

INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA
GOIANO - CAMPUS RIO VERDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS -
AGRONOMIA

PRODUÇÃO E CARACTERIZAÇÃO DE EMBALAGENS
BIODEGRADÁVEIS ATIVAS E INTELIGENTES COM ÓLEO DE
Hymenaea stigonocarpa, *H. courbaril*, *Annona crassiflora* E EXTRATO
DE *Syzygium cumini*

Doutorando: Antonio Carlos Pereira de Menezes Filho
Orientador: Prof. Dr. Frederico Antônio Loureiro Soares

RIO VERDE, GO
MARÇO, 2025

INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA
GOIANO - CAMPUS RIO VERDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS -
AGRONOMIA

PRODUÇÃO E CARACTERIZAÇÃO DE EMBALAGENS
BIODEGRADÁVEIS ATIVAS E INTELIGENTES COM ÓLEO DE
Hymenaea stigonocarpa, *H. courbaril*, *Annona crassiflora* E EXTRATO
DE *Syzygium cumini*

Doutorando: Antonio Carlos Pereira de Menezes Filho
Orientador: Prof. Dr. Frederico Antônio Loureiro Soares

Tese apresentada como parte das exigências para obtenção do título de DOUTOR EM CIÊNCIAS AGRÁRIAS - AGRONOMIA no Programa de Pós-Graduação em Ciências Agrárias - Agronomia do Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde – Área de concentração em Produção Vegetal Sustentável do Cerrado.

RIO VERDE, GO
MARÇO, 2025

**Ficha de identificação da obra elaborada pelo autor, através do
Programa de Geração Automática do Sistema Integrado de Bibliotecas do IF Goiano - SIBi**

M543p Menezes Filho, Antonio Carlos Pereira de
PRODUÇÃO E CARACTERIZAÇÃO DE EMBALAGENS
BIODEGRADÁVEIS ATIVAS E INTELIGENTES COM
ÓLEO DE *Hymenaea stigonocarpa*, *H. courbaril*, *Annona*
crassiflora E EXTRATO DE *Syzygium cumini* / Antonio Carlos
Pereira de Menezes Filho. Rio Verde 2025.

79f. il.

Orientador: Prof. Dr. Frederico Antônio Loureiro Soares.
Coorientador: Prof. Dr. Marconi Batista Teixeira.
Tese (Doutor) - Instituto Federal Goiano, curso de 0232014 -
Doutorado em Ciências Agrárias - Agronomia - Rio Verde
(Campus Rio Verde).
1. Polímeros naturais. 2. Biodegradáveis. 3. Embalagens. I.
Título.

TERMO DE CIÊNCIA E DE AUTORIZAÇÃO PARA DISPONIBILIZAR PRODUÇÕES TÉCNICO-CIENTÍFICAS NO REPOSITÓRIO INSTITUCIONAL DO IF GOIANO

Com base no disposto na Lei Federal nº 9.610, de 19 de fevereiro de 1998, AUTORIZO o Instituto Federal de Educação, Ciência e Tecnologia Goiano a disponibilizar gratuitamente o documento em formato digital no Repositório Institucional do IF Goiano (RIIF Goiano), sem ressarcimento de direitos autorais, conforme permissão assinada abaixo, para fins de leitura, download e impressão, a título de divulgação da produção técnico-científica no IF Goiano.

IDENTIFICAÇÃO DA PRODUÇÃO TÉCNICO-CIENTÍFICA

- ☒ Tese (doutorado)
☐ Dissertação (mestrado)
☐ Monografia (especialização)
☐ TCC (graduação)
☐ Produto técnico e educacional - Tipo:

☐ Artigo científico
☐ Capítulo de livro
☐ Livro
☐ Trabalho apresentado em evento

Nome completo do autor:

Antonio Carlos Pereira de Menezes Filho

Matrícula:

2021102320140019

Título do trabalho:

Produção e caracterização de embalagens biodegradáveis ativas e inteligentes com óleo de *Hymenaea stigonocarpa*, *H. courbaril*, *Annona crassiflora* e extrato de *Syzygium cumini*

RESTRIÇÕES DE ACESSO AO DOCUMENTO

Documento confidencial: ☒ Não ☐ Sim, justifique:

Informe a data que poderá ser disponibilizado no RIIF Goiano: 10 / 04 / 2025

O documento está sujeito a registro de patente? ☐ Sim ☒ NãoO documento pode vir a ser publicado como livro? ☐ Sim ☒ Não

DECLARAÇÃO DE DISTRIBUIÇÃO NÃO-EXCLUSIVA

O(a) referido(a) autor(a) declara:

- Que o documento é seu trabalho original, detém os direitos autorais da produção técnico-científica e não infringe os direitos de qualquer outra pessoa ou entidade;
- Que obteve autorização de quaisquer materiais incluídos no documento do qual não detém os direitos de autoria, para conceder ao Instituto Federal de Educação, Ciência e Tecnologia Goiano os direitos requeridos e que este material cujos direitos autorais são de terceiros, estão claramente identificados e reconhecidos no texto ou conteúdo do documento entregue;
- Que cumpriu quaisquer obrigações exigidas por contrato ou acordo, caso o documento entregue seja baseado em trabalho financiado ou apoiado por outra instituição que não o Instituto Federal de Educação, Ciência e Tecnologia Goiano.

Documento assinado digitalmente
 **ANTONIO CARLOS PEREIRA DE MENEZES FILHO**
 Data: 27/03/2025 13:52:39-0300
 Verifique em: <https://validar.ifgoiano.gov.br>

 Rio Verde, Goiás, Brasil
Local

 10 / 04 / 2025
Data

Assinatura do autor e/ou detentor dos direitos autorais

Ciente e de acordo:

Assinatura do(a) orientador(a)

Documento assinado digitalmente
 **FREDERICO ANTONIO LOUREIRO SOARES**
 Data: 27/03/2025 13:27:43-0300
 Verifique em: <https://validar.ifgoiano.gov.br>



SERVIÇO PÚBLICO FEDERAL
MINISTÉRIO DA EDUCAÇÃO
SECRETARIA DE EDUCAÇÃO PROFISSIONAL E TECNOLÓGICA
INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA GOIANO

Documentos 12/2025 - SREPG/CMPR/CPG-RV/DPGPI-RV/CMPRV/IFGOIANO

PRODUÇÃO E CARACTERIZAÇÃO DE EMBALAGENS BIODEGRADÁVEIS
ATIVAS E INTELIGENTES COM ÓLEO DE *Hymenaea stigonocarpa*, *H. courbaril*,
Annona crassiflora E EXTRATO DE *Syzygium cumini*

Autor: Antônio Carlos Pereira de Menezes Filho

Orientador: Dr. Frederico Antônio Loureiro Soares

TITULAÇÃO: Doutorado em Ciências Agrárias-Agronomia - Área de Concentração em
Produção Vegetal Sustentável no Cerrado

APROVADA em, 14 de março de 2025.

Prof. Dr. Frederico Antônio Loureiro Soares (Presidente)

Prof. Dr. Marconi Batista Teixeira (Avaliador interno)

Prof. Dr. Tiago Carnevalle Romão (Avaliador externo)

Prof. Dr. Matheus Vinícius Abadia Ventura (Avaliador externo)

Prof.^a Dr.^a Raquel Aparecida Loss (Avaliadora externa)

Documento assinado eletronicamente por:

- Frederico Antonio Loureiro Soares, PROFESSOR ENS BASICO TECN TECNOLOGICO, em 14/03/2025 11:32:48.
- MATHEUS VINÍCIUS ABADIA VENTURA CREA-GO 1019498617D, 048.979.021-69 - Usuário Externo, em 14/03/2025 11:40:17.
- Raquel Aparecida Loss, Raquel Aparecida Loss - Professor Avaliador de Banca - Ufmt (33004540000100), em 14/03/2025 11:41:08.
- Tiago Carnevalle Romao, PROFESSOR ENS BASICO TECN TECNOLOGICO, em 14/03/2025 11:51:39.
- Marconi Batista Teixeira, COORDENADOR(A) DE CURSO - FUC0001 - CCMDAGRO-R, em 14/03/2025 15:18:21.

Este documento foi emitido pelo SUAP em 12/03/2025. Para comprovar sua autenticidade, faça a leitura do QRCode ao lado ou acesse <https://suap.ifgoiano.edu.br/autenticar-documento/> e forneça os dados abaixo:

Código Verificador: 685626

Código de Autenticação: d6a859a468



INSTITUTO FEDERAL GOIANO

Campus Rio Verde

Rodovia Sul Goiana, Km 01, Zona Rural, 01, Zona Rural, RIO VERDE / GO, CEP 75901-970

(64) 3624-1000

Aos meus pais Antônio Carlos Pereira de Menezes e Sônia Gláucia Arantes Leão de
Menezes

As minhas irmãs Wíviám Leão de Menezes e Suyanne Leão de Menezes

OFEREÇO

A minha avó Zenith Arantes de Farias[†] e avô Sebastião Arantes de Farias[†]

DEDICO

AGRADECIMENTOS

Agradeço ao dom da vida e ao vasto Universo ao qual faço uma minúscula parte!

