H. Blue light combined with salicylic acid treatment maintained the postharvest quality of strawberry fruit during refrigerated storage. *Food Chemistry: X*, v. 15, p. 100384, 30 out. 2022.

ZHAO, L.; LAN, C.; TANG, X.; LI, B.; ZHANG, X.; GU, X.; ZHANG, H. Efficacy of *Debaryomyces hansenii* in the biocontrol for postharvest soft rot of strawberry and investigation of the physiological mechanisms involved. *Biological Control*, v. 174, p. 105011, 1 nov. 2022.

ZHAO, Y.; XIE, J. Practical applications of vacuum impregnation in fruit and vegetable processing. *Trends in Food Science & Technology*, v. 15, n. 9, p. 434-451, 2004.

4. MANUSCRIPT 1

QUALITY MAINTENANCE AND INCREASED FIRMNESS OF FRESH-CUT STRAWBERRIES USING VACUUM INFUSION OF CALCIUM LACTATE AND PECTIN METHYLESTERASE

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ABSTRACT

The demand for healthy foods has led to an increase in the consumption of fresh fruits, especially fresh-cut fruits. However, fresh-cut processing negatively affects fruit firmness, an important parameter for consumer acceptance. The objective of this work was to evaluate the effectiveness of the combined use of vacuum, calcium lactate and PME to increase the shelf life and quality of fresh cut strawberries. Fresh-cut strawberry halves were subjected to vacuum infusion with water, PME (10UI), PME plus 1% calcium lactate, or 1% calcium lactate alone; fresh-cut strawberries without infusion were used as the control. Firmness, loss of fresh mass, anthocyanin, color, soluble solids content (SSC), total acidity (TA) SSC/TA ratio, pH, visible spoilage and damage, visual quality, galacturonic acid content, degree of methylation, electrolyte efflux, PME activity, and respiration were measured initially and every 3 days for 9 days during 5°C storage. Vacuum infiltration of PME plus calcium lactate increased firmness by 90.7% for LCa+PME and 119.8% for LCa on day 3, delayed weight loss to below 0.8% for all except PME treatments, maintained freshness and anthocyanin content, reduced electrolyte efflux by 38% compared to the control, and lowered respiration rates to 134.92 and 135.62 mg.kg⁻¹.h⁻¹ CO₂ for LCa+PME and LCa, respectively, while the control reached 163.36 mg.kg⁻¹.h⁻¹ CO₂. Additionally, it minimized fruit damage. Therefore, vacuum infusion of 1% Lca+PME provided promising applications in extending the shelf life of cut strawberries by up to three days.

Key-words: Enzyme activity, Firming, *Fragaria x ananassa*, Postharvest.

4.1. Introduction

The growing demand for healthy, safe, and convenient foods has driven increased consumption of fresh fruits and vegetables, which provide essential nutrients, support balanced diets, and are associated with reduced risks of chronic diseases like type 2 diabetes and certain cancers (Aune et al., 2017). Furthermore, the relationship between a healthy diet, adequate nutritional status and strengthening the immune system has been highlighted as a relevant factor for the consumer (Gonella et al., 2022), with strawberries considered an important component in this context.

Maintaining the nutritional quality and safety of fresh products is a challenge for the food industry. Given the demand for fresh products, the application of minimal or fresh-cut processing has been investigated for many fruits and vegetables, such as apple (Perinban et al., 2022), dragon fruit (Li et al., 2022), kiwi (Hu et al., 2022), carrot (Li et al., 2021), yam (Teoh et al., 2016), mango (Kirtil et al., 2014) and strawberry (Méndez-Galarraga et al., 2022). Strawberry (*Fragaria x ananassa*), belonging to the Rosacea family, is widely consumed fresh due to its aroma, color and pleasant flavor. However, fresh-cut processing can negatively alter

the firmness of the fruits by accelerating cell wall degradation and loss of turgidity (Pinheiro and Almeida, 2008).

The decrease in firmness following fresh-cut fruit processing is associated with modifications and degradation of cell wall components such as cellulose, hemicellulose, and most notably pectins, due to their abundance and high sensitivity to chemical reactions. The enzymes pectin methylesterase (PME) and polygalacturonase (PG) play key roles in pectin breakdown. PME catalyzes the removal of methyl ester groups, exposing negatively charged carboxyl groups that serve as substrates for PG-mediated depolymerization (Li et al., 2021). Demethylation of pectin by PME exposes negatively charged carboxylic acid groups which can subsequently bind calcium ions. These de-esterified pectins can bind calcium ions, forming cross-links that strengthen the pectin network and reinforce cell wall structure, contributing to improved texture retention during storage (Li et al., 2024; Kirtil et al., 2014). As a result, calcium treatments can reduce softening, extend shelf life (Sirijariyawat et al., 2012), and consequently improve consumer acceptance of fresh-cut fruit.

Although calcium chloride (CaCl_2) is commonly applied postharvest (Langer et al., 2019; Prajapati et al., 2021), its sensory drawbacks, especially bitterness to residual chlorine (Luna-Guzmán and Barrett, 2000), can limit consumer acceptance and commercial viability. Calcium lactate, a compound with Generally Recognized As Safe (GRAS) status (U.S. FDA, 21 CFR § 184.1207), has been proposed as a viable alternative, offering comparable structural benefits with fewer sensory issues (Hu et al., 2022). Luna-Guzmán and Barrett (2000) demonstrated that calcium lactate maintained superior firmness in fresh-cut produce compared to calcium chloride, without inducing the undesirable bitterness associated with chloride-based treatments. The firming effect of calcium can be further enhanced by exogenous PME, which increases calcium binding sites via pectin demethylation (Huang et al., 2023). The effect of applying calcium and/or exogenous PME on firmness has been evaluated in different fruits such as papaya (Yang et al., 2017), mango (Kirtil et al., 2014), strawberry (Carnelossi et al., 2018), apricot (Liu et al., 2017), guava (Werner et al., 2009) and others.

Vacuum infusion has been used to improve the quality, physical-chemical characteristics and mainly the firmness of fresh-cut products, which in porous matrices, allows the exchange of gases and liquids with an impregnation solution under the action of the hydrodynamic mechanism, enabling the transfer of compounds of interest, such as PME, to intercellular spaces (Derossi et al., 2013; Guillemin et al., 2008). Combined infusion of PME and calcium has been reported to improve the firmness of fresh mangoes (Sirijariyawat et al., 2012). In the same way, infusion of CaCl_2 and PME into raspberries has been verified to maintain the fruit firmness (Yan et al., 2022). In addition, the isolated application of PME by vacuum impregnation was observed to result in an increase in the rigidity of pectins and the mechanical rigidity of apple tissue by Guillemin et al. (2008). In view of the above, the objective of this study was to evaluate the effectiveness of the combined use of vacuum, calcium lactate and PME to increase the shelf life and quality of fresh cut strawberries.

4.2. Material and Methods

4.2.1. Plant material

The strawberries (*Fragaria x ananassa*) were purchased from a local store at the full commercial development stage, with more than 75% red color (PBMH and PIMo, 2009) and were transported to the Fruit and Vegetable Laboratory of the Department of Food Technology at the Federal University of Sergipe.

4.2.2. Fresh-cut processing

Whole strawberries were selected based on physical integrity, appearance and absence of mechanical damage (Carnelossi et al., 2018). The fruit were previously washed with running water to remove residues. Both the removal of the calyx (3 mm from its base) and a longitudinal

cut were performed manually with the aid of a sharp blade. Sanitization was carried out by immersion of strawberry halves in chlorinated water cooled to $5 \pm 0.5^\circ\text{C}$ at a concentration of 200 ppm for 10 min (Carnelossi et al., 2018). After the initial chlorine submersion, additional rinsing was carried out in 5 ppm chlorinated water cooled to $5 \pm 0.5^\circ\text{C}$ with ice for 3 min. After rinsing, the strawberry halves were placed in sanitized sieves to drain in a controlled environment at the processing temperature $5 \pm 0.5^\circ\text{C}$ under $79 \pm 2\%$ RH (Carnelossi et al., 2018).

4.2.3. Vacuum infusion of fresh cut strawberry

Vacuum infusion was performed in a desiccator equipped with a pressure gauge and connected to a vacuum pump, where pressure was rapidly reduced to 12 kPa. Strawberry halves (100 g) in 250 mL beakers with 125 mL of solution ($5 \pm 0.5^\circ\text{C}$) underwent 10 min of vacuum exposure, followed by 1 min of gradual atmospheric pressure restoration. The following treatments were carried out: control (without infusion); infusion with water only; infusion with aqueous solution containing commercial PME; infusion with an aqueous solution containing commercial PME and calcium lactate (1%) and an aqueous solution containing only calcium lactate. The commercial PME enzyme (NovoShape) used, has a declared activity of 10 PEU/mL, manufactured by Novozymes, Denmark. The concentrations of PME and calcium lactate ($\text{C}_6\text{H}_{10}\text{CaO}_6$) were each 1% as used previously by Carnelossi et al. (2018).

The fresh-cut strawberries were divided into 5 treatments, namely: fresh-cut fruit (FC) without infusion (C); FC fruit infused with water (W); FC fruit infused with commercial PME; FC fruit infused with PME and calcium lactate (PME + LCa - 1%); FC fruit infused with only calcium lactate (LCa).

After the infusion process, the solution was drained using domestic sieves before being packaged and stored. After infusion, pieces were packaged in polypropylene packaging and stored in a vertical display with air circulation (Springer) at a temperature of $5^\circ\text{C} \pm 1^\circ\text{C}$ for 6 days, under a relative humidity of 79%. Samples were collected every 3 days to carry out biochemical and physical-chemical analyses, being previously crushed and homogenized with the aid of the Sorvall Omnimixer equipment and stored at -18°C until the moment of analysis. Analyses to determine pH, titratable acidity (TA), soluble solids content (SSC), firmness, color, visible spoilage, freshness, damage, and electrolyte efflux, as well as physiological analyses, were carried out on the fresh sample, that is, before the freezing process.

4.2.4. Firmness

The firmness of the fresh-cut fruit halves was measured using a digital penetrometer (TR Turoni, model 53205, Forli, Italy), with an 8-mm diameter tip according to the manufacturer. The results were expressed in Newtons (N).

4.2.5. Weight loss

Weight loss was determined during storage by subtracting the sample weight from their previously recorded initial weight of storage (day 0) and expressed as % of weight loss compared to the initial fruit weight (Zainal et al., 2019).

$$\text{Weight loss (\%)} = \frac{\text{Fruit initial weight} - \text{fruit weight}}{\text{Fruit initial weight}} \times 100$$

4.2.6. Electrolyte efflux

Cellular integrity analysis was performed according to the methodology described by Villalta and Sargent (2004). The strawberry mesocarp was minimally cut into slices (5 mm x 2 mm), 2 g of this tissue was rinsed with distilled water, dried on paper and placed in closed glass tubes with 35 mL of 0.4 M isotonic mannitol solution and kept at 23°C for 4 h. The conductivity of the bath solution was measured after 4 h of incubation at 23°C by a conductivity meter (Model 3403, Yellow Springs, OH, USA). The total electrolyte content was determined

after freezing for 24 h at -20°C, thawing for 1 h at 23°C, and then heating the pieces in a boiling water bath for 30 min. Electrolyte efflux of the fresh tissue was expressed as a percentage of total tissue electrolytes after freezing, thawing, and boiling.

4.2.7. Respiratory rate

The physiological analysis was carried out according to the methodology described by Furtado et al. (2018) with adaptations. The closed system CO₂ analysis was performed using approximately 25 g of fresh-cut strawberries. The respiratory rate was determined in glass bottles of approximately 120 mL previously prepared with rubber caps. The bottles were closed hermetically and aliquots of 0.1 cm³ were removed after 0.5 hours of closing the bottles with the aid of a 1000 microliter disposable syringe to quantify the gas composition. The determination of sampling time was carried out experimentally. The analysis of accumulated CO₂ was carried out on a gas chromatograph (Varian Model CP-3380), equipped with a thermal conductivity detector (TCD) and an Rt® capillary column -Q-BOND (RESTEK) (30m x 0.32mm ID x 10µm). The carrier gas used was Helium, with a flow of 5 mL/min. The temperatures of the column, injector, and TCD detector were 30, 200, and 120°C, respectively. The chromatographic analysis time was 120 s and the CO₂ retention time was 60 s.

Quantification of CO₂ was done by comparing the peak areas of the samples obtained in the Software (Galaxie Workstation) coupled to the chromatograph, with peak areas of the CO₂ standard of known concentration. The results were expressed in % CO₂. The % CO₂ was converted to respiration rate (mL/Kg.h) by calculation from the tissue weight, closure time, and the void space in the bottle.

4.2.8. Isolation of alcohol insoluble solids (AIS)

Isolation of alcohol insoluble solids (AIS) were isolated according to the method described by Huber (1984). Strawberry tissue (20 g) was homogenized in 80 mL of 100% ethanol for 3 min using a Sorvall Omnimixer. The homogenate was refluxed for 30 min in a boiling water bath. When necessary, 100% ethanol was added to maintain ethanol at the initial level. It was then stored overnight at -20° C. The suspension was filtered through a 0.6 µm retention capacity glass microfiber filter (MN GF-3, 55.5 mm, Machery-Nagel, Germany) in an aspiration bottle, followed by washing with 200 ml of 80% ethanol under slow aspiration. Then, it was washed with 100 mL of 100% acetone. The acetone was removed by vacuuming and the powder was air dried in an oven (39° C) for 1 day.

4.2.9. Degree of methylation and galacturonic acid

Methanol content was determined following the method of Wood and Siddiqui (1971), with modifications. Briefly, 5 mg of alcohol-insoluble solids (AIS) were dissolved in 2 mL of distilled water and sonicated for 10 min. Then, 0.8 mL of NaOH (2 M) was added for saponification, followed by incubation at 20 °C for 1 h with occasional shaking. Neutralization was performed with 0.8 mL of HCl (2 M), and the mixture was kept at 25 °C for 15 min (Ng and Waldron, 1997). For quantification, 1 mL of the sample or methanol standard was transferred to chilled test tubes containing 1 mL of H₂SO₄ (0.1 N). Next, 0.2 mL of KMnO₄ (2%, w/v) was added, and the mixture was gently stirred in an ice bath for 15 min. Then, 0.2 mL of sodium arsenite solution (0.5 M in H₂SO₄ 0.12 N) and 0.6 mL of distilled water were added, and the reaction was allowed to proceed at room temperature for 1 h. Finally, 2 mL of a 0.02 M acetylacetone solution in ammonium acetate (2.0 M) and acetic acid (0.05 M) was added, and the mixture was heated at 58–60 °C for 15 min.

Total galacturonic acid content was measured according to Ahmed and Labavitch (1978). AIS (5 mg) was homogenized in 2 mL of cold H₂SO₄ in a 20 mL beaker, followed by gradual addition of 0.5 mL of chilled distilled water under magnetic stirring for 5 min. The volume was then adjusted to 20 mL. For analysis, 0.5 mL of the sample was mixed with 3.6 mL of cold tetraborate in H₂SO₄ solution, heated in a boiling water bath for 5 min, and cooled

under running water. The degree of methylation (DM) was calculated as the molar ratio between methanol released and total galacturonic acid, and expressed as a percentage.

4.2.10. PME activity

The FC fruit tissue (1.00 g) was crushed in a pre-cooled (4°C) mortar and homogenized with 6 mL of 8.8% NaCl containing 10 g L⁻¹ of PVPP (Polyvinylpolypyrrolidone). After extraction at 4°C for 1 h, the solution was centrifuged at 12,000 x g for 20 min (Ren et al., 2020). The supernatant was obtained and used to measure enzyme activity. The PME activity was evaluated as described by Vicente et al. (2005). Activity was measured in a mixture containing 0.6 mL of 0.5% pectin (w/v), 0.15 mL of 0.05% bromothymol blue, 0.1 mL of distilled water and 0.1 mL of enzyme extract (supernatant). The mixture was incubated at 37°C for 30 min and the absorbance at 620 nm was recorded. Enzymatic activity was defined as a reduction of 0.01 units of OD 620 nm per minute per kilogram of tissue.

4.2.11. Visual quality (freshness, visible spoilage, and mechanical damage)

The evaluation of fresh-cut fruit halves for visual appearance (freshness), visible spoilage, and mechanical damage was performed using methodologies adapted from Jacomino et al. (2011). For freshness, randomly selected fresh-cut strawberry halves were evaluated independently by the authors using the following standardized scale: 9 = Excellent: completely fresh appearance, high gloss; 7 = Good: still fresh appearance, shiny; 5 = Fair: not fresh appearance, low brightness, marketability limit; 3 = Poor: dull appearance, usability limit; 1 = Extremely poor: wrinkled appearance. Results were calculated as mean scores for each experimental replicate. Visible spoilage was assessed by enumerating strawberry halves exhibiting postharvest rot or visible fungal growth. The incidence was expressed as a percentage relative to the total number of FC strawberry halves. Mechanical damage was quantified by counting fruit halves displaying postharvest physical injuries, with results expressed as a percentage in relation to the total number of FC fruit halves.

4.2.12. Color

Color parameters were directly measured using a Minolta CR-300 colorimeter (Konica Minolta Sensing, Osaka, Japan) in the CIE L*a*b* and hue angle (h°) value automatically calculated by the instrument's software: L* (Lightness): Ranges from 0 (black) to 100 (white); a: Red-green axis (+a = red, -a* = green); b: Yellow-blue axis (+b = yellow, -b* = blue); Hue angle (h°) = arctan(b*/a*): Describes color tone (0° = red, 90° = yellow, 180° = green). Measurements were performed according to manufacturer specifications (Konica Minolta, 2007) and standardized protocols (Gaya and Stermann Ferraz, 2006; HunterLab, 2008; Menezes, 2012).

4.2.13. Determination of pH, Soluble solids content (SSC), titratable acidity (TA), and RATIO (SSC/TA).

A 5-g sample of strawberry tissue was weighed, homogenized and mixed with 50 mL of distilled water. The pH of the resulting liquid was measured using the potentiometric method. After calibrating the pH meter, the electrode was immersed in the homogenized aqueous mixture, and the measurement was carried out (Instituto Adolfo Lutz, 2008).

The SSC of the strawberry tissue was determined by applying a few drops of juice onto the prism of a digital refractometer model HI 96801 (Hanna Instruments, Romania) according to the official method (AOAC, 1997). The results were expressed in °Brix.

The TA of the strawberry tissue was determined using 10 mL of the aqueous homogenate used to determine the pH, adding 1% phenolphthalein indicator and titrating with standardized NaOH (0.1N) to pH 8.2. The TA was expressed as % citric acid (Instituto Adolfo Lutz, 2008).

The ratio of SSC/TA was calculated using the ratio of the °Brix ratio to the percent acidity expressed as % citric acid (Instituto Adolfo Lutz, 2008).

4.2.14. Determination of total anthocyanin content

The determination of total anthocyanins was carried out according to Nunes et al. (2006). Aliquots (2 g) of homogenized strawberry tissue were mixed with 18 mL of 0.5% HCl in methanol (v/v). Anthocyanins were extracted by keeping the samples at 4 °C for 1 h in the dark. Samples were then filtered using single-layer fabric (Kimwipe) to remove flocculant. The absorbance of the solution was measured at 520 nm. The results were expressed as mg/100 g fresh weight of pelargonidin-3-glucoside.