Ao Programa de Pós-Graduação em Ciências Agrárias – Agronomia do Instituto Federal Goiano – Campus Rio Verde, por abrir às portas para realização do meu doutoramento.

Aos laboratórios de Hidráulica e Irrigação e Química Tecnológica do Instituto Federal Goiano – Campus Rio Verde; de Química Orgânica da Universidade Federal de Jataí por fornecer a infraestrutura e materiais para o desenvolvimento das atividades.

Ao meu grande e ilustríssimo orientador Dr. Frederico Antônio Loureiro Soares, pelas orientações sempre pertinentes e por ter acreditado em minha capacidade.

Ao meu “segundo Pai” e coorientador Prof. Dr. Marconi Batista Teixeira, pela grande paciência e palavras gentis quando eu mais precisei.

Aos colegas de pós-graduação e amigos que de forma direta ou indireta contribuíram para o desenvolvimento dessa dissertação.

Agradeço, por fim, aos integrantes da banca de qualificação e examinadora da tese pelas contribuições pertinentes.

BIOGRAFIA DO AUTOR

Antonio Carlos Pereira de Menezes Filho, nascido em Rio Verde, GO em 09 de junho de 1986. Concluiu os anos iniciais e finais do ensino fundamental no Colégio Estadual Eugênio Jardim e ensino médio no Colégio Estadual Martins Borges, ambos no município de Rio Verde, GO. Concluiu a Licenciatura e Bacharelado em Biologia em dezembro de 2010, na Universidade de Rio Verde, em Rio Verde, GO. Conclui o Mestrado em Agroquímica pelo Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde em fevereiro de 2019. Em março de 2021 após Pandemia do Covid-19, ingressou no nível de doutorado no Programa de Pós-Graduação em Ciências Agrárias – Agronomia do Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde.

ÍNDICE GERAL

Página

ÍNDICE DE TABELAS	vii
LISTA DE FIGURAS	ix
LISTA DE SÍMBOLOS, SIGLAS, ABREVIACÕES.....	xi
INTRODUÇÃO GERAL	1
1. EMBALAGENS SINTÉTICAS E BIODEGRADÁVEIS.....	1
2. BIOPOLÍMEROS NA PRODUÇÃO DE EMBALAGENS (BIOPLÁSTICOS)	2
3. AMIDO DE ARARUTA (<i>MARANTA ARUNDINACEA</i>)	4
4. <i>HYMENAEA STIGONOCARPA</i>	5
5. <i>HYMENAEA COURBARIL</i>	6
6. <i>ANNONA CRASSIFLORA</i>	7
7. <i>SYZYGium CUMINI</i>	9
OBJETIVOS	11
1. Geral	11
2. Específicos.....	11
REFERÊNCIAS BIBLIOGRÁFICAS.....	12
CHAPTER I – INCORPORATION OF OIL FROM <i>Hymenaea stigonocarpa</i> and <i>Hymenaea courbaril</i> INTO BIOFILMS MADE FROM ARROWROOT STARCH: PHYSICOCHEMICAL, BIODEGRADABILITY AND ANTIFUNGAL ACTIVITY	19
1. INTRODUCTION.....	21
2. MATERIAL AND METHODS	22
3. RESULTS AND DISCUSSION	26
4. CONCLUSIONS	37
5. REFERENCES	37
CHAPTER II – ACTIVE AND INTELLIGENT BIODEGRADABLE PACKAGING WITH <i>Hymenaea stigonocarpa</i> , <i>H. courbaril</i> AND <i>Annona crassiflora</i> OIL AND <i>Syzygium cumini</i> EXTRACT FOR CHEESE	44
1. INTRODUCTION.....	46
2. MATERIALS AND METHODS	47
2.1 FIXED OIL EXTRACTION	47
2.2 PRODUCTION OF JAMBOLAN EXTRACT	48
2.2.1 ANTHOCYANINS CONTENT	48
2.3 PRODUCTION OF BIODEGRADABLE PACKAGING.....	48
2.4 APPLICATION OF BIODEGRADABLE PACKAGING IN CHEESE	49

2.5 PHYSICOCHEMICAL ANALYSIS	50
2.5.1 BIOFILMS	50
2.5.2 ANTIOXIDANT ASSAY OF THE EXTRACT	51
2.5.3 ANTIBACTERIAL ACTIVITY	52
2.5.3 CHEESE COATED WITH BIOFILM	53
2.6 STATISTICAL ANALYSIS	54
3. RESULTS AND DISCUSSION	54
3.1 ANTHOCYANINS CONTENT	54
3.2 PHYSICOCHEMICAL PARAMETERS OF THE PACKAGING	54
3.3 PHYSICOCHEMICAL PARAMETERS OF COATED CHEESE	65
3.4 MICROBIOLOGICAL PARAMETERS OF COATED CHEESE	69
4. CONCLUSIONS	70
5. REFERENCES	70
CONCLUSÃO GERAL	78

ÍNDICE DE TABELAS

CHAPTER I – INCORPORATION OF OIL FROM *Hymenaea stigonocarpa* and *Hymenaea courbaril* INTO BIOFILMS MADE FROM ARROWROOT STARCH: PHYSICOCHEMICAL, BIODEGRADABILITY AND ANTIFUNGAL ACTIVITY

Table 1. Thickness, moisture, and solubility of arrowroot biofilms incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*..... 28

Table 2. Measurements of biofilm colors incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*..... 30

Table 3. Antifungal activity of biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril* at different concentrations on phytopathological microorganisms..... 36

CHAPTER II – ACTIVE AND INTELLIGENT BIODEGRADABLE PACKAGING WITH *Hymenaea stigonocarpa*, *H. courbaril* AND *Annona crassiflora* OIL AND *Syzygium cumini* EXTRACT FOR CHEESE

Table 1. Thickness, humidity, and solubility parameters of arrowroot biofilms composed of *Hymenaea stigonocarpa*, *Hymenaea courbaril* and *Annona crassiflora* oil, and *Syzygium cumini* extract56

Table 2. Color parameters in biofilms composed of oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril* and *Annona crassiflora*, and *Syzygium cumini* extract..... 57

Table 3. Antioxidant activities of <i>Syzygium cumini</i> extract.....	64
Table 4. Antibacterial activity of <i>Syzygium cumini</i> extract.....	65
Table 5. Total titratable acidity of refrigerated cheeses coated with biodegradable packaging containing oils from <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> , and <i>Syzygium cumini</i> extract, expressed as % lactic acid.....	66
Table 6. Color parameters in refrigerated Mozzarella-type cheese coated with oils of <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> , and <i>Syzygium cumini</i> extract.....	68

LISTA DE FIGURAS

CHAPTER I – INCORPORATION OF OIL FROM *Hymenaea stigonocarpa* and *Hymenaea courbaril* INTO BIOFILMS MADE FROM ARROWROOT STARCH: PHYSICOCHEMICAL, BIODEGRADABILITY AND ANTIFUNGAL ACTIVITY

Figure 1. UV-Vis light transmittance rate in arrowroot biofilms incorporating different doses of fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B)..... 29

Figure 2. Biodegradability assay of arrowroot biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*..... 32

Figure 3. Micrographs and 3-D surface area imaging analysis of arrowroot starch biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*..... 33

Figure 4. FTIR-ATR spectra of the arrowroot biofilms incorporated with fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B) 35

CHAPTER II – ACTIVE AND INTELLIGENT BIODEGRADABLE PACKAGING WITH *Hymenaea stigonocarpa*, *H. courbaril* AND *Annona crassiflora* OIL AND *Syzygium cumini* EXTRACT FOR CHEESE

Figure 1. UV-Vis light transmission rate in biodegradable arrowroot films composed of different concentrations of <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> oils, and <i>Syzygium cumini</i> extract	59
Figure 2. The behavior of biodegradable packaging composed of <i>Syzygium cumini</i> extract in different pH levels	61
Figure 3. Biodegradability of biodegradable films composed of oils from <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> , and extract from <i>Syzygium cumini</i> in natural soil	62
Figure 4. Biodegradability of biodegradable films composed of oils from <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> , and extract from <i>Syzygium cumini</i> in sand and seawater	63
Figure 5. Color parameters in refrigerated Mozzarella-type cheese coated with oils of <i>Hymenaea stigonocarpa</i> , <i>Hymenaea courbaril</i> , and <i>Annona crassiflora</i> , and <i>Syzygium cumini</i> extract.....	69

LISTA DE SÍMBOLOS, SIGLAS, ABREVIACÕES

PET	polietileno tereftalato
PEAD	polietileno de alta densidade
PEBD	polietileno de baixa densidade
PP	polipropileno
PVC	policloreto de vinila
PS	poliestireno
PLA	ácido polilático
ASTM	american Society for testing and materials
CPLA	copolímero alifático
BU	base úmida
G'	módulo de armazenamento
G''	módulo de perda
DAP	diâmetro a altura do peito
cm	centímetros
mm	milímetros
CI ₅₀	concentração de inibição
DPPH	2,2-difenil-1-picrilhidrazil
Fe	ferro
O ₂	oxigênio
C ₂ H ₄	gás etileno
HIV	vírus da imunodeficiência adquirida
CD ₄	linfócitos T auxiliares
g	gramas
h	hora
°C	grau Celsius
HCl	ácido clorídrico
TLC	cromatografia em camada delgada
R _f s	fator de retenção
v/v	volume/volume
s	segundos
nm	nanômetros
FT-IR	infravermelho com transformada de Fourier

ATR	reflectância atenuada total
RU	umidade relativa
kg	quilogramas
L	litro
CFU	unidade formadora de colônias
min	minutos
mg	miligramas
mol	número de moléculas
mL	mililitros
BOD	demanda bioquímica de oxigênio
FRAP	poder antioxidante redutor do ferro
mM	millimole
w/v	massa/volume
TPTZ	2,4,6-Tris(2-piridil)-s-Triazina
NaOH	hidróxido de sódio
EDTA	ácido etilenodiamino tetra-acético
H ₂ O ₂	peróxido de hidrogênio
ETB	enterobactéria
ROS	espécies reativas de oxigênio
pH	potencial hidrogeniônico
RNS	espécies reativas de nitrogênio
M _i	massa inicial
M _f	massa final
AAE	expresso em ácido ascórbico
a*	cromaticidade
b*	cromaticidade
L*	luminosidade/brilho
3D	terceira dimensão
Abs	absorção
ε	valor fixo de antocianinas
CO	monóxido de carbono
CO ₂	dióxido de carbono
%	por cento
µg	microgramas

RESUMO

MENEZES FILHO, A. C. P. Instituto Federal de Educação, Ciência e Tecnologia Goiano - Campus Rio Verde, fevereiro de 2025. **Produção e caracterização de embalagens biodegradáveis ativas e inteligentes com óleo de *Hymenaea stigonocarpa*, *H. courbaril*, *Annona crassiflora* e extrato de *Syzygium cumini*** Orientador: Dr. Frederico Antônio Loureiro Soares.

Embalagens biodegradáveis é o futuro para conservação e manutenção do ecossistema sustentável. O uso de biopolímeros naturais como fécula de araruta apresenta excepcional capacidade de formar misturas com óleos fixos e extratos vegetais. Embalagens biodegradáveis apresentam bons resultados equiparáveis a embalagens sintéticas que apresentam sérios problemas ambientais. Objetivou-se com este trabalho, produzir embalagens biodegradáveis compostas com óleo fixo de *Hymenaea stigonocarpa*, *Hymenaea courbaril* e *Annona crassiflora*, e extrato de *Syzygium cumini* em matriz polimérica de araruta. Foram utilizadas concentrações variáveis de óleo e extrato na produção das embalagens. Foram avaliadas características morfológicas, físico-químicas, bioativas, mecânicas e microbiológicas de embalagens biodegradáveis com e sem revestimento em queijo tipo Muçarela. Os resultados demonstram que concentrações crescentes de óleo e extrato influenciaram na espessura, teor de umidade, solubilidade, cor e transparência das embalagens compostas por óleos e extrato. As embalagens compostas por óleo exibiram atividade antifúngica sobre *Colletotrichum acutatum*, *C. gloeosporioides*, *Aspergillus tubingensis*, *A. fumigatus*, *A. niger* e *Rhizopus stolonifer*, além disso o tempo de biodegradabilidade não ultrapassou 35 dias em solo *in natura*. O extrato de *S. cumini* apresentou alto teor de antocianinas, a cor das embalagens compostas com extrato apresentou maior L*, na transparência embalagens com extrato apresentaram menor transparência, houve efeito ativo de embalagens em diferentes pHs com extrato de *S. cumini*, potencial atividade antioxidante e antibacteriana também foram observadas, queijos revestidos apresentaram baixa acidez total, a cor manteve-se constante com pequenas variações, biodegradabilidade acima de 90% em solo *in natura* e não foi descrito contaminações com microrganismos.

PALAVRAS-CHAVE: bioplásticos; gênero *Hymenaea*; biodegradabilidade, acidez titulável total; extrato vegetal; antocianinas.

ABSTRACT

MENEZES FILHO, A. C. P. Instituto Federal de Educação, Ciência e Tecnologia Goiano – Campus Rio Verde, February, 2025. **Production and characterization of active and smart biodegradable packaging with oil from *Hymenaea stigonocarpa*, *H. courbaril*, *Annona crassiflora*, and extract from *Syzygium cumini*.** Advisor: Dr. Frederico Antônio Loureiro Soares.

Biodegradable packaging is the future for preserving and maintaining a sustainable ecosystem. The use of natural biopolymers, such as arrowroot starch, demonstrates exceptional capacity to form mixtures with fixed oils and plant extracts. Biodegradable packaging shows promising results comparable to synthetic packaging, which has serious environmental issues. This study aimed to produce biodegradable packaging composed of fixed oil from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, as well as extract from *Syzygium cumini*, using an arrowroot starch polymer matrix. Variable concentrations of oil and extract were used in the packaging production. Morphological, physicochemical, bioactive, mechanical, and microbiological characteristics were evaluated for biodegradable packaging, both coated and uncoated, in Mozzarella cheese. The results demonstrate that increasing oil and extract concentrations influenced the thickness, moisture content, solubility, color, and transparency of the composite packaging. Packaging with oil exhibited antifungal activity against *Colletotrichum acutatum*, *C. gloeosporioides*, *Aspergillus tubingensis*, *A. fumigatus*, *A. niger*, and *Rhizopus stolonifer*. Furthermore, the biodegradability time did not exceed 35 days in natural soil. The *S. cumini* extract showed a high anthocyanin content. Packaging with the extract displayed higher L* values (lightness), reduced transparency, and active effects at different pH levels. Additionally, the extract contributed to potential antioxidant and antibacterial activities. Coated cheeses exhibited low total acidity, stable color with minor variations, biodegradability above 90% in natural soil, and no contamination by microorganisms was reported.

KEYWORDS: Bioplastics; *Hymenaea* genus; biodegradability; total titratable acidity; plant extract; anthocyanins.

INTRODUÇÃO GERAL

1. Embalagens sintéticas e biodegradáveis

As primeiras embalagens remetem ao uso de polímeros à base de combustíveis fósseis (petróleo) após o beneficiamento. Desde os primórdios da criação da primeira embalagem sintética, foram sendo aprimoradas ao longo dos anos e hoje existem diversos tipos de embalagens não biodegradáveis amplamente utilizadas em uma gama de ramos industriais como alimentícia, biomédica, biológica, agrícola e mecânica como: polietileno tereftalato (PET), polietileno de alta densidade (PEAD), polietileno de baixa densidade (PEBD), polipropileno (PP), policloreto de vinila (PVC), poliestireno (PS) e ácido polilático (PLA) (PUSCASELU *et al.*, 2021; TEIXEIRA-COSTA; ANDRADE, 2022; SHIRZADI; SEDAGHAT, 2022).

Anualmente são produzidas grandes quantidades dessas embalagens, superior a 200 milhões de toneladas para atender a necessidade humana, no entanto, problemas ambientais são relatados a partir do tempo de vida dessas embalagens que perduram no ambiente em média aproximada de 500 a 1000 anos ou superior a essa estimativa temporal (SIRACUSA *et al.*, 2008). As primeiras embalagens sintéticas produzidas pelo homem, ainda estão contaminando o ambiente, sendo seu fim em ambientes marinhos (TARIQUE *et al.*, 2022).