4.2.15. Statistical analysis

The design was completely randomized set up in a factorial scheme (5 x 3) with 5 treatments [FC fruit without infusion (SI); FC fruit infused with water (W); FC fruit infused with PME; FC fruit infused with PME + calcium lactate (PME + LCa - 1%); FC fruit infused with only calcium lactate (LCa)] and 3 evaluation times (0, 3, and 6 days) with 4 repetitions for each treatment. The results were subjected to analysis of variance (ANOVA) using the F test and comparison of means using the Tukey test ($p < 0.05$), expressed as the mean $\pm$ standard deviation, with the aid of the computer program SISVAR version 5.8 (Ferreira, 2019).

4.3. Results

Fruit halves treated with LCa+PME presented on “day 0” (Figure 1A) higher firmness values ($p < 0.05$) compared to the control group (C). The treatments with LCa and LCa+PME showed an increase in firmness relative to the pre-treatment value on the 3rd day of storage (2.5 N for LCa and 3.0N for LCa+PME), being superior throughout the storage time in relation to the other treatments. A decrease in firmness was observed over time in the control water-treated samples.

There was an increase in weight loss throughout the storage period for the evaluated treatments, with significant losses observed from the third day of storage (0.66%) (Figure 1B). Greater mass losses were observed in strawberries treated with PME ($p < 0.05$) while for the other treatments, a loss of less than 0.8% was observed over time.

Electrolyte efflux from FC strawberries increased significantly ($p < 0.05$) as a function of time (Figure 1C). However, on the sixth day of storage, it was observed that FC strawberries treated with LCa+PME showed less leakage compared to FC strawberries treated without infusion and with infusion with water alone. Strawberries treated with LCa+PME showed greater electrolyte efflux stability (Figure 1C).

Fresh-cut strawberries treated with LCa and LCa+PME showed lower respiratory rates (135.62 and 134.92 mg.kg⁻¹.h⁻¹ of CO₂) than those treated with water alone (163.36 mg.kg⁻¹.h⁻¹ of CO₂) (Figure 1D). The respiratory rate of FC strawberries did not vary until day 3, with a significant increase in the respiratory rate only being observed on day 6 of storage ($p < 0.05$). The effect of treatments did not vary significantly with storage duration ($p < 0.05$).

There were no significant differences during storage in GalAc acid levels for the C, water, and PME+LCA treatments ($p < 0.05$) (Figure 4). On day 3, higher GalAc values were observed for the LCa treatment, differing only from the PME+LCA treatment (Figure 2A).

Higher values of the degree of methylation (Figure 2B) for treatments with LCa+PME (72%) were verified on day 0 in relation to the other treatments ($p < 0.05$) remaining higher on day 3 of treatments with only LCa and SME. A reduction in the degree of methylation was observed in all treatments throughout storage ($p < 0.05$) (Figure 5).

Significant increases ($p < 0.05$) in PME activity were observed during the 6-day storage period: 113% for control (C), 37% for water-treated, and 22% for PME-treated samples (Figure 2C). In contrast, the LCa+PME treatment showed reduced PME activity, reaching its lowest

values on day 3. The LCa treatment maintained consistently lower PME activity throughout storage, showing no significant difference ($p > 0.05$) only when compared to the LCa+PME treatment.

When evaluating freshness (Figure 3A), a significant reduction was observed in all treatments over time. However, the LCa and LCa+PME samples showed greater freshness compared to samples infused with water alone (Figure 3A). The LCa+PME treatment showed greater freshness retention with a good appearance, fresh and still shiny appearance, maintained throughout the storage time (Figure 4).

There was no significant difference between treatments when evaluating the percent of visible spoilage of MP strawberries ($p < 0.05$). During storage, an increase in the percent of damage was also observed. However, for samples treated with calcium sources (LCa and PME+LCa), this variation was smaller (Figure 3B). On the other hand, the C, water and mainly PME treatments showed a high percentage of damage from day 3 onwards.

When evaluating the color index, there was a reduction in the luminosity (i.e., darkening) of the FC strawberries in the PME and LCa+PME treatments on day 3 (Figure 3C) ($p < 0.05$), while on day 6, an increase in the L^* color index was observed. The L^* for FC fruit treated with LCa, was higher than for the other treatments on day 6 ($p < 0.05$). Furthermore, over time, a significant reduction (reddening) in the Hue* angle (Figure 3D) was observed in fruit treated with water ($p < 0.05$), with higher (less red) values for treatments with LCa and LCa+PME of 54.4. When evaluating the color index “a*” (Figure 3E), lower levels of the red component were found in FC strawberries treated with calcium, regardless of the day, with values of 34.97 and 35.27 (average) for LCa and LCa+PME respectively ($p < 0.05$). An increase in b^* values was observed in all treatments (Figure 3F).

It was possible to observe an increase in pH values ($p < 0.05$) over time from the third day of storage of FC strawberries, with values of 3.77 for the first day and 3.91 and 3.96 for days 3 and 6 respectively (Table 1). No differences in TA were observed between treatments, with average values of 0.97 g/100g of citric acid (data not shown). An increase in SSC was observed on day 3 only for the C treatment (Table 1). The treatments did not differ on the same day of analysis. The SSC/TA ratio is the relationship between the °Brix and the acidity of the product, being an indicator of quality (Couto and Canniatti-Brazaca, 2010). In this study, no significant differences were observed when evaluating the SSC/TA ratio with average values of 5.36 for all FC strawberries.

A reduction in anthocyanin content in the FC strawberries was observed over time ($p < 0.05$) (Table 1). Strawberries treated with infusion with water alone and LCa+PME showed higher anthocyanin levels when compared to the other treatments.

4.4. Discussion

4.4.1. Firmness

Firmness is one of the most important parameters that determine the acceptability and shelf life of fruits and vegetables (Goulao and Oliveira, 2008). Calcium-treated strawberries exhibited greater firmness over time. This is attributed to the association of calcium ions, which form cross-links between pectin chains by binding to free carboxyl groups, thereby strengthening the cell wall (Udomkun et al., 2014). Additionally, the rapid formation of calcium-pectate cross-links during processing likely contributed to enhanced tissue cohesion and reduced softening immediately after cutting, thus explaining the significant differences in parameters such as firmness observed already at day 0 among treatments.

The loss of firmness of strawberries treated only with PME may be associated with the absence of sufficient endogenous calcium ions to crosslink pectic polymers by binding to the free carboxyl groups created by PME, making the demethylated pectin polymers susceptible to degradation by other pectolytic enzymes, such as PG (Chávez-Sánchez et al., 2013; Lara et al., 2004). Therefore, the vacuum infusion of calcium (LCa and LCa+PME) was effective in

maintaining the firmness of fresh-cut strawberries. These results corroborate those obtained by Yang et al. (2017), who found that the application of PME and calcium lactate increased the hardness of cut papaya. Tappi et al. (2016) also found that the infusion of calcium lactate in fresh-cut melon maintained the texture of the fruit. Vacuum impregnation with calcium in Kyoho grapes resulted in an increase in firmness from 12.93 to 14.4 N during storage (Mao et al., 2017). Thus, the availability of calcium ion through infusion with calcium lactate maintains the structure since the methyl groups of hydrolyzed pectin bind adjacent pectin polymers, making the cell wall firmer (Kirtil et al., 2014). Therefore, the application of LCa and LCa+PME can be an efficient alternative in improving the firmness of fresh-cut strawberries.

4.4.2. Weight loss

Water loss is one of the factors that can affect the texture of fruits and vegetables as it leads to loss of turgor and crunchiness, giving the product a withered appearance and flabby texture, which can harm its market value and acceptability by the consumer (Prajapati et al., 2021). Furthermore, water loss tends to occur more quickly in FC products due to the absence of epidermal layers and the exposure of internal tissues caused by tissue rupture during the cutting and peeling operations, which was observed in all treatments from the day 3 (Rolle and Chism, 1987; Toivonen and Brummell, 2008). However, excessive weight loss in PME treatments may be associated with physiological processes, such as respiration, throughout the storage period, and enzymatic activity, since an increase in respiratory rate and higher values of enzymatic activity were observed in relation to calcium treatments (Figure 6) (Ndukwu and Chinenye, 2011). These results corroborate those found by Zhang et al. (2019), who found a positive effect on the weight loss of jujube fruit treated with vacuum infiltration of Ca + PME and vacuum infiltration of Ca. Therefore, the application of the vacuum infusion of calcium lactate combined with refrigerated storage of FC strawberries in the current work contributed to maintaining quality and reducing weight loss.

4.4.3. Electrolyte efflux

With the increase in enzymatic activity and substantial changes in firmness, changes occur in the cellular structure of FC fruit. Thus, an increase in electrolyte leakage was observed on the third day of storage for FC strawberries (Figure 3). This was likely due to a loss of membrane integrity that allowed effusion of cellular osmotic solutes into the apoplastic space, which resulted in water movements out of the tissue and loss of turgor (Toivonen and Brummell, 2008).

The action of PME enables the formation of a complex of calcium and pectin, providing increased cell wall rigidity and tissue firmness, which was verified in this study in treatments with calcium ion, especially the treatment with LCa+PME, which showed less electrolyte efflux in relation to the control on the day 6 (Aghdam et al., 2012). This study is in line with that of Ngamchuachit et al. (2014) who found less effusion of electrolytes in FC mangoes treated with a calcium source, promoting a stabilizing effect. Similarly, Phanumong et al. (2016) found that calcium immersion treatment reduced electrolyte efflux when compared to the control in FC lychees. In the present study, a lower percentage of electrolyte leakage from FC strawberries subjected to infusion with LCa+PME was evidenced on the last day of storage, indicating that the use of these substances can help maintain the structural integrity of the FC strawberry tissues.

4.4.4. Respiratory rate

The results indicate that treatments with a source of calcium promoted the lowest respiratory rate among the treatments applied. This effect can be attributed to the role of calcium in the physiological pattern of fruits, where the reduction in cell wall degradation resulting from the formation of the calcium-pectin bonds and pectin crosslinking results in less solubilization of substrates necessary for enzyme activities, including those involved in the respiratory

process, consequently decreasing the respiratory rate (Batista-Silva et al., 2018; Kirtil et al., 2014). This enhanced tissue cohesion, evident from day 0, can also influence gas diffusion and result in a slightly different respiration pattern. The results corroborate those found by Perez-Cabrera et al. (2011) when evaluating the effect of infusing FC pears with salts, found that impregnation with calcium lactate had the most notable effect in that the rate of oxygen consumption was reduced compared to untreated samples, as was the rate of CO₂ generation. Luna-Guzmán et al. (1999) also found that calcium decreased metabolism as it reduced the respiratory rate of FC melon, in addition, calcium salts maintained the firmness of the melons throughout refrigerated storage.

However, an increase in the respiratory rate was observed on day 6. This increase may be associated with microbial activity since the presence of rot (3%) was verified in the samples on the last day of storage. Luna-Guzmán et al. (1999) observed that cutting and slicing makes FC products more prone to microbial contamination. Fresh-cut processing also increases enzymatic degradation (Rybak et al., 2020). This is due to the increased exposed surface area that allows oxygen to diffuse more quickly into the cells and the increased metabolic activity of injured cells (Zagory, 1995). Therefore, infusion with LCa and PME can be an effective approach for reducing the respiratory rate and consequently maintaining the quality of FC strawberries.

4.4.5. Degree of methylation and galacturonic acid

The degree of methylation provides an estimate of the carboxyl groups that are methylated, thus not available to bind with calcium ions (Ciriminna et al., 2022). Thus, an increase in the degree of methylation can reduce the formation of calcium bridges and consequently destabilize pectins (Lara et al., 2004). In this study, a higher degree of methylation was verified for treatments with LCa+PME, indicating less availability for the formation of the pectin network, with a reduction in the values of the degree of methylation in the subsequent days. Consequently, firmness increased on these days, indicating that calcium ions may have bonded to unmethylated galacturonic acid residues forming the calcium bridge (Lara et al., 2004). Langer et al. (2019), when evaluating the effects of calcium on the metabolism of the strawberry cell wall, they observed that the calcium treatment demonstrated a lower value in the degree of esterification of pectins when compared to the control and was verified for the LCa treatment +PME in the first days of storage. Therefore, a connection with calcium may have occurred, helping to improve the firmness of minimally processed strawberries.

4.4.6. PME activity

The loss or increase in fruit firmness is directly linked to the activity of the enzymes responsible for pectin degradation (Posé et al., 2019). During storage, PME activity increased in strawberries, due to the rupture of the cell wall during cutting, which releases pectolytic enzymes that promote the breakdown of pectins and compromise the structure of the fruits (Toivonen and Brummell, 2008; Yang et al., 2017). It was shown that vacuum infusion with PME may have accelerated the impregnation of enzymes in the fruits, resulting in greater PME activity (Guillemin et al., 2006). Furthermore, the increase in enzyme activity in water treatment may indicate a negative effect of PME Infusion in strawberries, while in calcium treatments this effect was not observed. With the increase in enzymatic activity, there was a loss of firmness for treatments with and without infusion without the application of LCa. Anjongsinsiri et al. (2006) and Guillemin et al. (2006) who verified an increase in PME activity after vacuum infusion of strawberries and apples respectively.

However, an improvement in the firmness of the LCa and PME + LCa samples was verified, which may be associated with the response of the PME action with the availability of calcium in solution, which allows the calcium ion to bind to the pectin molecules that make up the cell wall, thus extending fruit firmness (Huang et al., 2023). This immediate and efficient cross-linking during initial processing may significantly contribute to the enhanced tissue

cohesion observed from day 0, possibly explaining the early differences in firmness among treatments. Similar results were found by Sirijariyawat et al. (2012), when applying PME and calcium by infusion, they found that the PME activity of mango cubes was significantly higher after infusion at all pressure levels. Langer et al. (2019) observed that calcium treatment increased the activity of pectin methylesterase while inhibiting those corresponding to pectin hydrolases such as polygalacturonase and β -galactosidase. Therefore, vacuum infusion with LCa+PME can be effective in maintaining or improving the quality of fresh-cut strawberries, promoting the formation of calcium pectate and inhibiting the activity of pectolytic enzymes.

4.4.7. Visual quality (visible spoilage, freshness and damage)

A reduction in freshness and an increase in the percentage of damage were observed mainly in the groups treated with PME. Weight loss is a crucial factor in the quality and commercialization of vegetable products, as it directly affects firmness and appearance, which can lead to wilting of the product (El-Beltagi et al., 2022). In this study, an increase in water loss was observed over time in all treatments, being more significant in the group treated with PME. This treatment also presented the highest percentage of damage and loss of freshness throughout the storage period, differing from the samples treated with LCa+PME on the sixth day.

The presence of calcium can inhibit the activity of endogenous PME, preserving the structure of the strawberry cell wall. The endogenous calcium present in PME treatments may have been insufficient to promote this inhibition, resulting in reduced fruit firmness (Kirtil et al., 2014). Vacuum infusion of enzymes and calcium leads to the formation of calcium pectate, which improves and maintains fruit firmness, preventing damage (Carnelessi et al., 2018).

The low incidence of rot observed (3%) can be attributed to the possible microbial resistance promoted by LCa, as it is an organic acid salt, and to mechanical resistance, since mechanical damage can be a means of entry for microorganisms (Aguayo et al., 2015; Khademi and Khoveyteri-Zadeh, 2022). For example, Phanumong et al. (2016) evaluated the application of various calcium salts on the quality and texture of fresh-cut lychee fruits and verified an increase in the product's shelf life to 9 days based on sensory scores. It was found in minimally processed jackfruit that the application of calcium lactate with vacuum infusion showed positive results in reducing contamination during storage compared to control and that vacuum infusion prolonged shelf life (Sandianysamy et al., 2023). Nasri et al. (2023) when evaluating the effect of calcium lactate and ultrasound on mushrooms, observed a significant reduction in contamination. Therefore, infusion with LCa and PME appears as an effective alternative in maintaining freshness and reducing damage in fresh-cut strawberries during storage.

4.4.8. Color

The tendency towards a reduction in luminosity parameters observed in treatments over time may be associated with an increase in vacuum, which in turn leads to greater impregnation of the solution and greater gas-liquid exchange, promoting changes in color coordinates, which may cause changes in the refractive index (Tapia et al., 1999). The index a^* represents the color from green to red, which may reflect the degree of maturation, however, no variation was observed over time. Likewise, Tappi et al. (2016), when evaluating minimally processed melon with calcium lactate vacuum impregnation, observed a reduction in L^* (luminosity) in all treatments applied. Yang et al. (2017) when evaluating the vacuum impregnation of calcium lactate and PME in minimally processed papaya, they found a decrease in the L^* and C^* index in all treatments as a result of pigment degradation, in addition, they found that the infusion was able to delay the degradation of pigments once the gaseous phase of the fruit was removed. Perez-Cabrera et al. (2011) found that calcium lactate applied to freshly vacuum-processed pears maintained their color and firmness during storage. Thus, the joint and efficient action of PME and calcium allows the structure of the cell wall to remain unchanged, maintaining the color and visual characteristics of fresh-cut strawberries. These effects are directly related to

fruit freshness perception by consumers, as color stability is a key indicator of ripeness and overall visual quality. This relationship is supported by the results in Fig. 3A, which showed reduced freshness and increased damage mainly in PME-treated groups, while LCa+PME preserved visual quality, aligning with the maintenance of color and structural integrity described above.

4.4.9. pH

The observed pH increase across treatments from day 3 of storage likely reflects cellular damage responses during minimal processing. Cutting induces pH changes in fresh-cut fruit: Immediate acidification from vacuolar content leakage (lowering pH), followed by progressive alkalization due to metabolic shifts including ammonia release from deamination reactions and membrane lipid degradation (Beaulieu, 2002; Toivonen and Brummell, 2008). Furthermore, lower values observed in PME treatments may indicate the loss of cellular content. Results that corroborate this finding were reported by Sandianysamy et al. (2023) who, when evaluating the infusion of LCa in minimally processed jackfruit, verified the same behavior, where an increase in pH was observed after the application of treatment and during storage.

4.4.10. Soluble solids content (SSC), titrable acidity (TA), and RATIO

After harvest and during storage, there is a tendency for fruits to decrease in the quantities of the main precursors of respiratory metabolism, such as sugars and acids. However, in this study, since the respiratory rate was stable until day 3 of storage, no significant variations in these parameters were observed, in agreement with Zhang et al. (2008). The SSC is an important quality index related to fruit flavor that develops during ripening, namely sweetness. Despite the increase in SSC being observed from day 3 onwards, the treatments applied did not differ from each other. Thus, this may indicate that the treatments do not cause significant changes in this quality parameter, since the greater the decline in SSC, the greater the degradation in fruit quality (Li et al., 2015). Zhang et al. (2019), when evaluating the effects of methylesterase and calcium on the quality attributes of vacuum-impregnated jujube fruit, found that the application of Ca and Ca+PME by vacuum infiltration could preserve the quality of the jujubes during storage. On the other hand, Sun et al. (2023) when evaluating changes in the forms of Ca and soluble pectin in chelate of cherry tomatoes treated with ultrasound-assisted calcium lactate, found no differences in the levels of SSC between treatments. Furthermore, when evaluating the SSC/TA ratios, no differences were found between treatments. The SSC/TA can be an indication of the degree of ripeness of many fruits, being a parameter used to indicate a possible pleasant sensation on the consumer's palate, however, this parameter proved to be stable during storage of guava fruit (Azzolini et al., 2004). Thus, we can conclude that the organic acid content and soluble solids content of the FC strawberries and the SSC/TA do not undergo significant changes, causing no noticeable sensory changes, since the products remained stable. Thus, the results suggest that the application of the LCa and LCa+PME treatments in the current work maintain taste the characteristics of the product.