A indústria química está em estado alarmante quanto a produção e desenvolvimento de embalagens que favorecem os seres humanos, no entanto, agredem a natureza. A Química Verde surgiu a partir do preceito sobre a contaminação causada pelas embalagens sintéticas nos ambientes terrestre e marítimo (DENG *et al.*, 2022). Visto que, pesquisas mostram a contaminação de plásticos em seres vivos marinhos e terrestres. Pesquisadores encontraram uma forma microscópica de plásticos de origem sintética em carne de peixes, frutos do mar e em humanos prejudicando a vida dos seres em diversos ambientes (PUSCASELU *et al.*, 2021). Além disso, a incineração de plásticos sintéticos leva a liberação de quantidades significativas de gases nocivos como dióxido de carbono,

monóxido de carbono, cloro, 1,3-butadieno, furanos, aminas, dioxinas entre outros, que degradam a qualidade do ar e influenciam negativamente sobre o aquecimento global (SHAIKH *et al.*, 2021).

Biopolímeros ou bioplásticos são ambientalmente corretos, geram baixo conteúdo de carbono, contaminação e são absorvidos por microrganismos naturais servindo de fonte de carbono para o desenvolvimento natural, não interferindo diretamente no ambiente. Além disso, são fontes naturais renováveis, sustentáveis e geram empregos para comunidades locais (NANDA *et al.*, 2021). Conforme descrito por Shirzadi & Sedaghat (2022) os biopolímeros são classificados em categorias como polímeros extraídos de compostos de biomassa, polímeros sintetizados a partir de monômeros e polímeros produzidos a partir de microrganismos. A qualidade dos biopolímeros depende das propriedades físicas, químicas, mecânicas e térmicas.

Atualmente, os diversos polímeros naturais empregados no desenvolvimento e produção de embalagens biodegradáveis ativas e inteligentes são exploradas como alternativas potenciais contra o uso descontrolado de plásticos sintéticos. Consequentemente, vários compostos termoplásticos são amplamente empregados na produção de bioplásticos ecologicamente corretos, respeitando o meio ambiente (ILYAS *et al.*, 2018; ABRAL *et al.*, 2020).

2. Biopolímeros na produção de embalagens (bioplásticos)

Conforme Yadav *et al.* (2018) a embalagem é um componente integral e importante no setor de processamento de alimentos, agrícola, farmacêutico e mecânico no mundo. Na indústria de alimentos, a embalagem é uma combinação entre a beleza, ciência e tecnologia que reveste um produto para transporte, armazenamento e manutenção contra diversos fatores bióticos e abióticos (ROBERTSON, 2016).

Os primeiros plásticos ou embalagens plásticas como citado anteriormente, vieram a partir do beneficiamento do petróleo. As embalagens mais utilizadas no mundo são: PVC, PET, poliestireno (PS), polipropileno (PP) e poliamida (PA) (AVEROUS; POLLET, 2012; SILVA; BLUMBERGA, 2019). O problema do uso de embalagens sintéticas é a resistência à degradação no ambiente e a contaminação em solo e água que afetam significativamente a fauna e flora terrícola e aquática (WEBB *et al.*, 2013). Esse

problema agrava-se ainda mais em países subdesenvolvidos que recebem lixo de outros países inclusive desenvolvidos.

De acordo com ASTM D-5488-94d, um produto (embalagem) biodegradável é definida como a capacidade de sofrer decomposição em dióxido de carbono, metano, água, compostos inorgânicos e biomassa que são utilizados pelos microrganismos deterioradores desses produtos (AVEROUS; POLLET, 2012; MANGARAJ *et al.*, 2015). Com os péssimos índices projetados sobre a produção de lixo até 2050, pesquisadores viram a necessidade de desenvolver novos produtos capazes de promoverem a biodegradabilidade em menor tempo, e que os subprodutos não alterem a biodiversidade, favorecendo os microrganismos no ambiente em que esse produto foi descartado (PORTA *et al.*, 2020; YU *et al.*, 2023).

Nesse sentido, a Química Verde foi criada na necessidade de promover o uso de produtos naturais e evitar o uso de produtos sintéticos. Nessa conscientização crescente sobre sustentabilidade, as indústrias de embalagens no mundo estão procurando biopolímeros capazes de substituir os polímeros sintéticos (SHELDON; BRADY, 2022). Biopolímeros são definidos como polímeros de natureza natural vindas de plantas, animais e microrganismos que apresentam ação biodegradável em curto período (KUIKAR; WARKAR, 2023).

Existem três grupos: o primeiro grupo é composto por polímeros que são diretamente extraídos ou removidos da biomassa. Dentre esses materiais encontram polissacarídeos como amido, celulose e proteínas como caseína e glúten. Esses, apresentam natureza hidrofílica e com morfologia cristalina e apresentam ponto negativo durante o processamento, armazenamento em umidade relativa alta. O desempenho também apresenta alguns problemas, principalmente em alimentos úmidos. Embora apresentem diversos pontos positivos, devido as excelentes propriedades de barreira a gases que os tornam adequados para utilização na indústria de embalagens para alimentos (GRUJIĆ *et al.*, 2017; YADAV *et al.*, 2018; AGARWAL *et al.*, 2022).

O segundo grupo inclui materiais poliméricos que são sintetizados por procedimento clássico de polimerização, sendo copolímeros aromáticos alifáticos, poliésteres alifáticos, poli(lactídeo), copolímero alifático (CPLA), aplicando monômeros renováveis a base biológica como poli (ácido láctico) e monômeros à base de óleos, como policaprolactonas. Por exemplo, o PLA pode ser formado em embalagem soprada, objetos de molde injetados e revestimento, todos juntos explicando por que o PLA é o primeiro

novo material com base biológica produzida em escala comercial (GRUJIĆ *et al.*, 2017; AGARWAL *et al.*, 2022).

No terceiro grupo estão classificados os polímeros produzidos por microrganismos (fungos e bactérias) geneticamente modificados que constituem esse grupo. Esse grupo de polímeros naturais consiste principalmente de poli-hidroxi-alcanoatos, embora embalagens à base de celulose bacteriana e outros polissacarídeos também estão sendo elaborados (GRUJIĆ, 2017; YADAV *et al.*, 2018; AGARWAL *et al.*, 2022).

3. Amido de araruta (*Maranta arundinacea*)

Maranta arundinacea (L.) é um vegetal herbáceo terrícola formadora de rizomas (tubérculos) longos, cilíndricos ricos em amido solúvel, amplamente cultivado nas ilhas do Caribe, Sudeste Asiático, América do Sul, Filipinas e Índia (VALENCIA *et al.*, 2015). É uma planta tropical e perene pertencente à família Marantaceae, que inclui 31 gêneros e 530 espécies com inúmeras espécies que apresentam interesse comercial potencial ou real (BRITO *et al.*, 2005; JAYAKUMAR; SUGANTHI, 2017). No Brasil, são encontrados 12 gêneros e 150 espécies. Naturalmente, a forma de armazenamento de produtos do metabolismo especial por *M. arundinacea* é através de carboidratos na forma de amido. O rendimento de amido coletado dessa tuberosa é de > 85% amplamente utilizado na indústria alimentícia (MADINENI *et al.*, 2012).

Resumidamente o amido é um grupo de carboidratos amplamente estudados em diversos grupos vegetais tuberosos. *Maranta arundinacea* possui amido com baixa composição de proteínas, gorduras, cinzas e fibras com tamanho de partícula entre 4-42 µm, temperatura de gelatinização entre 68-75°C, facilmente digerível (ERDMAN, 1986; ARACHCHIGE *et al.*, 2009; MADINENI *et al.*, 2012; TARIQUE *et al.*, 2021), alto teor de amilose (35,20%) que o torna adequado para a produção de embalagens biodegradáveis (NOGUEIRA *et al.*, 2018).

Pouco se conhece sobre as características físico-químicas do amido de araruta, no estudo de Mandineni *et al.* (2012) os pesquisadores descreveram algumas características físico-químicas do amido de *M. arundinacea*, em que a viscosidade de pico foi de 498 BU e viscosidade de pasta fria de 669 BU, além de maior estabilidade de congelamento-degelo e por fim, as propriedades reológicas dinâmicas do amido, aferidas usando

geometria de placa paralela, demonstraram valores de módulo de armazenamento (G') aumentados, enquanto para valores de módulo de perda (G'') observaram diminuição dos valores de frequência crescentes (0-100 Hz). A baixa temperatura de gelatinização e a alta estabilidade de congelamento-degelo do amido indicam o potencial para aplicação como espessante em indústrias alimentícias como defendido pelos autores anteriormente citados e por Raja & Sindhu (2000) e Malki *et al.* (2023).

Diversas características classificam o amido de *M. arundinacea* como um potencial uso industrial. Além disso, o amido de araruta não contém glúten como observado em amidos tradicionais do trigo, aveia, centeio ou cevada. Essa importante característica torna-o como potencial de atuar como substituto para produtos que contêm glúten para clientes com intolerância ao glúten ou preferem dietas sem glúten (CARDOSO *et al.*, 2022).

4. *Hymenaea stigonocarpa*

Hymenaea stigonocarpa Mart. ex Hayne pertence à família Fabaceae (Leguminosae) e ao gênero *Hymenaea*, decídua, heliófita, seletiva xerófito, característica de formações abertas do Cerrado e Campo-Cerrado e período de floração entre outubro a abril e frutificação entre abril a junho (ALMEIDA *et al.*, 1998; DE-CARVALHO *et al.*, 2005). Essa espécie arbórea de grande porte é encontrada principalmente no Domínio Cerrado brasileiro e todos os anos apresenta grandes quantidades de frutos que servem de alimento para animais selvagens e ao homem do campo. O jatobá como é popularmente conhecido é uma árvore decídua que atinge até 20 metros de altura com 50 cm de DAP (diâmetro à altura do peito) na idade adulta. O tronco é tortuoso com fuste curto, a ramificação é do tipo dicotômica e a copa é baixa.

A casca mede até 3 cm de espessura; as folhas são alternas, compostas bifoliadas, pecioladas, com estípulas caducas; folíolos curto-peciolados e subsésseis; a inflorescência é do tipo cimeira terminal, bracteada com até 30 flores; as flores são grandes, com pétalas pouco excedentes ao cálice; o fruto é do tipo legume seco, indeiscente, monospermico ou polispermico, alongado, ápice arredondado ou levemente retuso, base arredondada e margem inteira ou levemente ondulada, mede entre 8,7 cm a 20 cm de comprimento; a textura é rugosa pela presença de pontuações, pequenas, salientes e arredondadas, apresenta a linha de sutura proeminente circundando todo o fruto, a cor varia do marrom-

claro ao marrom-escuro, em cada fruto ocorrem de uma a seis sementes; as sementes são do tipo globosa, largo-oblonga, obovada, comprida, com ápice arredondado ou levemente truncado e base arredondada ou afinada, superfície irregular, com algumas depressões, medindo 17,8 mm a 28,4 mm de comprimento. Envolvendo as sementes, há o arilo, amarelo-esverdeado, macio, fibroso-farináceo, com cheiro característico e sabor doce, constituindo a polpa (CARVALHO, 2007).

A semente de *H. stigonocarpa* possui óleo fixo, xiloglucanas e galactomananas principais hemiceluloses encontradas na parede celular de plantas dicotiledôneas. No estudo de Matuda & Maria Netto (2005) os pesquisadores descreveram conteúdos expressivos de ácidos linoleico (18:2 (n-6)) 52,8%, oleico (18:1 (n-9)) 31,6%, palmítico (16:0) 8,9%, esteárico (18:0) 4,7%, linolênico (18:3 (n-3)) 1,2%, eicosanoico (20:0) 0,8% e conjuntos de insaturados com 85,6% e saturados com 14,4%.

Em outros estudos com o óleo de *H. stigonocarpa*, Menezes Filho *et al.* (2022) verificaram que o óleo fixo de *H. stigonocarpa* incorporado em matriz polimérica de araruta apresentaram atividade antifúngica sobre *Colletotrichum acutatum*, *C. gloeosporioides*, *Aspergillus tubingensis*, *A. flavus*, *A. niger* e *Rhizopus stolonifer*, além de atividade antioxidante na redução do radical livre DPPH com concentração de inibição (CI₅₀) de 398,17 µg mL⁻¹. O mesmo foi verificado por Alves-Silva *et al.* (2022) que avaliaram embalagens biodegradáveis da polpa do fruto de *H. stigonocarpa* que apresentaram potencial aplicabilidade industrial, principalmente para a indústria de alimentos. As embalagens apresentaram atividade antioxidante, boa biodegradabilidade em solo e água salina + areia de praia, e em especial para propriedades mecânicas interessantes.

5. *Hymenaea courbaril*

Hymenaea courbaril L. é uma espécie arbórea frutífera, tropical, hermafrodita, polinizada por morcegos e amplamente distribuída nos trópicos pertencente à família Fabaceae com 30 espécies, subfamília Caesalpiniaceae e gênero *Hymenaea* (SILVA *et al.*, 2014). É uma espécie típica do Cerrado brasileiro que atinge entre 15-20 metros de altura, com copa ampla e densa, tronco cilíndrico de até 1 metro de diâmetro. Espécie facilmente adaptável a diversos tipos de solos, arenosos, argilosos, continentais e de várzea alta, sendo encontrada em diversas regiões do Brasil desde o estado do Piauí até

Norte do Paraná (DIAS *et al.*, 2013). É uma espécie vegetal amplamente utilizada em parques e jardins, bem como, em composição de reflorestamentos heterogêneos (CARVALHO FILHO *et al.*, 2003).

As folhas são alternas, pecioladas, compostas de dois folíolos (bifoliadas), coriáceos e falcados medindo entre 6-10 cm de comprimento, as flores com cálice campanulado formado por 4 sépalas unidas na base e corola formada por 5 sépalas obovadas brancas e cremes. Os frutos são do tipo legume achatado em forma de vagem elipsoide, indeiscente com casca lenhosa marrom-escura com 5-15 cm de comprimento, a polpa (arilo) apresenta coloração amarelo-intensa, com sabor adocicado e aroma agradável, sendo usado *in natura* ou em salgadinhos extrusados e como alimento rico em fibras naturais. As sementes são achatadas e de coloração marrom-escura (CHANG *et al.*, 1998), em número de 2-6 por fruto ou mais, são obovoide a elipsoide, envoltas em material farináceo (MENEZES FILHO *et al.*, 2022). Sabe-se que, o extrato metanólico da casca de *H. courbaril* e da polpa do fruto apresenta atividade cicatrizante em úlceras, dores gastrintestinais e é um potente agente antidiarreico devido aos agentes antioxidantes do metabolismo especial presentes como taninos e flavonoides (ORSI *et al.*, 2012).

Em um estudo realizado por Dias *et al.* (2013) verificaram através do perfil químico a presença dos ácidos C14:0 traços, C16:0 (6,04%), C16:1 (0,11%), C18:0 (4,58%), C18:1 (n-9) (24,96%), C18:2 (n-6) (47,91%), C20:0 (1,57%), C18:3 (n-3) (0,54%), C22:0 (6,41%) e C24:0 (7,84%) no óleo da semente de *H. courbaril*. Além disso, foi observado que o óleo apresenta atividade antioxidante na redução do radical livre DPPH de 48,56% em CI₅₀ g óleo/g⁻¹. No estudo de Menezes Filho *et al.* (2022), foram elaborados embalagens biodegradáveis compostas com óleo da semente de *H. courbaril* que demonstram potencial para o desenvolvimento de novos bioplásticos ativos, com ação antioxidante na redução do radical DPPH e com atividade antifúngica sobre *Colletotrichum acutatum*, *C. gloeosporioides*, *Aspergillus fumigatus*, *A. niger* e *Rhizopus stolonifer*.

6. *Annona crassiflora*

Dentre as frutíferas do Cerrado brasileiro, destaca *Annona crassiflora* Mart. popularmente conhecida por “marolo, araticum” incluída na família Annonaceae que anualmente produz frutos saborosos que alimentam animais selvagens e o ser humano.

Naturalmente nos cerrados do Brasil, *A. crassiflora* apresenta as seguintes características de uma árvore caducifolia, heliófita, xerófita, hermafrodita e de médio porte atingindo entre 4-8 metros de altura e aproximadamente 4 metros de diâmetro de copa (não forma docel) (ARRUDA; PASTORE, 2019).

Essa espécie da flora brasileira apresenta maior ocorrência em solos classificados como Latossolo Amarelo e Vermelho-Amarelo, um indivíduo de *A. crassiflora* pode atingir até 8 metros de altura, sendo um vegetal alógama, com flores hermafroditas, com autocompatibilidade protogínica, com gineceu amadurecendo primeiro que o androceu, são solitárias, axilares, actinomórficas com três sépalas, seis pétalas e numerosos estames e carpelos (SOARES *et al.*, 2009), folhas crasso-membranosas, ferruginosas hirsutas quando jovens e coriáceas quando maduras, dispostas de forma intercambiável na posição horizontal ao longo dos ramos (SOARES *et al.*, 2009), além disso, são hipostomáticas com estômatos paracíticos, mesofilo dorsiventral, tricomas simples e com grande quantidade de tecido esclerênquima (CORRÊA *et al.*, 2007) e ramos jovens apresentando densa pilosidade marrom-avermelhada e órgãos reprodutivos glabrescentes com a idade (BRAGA FILHO *et al.*, 2014).