4.4.11. Anthocyanin

The anthocyanin content of strawberries is a key quality attribute, affecting both visual appeal (red color intensity) and antioxidant capacity, but is highly vulnerable to postharvest handling (Crecente-Campo et al., 2012; Nunes et al., 2006). In our study, anthocyanin levels declined by day 3 in all treatments, consistent with earlier reports that fresh-cut (FC) processing accelerates pigment degradation due to increased surface area enhancing oxygen diffusion and promoting anthocyanin oxidation (Zagory, 1995), cutting injury upregulates polyphenol oxidase (PPO) and peroxidase (POD), as observed by Van de Velde et al. (2018) in FC strawberries and cellular damage altering apoplastic pH, which destabilizes anthocyanins (Gil et al., 1997; Odriozola-Serrano et al., 2009). However, the subsequent anthocyanin rebound by

day 6 (Table 1) suggests that though weight loss was statistically insignificant (Fig 1B), even marginal moisture evaporation could concentrate cellular pigments, artificially elevating spectrophotometric readings. The stress may trigger anthocyanin synthesis via the upregulation of phenylalanine ammonia-lyase (PAL) (Van de Velde et al., 2018). Additionally, the observed increase in pH could facilitate conversion of colorless chalcones to colored anthocyanidins and shift equilibrium toward more stable flavylum cations at moderately acidic pH, although this effect may have been mitigated by calcium-induced pigment stabilization in the LCa treatment (Khoo et al., 2017). Therefore, studies are needed to further evaluate the effect of the treatments evaluated here on FC strawberries.

4.5. Conclusion

The infusion of calcium lactate into fresh-cut (FC) strawberry halves proved highly effective in preserving fruit quality and extending shelf life by up to 3 days. Vacuum infiltration with calcium lactate increased firmness by 119.8% on day three, while the combination with PME further enhanced firmness by 90.7%, reduced electrolyte efflux by 38% compared to the control, and maintained respiration rates significantly lower than untreated samples. Both treatments delayed weight loss to below 0.8% (except PME alone), preserved freshness and anthocyanin content, and minimized fruit damage, highlighting their potential for improving post-harvest quality in the fresh-cut fruit industry.

4.6. References

- Aghdam, M. S., Hassanpouraghdam, M. B., Paliyath, G., & Farmani, B. (2012). The language of calcium in postharvest life of fruits, vegetables and flowers. *Scientia Horticulturae*, 144, 102–115. <https://doi.org/10.1016/J.SCIENTA.2012.07.007>
- Aguayo, E., Requejo-Jackman, C., Stanley, R., & Woolf, A. (2015). Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality. *Postharvest Biology and Technology*, 110, 158–165. <https://doi.org/10.1016/J.POSTHARVBIO.2015.07.001>
- Ahmed, A. E. R., & Labavitch, J. M. (1978). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry*, 1(4), 361–365. <https://doi.org/10.1111/j.1745-4514.1978.tb00193.x>
- Anjongsinsiri, P. B., Kenney, J., & Wicker, L. (2006). Detection of Vacuum Infusion of Pectinmethylesterase in Strawberry by Activity Staining. *Journal of Food Science*, 69(3), FCT179–FCT183. <https://doi.org/10.1111/j.1365-2621.2004.tb13354.x>
- AOAC. (1997). Official methods of analysis of the Association of Official Analytical Chemists (method 945.46). In *Official methods of analysis of the Association of Official Analytical Chemists*.
- Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N. N., Norat, T., Greenwood, D. C., Riboli, E., Vatten, L. J., & Tonstad, S. (2017). Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. *International Journal of Epidemiology*, 46(3), 1029–1056. <https://doi.org/10.1093/IJE/DYW319>

- Azzolini, M., Jacomino, A. P., & Spoto, M. H. F. (2004). Estádios de maturação e qualidade pós-colheita de goiabas “Pedro Sato”. *Revista Brasileira de Fruticultura*, 26(1), 29–31. <https://doi.org/10.1590/S0100-29452004000100009>
- Batista-Silva, W., Nascimento, V. L., Medeiros, D. B., Nunes-Nesi, A., Ribeiro, D. M., Zsögön, A., & Araújo, W. L. (2018). Modifications in Organic Acid Profiles During Fruit Development and Ripening: Correlation or Causation? *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/FPLS.2018.01689>
- Beaulieu, J. C., & Gorny, J. R. (2002). Fresh-cut fruits. *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*, 604.
- Carnelossi, M., Brecht, J. K., Huber, D., Carvalho, L. M., & Sargent, S. A. (2018). Vacuum infusion of pectin methylesterase and calcium maintains firmness of the fresh-cut strawberry. *I Congresso Luso-Brasileiro de Horticultura (I CLB Hort)*, Lisboa, Portugal, 1-4 de Novembro de 2017, *Acta Portuguesas de Horticultura* 29, 132-140
- Chávez-Sánchez, I., Carrillo-López, A., Vega-García, M., & Yahia, E. M. (2013). The effect of antifungal hot-water treatments on papaya postharvest quality and activity of pectinmethylesterase and polygalacturonase. *Journal of Food Science and Technology*, 50(1), 101–107. <https://doi.org/10.1007/S13197-011-0228-0/FIGURES/3>
- Ciriminna, R., Fidalgo, A., Scurria, A., Ilharco, L. M., & Pagliaro, M. (2022). Pectin: New science and forthcoming applications of the most valued hydrocolloid. *Food Hydrocolloids*, 127, 107483. <https://doi.org/10.1016/J.FOODHYD.2022.107483>
- Couto, M. A. L., & Canniatti-Brazaca, S. G. (2010). Quantificação de vitamina C e capacidade antioxidante de variedades cítricas. *Ciencia e Tecnologia de Alimentos*, 30(SUPPL. 1), 15–19. <https://doi.org/10.1590/s0101-20612010000500003>
- Crecente-Campo, J., Nunes-Damaceno, M., Romero-Rodríguez, M. A., & Vázquez-Odériz, M. L. (2012). Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (*Fragaria* × *ananassa* Duch, cv Selva). *Journal of Food Composition and Analysis*, 28(1), 23–30. <https://doi.org/10.1016/J.JFCA.2012.07.004>
- Derossi, A., De Pilli, T., & Severini, C. (2013). Application of Vacuum Impregnation Techniques to Improve the pH Reduction of Vegetables: Study on Carrots and Eggplants. *Food and Bioprocess Technology*, 6(11), 3217–3226. <https://doi.org/10.1007/S11947-012-0994-3/FIGURES/5>
- El-Beltagi, H. S., Ali, M. R., Ramadan, K. M. A., Anwar, R., Shalaby, T. A., Rezk, A. A., El-Ganainy, S. M., Mahmoud, S. F., Alkafafy, M., & El-Mogy, M. M. (2022). Exogenous Postharvest Application of Calcium Chloride and Salicylic Acid to Maintain the Quality of Broccoli Florets. *Plants* 2022, Vol. 11, Page 1513, 11(11), 1513. <https://doi.org/10.3390/PLANTS11111513>
- Ferreira, D. F. (2019). SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista brasileira de biometria*, 37(4), 529–535. <https://doi.org/10.28951/rbb.v37i4.450>

- Furtado, M. C., De Souza, J. F., Matos, P. N., De Araújo, H. G. G. S., De Jesus, M. S., Narain, N., & Carnelossi, M. A. G. (2018). Influence of the coating of edible starch and antioxidant on physiological characteristics of minimally processed yam (*Dioscorea* sp.). *Acta Horticulturae*, 1198, 213–216. <https://doi.org/10.17660/ActaHortic.2018.1198.34>
- Gaya, L. D. G., & Sterman Ferraz, J. B. (2006). Aspectos genético-quantitativos da qualidade da carne em frangos. *Ciencia Rural*, 36(1), 349–356. <https://doi.org/10.1590/s0103-84782006000100058>
- Gil, M. I., Holcroft, D. M., & Kader, A. A. (1997). Changes in Strawberry Anthocyanins and Other Polyphenols in Response to Carbon Dioxide Treatments. *Journal of Agricultural and Food Chemistry*, 45(5), 1662–1667. <https://doi.org/10.1021/JF960675E/ASSET/IMAGES/LARGE/JF960675EF00004.JPEG>
- Gonella, K., Nayak, S. P., Garg, M., & Kotebagilu, N. P. (2022). Impact of the COVID-19 pandemic on immune boosting food consumption and overall dietary pattern among selected Indian adults: An observational study. *Clinical Epidemiology and Global Health*, 15. <https://doi.org/10.1016/J.CEGH.2022.101056>
- Goulao, L. F., & Oliveira, C. M. (2008). Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science & Technology*, 19(1), 4–25. <https://doi.org/10.1016/J.TIFS.2007.07.002>
- Guillemin, A., Degraeve, P., Guillon, F., Lahaye, M., & Saurel, R. (2006). Incorporation of pectinmethylesterase in apple tissue either by soaking or by vacuum-impregnation. *Enzyme and Microbial Technology*, 38(5), 610–616. <https://doi.org/10.1016/j.enzmictec.2005.07.028>
- Guillemin, A., Guillon, F., Degraeve, P., Rondeau, C., Devaux, M. F., Huber, F., Badel, E., Saurel, R., & Lahaye, M. (2008). Firming of fruit tissues by vacuum-infusion of pectin methylesterase: Visualisation of enzyme action. *Food Chemistry*, 109(2), 368–378. <https://doi.org/10.1016/J.FOODCHEM.2007.12.050>
- Hu, X., Chen, Y., Wu, X., Liu, W., Jing, X., Liu, Y., Yan, J., Liu, S., & Qin, W. (2022). Combination of calcium lactate impregnation with UV-C irradiation maintains quality and improves antioxidant capacity of fresh-cut kiwifruit slices. *Food Chemistry: X*, 14, 100329. <https://doi.org/10.1016/j.fochx.2022.100329>
- Huang, W., Shi, Y., Yan, H., Wang, H., Wu, D., Grierson, D., & Chen, K. (2023). The calcium-mediated homogalacturonan pectin complexation in cell walls contributes the firmness increase in loquat fruit during postharvest storage. *Journal of Advanced Research*, 49, 47–62. <https://doi.org/10.1016/j.jare.2022.09.009>
- Huber, D. J. (1984). Strawberry Fruit Softening: The Potential Roles of Polyuronides and Hemicelluloses. *Journal of Food Science*, 49(5), 1310–1315. <https://doi.org/10.1111/j.1365-2621.1984.tb14976.x>
- HunterLab. (2008). CIE L*C*h Color Scale. Insight on Color. <https://doi.org/10.1172/JCI45563.Changes>
- Instituto Adolfo Lutz. (2008). Métodos físico-químicos para análise de alimentos. In Instituto Adolfo Lutz. <https://doi.org/10.1017/CBO9781107415324.004>

Jacomino, A. P., Sargent, S. A., Berry, A. D., & Brecht, J. K. (2011). Potential for Grading, Sanitizing, and Hydrocooling Fresh Strawberries. *Proc. Fla. State Hort. Soc. Proc. Fla. State Hort. Soc.*

Khademi, O., & Khoveyteri-Zadeh, S. (2022). Determination the best source of calcium for button mushroom conservation. *Journal of Horticulture and Postharvest Research*, 5(Issue 2), 177–186. <https://doi.org/10.22077/JHPR.2022.4797.1246>

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*. <https://doi.org/10.1080/16546628.2017.1361779>

Kirtil, E., Oztop, M. H., Sirijariyawat, A., Ngamchuachit, P., Barrett, D. M., & McCarthy, M. J. (2014). Effect of pectin methyl esterase (PME) and CaCl₂ infusion on the cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study. *Food Research International*, 66, 409–416. <https://doi.org/10.1016/J.FOODRES.2014.10.006>

Langer, S. E., Marina, M., Burgos, J. L., Martínez, G. A., Civello, P. M., & Villarreal, N. M. (2019). Calcium chloride treatment modifies cell wall metabolism and activates defense responses in strawberry fruit (*Fragaria × ananassa*, Duch). *Journal of the Science of Food and Agriculture*, 99(8), 4003–4010. <https://doi.org/10.1002/JSFA.9626>

Lara, I., García, P., & Vendrell, M. (2004). Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest Biology and Technology*, 34(3), 331–339. <https://doi.org/10.1016/J.POSTHARVBIO.2004.05.018>

Li, L., Li, C., Sun, J., Xin, M., Yi, P., He, X., Sheng, J., Zhou, Z., Ling, D., Zheng, F., Li, J., Liu, G., Li, Z., Tang, Y., Yang, Y., & Tang, J. (2021). Synergistic effects of ultraviolet light irradiation and high-oxygen modified atmosphere packaging on physiological quality, microbial growth and lignification metabolism of fresh-cut carrots. *Postharvest Biology and Technology*, 173, 111365. <https://doi.org/10.1016/J.POSTHARVBIO.2020.111365>

Li, L., Luo, Z., Huang, X., Zhang, L., Zhao, P., Ma, H., Li, X., Ban, Z., & Liu, X. (2015). Label-free quantitative proteomics to investigate strawberry fruit proteome changes under controlled atmosphere and low temperature storage. *Journal of Proteomics*, 120, 44–57. <https://doi.org/10.1016/J.JPROT.2015.02.016>

Li, X., Li, B., Li, M., Fu, X., Zhao, X., Min, D., Li, F., & Zhang, X. (2022). Ethylene pretreatment induces phenolic biosynthesis of fresh-cut pitaya fruit by regulating ethylene signaling pathway. *Postharvest Biology and Technology*, 192, 112028. <https://doi.org/10.1016/j.postharvbio.2022.112028>

Liu, H., Chen, F., Lai, S., Tao, J., Yang, H., & Jiao, Z. (2017). Effects of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*). *Food Chemistry*, 225, 87–97. <https://doi.org/10.1016/J.FOODCHEM.2017.01.008>

Luna-Guzmán, I., & Barrett, D. M. (2000). Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19(1), 61–72. [https://doi.org/10.1016/S0925-5214\(00\)00079-X](https://doi.org/10.1016/S0925-5214(00)00079-X)

- Luna-Guzmán, I., Cantwell, M., & Barrett, D. M. (1999). Fresh-cut cantaloupe: effects of CaCl₂ dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology*, 17(3), 201–213. [https://doi.org/10.1016/S0925-5214\(99\)00048-4](https://doi.org/10.1016/S0925-5214(99)00048-4)
- Mao, J., Zhang, L., Chen, F., Lai, S., Yang, B., & Yang, H. (2017). Effect of Vacuum Impregnation Combined with Calcium Lactate on the Firmness and Polysaccharide Morphology of Kyoho Grapes (*Vitis vinifera* x *V. labrusca*). *Food and Bioprocess Technology*, 10(4), 699–709. <https://doi.org/10.1007/S11947-016-1852-5/FIGURES/4>
- Méndez-Galarraga, M. P., van de Velde, F., Piagentini, A. M., & Pirovani, M. É. (2022). Kinetic modeling of the changes in bioactive compounds and quality attributes of fresh-cut strawberries stored in controlled atmospheres with high oxygen alone or with carbon dioxide. *Postharvest Biology and Technology*, 190, 111947. <https://doi.org/10.1016/J.POSTHARVBIO.2022.111947>
- Menezes, Y. B. S. (2012). Resfriamento hídrico, ar forçado e utilização de revestimento comestível na conservação de mangaba (*Hancornia speciosa* Gomes). Universidade Federal de Sergipe.
- Nasri, E., Khademi, O., Saba, M. K., & Ebrahimi, R. (2023). Extension of button mushroom storability by ultrasound treatment in combination with calcium lactate. *Journal of Food Measurement and Characterization*, 17(1), 54–62. <https://doi.org/10.1007/S11694-022-01560-6/FIGURES/7>
- Ndukwu, M. C., & Chinenye, N. M. (2011). Development of Clay Evaporative Cooler for Fruit and Vegetables Preservation Development of clay evaporative cooler for fruits and vegetables preservation. 13(1). <http://www.cigrjournal.org>
- Ng, A., & Waldron, K. W. (1997). Effect of Cooking and Pre-Cooking on Cell-Wall Chemistry in Relation to Firmness of Carrot Tissues. *Journal of the Science of Food and Agriculture*, 73(4), 503–512. [https://doi.org/10.1002/\(SICI\)1097-0010\(199704\)73:4<503::AID-JSFA762>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1097-0010(199704)73:4<503::AID-JSFA762>3.0.CO;2-Z)
- Ngamchuachit, P., Sivertsen, H. K., Mitcham, E. J., & Barrett, D. M. (2014). Effectiveness of Calcium Chloride and Calcium Lactate on Maintenance of Textural and Sensory Qualities of Fresh-Cut Mangos. *Journal of Food Science*, 79(5). <https://doi.org/10.1111/1750-3841.12446>
- Nunes, M. C. N., Brecht, J. K., Morais, A. M. M. B., & Sargent, S. A. (2006). Physicochemical changes during strawberry development in the field compared with those that occur in harvested fruit during storage. *Journal of the Science of Food and Agriculture*, 86(2), 180–190. <https://doi.org/10.1002/JSFA.2314>
- Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT - Food Science and Technology*, 42(1), 93–100. <https://doi.org/10.1016/J.LWT.2008.05.008>
- PBMH, & PIMo. (2009). Programa Brasileiro para a Modernização da Horticultura & Produção Integrada de Morango. Normas de Classificação de Morango. São Paulo: CEAGESP, 2009. (Documentos, 33). <https://ceagesp.gov.br/wp-content/uploads/2015/07/morango.pdf>

- Perez-Cabrera, L., Chafer, M., Chiralt, A., & Gonzalez-Martinez, C. (2011). Effectiveness of antibrowning agents applied by vacuum impregnation on minimally processed pear. *LWT - Food Science and Technology*, 44(10), 2273–2280.
<https://doi.org/10.1016/J.LWT.2011.04.007>
- Perinban, S., Orsat, V., & Raghavan, V. (2022). Influence of plasma activated water treatment on enzyme activity and quality of fresh-cut apples. *Food Chemistry*, 393, 133421.
<https://doi.org/10.1016/J.FOODCHEM.2022.133421>
- Phanumong, P., Sangsuwan, J., Kim, S. M., & Rattanapanone, N. (2016). The Improvement of Texture and Quality of Minimally Processed Litchi Fruit Using Various Calcium Salts. *Journal of Food Processing and Preservation*, 40(6), 1297–1308.
<https://doi.org/10.1111/JFPP.12715>
- Pinheiro, S. C. F., & Almeida, D. P. F. (2008). Modulation of tomato pericarp firmness through pH and calcium: Implications for the texture of fresh-cut fruit. *Postharvest Biology and Technology*, 47(1), 119–125. <https://doi.org/10.1016/J.POSTHARVBIO.2007.06.002>
- Posé, S., Paniagua, C., Matas, A. J., Gunning, A. P., Morris, V. J., Quesada, M. A., & Mercado, J. A. (2019). A nanostructural view of the cell wall disassembly process during fruit ripening and postharvest storage by atomic force microscopy. *Trends in Food Science & Technology*, 87, 47–58. <https://doi.org/10.1016/J.TIFS.2018.02.011>
- Prajapati, U., Asrey, R., Varghese, E., & Sharma, R. R. (2021). Effects of calcium lactate on postharvest quality of bitter melon fruit during cold storage. *Physiology and Molecular Biology of Plants*, 27(8), 1811–1821. <https://doi.org/10.1007/S12298-021-01045-8/FIGURES/3>
- Quiles, A., Hernando, I., Pérez-Munuera, I., & Lluch, M. Á. (2007). Effect of calcium propionate on the microstructure and pectin methylesterase activity in the parenchyma of fresh-cut Fuji apples. *Journal of the Science of Food and Agriculture*, 87(3), 511–519.
<https://doi.org/10.1002/JSFA.2749>
- Ren, Y., Sun, P., Wang, X., & Zhu, Z. (2020). Degradation of cell wall polysaccharides and change of related enzyme activities with fruit softening in *Annona squamosa* during storage. *Postharvest Biology and Technology*, 166, 111203.
<https://doi.org/10.1016/j.postharvbio.2020.111203>
- Rolle, R. S., & Chism, G. W. (1987). Physiological consequences of minimally processed fruits and vegetables. *Journal of Food Quality*, 10(3), 157–177.
<https://doi.org/10.1111/J.1745-4557.1987.TB00856.X>
- Rybak, K., Wiktor, A., Witrowa-Rajchert, D., Parniak, O., & Nowacka, M. (2020). The Effect of Traditional and Non-Thermal Treatments on the Bioactive Compounds and Sugars Content of Red Bell Pepper. *Molecules* 2020, Vol. 25, Page 4287, 25(18), 4287.
<https://doi.org/10.3390/MOLECULES25184287>
- Sandianysamy, M. A., Koh, S. P., Mohd Adzahan, N., & Yusof, N. L. (2023). Effect of calcium lactate and sucrose on the quality attributes and storage stability of vacuum impregnated jackfruits. *Volume 6, Pages 90 - 101*, 6, 90–101.
[https://doi.org/10.26656/fr.2017.6\(S4\).013](https://doi.org/10.26656/fr.2017.6(S4).013)