O tronco é tortuoso com diâmetro entre 20-30 cm e coberto por uma casca áspera e muito espessa resistente à ação do fogo (SOARES *et al.*, 2009). Os frutos são sincárpicos, estrobiliformes múltiplos, glabro, forma subglobosa, composto por gemas em forma de cone contendo apenas uma única semente, podendo ser consumidos *in natura*, como também, ser utilizados na fabricação de compotas, doces, geleias, sorvetes, picolés, sucos, licores e vinagres. As sementes são obovoides achatadas, o tegumento é glabro, marrom-claro, com textura lisa e consistência óssea (ARRUDA; PASTORE, 2019). O período de frutificação é entre os meses de fevereiro e março. A polpa do fruto de *A. crassiflora* apresenta fácil digestibilidade, alto valor nutritivo, bons teores de minerais e elevados teores de açúcares, proteínas e vitaminas (PIMENTA *et al.*, 2014). *A. crassiflora* é uma espécie que além do uso culinários, apresenta diversas atividades biológicas, sendo utilizada na medicina tradicional no tratamento da diarreia (sementes) e como antimicrobiana pelas propriedades antifúngica e antibacteriana (TELLES *et al.*, 2003).

Além do uso medicinal, estudo comprovam que extratos de *A. crassiflora* apresentam atividade alelopática sobre plantas daninhas inibindo a germinação de *Brachiaria brizantha* e *Euphorbia heterophylla* (INOUE *et al.*, 2010). A polpa e sementes do fruto de *A. crassiflora* apresentam potencial atividade antioxidante na redução dos

radicais livres DPPH, FRAP, ABTS⁺ entre outros (ARRUDA; PASTORE, 2019). No estudo de Braga *et al.* (2024) os pesquisadores descreveram diversos ácidos quem compõem o perfil químico do óleo das sementes de *A. crassiflora*. Dentre eles estão láuricos, mirísticos, palmítico com 8,66%, palmitoleicos, esteárico, oleicos com 47,81%, linoleicos com 35,64%, linolênico, aracnoicos, gadoleicos, behenicos, cetoleicos e lignocéricos. Ainda nesse estudo, os autores demonstraram potencial atividade antioxidante na redução do radical livre ABTS⁺ com redução de 10,48 µM Trolox/g⁻¹.

7. *Syzygium cumini*

Syzygium cumini é uma espécie arbórea frutífera pertencente à família Myrtaceae comumente conhecida por jambolão ou “Black Plum, Madan”. É uma arbórea perene com troncos glabros com 6-20 metros de altura distribuída no subcontinente indiano, países do Sudoeste Asiático, Leste da África, e adaptada a ambientes do trópico e subtropical (SINGH *et al.*, 2013; DISSANYAKE *et al.*, 2022). No Brasil é introduzida, e estabeleceu bem nos diversos tipos de solos e climas. Folhas simples e opostas, lâminas de oblongas a elípticas, com margem inteira, coriáceas, 7-18 cm de comprimento e 3-8 cm de largura, com ápices finos. Inflorescências axilares e cimosas-paniculadas, de 3 a 4 vezes compostas, com flores sésseis em grupos de 3 ou mais nas pontas.

Apresenta flores pequenas com 1-1,5 cm tetrâmeras, hipanto obconico, cálice truncado, rosa acastanhado, sépalas livres e triangulares, corola pseudocaliptrada, pétalas creme a brancas e orbiculares. Estames numerosos com 4-5 mm de comprimento, anteras dorsifixas, estilete com 2-5 mm de comprimento, ovário ínfero, bilocular e com numerosos óvulos com placentação axilar. Os frutos são carnosos, cor esverdeada quando imaturos, passando a roxo, e negro quando maduros, apresentam casca e polpa de cor vinho, levemente adstringente (PIZZARDO; ANTONICELLI, 2024), sendo utilizados em tratamentos da diabetes, faringite, esplenopatia, uretrorreia e infecções ocasionadas por fungos (micoses). A semente é solitária de testa dura medindo entre 1-1,5 cm de comprimento. Além de apresentarem como importante fonte de Ferro (Fe), diversos outros minerais, açúcares e proteínas (SWAMY *et al.*, 2017). A casca do tronco apresenta efeitos carminativo, digestivo, anti-hiperglicêmica, anti-helmíntica e antibacteriana (SRIVASTAVA; CHANDRA, 2013).

No estudo de revisão proposto por Srivastava & Chandra (2013) na casca e polpa do fruto de *S. cumini* o ácido málico é o ácido com maior teor em percentagem, e como ácido traço os ácidos oxálico e gálico, e taninos que são responsáveis pela adstringência. A cor roxa do fruto é pela presença de cianidina diglicosídica, os açúcares presentes são glicose, frutose, manose e galactose, além de ser uma fruta rica em minerais e vitaminas essenciais.

Além do seu uso medicinal, *S. cumini* é utilizado na alimentação, o suco da fruta pode ser ingerido *in natura* ou polpa congelada apresentando diversas atividades biológicas em especial, antioxidantes (QAMAR *et al.*, 2022). O extrato do fruto de *S. cumini* por apresentar compostos antocianidínicos são utilizados como indicadores naturais ácido-base, por serem sensíveis ao pH e que podem ser utilizadas incorporadas em alimentos. Nesse sentido, Filipini *et al.* (2020) desenvolveram embalagens biodegradáveis compostas com extrato do fruto de *S. cumini* e demonstraram serem agentes inteligentes demonstraram a alteração de produtos alimentícios que sofreram alguma interferência por exemplo, enzimática, alterando a coloração da embalagem incorporada com extrato.

OBJETIVOS

1. Geral

Produzir embalagens ativas biodegradáveis a partir da matriz polimérica de araruta incorporada com diferentes concentrações de óleo de *Hymenaea stigonocarpa*, *H. courbaril* e *Annona crassiflora* e inteligentes com extrato do fruto de *Syzygium cumini* e aplicação em queijo tipo Muçarela.

2. Específicos

Produzir embalagens biodegradáveis ativas compostas com óleo fixo das sementes de *Hymenaea stigonocarpa*, *Hymenaea courbaril* e *Annona crassiflora* e avaliar a morfologia, estrutura microscópica, cristalina, molecular e mecânica.

Verificar a ação inteligente de embalagens biodegradáveis produzidas a partir do extrato do fruto de *Syzygium cumini*.

Avaliar as atividades biológicas antioxidante, antifúngica e antibacteriana dos óleos fixos e extrato *in natura* e incorporados em matriz polimérica de araruta.

Propor novos produtos à base de óleos fixos de espécies do Cerrado e extrato vegetal incorporados em embalagens biodegradáveis em queijo tipo Muçarela.

Determinar os parâmetros morfológicos, mecânicos e antimicrobianos de embalagens biodegradáveis ativas e inteligentes em queijo tipo Muçarela.

REFERÊNCIAS BIBLIOGRÁFICAS

ABRAL, H.; ATMAJAYA, A.; MAHARDIKA, M.; HAFIZULHAQ, F.; KADRIADI; HANDAYANI, D.; SAPUAN, S. M.; & ILYAS, R. A. Effect of ultrasonication duration of polyvinyl alcohol (PVA) gel on characterizations of PVA film. **Journal of Materials Research and Technology**, v. 9, p. 2477-2486, 2020.

AGARWAL, A.; SHAIDA, B.; RASTOGI, M.; & SINGH, N. B. Food packaging materials with special reference to biopolymers-properties and applications. **Chemistry Africa**, v. 6, p. 117-144, 2022.

ALMEIDA, S. P.; PROENÇA, C. E. B.; SANO, S. M.; & RIBEIRO, J. F. Cerrado: espécies vegetais úteis. Planaltina: EMBRAPA- CPAC, 1998.

ALVES-SILVA, G. F.; ROMANI, V. P.; & MARTINS, V. G. Jatobá (*Hymenaea stigonocarpa*) pulp films: Properties, antioxidant potential and biodegradability. **Food Packaging and Shelf Life**, v. 34, p. 100923, 2022.

ARACHCHIGE, H.; WICKRAMASINGHE, M.; TAKIGAWA, S.; MATSUURA-ENDO, C.; YAMAUCHI, H.; & NODA, T. Comparative analysis of starch properties of different root and tuber crops of Sri Lanka. **Food Chemistry**, v. 112, n. 1, p. 98-103, 2009.

ARRUDA, H. S.; & PASTORE, G. M. Araticum (*Annona crassiflora* Mart.) as a source of nutrients and bioactive compounds for food and non-food purposes: A comprehensive review. **Food Research International**, v. 123, p. 450-480, 2019.

AVEROUS, L.; & POLLET, E. Environmental Silicate nano-biocomposites. Springer, London Heidelberg, New York Dordrecht, 2012.