- Sirijariyawat, A., Charoenrein, S., & Barrett, D. M. (2012). Texture improvement of fresh and frozen mangoes with pectin methylesterase and calcium infusion. *Journal of the Science of Food and Agriculture*, 92(13), 2581–2586. <https://doi.org/10.1002/jsfa.5791>
- Sun, X., Wang, P., Shen, X., Chen, F., & Zhang, L. (2023). Changes of Ca forms and chelate-soluble pectin in cherry tomatoes treated with ultrasound and calcium lactate. *LWT*, 173, 114393. <https://doi.org/10.1016/J.LWT.2022.114393>
- Tapia, M. S., López-Malo, A., Consuegra, R., Corte, P., & Welte-Chanes, J. (1999). Minimally processed papaya by vacuum osmotic dehydration (VOD) techniques / Papaya mínimamente procesada mediante técnicas de deshidratación osmótica al vacío (VOD). [Http://Dx-Doi.Ez20.Periodicos.Capes.Gov.Br/10.1177/108201329900500104](http://Dx-Doi.Ez20.Periodicos.Capes.Gov.Br/10.1177/108201329900500104), 5(1), 41–49. <https://doi.org/10.1177/108201329900500104>
- Tappi, S., Tylewicz, U., Romani, S., Siroli, L., Patrignani, F., Dalla Rosa, M., & Rocculi, P. (2016). Optimization of Vacuum Impregnation with Calcium Lactate of Minimally Processed Melon and Shelf-Life Study in Real Storage Conditions. *Journal of Food Science*, 81(11), E2734–E2742. <https://doi.org/10.1111/1750-3841.13513>
- Teoh, L. S., Lasekan, O., Adzahan, N. M., & Hashim, N. (2016). The effect of combinations of UV-C exposure with ascorbate and calcium chloride dips on the enzymatic activities and total phenolic content of minimally processed yam slices. *Postharvest Biology and Technology*, 120, 138–144. <https://doi.org/10.1016/J.POSTHARVBIO.2016.06.008>
- Toivonen, P. M. A., & Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*, 48(1), 1–14. <https://doi.org/10.1016/J.POSTHARVBIO.2007.09.004>
- Udomkun, P., Mahayothee, B., Nagle, M., & Müller, J. (2014). Effects of calcium chloride and calcium lactate applications with osmotic pretreatment on physicochemical aspects and consumer acceptances of dried papaya. *International Journal of Food Science & Technology*, 49(4), 1122–1131. <https://doi.org/10.1111/IJFS.12408>
- U.S. Food and Drug Administration. (n.d.). 21 CFR § 184.1207: Calcium lactate. eCFR. Retrieved from <https://www.ecfr.gov/current/title-21/part-184/section-184.1207>
- Van de Velde, F., Fenoglio, C., Piagentini, A. M., & Pirovani, M. E. (2018). Modeling the impact of the type of cutting and storage temperature on the bioactive compound content, phenylpropanoid metabolism enzymes and quality attributes of fresh-cut strawberries. *Food and Bioprocess Technology*, 11(1), 96–109. <https://doi.org/10.1007/S11947-017-1996-Y/TABLES/6>
- Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R., & Civello, P. M. (2005). Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology and Technology*, 38(3), 213–222. <https://doi.org/10.1016/j.postharvbio.2005.06.005>
- Villalta, A. M., & Sargent, S. A. (2004). Response of beit alpha-type cucumbers (*Cucumis Sativus* L., 'Manar') to continuous ethylene exposure. In *Proc. Fla. State Hort. Soc* (Vol. 117).
- Werner, E. T., de Oliveira Junior, L. F. G., de Bona, A. P., Cavati, B., & Gomes, T. D. U. H. (2009). Efeito do cloreto de cálcio na pós-colheita de goiaba Cortibel. *Bragantia*, 68(2), 511–518. <https://doi.org/10.1590/S0006-87052009000200026>

Wood, P. J., & Siddiqui, I. R. (1971). Determination of Methanol and Its Application to Measurement of Pectin Ester Content and Pectin Methyl Esterase Activity'. In ANALYTICAL BIOCHEMISTRY (Vol. 39).

Yan, R., Han, C., Fu, M., Jiao, W., & Wang, W. (2022). Inhibitory effects of calcium and pectin methylesterase on fruit softening of raspberry during cold storage. *Horticulturae*, 8(1).
<https://doi.org/10.3390/HORTICULTURAE8010001>

Yang, H., Wu, Q., Ng, L. Y., & Wang, S. (2017). Effects of Vacuum Impregnation with Calcium Lactate and Pectin Methylesterase on Quality Attributes and Chelate-Soluble Pectin Morphology of Fresh-Cut Papayas. *Food and Bioprocess Technology*, 10(5), 901–913.
<https://doi.org/10.1007/S11947-017-1874-7>

Zagory, D. (1995). Controlled and modified atmospheres for fresh-cut products: film technology and selection. University of California Davis. *Perishables Handling Newsletter*, 81, 20–22.

Zainal, B., Ding, P., Ismail, I. S., & Saari, N. (2019). Physico-chemical and microstructural characteristics during postharvest storage of hydrocooled rockmelon (*Cucumis melo* L. *reticulatus* cv. Glamour). *Postharvest Biology and Technology*, 152(March), 89–99.
<https://doi.org/10.1016/j.postharvbio.2019.03.001>

Zhang, H., Wang, J., & Ye, S. (2008). Predictions of acidity, soluble solids and firmness of pear using electronic nose technique. *Journal of Food Engineering*, 86(3), 370–378.
<https://doi.org/10.1016/J.JFOODENG.2007.08.026>

Zhang, L., Wang, P., Chen, F., Lai, S., Yu, H., & Yang, H. (2019). Effects of calcium and pectin methylesterase on quality attributes and pectin morphology of jujube fruit under vacuum impregnation during storage. *Food Chemistry*, 289, 40–48.
<https://doi.org/10.1016/J.FOODCHEM.2019.03.008>

Table 1. Physico-chemical analyses of fresh-cut strawberry halves stored at 5°C±1°C.

	Treatment	Days		
		0	3	6
pH	C	3.8±0.02 Ab	3.91±0.01 Aa	3.96±0.04 Aa
	WI	3.81±0.04 Ab	3.95±0.01 Aa	3.91±0.02 Aa
	LCa	3.82±0.03 Ab	3.92±0.04 Aa	3.99±0.01 Aa
	PME	3.73±0.03 Ab	3.9±0.01 Aa	3.88±0 Aa
	LCa + PME	3.79±0 Ab	3.93±0.02 Aa	3.97±0.01 Aa
SSC (°Brix)	C	4.73±0.22 Ab	6.07±0.05 Aa	5.33±0.05 Aab
	WI	4.93±0.6 Aa	5.07±0.03 Aa	4.67±0.12 Aa
	LCa	5.2±0.52 Aa	5.3±0.08 Aa	5.27±0.11 Aa
	PME	4.93±0.6 Aa	5.77±0.27 Aa	5.23±0.11 Aa
	LCa + PME	4.73±0.22 Aa	5.27±0.07 Aa	5.4±0.08 Aa
SSC/TA	C	5.06±0.22 Aa	6.04±0.07 Aa	5.31±0.12 Aa
	WI	5.53±0.57 Aa	5.4±0.21 Aa	4.95±0.1 Aa
	LCa	5.64±0.55 Aa	5.21±0.14 Aa	5.17±0.07 Aa
	PME	5.14±0.72 Aa	5.77±0.4 Aa	5.28±0.19 Aa
	LCa + PME	4.99±0.28 Aa	5.33±0.27 Aa	5.53±0.3 Aa
Anthocyanin (mg/100g fresh fruit)	C	14.61±0.44 Aa	5.09±0.17 BCc	9.76±0.31 Ab
	WI	13.36±0.04 Aa	6.92±0.35 Ac	8.23±0.25 Bb
	LCa	13.96±0.32 Aa	3.8±0.29 Cc	5.71±0.21 Cb
	PME	13.41±0.19 Aa	3.85±0.36 Cc	5.94±0.46 Cb
	LCa + PME	13.66±0.2 Aa	6.42±0.37 ABb	4.83±0.16 Cc

C – No treatment and infusion; Water – Infusion with water; LCa – Infusion with calcium lactate; PME – Infusion with pectinmethylesterase; LCa + PME – Infusion with calcium lactate and pectinmethylesterase. Different uppercase letters indicate a statistical difference between treatments on the same day and different lowercase letters indicate a statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

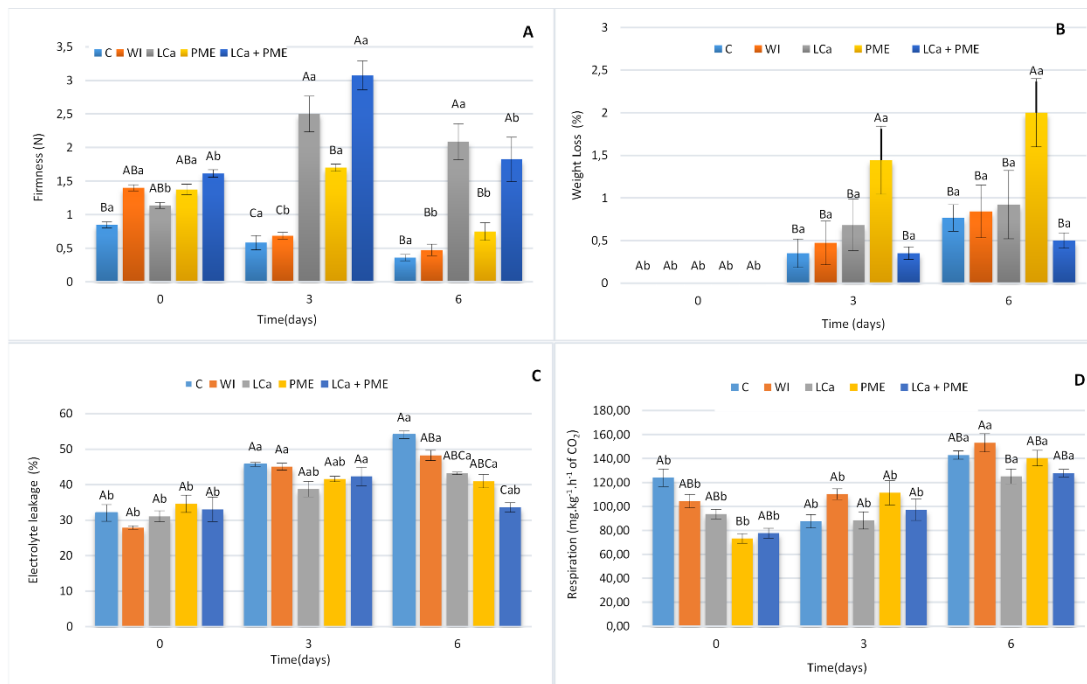


Figure 1 - (A) Firmness (B) Weight loss (C) Electrolyte leakage (D) Respiratory rate of fresh-cut strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. C– No treatment and infusion; Water – Infusion with water; LCa- Infusion with Calcium Lactate; PME - Infusion with pectinmethylesterase; LCa + PME – Infusion with Calcium Lactate and Pectinmethylesterase. Different uppercase letters indicate statistical difference between treatments on the same day and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

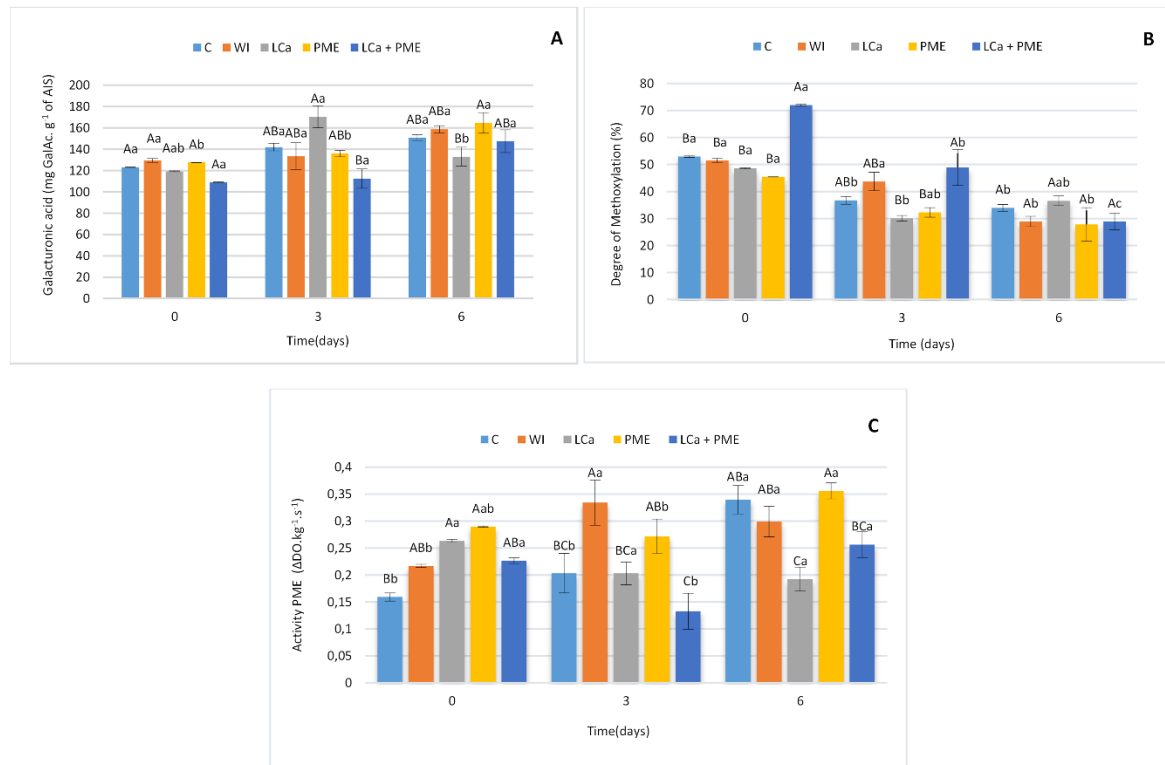


Figure 2 - (A) Galacturonic acid (B) Degree of Methoxylation and (C) PME activity of fresh-cut strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. C– No treatment and infusion; Water – Infusion with water; LCa- Infusion with Calcium Lactate; PME - Infusion with pectinmethylesterase; LCa+PME – Infusion with Calcium Lactate and Pectinmethylesterase. Different uppercase letters indicate statistical difference between treatments on the same day and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).



Figure 3 - (A) Freshness, (B) Damage, (C) Brightness (*L), (D) Hue, (E) Color (a*) and Color (b*) of fresh-cut strawberries stored at 5°C±1°C. C – No treatment and infusion; Water – Infusion with water; LCa- Infusion with Calcium Lactate; PME - Infusion with pectinmethylesterase; LCa + PME – Infusion with Calcium Lactate and Pectinmethylesterase. Different uppercase letters indicate statistical difference between treatments on the same day and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

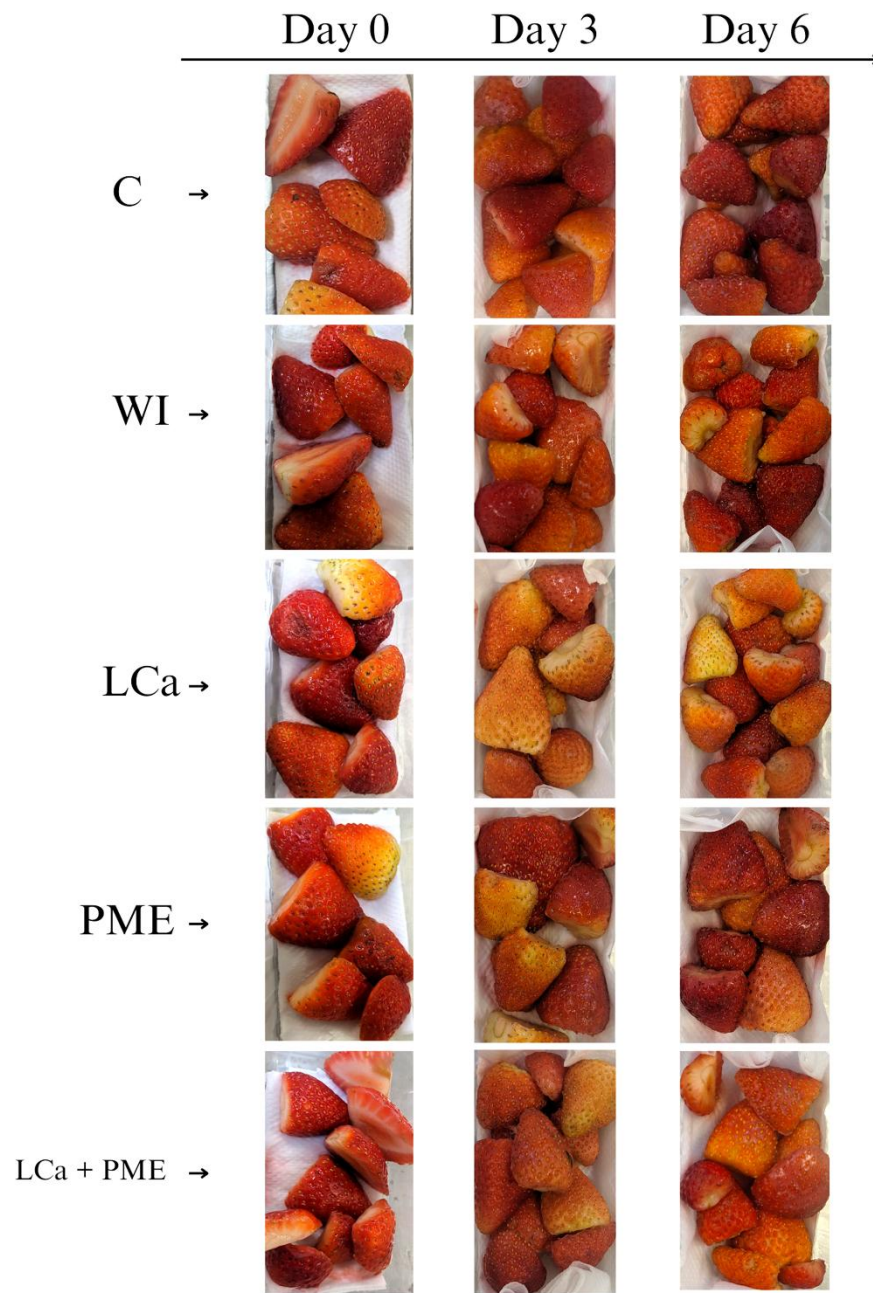


Figure 4 - Visual quality of fresh-cut strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. C – No treatment and infusion; Water – Infusion with water; LCa- Infusion with Calcium Lactate; PME - Infusion with pectinmethylesterase; LCa + PME – Infusion with Calcium Lactate and Pectinmethylesterase.

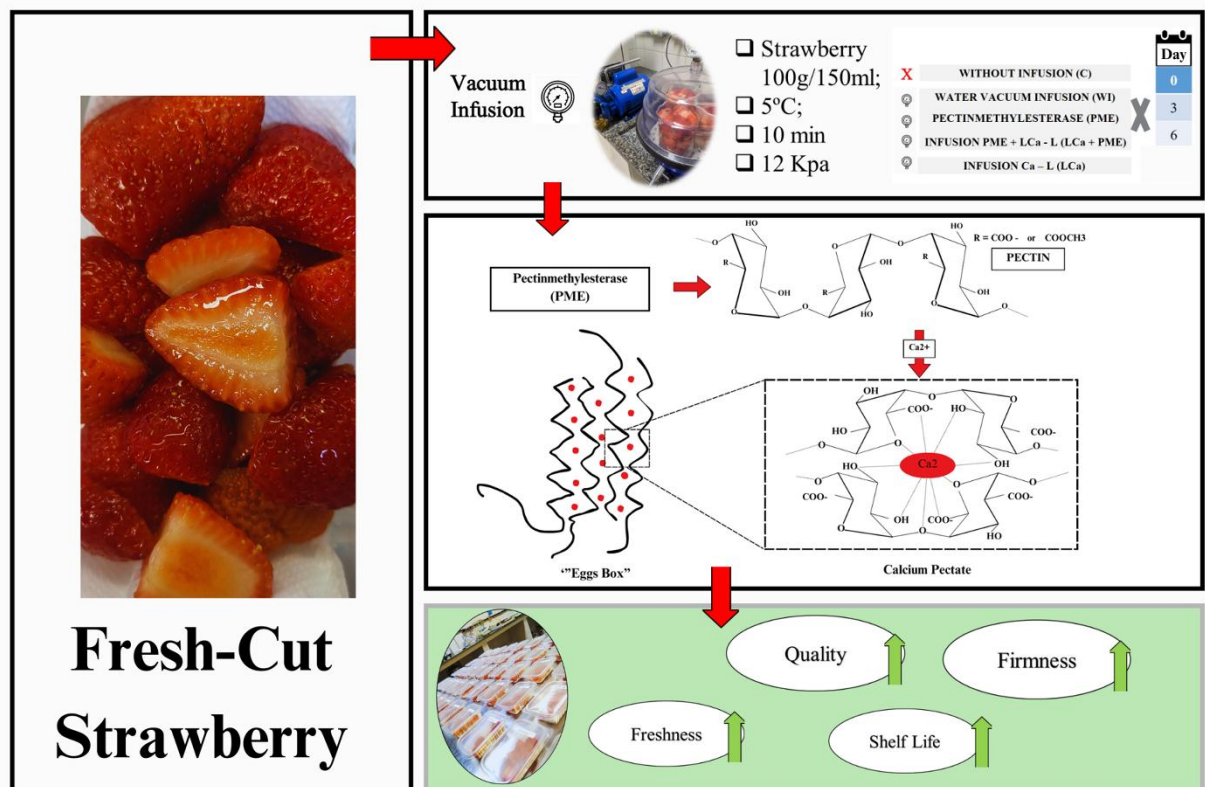


Figure 5 - Shelf life extension scheme for fresh cut strawberries with vacuum application of calcium lactate and PME.

5. MANUSCRIPT 2

Synergistic effects of calcium lactate and pectin methylesterase on cell wall stability and firmness of strawberries during postharvest storage

Artigo formatado de acordo com as normas do periódico *Postharvet Biology and Technology*

ABSTRACT

Strawberry is a highly perishable fruit, with limited post-harvest shelf life due to rapid softening and deterioration. This study aimed to evaluate the effectiveness of vacuum infusion of Calcium Lactate (LCa) and Pectin Methylesterase (PME) in maintaining firmness, cell integrity, and quality of strawberries during 12 days of storage at 5°C. The treatments applied were Control (NI), Water (W), PME, LCa, and LCa + PME. Principal Component Analysis (PCA) established the LCa + PME treatment as the most effective, segregating it into the high-quality cluster, positively correlated with firmness and freshness. LCa + PME increased fruit firmness by 48% after 3 days of storage compared to control fruits (W), maintaining it up to 12 days of storage. This stability was attributed to the synergy of the treatment between LCa and exogenous PME, which increases free carboxylic groups, optimizing the formation of Ca-pectate cross-links, resulting in the stabilization of CSP (Sodium Carbonate Soluble Pectin). Additionally, treatments using calcium reduced electrolyte leakage by 22% and limited weight loss to about 2.7% for LCa + PME, resulting in better freshness and lower incidence of damage and contamination. LCa + PME significantly preserved color parameters (L^* , C^*) and extended shelf life by at least 3 days. It is concluded that vacuum infusion of Calcium Lactate and PME is a promising technology for pectic reinforcement and extension of strawberry post-harvest quality.

Key-words: Enzymatic Activity, Vacuum infusion, *Fragaria x ananassa*, Postharvest.

5.1. Introduction

The strawberry (*Fragaria x ananassa* Duch.), belonging to the Rosaceae family, stands out as one of the most consumed fruits due to its attractive sensory properties and nutritional quality. It is a significant source of fiber, vitamins, minerals, anthocyanins, and phenolic compounds (Bertolo et al., 2025; Dhital et al., 2018). However, the strawberry is highly perishable, with a shelf life of around 7 days under refrigeration, which leads to significant economic losses and limits its commercialization and consumer acceptance (Ikegaya et al., 2020; Yao et al., 2025).

The short durability of strawberry fruits is attributed to rapid pulp softening, accelerated deterioration, vulnerability to infections caused by pathogens, and mechanical damage (Kahramanoğlu, 2019). Among quality indicators, pulp firmness is one of the most relevant parameters, and its loss is directly associated with the strawberry senescence process (Kahramanoğlu, 2019). The softening process, natural during post-harvest ripening, results from structural transformations in the cell wall, particularly in the matrix of pectins, cellulose, and hemicelluloses. Enzymes such as pectin methylesterase (PME) and polygalacturonase (PG) play central roles in pectin depolymerization, causing cell wall weakening and consequent loss of firmness (Langer et al., 2019).

Given these challenges, the development and application of post-harvest technologies that can extend strawberry shelf life while simultaneously preserving its nutritional and sensory qualities become essential. In this context, the application of calcium-based compounds (Ca^{2+}) has stood out as an effective strategy. Calcium acts on the maintenance of cell turgor, plasma

membrane integrity, and cell wall cohesion by forming cross-links with the carboxylic groups of de-esterified pectins. These bonds stabilize the middle lamella, conferring greater structural resistance to the tissue (Jacuinde-Guzmán et al., 2024). Calcium, by interacting with pectates, contributes to reinforcing cell architecture in various fruits and vegetables such as strawberry, mango, loquat, kiwi, and tomato (Huang et al., 2023; Hu et al., 2022a; Langer et al., 2019; Sun et al., 2023; Suriati et al., 2021). In strawberries, calcium-mediated maintenance of firmness has been linked to the modulation of genes involved in cell wall metabolism, resulting in the suppression of softening-related processes and enhanced structural stability of the tissue (Langer et al., 2019).

However, although calcium chloride application is effective, its use may be associated with inducing bitter flavors and unwanted effects (Abd-Elhady, 2014). As an alternative, calcium lactate, an organic salt, offers film-forming properties, good calcium bioavailability, and a more neutral sensory profile, minimizing bitterness (Abd-Elhady, 2014). In addition to preserving color, regulating acidity, and providing antioxidant action, calcium lactate can avoid the risks of chlorinated residue formation, expanding its applicability in fresh fruits (Hu et al., 2022b; Luna-Guzmán & Barrett, 2000). When combined with the application of exogenous PME and vacuum infusion, its firming potential is enhanced, potentially retarding electrolyte efflux, reducing metabolism, and minimizing fruit damage (Soares et al., 2025).

Despite advances, there is still a great demand for strategies that simultaneously preserve texture, sensory properties, and biochemical aspects of strawberry fruits. Given this scenario, the main objective of this study was to evaluate the effects of vacuum infusion of calcium lactate and PME on firmness, physicochemical quality, and enzymatic activity of strawberries subjected to refrigerated storage

5.2. Materials and Methods

5.2.1. Material vegetal

Strawberries were sourced from the State Supply Center of Sergipe (CEASA) at the commercial ripening stage, characterized by more than 75% red surface coloration, according to PBMH and PIMO (2009) standards. The samples were immediately transported to the Fruit and Vegetable Laboratory at the Federal University of Sergipe (UFS) for selection and experimental setup. Upon arrival at the laboratory, strawberries were selected for uniform size, color, and absence of physical injuries or decay (Carnelossi et al., 2018). The fruits were washed under running water to remove surface debris. Sanitization was performed by immersing the samples in a 200 ppm chlorinated solution at 5 ± 0.5 °C for 10 min. Subsequently, a second rinse was conducted in 5 ppm chlorinated water at the same temperature for 3 min to remove excess chlorine before experimental processing.

5.2.2. Vacuum infusion treatments

Whole strawberries (100 g) strawberries were immersed in 125 mL of aqueous solution in 250 mL beakers and subjected to a pressure of 12 kPa for 10 min. The Vacuum Infusion (VI) process was conducted in a desiccator coupled to a manometer and a vacuum pump. Five distinct treatments were defined: C (Control): No Infusion; W (Water): infusion with water only; PME: Infusion with aqueous solution containing commercial Pectinmethylesterase (10 UI); LCa: Aqueous solution containing only calcium lactate; LCa+PME: infusion with aqueous solution containing commercial PME (10 UI) and calcium lactate (1%). The commercial PME enzyme (NovoShape, Novozymes, Denmark), with a declared activity of 10 PEU/mL, was used at the concentration recommended by the manufacturer (1 mL/kg of fruit). The 1% calcium lactate concentration was based on previous studies (Carnelossi et al., 2018; Soares et al., 2025).

After infusion, the fruits were drained and briefly rinsed under running water using sieves. They were then packed in polypropylene containers and stored in a refrigerated vertical

display case with air circulation (Springer) at 5 ± 1 °C and 79% relative humidity. Analyses were performed at 0, 3, 6, 9, and 12 days of storage.

5.2.3. Firmness

Fruit firmness was determined using a digital penetrometer (TR Turoni, model 53205, Forli, Italy) equipped with an 8 mm diameter cylindrical probe. Measurements were taken at two opposite points on the equatorial region of each strawberry. The probe was driven into the fruit tissue at a constant speed until maximum resistance was reached, without prior removal of the skin. Results were expressed as the maximum force in Newtons (N).

5.2.4. Weight loss

Fresh weight loss was calculated by the difference between the initial weight and the weight at each storage time, expressed as the percentage of loss in relation to the initial weight (Zainal et al., 2019).

5.2.5. Electrolyte Leakage

Cell membrane integrity was evaluated through electrolyte leakage, as adapted from (Villalta & Sargent, 2004). The mesocarp (2g in 5 mm x 2 mm slices), previously washed with distilled water, was incubated in 0.4M mannitol isotonic solution for 4h at 23°C. Initial conductivity was measured (Model 3403, Yellow Springs, OH, USA). Total conductivity was obtained after freezing the samples (24h at -20°C) and heating in a boiling water bath for 30 min. Electrolyte leakage was expressed as the percentage of initial conductivity in relation to total conductivity.

5.2.6. Cell Wall Pectin Components

Extraction of pectin components was conducted according to (Zhi et al., 2017) with adaptation. The fruit pulp (5 g) was ground and heated with 12 mL of ethanol (80%, v/v) at 80 °C for 20 min, then cooled to room temperature (RT). The mixture was centrifuged at $8000 \times g$ for 15 min, then the supernatant was decanted. The residue was mixed with 12 mL of chloroform-methanol solution (1:1, v/v), stirred for 10 min, centrifuged, and the supernatant removed. The final residue was then washed twice with 12 mL of 80% ethanol and 12 mL of acetone, respectively, and dried to constant weight at 45 °C to obtain Cell Wall Material (CWM).

CWM (100 mg) was suspended in 8 mL of distilled water and stirred continuously at room temperature for 2 h, then centrifuged at $8000 \times g$ for 15 min. The supernatants were collected as water-soluble pectin (WSP). Subsequently, the residue was resuspended in 8 mL of 50 mmol trans-1,2-cyclohexanediaminetetraacetic acid (CDTA) (pH 6.5 with acetate buffer) and stirred continuously at room temperature for 6 h. The supernatants were collected as CDTA-soluble pectin (ISP). Then, the residue was resuspended in 8 mL of 50 mmol (containing 2 mmol CDTA) at 4 °C for 12 h. The supernatants were collected as CSP. The content of pectin components was determined by carbazole colorimetry using galacturonic acid as a standard. Pectin components were determined as a measure of galacturonic acid (Ahmed & Labavitch, 1978).

5.2.7. Pectinmethylesterase (PME) Activity

PME activity was determined by a colorimetric method as described by (Vicente et al., 2005), with modification in extraction by (Ren et al., 2020). The enzymatic extract was obtained by macerating 1.0 g of the sample in 6 mL of 8.8% NaCl containing 10 g/L of PVPP. After extraction at 4°C for 1 h, the solution was centrifuged at $12,000 \times g$ for 20 min. Activity was measured in a mixture containing 0.6 mL of 0.5% (w/v) pectin, 0.15 mL of 0.05% bromothymol blue, 0.1 mL of distilled water, and 0.1 mL of enzymatic extract. The mixture was incubated at

37°C for 30 min, and absorbance at 620 nm was recorded. Enzymatic activity was defined as the reduction of 0.01 units of OD 620nm per minute per kilogram of flesh.

5.2.8. Visual Quality (Freshness, Visual Deterioration, and Mechanical Damage)

The visual quality of individual strawberries was evaluated using methodologies adapted from (Jacomino et al., 2011). Visible appearance was subjectively rated using the scale: 9 = excellent: totally fresh appearance, high gloss; 7 = good: still looks fresh, still glossy; 5 = fair: not fresh appearance, low gloss, marketability limit; 3 = poor: dull, usability limit; 1 = extremely poor: shriveled appearance. Results were expressed as the average score for each replicate. Visible Deterioration (Contamination) was quantified by enumerating fruits showing post-harvest rot or visible fungal growth. Results were expressed as a percentage relative to the total fruits evaluated. Mechanical damage was quantified by visual inspection, identifying fruits that exhibited physical lesions such as bruising, skin abrasions, or cracks. The incidence of damage was calculated as the ratio of affected fruits to the total number of samples per treatment, with results expressed as a percentage (%).

5.2.9. Color

Surface color was determined by direct measurements taken at the equatorial region of each fruit using a Minolta CR-300 colorimeter (Konica Minolta Sensing) in the CIELab* system, where L* (Lightness): Ranging from 0 (black) to 100 (white); a* (Red-green axis): Where +a* indicates red and -a* indicates green; b* (Yellow-blue axis): Where +b* indicates yellow and -b* indicates blue. The hue angle (h°) was calculated automatically by the instrument software using the formula $h^\circ = \arctan(b^*/a^*)$, describing the color tone (0° = red, 90° = yellow) (Gaya & Serman Ferraz, 2006; HunterLab, 2008; Menezes, 2012).

5.2.10. Physicochemical Analyses

pH was measured by a potentiometer, using a calibrated pH meter, in a solution obtained from macerating 5g of sample in 50 mL of distilled water. Titratable Acidity (TA) was determined by titration of the aliquot of this solution with 0.1 N NaOH, using phenolphthalein as an indicator. Results were expressed as % citric acid (Instituto Adolfo Lutz, 2008).

Total Soluble Solids (SSC) were determined in °Brix using a digital refractometer (Hanna Instruments, model HI 96801), according to the official method (AOAC, 1997). The RATIO (SSC/TA relationship) was calculated (Instituto Adolfo Lutz, 2008).

Total anthocyanin was quantified according to (Nunes et al., 2006). Pigments were extracted from 2 g of tissue homogenized in 18 mL of 0.5% HCl in methanol (v/v), keeping samples at 4 °C in the dark for 1 h. The absorbance of the filtrate was measured at 520 nm. Results were expressed in mg 100 g⁻¹ fresh weight of PGN (Pelargonidin-3-Glucoside).

5.2.11. Statistical Analysis

The experimental design was completely randomized (CRD), in a 5 x 5 factorial scheme, combining 5 treatments (NI, W, PME, LCa, LCa+PME) and 5 evaluation times (0, 3, 6, 9, 12 days), with 4 replicates for each combination. Data were subjected to Analysis of Variance (ANOVA) by the F-test, followed by mean comparison using the Tukey test ($p < 0.05$), using the SISVAR software version 5.8 (Ferreira, 2019). Additionally, Principal Component Analysis (PCA) was conducted for post-harvest quality variables, followed by cluster analysis using relevant PCA dimensions. Variables (firmness, freshness, damage, contamination, and color parameters) were previously standardized. Multivariate analysis was performed using the Python language (v3.9) with the Pandas library.

5.3. Results

5.3.1. Principal Component and Cluster Analysis

The first two principal components (PC1 and PC2) collectively explained a substantial proportion of the total data variance, reaching approximately 70% of the explained variance (PC1: 45%; PC2: 25%) (Figure 1).

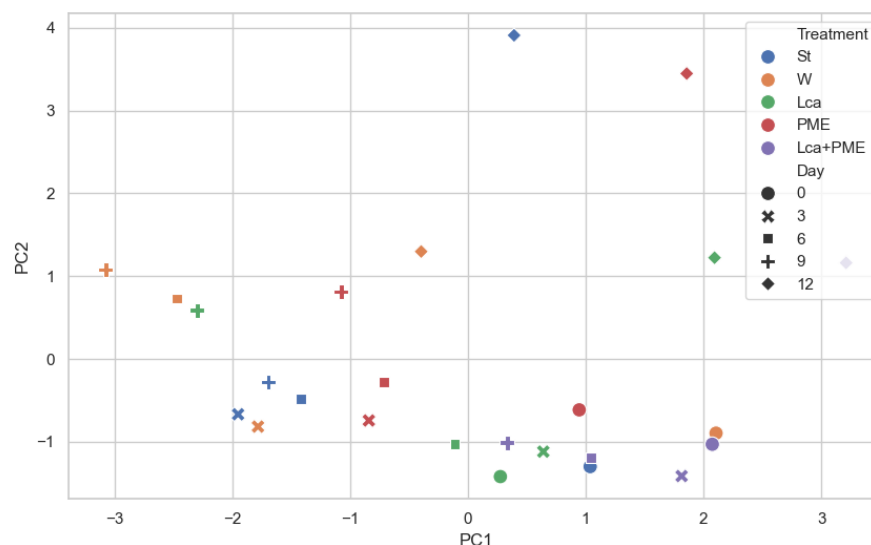


Figure 1 Principal Component Analysis of treated strawberries St (No treatment); W (Infusion with water); LCa (Infusion 1% calcium lactate); PME (Pectinmethylesterase); LCa+PME (Infusion 1% calcium lactate + Pectinmethylesterase).