BRAGA, L. P.; AMORIM, K. A.; GOULART, G. A. S.; ASQUIERI, E. R.; DAMIANI, C. Effect of different extraction methods on yield and quality of araticum (*Annona crassiflora* Mart.) seed oil. **Food Science and Technology**, v. 44, p. e00176, 2024.

BRAGA FILHO, J. R.; NAVES, R. V.; CHAVES, L. J.; PIRES, L. L.; & MAZON, L. T. Caracterização física e físico-química de frutos de araticum (*Annona crassiflora* Mart.) **Bioscience Journal**, v. 30, n. 1, p. 16-24, 2014.

BRITO, V.; NASCIMENTO, R.; NARCISA-OLIVEIRA, J.; JOFFER, N.; FATTORI, A.; CEREDA, M.; OLIVEIRA, C.; COSTA, R.; TIBURTINO-SILVA, L.; & MACIEL, J. Arrowroot (*Maranta arundinacea* L.): Botany, horticulture, and uses. **Horticultural Reviews**, v. 48, p. 233-274, 2005.

CARDOSO, G. J.; KIPP, S. D. M.; GARCIA, V. A. S.; CARVALHO, R. A.; & VANIN, F. M. Arrowroot starch (*Maranta arundinacea*) as a bread ingredient for product development. **Journal of Food Processing and Preservation**, v. 46, n. 2, p. e16251, 2022.

CARVALHO, P. E. R. Jatobá-do-cerrado *Hymenaea stigonocarpa*. **Circular Técnica 133**, Embrapa Florestas.

CARVALHO FILHO, J. L. S.; ARRIGONI-BLANK, M. F.; BLANK, A. F.; & RANGEL, M. S. A. Produção de mudas de jatobá (*Hymenaea courbaril* L.) em diferentes ambientes, recipientes e composições de substratos. **Revista Cerne**, v. 9, n. 1, p. 109-118, 2003.

CHANG, Y. K.; SILVA, M. R.; GUTKOSKI, L. C.; SEBIO, L.; & SILVA, M. A. A. P. Development of extruded snacks using jatobá (*Hymenaea stigonocarpa* Mart) flour and cassava starch blends. **Journal of the Science of Food and Agriculture**, v. 78, p. 59-66, 1998.

CORRÊA, P. G., DAS CHAGAS, M. G. S., & DE PIMENTEL, R. M. M. Caracterização morfoanatômica foliar de *Annona crassiflora* Mart. **Revista Brasileira de Biociências**, v. 5, n. S1, p. 816-818, 2007.

DE-CARVALHO, P. S.; MIRANDA, S. C.; & SANTOS, M. L. Germinação e dados biométricos de *Hymenaea stigonocarpa* Mart. ex Hayne (Leguminosae-Caesalpinoideae) – jatobá-do-cerrado. **Revista Anhangüera**, v. 6, n. 1, p. 101-116, 2005.

DENG, J.; ZHU, E-Q.; XU, G-F.; NAIK, N.; MURUGADOSS, V.; MA, M-G.; GUO, Z.; & SHI, Z-J. Overview of renewable polysaccharide-based composites for biodegradable food packaging applications. **Green Chemistry**, v. 24, n. 2, p. 480-492, 2022.

DIAS, L. S.; LUIZA, D. M. M.; & JORGE, N. Physicochemical and bioactive properties of *Hymenaea courbaril* L. pulp and seed lipid fraction. **Industrial Crops and Products**, v. 49, p. 610-618, 2013.

DISSANAYAKE, P. K.; WEKUMBURA, W. G. C.; WIJERATNE, A. W.; & WIJESUNDARA, D. S. A. Morphological characterization, antioxidant capacity and diversity of *Syzygium cumini* trees from Sri Lanka. **Horticultural Plant Journal**, v. 8, n. 1, p. 53-67, 2022.

ERDMAN, M. D. Starch from arrowroot (*Maranta arundinacea*) grown at Tifon, Georgia. **Cereal Chemistry**, v. 63, n. 3, p. 277-279, 1986.

FILIPINI, G. S.; ROMANI, V. P.; & MARTINS, V. G. Biodegradable and active-intelligent films based on methylcellulose and jambolão (*Syzygium cumini*) skins extract for food packaging. **Food Hydrocolloids**, v. 109, p. 1061139, 2020.

GRUJIĆ, R.; VUJADINOVIĆ, D.; & SAVANOVIĆ, D. Biopolymers as food packaging materials. **Advances in Applications of Industrial Biomaterials**, Chapter, p. 139-160, 2017.

INOUE, M. H.; SANTANA, D. C.; SOUZA FILHO, A. P. S.; POSSAMAI, A. C. S.; SILVA, L. E.; PEREIRA, M. J. B.; & PEREIRA, K. M. Potencial alelopático de *Annona crassiflora*: efeitos sobre plantas daninhas. **Planta Daninha**, v. 28, n. 3, p. 489-498, 2010.

ILYAS, R.A.; SAPUAN, S.M.; ISHAK, M.R.; & ZAINUDIN, E. S. Development and characterization of sugar palm nanocrystalline cellulose reinforced sugar palm starch bionanocomposites. **Carbohydrate Polymers**, v. 202, p. 186-202, 2018.

JAYAKUMAR, A.; & SUGANTHI, A. Biochemical and phytochemical analysis of *Maranta arundinacea* (L.) rhizome. **International Journal of Research in Pharmacy and Pharmaceutical Sciences**, v. 2, n. 3, p. 26-30, 2017.

JUIKAR, S. K.; & WARKAR, S. G. Biopolymers for packaging applications: An overview. **Packaging Technology and Science, An International Journal**, v. 36, n. 4, p. 229-251, 2023.

MADINENI, M. N.; FAIZA, S.; SUREKHA, R. S.; RAVI, R.; & GUHA, M. Morphological, structural, and functional properties of Maranta (*Maranta arundinacea* L.) starch. **Food Science and Biotechnology**, v. 21, n. 3, p. 747-752, 2012.

MALKI, M. K. S.; WIJESINGHE, J. A. A. C.; RATNAYAKE, R. H. M. K.; THILAKARATHNA, G. C. Characterization of arrowroot (*Maranta arundinacea*) starch as a potential starch source for the food industry. **Heliyon**, v. 9, n. 9, p. e20033, 2023.

MANGARAJ, S.; GOSWAMI, T. K.; & PANDA, D.K. Modeling of gas transmission properties of polymeric films used for MA Packaging of fruits. **Journal of Food Science and Technology**, v. 52, n. 9, p. 5456-5469, 2015.

MATUDA, T. G.; & MARIA NETTO, F. Caracterização química parcial da semente de jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.). **Revista Ciência e Tecnologia de Alimentos**, v. 25, n. 2, p. 353-357, 2005.

MENEZES FILHO, A. C. P.; VENTURA, M. V. A.; FAVARETO, R.; CASTRO, C. F. S.; TEIXEIRA, M. B.; & SOARES, F. A. L. Incorporation of oil from *Hymenaea stigonocarpa* and *Hymenaea courbaril* into biofilms made from arrowroot starch: physicochemical, biodegradability and antifungal activity. **Journal of Agricultural Science**, v. 14, n. 4, p. 156-169, 2022.

NANDA, S.; PATRA, B. R.; PATEL, R.; BAKOS, J.; & DALAI, A. K. Innovations in applications and prospects of bioplastics and biopolymers: a review. **Environmental Chemistry Letters**, v. 20, p. 379-395, 2021.

NOGUEIRA, G. F.; FAKHOURI, F. M.; & OLIVEIRA, R. A. Extraction and characterization of arrowroot (*Maranta arundinaceae* L.) starch and its application in edible films. **Carbohydrate Polymers**, v. 186, p. 64-72, 2018.

ORSI, P. R.; BONAMIN, F.; SEVERI, J. A.; SANTOS, R. C.; VILEGAS, W.; HIRUMA-LIMA, C. A.; STASI, L. C. D. *Hymenaea stigonocarpa* Mart. Ex Hayne: a brazilian medicinal plants with gastric and duodenal anti-ulcer and antidiarrheal effects in experimental rodent models. **Journal of Ethnopharmacology**, v. 143, p. 81-90, 2012.

PIMENTA, A. C.; ROSA E SILVA, P. S.; ZUFFELLATO-RIBAS, K. C.; & KOEHLER, H. S. Caracterização de plantas e de frutos de araticunzeiro (*Annona crassiflora* Mart.) nativos no cerrado matogrossense. **Revista Brasileira de Fruticultura**, v. 36, n. 4, p. 892-899, 2014.

PIZZARDO, R. C.; & ANTONICELLI, M. C. *Syzygium* in Flora e Funga do Brasil. *Syzygium cumini* (L.) Skeels, FB86017. Jardim Botânico do Rio de Janeiro, 2024.