Principal Component Analysis (PCA) was applied to explore the correlation structure among the multiple post-harvest quality variables and to visualize the impact of different treatments and storage time on the strawberry quality profile. The distribution of scores on the two-dimensional plane demonstrates a clear separation of samples based on storage duration and the applied treatment (Figure 1).

Samples treated with the LCa + PME combination are concentrated at the positive end of PC1, particularly during the initial and intermediate periods (days 0 to 6), displaying a superior quality profile compared to the control group. Conversely, control (W) and single-treatment samples (PME) in the final storage stages (days 9 and 12) cluster at the negative end of PC1, indicating a direct correlation with the loss of global fruit integrity. This separation pattern confirms the protective role of the LCa + PME synergy in preserving strawberry quality.

PC2 predominantly describes the temporal dynamics of the experiment. Most samples at days 9 and 12 (such as W, PME, and LCa) shift toward the positive region of PC2, signaling physiological changes typical of advanced senescence. A notable exception is the LCa+PME treatment, which remains at negative or near-zero PC2 values during the available periods, suggesting metabolic stabilization.

The application of the elbow method (Figure 2B) suggested $k=3$ as the ideal number of clusters, resulting in the classification of treatments into three distinct clusters (Figure 2A). The high-performance cluster concentrated samples from the LCa + PME and LCa treatments on the positive axis of PC1, as well as initial samples from other treatments (Day 0 and 3). This cluster is characterized by a profile of high firmness, excellent freshness, better coloration, and low contamination, representing the state of maximum quality preservation. The intermediate cluster predominantly grouped treatments with moderate efficacy (LCa, PME, W) at intermediate times (Day 6 to 9). This cluster reflects a transition state where firmness and freshness begin to decline sharply. The low-quality and advanced senescence cluster consistently grouped treatments at the final storage times (Day 9 and 12), being associated with a state of greater degradation, darkening, high incidence of deterioration, and the lowest

freshness scores, with a higher predominance of control groups (NI and W) and less effective treatments.

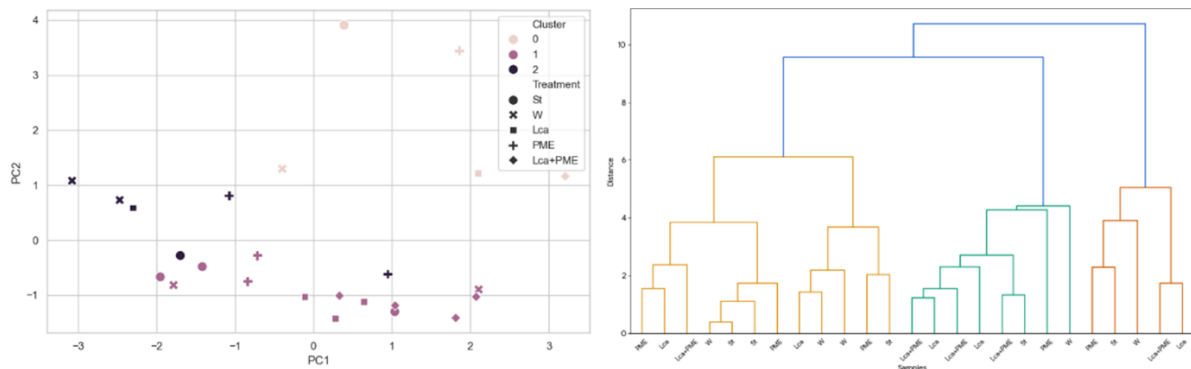


Figure 2 Score Plot PC1 x PC2 with Clusters (A); Hierarchical clustering analysis: Dendrogram (B) representing the responses of treated strawberries St (No treatment); W (Infusion with water); LCa (Infusion 1% calcium lactate); PME (Pectinmethylesterase); LCa+PME (Infusion 1% calcium lactate + Pectinmethylesterase).

The segregation of treatments in the Score Plot (Figure 2A) demonstrates that the LCa + PME treatment maintains the trajectory closest to High Quality for a longer period, while the controls are rapidly pushed into the Advanced Senescence Cluster, corroborating the conclusion of the PCA.

The correlation matrix (Figure 3) validated and deepened the relationships observed in the PCA, highlighting the critical variables of quality loss. A significant negative correlation was observed between firmness and mechanical damage ($r = -0.48$), and a strong negative correlation between freshness and contamination ($r = -0.80$). These results confirm that the loss of physical integrity (Texture and damage) is directly associated with the increase in biological deterioration and the loss of visual acceptability. The relationships between color and firmness were notable. Firmness presented a positive correlation with the color parameter b^* ($r = 0.46$), suggesting that firmer fruits maintained a more vivid and less dull coloration. Conversely, the L^* parameter (Lightness) presented a positive correlation with firmness ($r = 0.27$) and a negative correlation with damage ($r = -0.53$). This negative correlation is a strong indication of darkening in the most degraded and senescent fruits, probably due to enzyme action and loss of cell integrity.

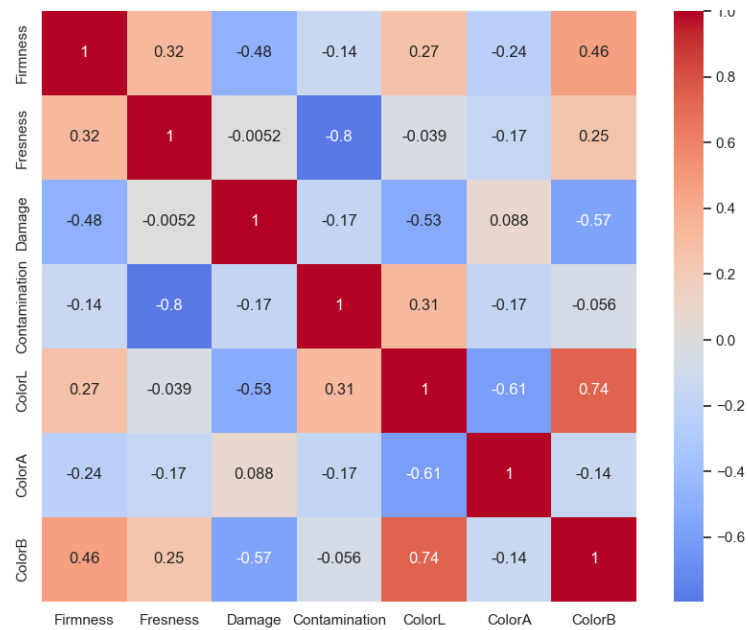


Figure 3 Correlation matrix of treated strawberries St (No treatment); W (Infusion with water); LCa (Infusion 1% calcium lactate); PME (Pectinmethylesterase); LCa+PME (Infusion 1% calcium lactate + Pectinmethylesterase).

The radar chart analysis (Figure 4) shows that the LCa + PME treatment presented the highest values for most quality parameters, particularly firmness, freshness, and color retention, while exhibiting the lowest indices of damage and contamination. The treatments ranked, in descending order of performance, as LCa + PME, LCa, W, NI, and PME.

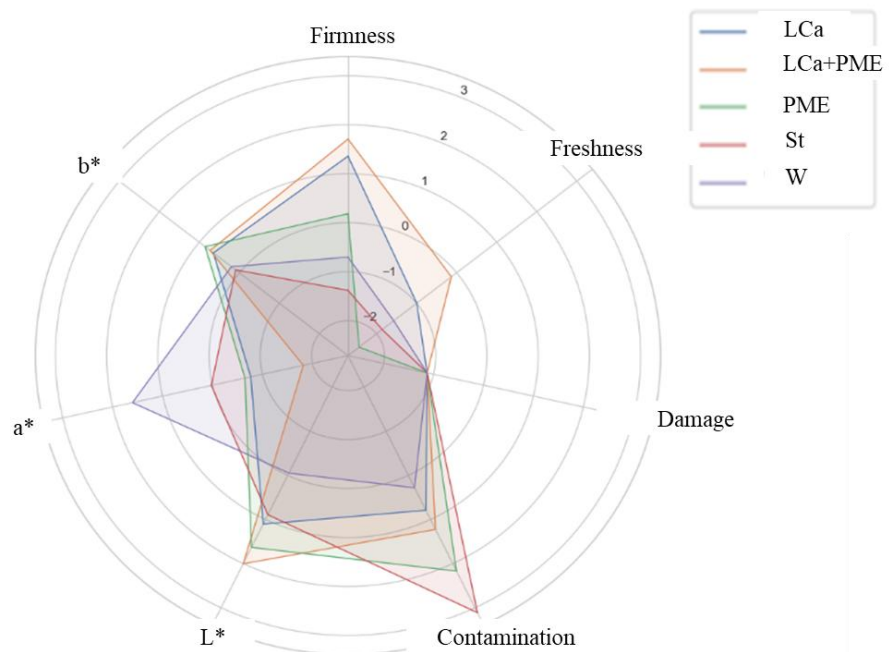


Figure 4 Radar chart comparing treated strawberries St (No treatment); W (Infusion with water); LCa (Infusion 1% calcium lactate); PME (Pectinmethylesterase); LCa+PME (Infusion 1% calcium lactate + Pectinmethylesterase).

Multivariate analysis established the vacuum infusion of Calcium Lactate and Pectin Methylesterase as the most effective treatment for preserving quality and extending the shelf

life of strawberries. The high correlation of LCa + PME samples with metrics such as firmness and freshness demonstrates that this combination is directly linked to quality retention and the delay of senescence in strawberry fruits. Furthermore, the strong negative correlation between firmness and damage, and between freshness and contamination, validates the premise that the loss of textural integrity is the main driver of rejection and deterioration in red fruits. The LCa + PME treatment resulted in a shelf-life extension of at least 3 days, maintaining commercial quality (score > 7, the acceptable limit for marketing) and presenting a good appearance up to Day 12.

The application of LCa+PME, as well as LCa alone, promoted greater firmness, stable coloration, and lower deterioration, probably due to the formation of calcium-pectin bridges, which reinforce cell wall integrity (Jacuinde-Guzmán et al., 2024). Thus, this study conducted experiments to evaluate the effects of the application of LCa and LCa+PME and comparing it to the control (W).

5.3.2. Firmness

The synergistic application of LCa + PME effectively increased fruit firmness compared to the water-treated control (W) throughout the storage period (Figure 5). From the 3rd day of storage, fruits treated with LCa + PME maintained an average firmness of 1.68 N, significantly higher than the 0.76 N observed in the control group. Furthermore, the LCa treatment alone also improved firmness retention compared to the W treatment during most of the experiment, except on the 9th day, when no significant difference was observed.

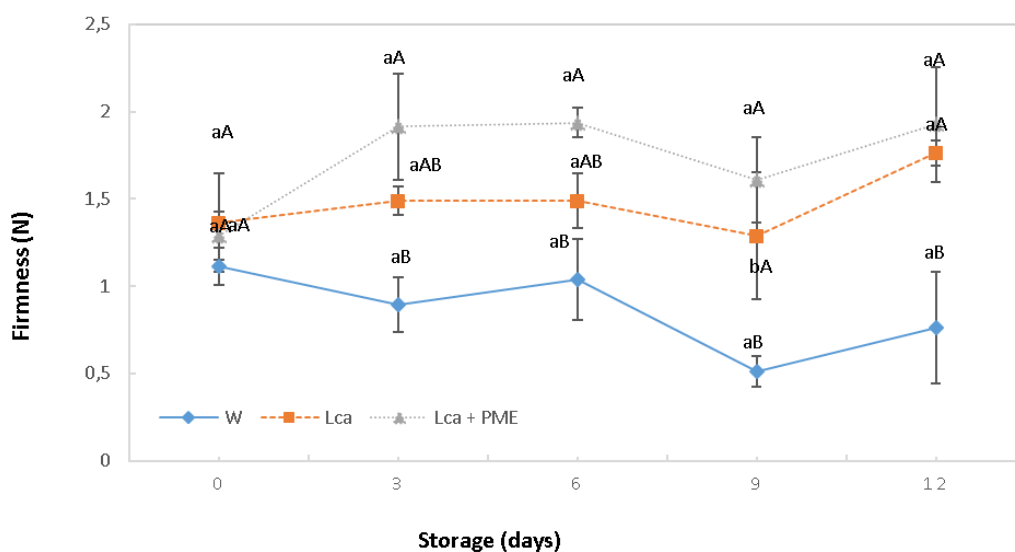


Figure 5 Firmness of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

5.3.3. Weight Loss

Fresh weight loss of fruits increased progressively in all treatments throughout the storage period (Figure 6). The water treatment (W) presented the highest cumulative loss, reaching about 4.4% at the end of the 12th day of refrigeration. Treatments containing calcium (LCa and LCa + PME) were more effective in weight retention. From the 3rd day onwards, both treatments significantly reduced weight loss compared to W, showing no statistical difference between each other until the 6th day. At the end of storage, the treatment using only Calcium Lactate (LCa) demonstrated the highest efficiency, with a weight loss of 2.01%. The combined treatment LCa + PME followed, recording a loss of 2.7%.

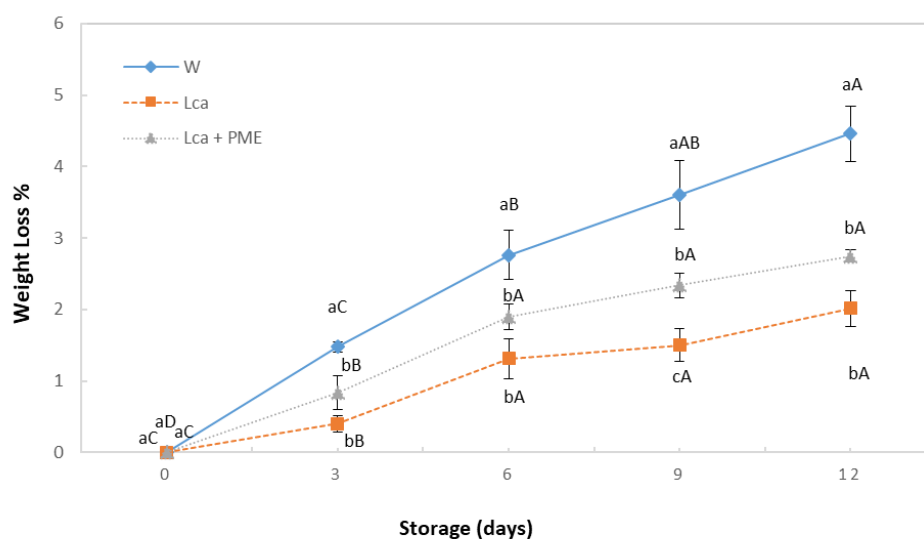


Figure 6 Weight loss of strawberries stored at $5^{\circ}\text{C}\pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

5.3.4. Electrolyte leak

Cell membrane integrity, evaluated by electrolyte leakage, was significantly influenced by treatments containing calcium (Figure 7). It was verified that, throughout the storage period, fruits treated with LCa and LCa + PME showed lower electrolyte leakage, keeping it below 40%. These treatments presented values statistically similar to each other ($p > 0.05$). Comparatively, calcium treatments (LCa and LCa+PME) resulted in an average reduction of 22% in electrolyte leakage in relation to fruits treated with water (W), indicating superior protection of plasma membrane integrity.

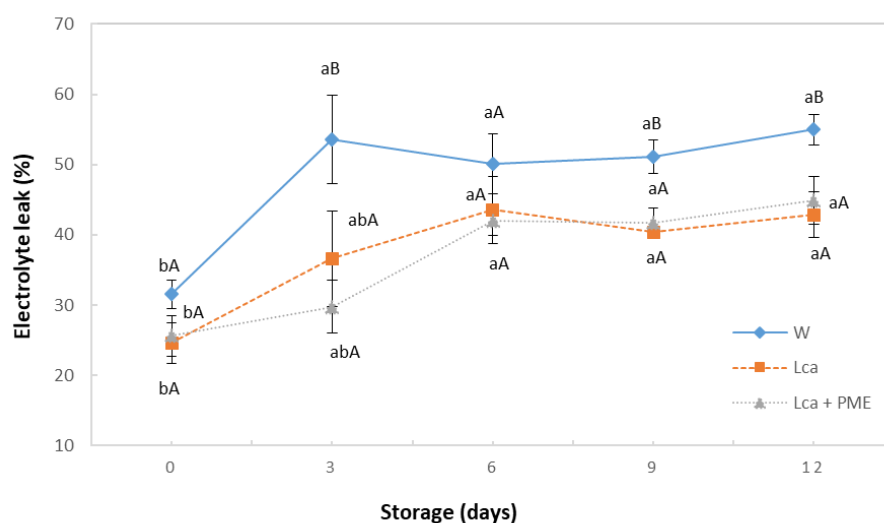


Figure 7 Electrolyte leakage of strawberries stored at $5^{\circ}\text{C}\pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

5.3.5. Pectin components WSP (Water Soluble Pectin), ISP (CDTA Soluble Pectin), and CSP (Sodium Carbonate Soluble Pectin).

In control fruits (W), the initial WSP content was high (Figure 8A) but decreased significantly within the first days of storage. The LCa treatment maintained WSP content at higher and more stable levels during storage, showing little fluctuation. The LCa + PME treatment, although starting at levels similar to the control, demonstrated the ability to maintain higher WSP levels than the control throughout the period, approaching the LCa profile at the end of storage.

The ISP content remained relatively stable for all treatments during storage (Figure 8B). The LCa treatment presented a slightly higher and more consistent profile until the 6th day.

A marked decrease was observed in CSP content (Figure 8C), which is the most tightly bound fraction, for the control (W) up to the 9th day, with the lowest levels recorded on the 12th day. The LCa and LCa + PME treatments showed the same declining trend, however, in a less pronounced manner. For these calcium treatments, no significant differences were observed between the levels on the initial day and the final day of storage. On the 9th and 12th days, the calcium treatments were significantly higher than the control.

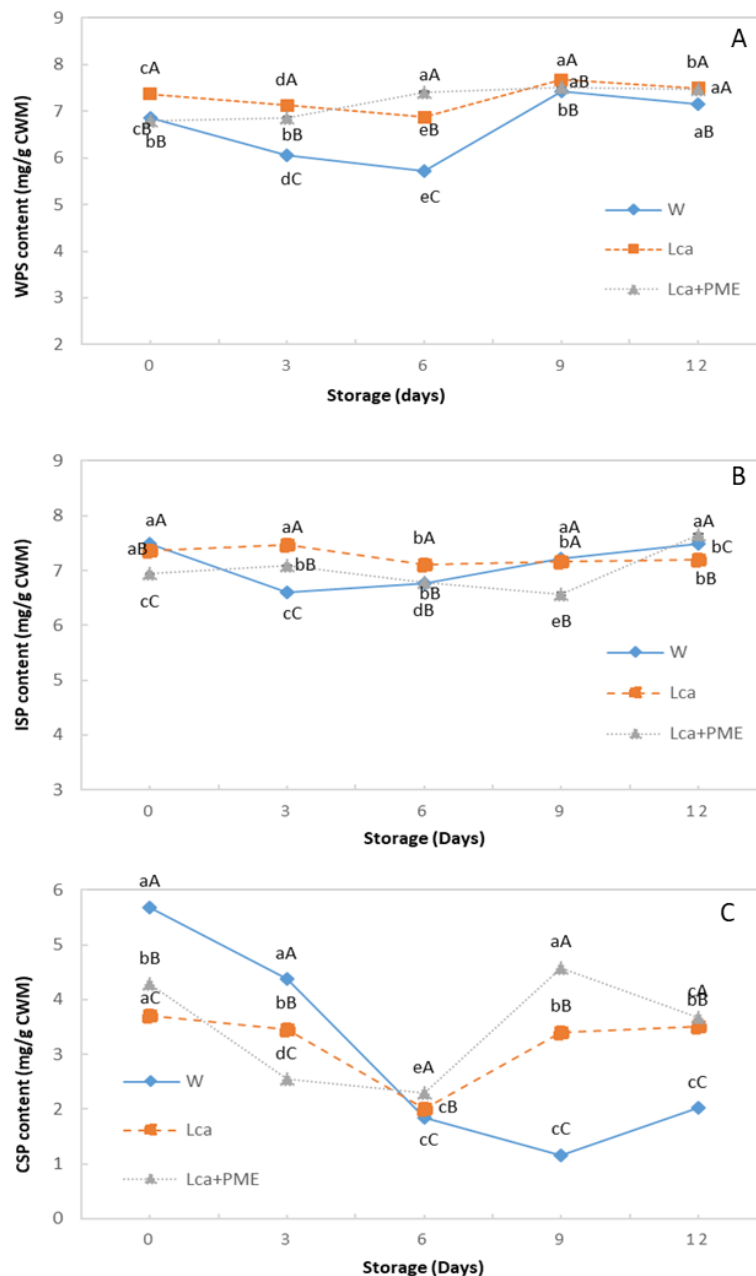


Figure 8 WSP (A); ISP (B) and CSP (C) of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference between the same treatment across days using the Tukey test ($p < 0.05$).

5.3.6. PME Activity

Pectin methylesterase (PME) activity was influenced by the treatments, especially during longer storage periods. The water treatment (W, Control) showed a constant and progressive increase in PME activity throughout the entire storage period (Figure 9). The treatments containing calcium (LCa and LCa + PME) demonstrated a similar activity profile to each other, with a slight increase over time, except on the 3rd day. However, from the 6th day onwards, the PME activity values observed in fruits treated with LCa and LCa + PME were consistently lower than the values of the control treatment (W).

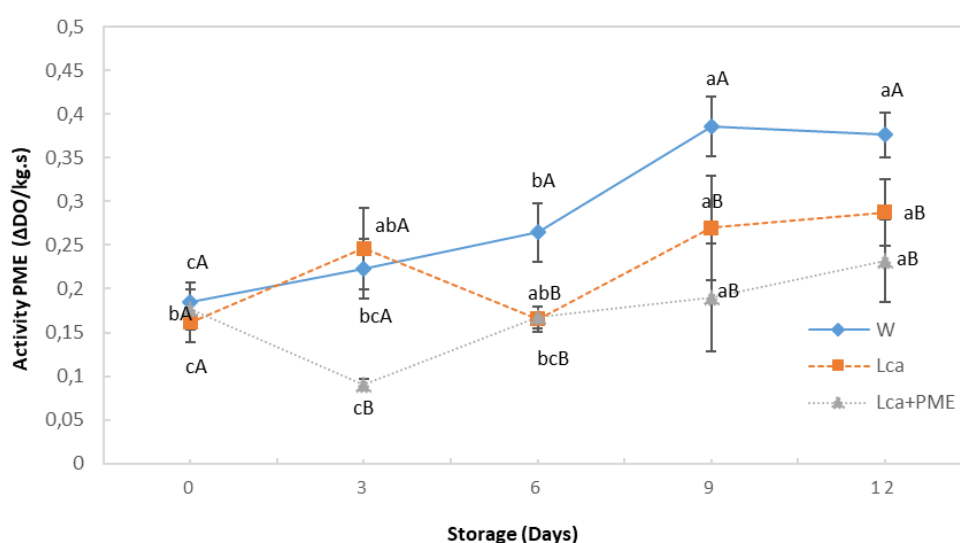


Figure 9 PME activity of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—water infusion; LCa—calcium lactate infusion; LCa + PME—calcium lactate and pectin methylesterase infusion. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference within the same treatment on different days, using Tukey's test ($p < 0.05$).

5.3.7. Visual quality (Freshness, color, and damage)

A sharp drop in freshness was observed in control fruits (W), which reached the lowest level of freshness on the 12th day (average score of 4.2) (Figure 10 and Figure 11A). The LCa + PME treatment proved to be superior, maintaining a significantly higher freshness appearance ($p < 0.05$) than control fruits from the 3rd day onwards and surpassing the LCa treatment alone from the 9th day onwards, maintaining the best visual aspect until the 12th day.



Figure 10 Storage of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—water infusion; LCa—calcium lactate infusion; LCa + PME—calcium lactate and pectin methylesterase infusion. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference within the same treatment on different days, using Tukey's test ($p < 0.05$).

Upon evaluating mechanical damage and deterioration during the storage period, calcium treatments (LCa and LCa + PME) were highly effective in reducing the incidence of damage (Figure 11B). The LCa + PME treatment exhibited the greatest protection against deterioration, with a slight increasing trend in the percentage of damage that was quickly contained. The percentage of damage remained at very low levels (below 10% on the 9th day and 20% on the 12th day) for this treatment, being significantly lower than the other treatments on the 9th and 12th day.

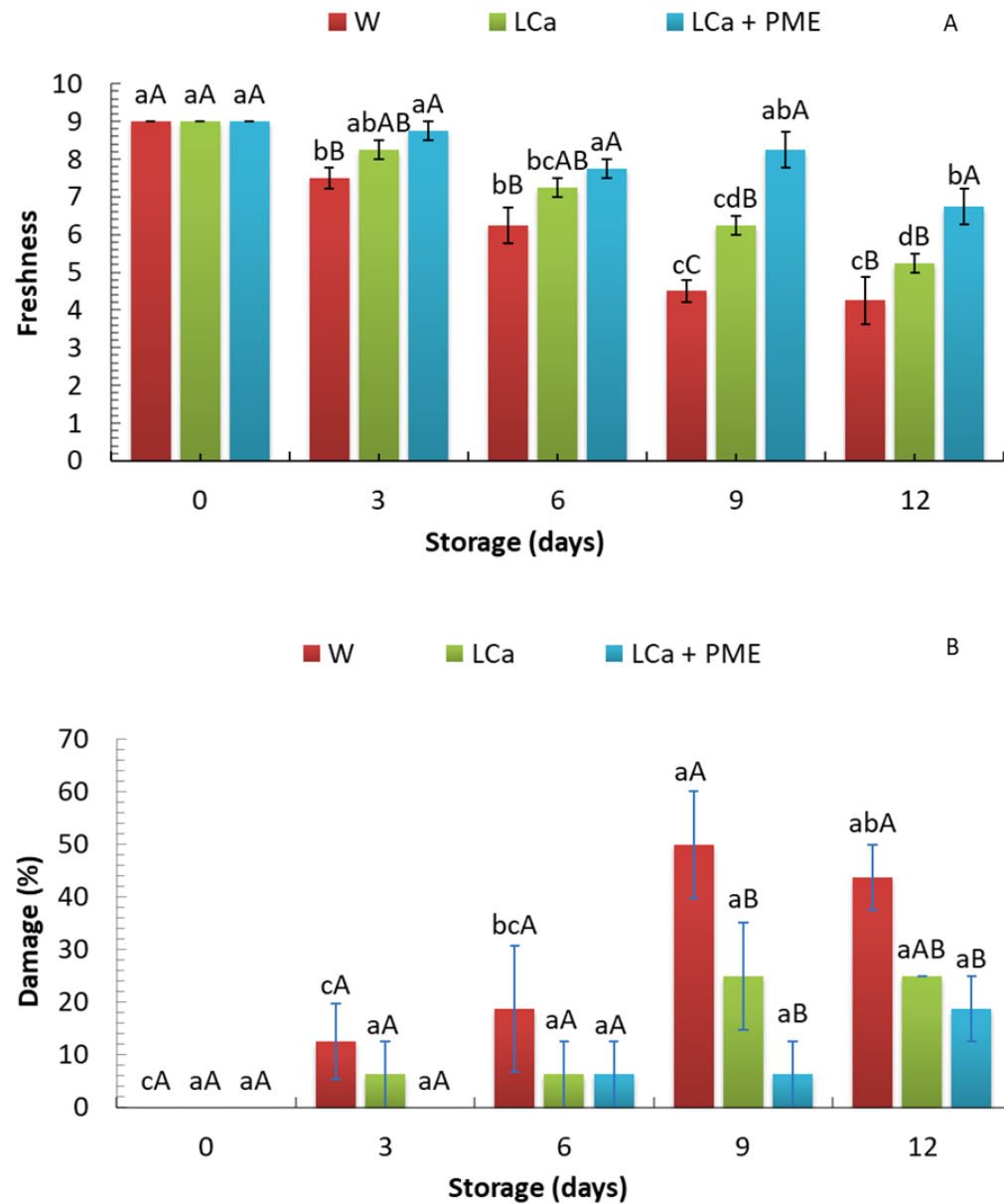


Figure 11 Freshness (A) and Damage (B) of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference between the same treatment across days, using the Tukey test ($p < 0.05$).

Fruit Lightness (L^*) generally decreased across all treatments during the first days of storage, reaching its lowest point on the 9th day (Figure 12A). However, fruits treated with LCa and LCa + PME maintained higher values during the storage period, indicating a lower tendency toward darkening compared to the controls. For the axis (red/green), variations were relatively stable among treatments (Figure 12B). On the 12th day, values for the control treatment were higher, suggesting a slightly redder tone for the control sample at the end of storage. There was a slight reduction in values (yellow/blue) over time, but the treatments did not show significant statistical differences among themselves (Figure 12C). Chroma (C^*), which represents color saturation, showed a marked decrease in control treatment, indicating a reduction in color (Figure 12D). Both calcium treatments (LCa and LCa + PME) demonstrated a positive effect on maintaining color saturation (Chroma), especially from the 6th day of storage onwards, compared to the control. Hue angle (h^*), which describes the color tone,

recorded a significant decrease in control fruits starting from the 6th day. In contrast, fruits treated with LCa and LCa + PME showed greater stability in Hue angle, suggesting better color preservation and less darkening or loss of the intense red tone (Figure 12E).

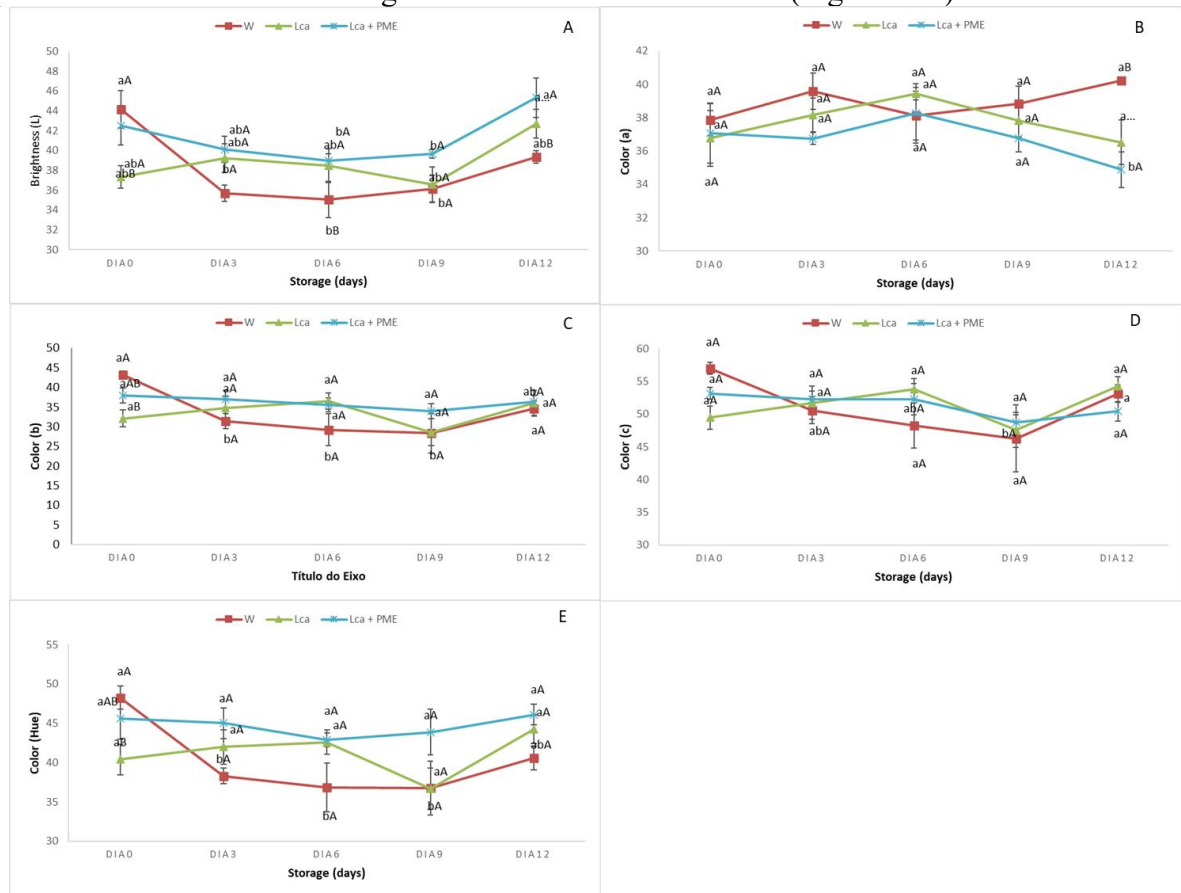


Figure 12 Color parameters L* (A), a* (B), b* (C), c* (D), and H (E) of strawberries stored at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$. W—infusion with water; LCa—infusion with calcium lactate; LCa + PME—infusion with calcium lactate and pectin methylesterase. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference between the same treatment across days, using the Tukey test ($p < 0.05$).

5.3.8. pH, Titratable Total Acidity, TSS, RATIO, and Anthocyanin.

The LCa + PME treatment resulted in fruits with lower pH values throughout the storage period, regardless of the analysis day. A reduction trend in pH values was observed in all treatments from the 3rd day onwards (Table 1), which was maintained until the last day of analysis, although the variations were not considered statistically significant ($p > 0.05$). Regarding Titratable Acidity (TA) content, no significant variations were verified in the fruits throughout the entire storage period.

Table 1. Physicochemical analyses of fresh strawberry halves cut and stored at 5 ± 1 °C.

		Storage				
		0	3	6	9	12
pH	W	3,94±0,006aA	3,9±0,051aB	3,82±0,037aAB	3,6±0,018aC	3,6±0,039aC
	Lca	3,96±0,02aA	3,82±0,038aB	3,9±0,063aAB	3,63±0,048aC	3,66±0,014aC
	Lca+PME	3,84±0,007bA	3,73±0,021bB	3,88±0,012bAB	3,62±0,019bC	3,47±0,023bC
AT (g/100g Citric acid)	W	0,69±0,016aB	0,97±0,05aA	1,07±0,092aA	1,28±0,114aA	0,75±0,039aB
	Lca	0,66±0,016aB	1,07±0,099aA	1,13±0,081aA	1,12±0,134aA	0,78±0,084aB
	Lca+PME	0,77±0,002aB	1,2±0,073aA	1,2±0,07aA	1,34±0,108aA	0,79±0,03aB
TSS (°Brix)	W	5,1±0,23aB	7,4±0,45aA	6,45±0,6aA	6,78±0,21aA	6,35±0,16aA
	Lca	5,13±0,31aB	6,05±0,32aA	7,4±0,36aA	7,3±0,41aA	6,7±0,24aA
	Lca+PME	5,45±0,42aB	7,3±0,53aA	7,38±0,36aA	7,13±0,64aA	6,28±0,09aA
RATIO	W	8,02±0,2aA	5,69±0,29aB	5,26±0,49aB	4,42±0,41aB	7,37±0,41aA
	Lca	8,4±0,2aA	5,26±0,45aB	4,93±0,35aB	5,14±0,63aB	7,25±0,68aA
	Lca+PME	7,17±0,02bA	4,63±0,26bB	4,62±0,25bB	4,19±0,29bB	6,97±0,28bA
Anthocyanin (mg/100g fresh fruit)	W	7,86±0,37aA	6,8±0,72aAB	8,09±0,89aAB	5,71±0,69aB	8,07±0,44aA
	Lca	8,94±0,22aA	7,9±0,91aAB	6,54±0,77aAB	5,17±0,32aB	7,99±0,7aA
	Lca+PME	9,29±0,28aA	7,09±0,47aAB	7,23±1,95aAB	7,54±0,7aB	8,04±0,4aA

W—Water infusion; LCa—calcium lactate infusion; LCa + PME—calcium lactate and pectin methylesterase infusion. Different uppercase letters indicate statistical difference between treatments on the same day, and different lowercase letters indicate statistical difference within the same treatment on different days, using Tukey's test ($p < 0.05$).

An increase in the Total Soluble Solids (SSC) content of the fruits was verified for all treatments from the 3rd day onwards, remaining constant until the last day of storage. No significant statistical difference was observed between treatments or over time ($p > 0.05$). The treatments did not affect the Ratio levels (SSC/TA) of the fruits, maintaining similar values at the beginning and end of the period ($p > 0.05$). Upon evaluating Anthocyanin content, a significant variation was found only on Day 9 when compared to the initial content on day 0 of storage, with no difference between treatments ($p > 0.05$).

5.4. Discussion

Multivariate approaches are essential for elucidating the complex relationships between post-harvest treatments and fruit quality attributes, particularly those associated with delayed senescence and commercial acceptability. In strawberries, quality preservation is intrinsically linked to the maintenance of firmness and freshness, critical determinants for consumer acceptance and shelf-life extension. The commonly reported inverse relationships between firmness and physical damage, as well as between freshness and microbial contamination, corroborate the premise that the loss of textural integrity is the primary driver of deterioration in soft berries, such as strawberries.

The maintenance of firmness was the most relevant indicator among treatments, attributed to the infusion of exogenous Pectin Methylesterase (PME). PME catalyzes the de-esterification of cell wall homogalacturonan (Langer et al., 2019). This increase in free carboxylic groups acts as an ideal substrate for cross-linking with exogenous Ca^{2+} , creating a denser and more rigid Ca-pectate network, the "egg-box model", that is more resistant to degradation (Wang et al., 2018; Kirtil et al., 2014). This structural reinforcement stabilizes the middle lamella and increases the mechanical resistance of the tissue (Quiles et al., 2007).

Investigations employing advanced approaches, such as transmission electron microscopy (TEM) and nuclear magnetic resonance (NMR), have further elucidated these mechanisms (Lara et al., 2004; Zhang et al., 2018). Furthermore, the application of Calcium Lactate (LCa) inhibited enzymatic activity that causes fruit softening, such as polygalacturonase (PG) and beta-galactosidase (Zhang et al., 2018; Langer et al., 2019). By limiting these hydrolases, the dissolution of the middle lamella is prevented, which is confirmed by the stabilization of CSP (sodium carbonate soluble pectin) content (Zhang et al., 2018; Kurz et al., 2008). The decrease in CSP is directly related to fruit softening (Chen et al., 2011). Similar synergistic successes have been reported in loquat (Huang et al., 2023), cherry tomato (Sun et al., 2023), raspberry (Yan et al., 2021), papaya (Yang et al., 2017), and melon (Tappi et al., 2016).

The maintenance of plasma membrane integrity represents another critical mechanism underlying quality preservation. Calcium ions stabilize membrane phospholipids, reducing permeability and limiting solute leakage, which delays cellular disorganization and senescence (Wang et al., 2023). This stability resulted in the retention of mass and freshness; reduced permeability limited water transport, resulting in significantly lower weight loss during storage (Chen et al., 2011).

Due to the absence of a protective waxy cuticle and a highly permeable epidermis composed of a thin layer of pectic polysaccharides, strawberries are extremely susceptible to rapid post-harvest dehydration and shriveling (Hernández-Muñoz et al., 2008). The rate of this loss is governed by the water vapor pressure deficit. To mitigate this, storage under low vapor pressure differences and maintenance of low temperatures is essential, as dehydration accentuates surface injuries (Hernández-Muñoz et al., 2008). Synergistic application of Ca + PME through vacuum infiltration effectively reinforces fruit structure and reduces mass loss by stabilizing the matrix (Zhang et al., 2019).

Stabilizing cellular structures preserves biochemical attributes related to flavor and quality. The maintenance of pH and titratable acidity observed in calcium-treated fruits is consistent with a reduced metabolic rate and delayed degradation of organic acids, processes closely linked to membrane integrity and respiration control (Mahmud et al., 2008; Soares et al., 2025). Calcium application effectively decelerates the senescence-associated metabolism, delaying changes in soluble solids and acidity (García et al., 1996; Lara et al., 2004a).

This metabolic deceleration is further supported by the dynamics of endogenous PME activity, where the LCa + PME treatment demonstrated lower activity during early storage stages, preventing premature tissue dissolution. The physical strengthening of the cell wall limits the accessibility of pathogen hydrolases to fruit tissues (Lara et al., 2004b). Calcium also induces defense enzymes such as PPO and POD (Langer et al., 2019). By preserving the middle lamella and inhibiting pectin degradation, the treatment created a biochemical barrier that explains the lower susceptibility to fungal deterioration and the preservation of anthocyanin levels during critical storage intervals.

Color is the first factor consumers consider (Wigati et al., 2023). Color change is a natural consequence occurring in the strawberry epidermis during ripening (Wigati et al., 2023) and is associated with cell wall integrity. By preventing decompartmentalization, calcium restricts the contact of oxidative enzymes with their phenolic substrates, mitigating browning (López-Serrano & Ros Barceló, 2001). Despite natural anthocyanin fluctuations, the maintenance of vibrant color and firm texture ensures prolonged sensory acceptability (Lara et al., 2004; Öcalan et al., 2022).

In conclusion, vacuum infusion of Calcium Lactate and PME is an effective post-harvest technology acting on structural pectic reinforcement and membrane stabilization, ensuring quality preservation and extending the shelf life of strawberries.

5.5. Conclusions

The LCa + PME treatment promoted a shelf-life extension of at least 3 days for the strawberries, maintaining commercial quality (visual score and freshness > 7) until the 12th day of storage. Exogenous PME, in synergy with Ca²⁺, resulted in a firmness increase of up to 48%, alongside a 22% reduction in electrolyte leakage and a consequent reduction in mass loss, preserving attributes such as pH and TA, which are crucial for flavor maintenance. Furthermore, it strengthened the physical barrier of the cell wall, conferring lower susceptibility to visible deterioration and color degradation. The vacuum infusion technique with LCa + PME represents, therefore, an alternative capable of maintaining quality and reducing post-harvest losses in strawberries.

5.6. References

- Abd-Elhady, M. (2014). Effect of citric acid, calcium lactate and low temperature prefreezing treatment on the quality of frozen strawberry. *Annals of Agricultural Sciences*, 59(1), 69–75. <https://doi.org/10.1016/J.AOAS.2014.06.010>
- Ahmed, A. E. R., & Labavitch, J. M. (1978). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry*, 1(4), 361–365. <https://doi.org/10.1111/j.1745-4514.1978.tb00193.x>
- AOAC. (1997). Official methods of analysis of the Association of Official Analytical Chemists (method 945.46). In *Official methods of analysis of the Association of Official Analytical Chemists*.
- Bertolo, M. R. V., de Oliveira Filho, J. G., Lamonica, G. C., de Oliveira Nobre Bezerra, C. C., da Conceição Amaro Martins, V., Ferreira, M. D., de Guzzi Plepis, A. M., & Bogusz Junior, S. (2025). Improvement of the physical-chemical, microbiological, volatiles and sensory quality of strawberries covered with chitosan/gelatin/pomegranate peel extract-based coatings. *Food Chemistry*, 471, 142755. <https://doi.org/10.1016/J.FOODCHEM.2025.142755>
- Carnelossi, Huber, D., & Carvalho. (2018). Vacuum infusion of pectin methylesterase and calcium maintains firmness of the fresh-cut strawberry.
- Chen, F., Liu, H., Yang, H., Lai, S., Cheng, X., Xin, Y., Yang, B., Hou, H., Yao, Y., Zhang, S., Bu, G., & Deng, Y. (2011). Quality attributes and cell wall properties of strawberries (*Fragaria annanassa* Duch.) under calcium chloride treatment. *Food Chemistry*, 126(2), 450–459. <https://doi.org/10.1016/J.FOODCHEM.2010.11.009>
- Dhital, R., Mora, N. B., Watson, D. G., Kohli, P., & Choudhary, R. (2018). Efficacy of limonene nano coatings on post-harvest shelf life of strawberries. *LWT*, 97, 124–134. <https://doi.org/10.1016/j.lwt.2018.06.038>
- Ferreira, D. F. (2019). SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, 37(4), 529–535. <https://doi.org/10.28951/rbb.v37i4.450>
- García, J. M., Herrera, S., & Morilla, A. (1996). Effects of Postharvest Dips in Calcium Chloride on Strawberry. *Journal of Agricultural and Food Chemistry*, 44(1), 30–33. <https://doi.org/10.1021/JF950334L>

Gaya, L. D. G., & Sterman Ferraz, J. B. (2006). Aspectos genético-quantitativos da qualidade da carne em frangos. *Ciência Rural*, 36(1), 349–356. <https://doi.org/10.1590/s0103-84782006000100058>

Hernández-Muñoz, P., Almenar, E., Valle, V. Del, Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria × ananassa*) quality during refrigerated storage. *Food Chemistry*, 110(2), 428–435. <https://doi.org/10.1016/J.FOODCHEM.2008.02.020>

Huang, W., Shi, Y., Yan, H., Wang, H., Wu, D., Grierson, D., & Chen, K. (2023). The calcium-mediated homogalacturonan pectin complexation in cell walls contributes the firmness increase in loquat fruit during postharvest storage. *Journal of Advanced Research*, 49, 47–62. <https://doi.org/10.1016/j.jare.2022.09.009>

HunterLab. (2008). CIE L*C*h Color Scale. Insight on Color. <https://doi.org/10.1172/JCI45563.Changes>

Hu, X., Chen, Y., Wu, X., Liu, W., Jing, X., Liu, Y., Yan, J., Liu, S., & Qin, W. (2022a). Combination of calcium lactate impregnation with UV-C irradiation maintains quality and improves antioxidant capacity of fresh-cut kiwifruit slices. *Food Chemistry: X*, 14, 100329. <https://doi.org/10.1016/j.fochx.2022.100329>

Hu, X., Chen, Y., Wu, X., Liu, W., Jing, X., Liu, Y., Yan, J., Liu, S., & Qin, W. (2022b). Combination of calcium lactate impregnation with UV-C irradiation maintains quality and improves antioxidant capacity of fresh-cut kiwifruit slices. *Food Chemistry: X*, 14, 100329. <https://doi.org/10.1016/J.FOCHX.2022.100329>

Ikegaya, A., Ohba, S., Nakajima, T., Toyozumi, T., Ito, S., & Arai, E. (2020). Practical long-term storage of strawberries in refrigerated containers at ice temperature. *Food Science and Nutrition*, 8(9), 5138–5148. <https://doi.org/10.1002/FSN3.1817;WEBSITE:WEBSITE:PERICLES;ISSUE:ISSUE:DOI>

Instituto Adolfo Lutz. (2008). Métodos físico-químicos para análise de alimentos. In Instituto Adolfo Lutz. <https://doi.org/10.1017/CBO9781107415324.004>

Jacomino, A. P., Sargent, S. A., Berry, A. D., & Brecht, J. K. (2011). Potential for Grading, Sanitizing, and Hydrocooling Fresh Strawberries. *Proc. Fla. State Hort. Soc. Proc. Fla. State Hort. Soc.*

Jacuinde-Guzmán, J. K., Escalona-Buendía, H. B., Barbosa-Martínez, C., Rivera-Cabrera, F., Raddatz-Mota, D., & Soriano-Melgar, L. de A. A. (2024). The potential of calcium nanoparticles in postharvest conservation of fresh-cut seedless watermelon (*Citrullus lanatus*). *Postharvest Biology and Technology*, 216, 113069. <https://doi.org/10.1016/J.POSTHARVBIO.2024.113069>

Kahramanoğlu, İ. (2019). Effects of lemongrass oil application and modified atmosphere packaging on the postharvest life and quality of strawberry fruits. *Scientia Horticulturae*, 256, 108527. <https://doi.org/10.1016/J.SCIENTA.2019.05.054>

KIRTIL, E.; OZTOP, M. H.; SIRIJARIYAWAT, A.; NGAMCHUACHIT, P.; BARRETT, D. M.; MCCARTHY, M. J. Effect of pectin methyl esterase (PME) and CaCl₂ infusion on the

cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study. *Food Research International*, v. 66, p. 409–416, 1 dez. 2014.

Kurz, C., Carle, R., & Schieber, A. (2008). Characterisation of cell wall polysaccharide profiles of apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* L.), and pumpkins (*Cucurbita* sp.) for the evaluation of fruit product authenticity. *Food Chemistry*, 106(1), 421–430. <https://doi.org/10.1016/J.FOODCHEM.2007.05.078>

Langer, S. E., Marina, M., Burgos, J. L., Martínez, G. A., Civello, P. M., & Villarreal, N. M. (2019). Calcium chloride treatment modifies cell wall metabolism and activates defense responses in strawberry fruit (*Fragaria × ananassa*, Duch). *Journal of the Science of Food and Agriculture*, 99(8), 4003–4010. <https://doi.org/10.1002/JSFA.9626>

Lara, I., García, P., & Vendrell, M. (2004a). Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest Biology and Technology*, 34(3), 331–339. <https://doi.org/10.1016/J.POSTHARVBIO.2004.05.018>

López-Serrano, M., & Ros Barceló, A. (2001). Histochemical Localization and Developmental Expression of Peroxidase and Polyphenol Oxidase in Strawberries. *Journal of the American Society for Horticultural Science*, 126(1), 27–32. <https://doi.org/10.21273/JASHS.126.1.27>

Luna-Guzmán, I., & Barrett, D. M. (2000). Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19(1), 61–72. [https://doi.org/10.1016/S0925-5214\(00\)00079-X](https://doi.org/10.1016/S0925-5214(00)00079-X)

Mahmud, T. M. M., Al Eryani-Raqeeb, A., Syed Omar, S. R., Mohamed Zaki, A. R., & Abdul-Rahman, A. E. (2008). Effects of Different Concentrations and Applications of Calcium on Storage Life and Physicochemical Characteristics of Papaya (*Carica Papaya* L.). *American Journal of Agricultural and Biological Sciences*, 3(3), 526–533. <https://doi.org/10.3844/AJABSSP.2008.526.533>

Menezes, Y. B. S. (2012). Resfriamento hídrico, ar forçado e utilização de revestimento comestível na conservação de mangaba (*Hancornia speciosa* Gomes). Universidade Federal de Sergipe.

Nunes, C. N., Brecht, J. K., Morais, A. M., & Sargent, S. A. (2006). Physicochemical changes during strawberry development in the field compared with those that occur in harvested fruit during storage. *Journal of the Science of Food and Agriculture J Sci Food Agric*, 86, 180–190. <https://doi.org/10.1002/jsfa.2314>

Öcalan, O. N., Çezik, F., Asi, A., Al-Salihi, M., Çiğdem, R., & Yıldız, K. (2022). The Effect of Post-Harvest Calcium Chloride Applications on the Shelf Life Quality of Strawberry. *Turkish Journal of Agriculture - Food Science and Technology*, 10(sp1), 2701–2707. <https://doi.org/10.24925/TURJAF.V10ISP1.2701-2707.5673>

Quiles, A., Hernando, I., Pérez-Munuera, I., & Lluch, M. Á. (2007). Effect of calcium propionate on the microstructure and pectin methylesterase activity in the parenchyma of fresh-cut Fuji apples. *Journal of the Science of Food and Agriculture*, 87(3), 511–519. <https://doi.org/10.1002/JSFA.2749>

- Ren, Y., Sun, P., Wang, X., & Zhu, Z. (2020). Degradation of cell wall polysaccharides and change of related enzyme activities with fruit softening in *Annona squamosa* during storage. *Postharvest Biology and Technology*, 166, 111203. <https://doi.org/10.1016/j.postharvbio.2020.111203>
- Soares, A. C., de Jesus, M. S., Sargent, S. A., Brecht, J. K., de Oliveira Junior, L. F. G., & Carnelossi, M. A. G. (2025). Quality Maintenance and Increased Firmness of Fresh-Cut Strawberries Using Vacuum Infusion of Calcium Lactate and Pectin Methylesterase. *Journal of Food Science*, 90(11), e70658. <https://doi.org/10.1111/1750-3841.70658>
- Sun, X., Wang, P., Shen, X., Chen, F., & Zhang, L. (2023). Changes of Ca forms and chelate-soluble pectin in cherry tomatoes treated with ultrasound and calcium lactate. *LWT*, 173, 114393. <https://doi.org/10.1016/J.LWT.2022.114393>
- Suriati, L., Utama, Im. S., Harsojuwono, B. A., Gunam, I. B. W., & Adnyana, Im. (2021). Differences in Physicochemical Characters of Fresh-Cut Mango, Mangosteen and Rambutan Due to Calcium Chloride Application. *Food Science and Nutrition*, 7(3), 1–6. <https://doi.org/10.24966/FSN-1076/100107>
- Tappi, S., Tylewicz, U., Romani, S., Siroli, L., Patrignani, F., Dalla Rosa, M., & Rocculi, P. (2016). Optimization of Vacuum Impregnation with Calcium Lactate of Minimally Processed Melon and Shelf-Life Study in Real Storage Conditions. *Journal of Food Science*, 81(11), E2734–E2742. <https://doi.org/10.1111/1750-3841.13513>
- Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R., & Civallo, P. M. (2005). Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology and Technology*, 38(3), 213–222. <https://doi.org/10.1016/j.postharvbio.2005.06.005>
- Villalta, A. M., & Sargent, S. A. (2004). Response of beit alpha-type cucumbers (*Cucumis sativus* L., ‘Manar’) to continuous ethylene exposure. In *Proc. Fla. State Hort. Soc* (Vol. 117).
- Wang, D., Yeats, T. H., Uluisik, S., Rose, J. K. C., & Seymour, G. B. (2018). Fruit Softening: Revisiting the Role of Pectin. *Trends in Plant Science*, 23(4), 302–310. <https://doi.org/10.1016/J.TPLANTS.2018.01.006>
- Wang, Y., Li, Y., Yang, S., Li, C., Li, L., Gao, S., & Wu, Z. (2023). Mechanism of ozone treatment in delayed softening of fresh-cut kiwifruit during storage. *Postharvest Biology and Technology*, 204, 112469. <https://doi.org/10.1016/J.POSTHARVBIO.2023.112469>
- Wigati, L. P., Wardana, A. A., Tanaka, F., & Tanaka, F. (2023). Strawberry preservation using combination of yam bean starch, agarwood Aetoxylon bouya essential oil, and calcium propionate edible coating during cold storage evaluated by TOPSIS-Shannon entropy. *Progress in Organic Coatings*, 175, 107347. <https://doi.org/10.1016/J.PORGCOAT.2022.107347>
- Yang, H., Wu, Q., Ng, L. Y., & Wang, S. (2017). Effects of Vacuum Impregnation with Calcium Lactate and Pectin Methylesterase on Quality Attributes and Chelate-Soluble Pectin Morphology of Fresh-Cut Papayas. *Food and Bioprocess Technology*, 10(5), 901–913. <https://doi.org/10.1007/S11947-017-1874-7/FIGURES/6>
- Yao, L., Tao, Q., Xian, F., Chen, Z., Huang, L., Zhong, N., & Gao, J. (2025). Development of pullulan/gellan gum films loaded with astaxanthin nanoemulsion for enhanced strawberry

preservation. Food Research International, 201, 115644.
<https://doi.org/10.1016/J.FOODRES.2024.115644>

YAN, R.; HAN, C.; FU, M.; JIAO, W.; WANG, W. Inhibitory effects of CaCl_2 and pectin methylesterase on fruit softening of raspberry during cold storage. *Horticulturae*, v. 8, n. 1, 1 jan. 2021.

Zainal, B., Ding, P., Ismail, I. S., & Saari, N. (2019). Physico-chemical and microstructural characteristics during postharvest storage of hydrocooled rockmelon (*Cucumis melo* L. reticulatus cv. Glamour). *Postharvest Biology and Technology*, 152(March), 89–99.
<https://doi.org/10.1016/j.postharvbio.2019.03.001>

Zhang, L., Wang, P., Chen, F., Lai, S., Yu, H., & Yang, H. (2019). Effects of calcium and pectin methylesterase on quality attributes and pectin morphology of jujube fruit under vacuum impregnation during storage. *Food Chemistry*, 289, 40–48.
<https://doi.org/10.1016/J.FOODCHEM.2019.03.008>

Zhang, L., Zhao, S., Lai, S., Chen, F., & Yang, H. (2018). Combined effects of ultrasound and calcium on the chelate-soluble pectin and quality of strawberries during storage. *Carbohydrate Polymers*, 200, 427–435. <https://doi.org/10.1016/J.CARBPOL.2018.08.013>

Zhi, H., Liu, Q., Xu, J., Dong, Y., Liu, M., & Zong, W. (2017). Ultrasound enhances calcium absorption of jujube fruit by regulating the cellular calcium distribution and metabolism of cell wall polysaccharides. *Journal of the Science of Food and Agriculture*, 97(15), 5202–5210.
<https://doi.org/10.1002/JSFA.8402>

6. CONSIDERAÇÕES FINAIS

O presente estudo demonstrou, de forma conclusiva, a eficácia da tecnologia de infusão a vácuo (IV) combinada de lactato de cálcio (LCa) e pectinametilesterase (PME) como uma estratégia superior para a preservação pós-colheita de morangos (*Fragaria x ananassa* Duch.).

A aplicação do tratamento LCa + PME resultou em uma proteção estrutural e fisiológica sistêmica do fruto, atingindo o objetivo principal do trabalho. Os dados multivariados (ACP e Análise de Cluster) validaram o tratamento combinado como o mais bem-sucedido na mitigação da senescência, prolongando o tempo de prateleira em, no mínimo, 3 dias, ao manter a qualidade comercial superior (nota > 7) até o Dia 12.

O Ca^{2+} fornecido pelo lactato de cálcio reforçou a estrutura da parede celular e estabilizou a membrana plasmática, conforme evidenciado pela manutenção da firmeza (aumento de 48% no Dia 3) e pela redução de 22% no vazamento de eletrólitos.

A PME exógena otimizou a formação de ligações cruzadas de Ca-pectato, justificando a superioridade do tratamento combinado sobre o LCa isolado, resultando em menor perda de massa e maior estabilidade na cor (L^* , C^* , h).

Em suma, a infusão a vácuo de lactato de cálcio e PME configura-se como uma tecnologia promissora, segura e eficiente para a agroindústria de frutas altamente perecíveis, pois atua na causa primária da deterioração, garantindo a integridade física e a aceitabilidade sensorial do morango no mercado.

Recomenda-se que pesquisas futuras explorem a otimização da concentração de PME e o tempo de IV para maximizar o desempenho, bem como a avaliação do impacto desta tecnologia no perfil de compostos voláteis e aceitabilidade sensorial em testes com consumidores.