PORTA, R.; SABBAH, M.; & DI PIERRO, P. Biopolymers as food packaging materials. **International Journal of Molecular Sciences**, v. 13, n. 14, p. 4942, 2020.

PUSCASELU, R. G.; BESLIU, I.; & GUTT, G. Edible biopolymer-based materials for food applications – the eco alternative to conventional synthetic packaging. **Polymers**, v. 12, n. 21, p. 3779, 2021.

QAMAR, M.; AKHTAR, S.; ISMAIL, T.; WAHID, M.; ABBAS, M. W.; MUBARAK, M. S.; YUAN, Y.; BARNARD, R. T.; ZIORA, Z. M.; & ESATBEYOGLU, T. Phytochemical profile, biological properties, and food applications of the medicinal plant *Syzygium cumini*. **Foods**, v. 11, n. 3, p. 378, 2022.

RAJA, M. K. C.; & SINDHU, P. Properties of steam-treated arrowroot (*Maranta arundinacea*) starch. **Starch**, v. 52, n. 12, p. 471-476, 2000.

ROBERTSON, G. L. Food Packaging: Principles and Practice. 3rd Ed, CRC Press, Boca Raton, 2012, 2016.

SHAIKH, S.; YAQOOB, M.; & AGGARWAL, P. Na overview of biodegradable packaging in food industry. **Current Research in Food Science**, v. 4, p. 503-520, 2021.

SHELDON, R. A.; BRADY, D. Green chemistry, biocatalysis, and the chemical industry of the future. **ChemSusChen**, v. 15, n. 9, p. 202102628, 2022.

SHIRZADI, A. G.; & SEDAGHAT, N. An overview of the packaging of synthetic biodegradable plastics. **Packaging Science and Art**, v. 13, n. 50, p. 61-72, 2022.

SILVA, S. M. M.; MESQUITA, A. G. G.; & WADT, L. H. O. Parâmetros genéticos para a conservação de *Hymenaea courbaril* L. na Amazônia Sul-Occidental. **Ciência Florestal**, v. 24, n. 1, p. 87-95, 2014.

SILVA, N.; BLUMBERGA, D. Why biopolymer packaging materials are better. **Environmental and Climate Technologies**, v. 23, n. 2, p. 366-384, 2019.

SINGH, J. P.; SINGH, A. K.; BAJPAI, A.; AHMAD, I. Z.; & MUTHUKUMAR, M. Genetic variability assessment among *Syzygium cumini* Skeels. based on dominant and co-dominant DNA marker systems. **Research Environmental Life Science**, v. 6, p. 13-16, 2013.

SIRACUSA, V.; ROCCULI, P.; ROMANI, S.; & ROSA, M. D. Biodegradable polymers for food packaging: a review. **Trends in Food Science & Technology**, v. 19, p. 634-643, 2008.

SOARES, F. P., PAIVA, R., NOGUEIRA, R. C., STEIN, V. C., & SANTANA, J. R. F. Marolo: Uma frutífera nativa do Cerrado. 82, Lavras: **Boletim Técnico**, p. 1-17, 2009.

SRIVASTAVA, S.; & CHANDRA, D. Pharmacological potentials of *Syzygium cumini*: a review. **Journal of the Science of Food and Agriculture**, v. 93, n. 9, p. 2084-2093, 2013.

SWAMY, G. S. K.; ANUSHMA, P. L.; JAGADEESHA, R. C. Morphological characterization of elite jamun (*Syzygium cumini* Skeels) genotypes. **International Journal of Minor Fruits, Medicinal and Aromatic Plants**, v. 3, n. 1, p. 09-15, 2017.

TARIQUE, J.; SAPUAN, S. M.; KHALINA, A.; SHERWANI, S. F. K.; YUSUF, J.; & ILYAS, R. A. Recent developments in sustainable arrowroot (*Maranta arundinacea* Linn) starch biopolymers, fibers, biopolymer composites and their potential industrial applications: A review. **Journal of Materials Research and Technology**, v. 13, p. 1191-1219, 2021.

TEIXEIRA-COSTA, B. E.; & ANDRADE, C. T. Natural polymers used in edible food packaging – history, function and application trends as a sustainable alternative to synthetic plastic. **Polysaccharides**, v. 3, n. 1, p. 32-58, 2022.

TELLES, M. P. C.; VALVA, F. D.; BANDEIRA, L. F.; & COELHO, A. S. G. Caracterização genética de populações naturais de araticunzeiro (*Annona crassiflora* Mart. Annonaceae) no estado de Goiás. **Revista Brasileira de Botânica**, v. 25, n. 1, p. 123-129, 2003.

VALENCIA, G. A.; MORAES, I. C. F.; LOURENÇO, R. V.; BITTANTE, A. M. Q. B.; & SOBRAL, P. J. A. Physicochemical properties of maranta (*Maranta arundinacea* L.) starch. **International Journal of Food Properties**, v. 18, n. 9, p. 1990-2001, 2025.

YADAV, A.; MANGARAJ, S.; SINGH, R.; DAS, S. K.; KUMAR, N. M.; & ARORA, S. Biopolymers as packaging material in food and allied industry. **International Journal of Chemical Studies**, v. 6, n. 2, p. 2411-1418, 2018.

YU, C.; JIN, D.; HU, X.; HE, W.; & LI, G. An overview of management status and recycling strategies for plastic packaging waste in China. **Recycling**, v. 8, n. 6, p. 90, 2023.

CHAPTER I – INCORPORATION OF OIL FROM *Hymenaea stigonocarpa* and *Hymenaea courbaril* INTO BIOFILMS MADE FROM ARROWROOT STARCH: PHYSICOCHEMICAL, BIODEGRADABILITY AND ANTIFUNGAL ACTIVITY

(Article published in the Journal of Agricultural Science)

Abstract: Incorporating fixed oils in biodegradable packaging has an important effect on the polymer matrix and biological activities of phytopathogens. This study aimed to evaluate the incorporation of fixed oils from the seeds of *Hymenaea stigonocarpa* and *Hymenaea courbaril* in the arrowroot starch biofilm matrix, evaluating the physicochemical parameters of biodegradability and antifungal activity on *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Aspergillus niger* and *Rhizopus stolonifer*. Both fixed oils from *Hymenaea* seeds showed biological antioxidant activity in reducing DPPH. Biofilms showed increasing variation in thickness ranging from 0.23-0.43 mm and decreasing moisture content and solubility 15.01-5.14% and 51.07-34.10%, respectively, as oil concentrations increased. The oil concentration also reduced the transparency rate, considerably varying the color and biodegradability parameters. However, the biofilms presented a mass reduction of more than 90% for this test. Biofilms still demonstrate considerable antifungal activity for the evaluated phytopathogens. The seed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril* played important roles in developing biopolymer matrices and special biological activity on potential phytopathological agents of fruits and grains.

Keywords: *Colletotrichum acutatum*, *Aspergillus niger*, antioxidant activity, *Rhizopus stolonifer*.

CAPÍTULO I – INCORPORAÇÃO DO ÓLEO DE *Hymenaea stigonocarpa* E *Hymenaea courbaril* EM BIOFILMES FEITOS DE AMIDO DE ARARUTA: ATIVIDADES FÍSICOQUÍMICAS, BIODEGRADABILIDADE E ATIVIDADE ANTIFÚNGICA

(Artigo publicado no Journal of Agricultural Science)

Resumo: A incorporação de óleos fixos em embalagens biodegradáveis tem ação importante na matriz polimérica e nas atividades biológicas sobre fitopatógenos. Este estudo teve como objetivo avaliar a incorporação de óleos fixos das sementes de *Hymenaea stigonocarpa* e *Hymenaea courbaril* na matriz de biofilme de amido de araruta, avaliando os parâmetros físico-químicos de biodegradabilidade e atividade antifúngica em *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Aspergillus tubingensis*, *Aspergillus fumigatus*, *Aspergillus niger* e *Rhizopus stolonifer*. Ambos os óleos fixos das sementes de *Hymenaea* mostraram atividade antioxidante biológica na redução de DPPH. Os biofilmes apresentaram variação crescente na espessura, variando de 0,23 a 0,43 mm, e redução no teor de umidade e solubilidade de 15,01% a 5,14% e de 51,07% a 34,10%, respectivamente, à medida que as concentrações de óleo aumentavam. A concentração de óleo também reduziu a taxa de transparência, com variação considerável entre os parâmetros de cor e biodegradabilidade. No entanto, os biofilmes apresentaram redução de massa de mais de 90% para este teste. Os biofilmes ainda demonstraram considerável atividade antifúngica para os fitopatógenos avaliados. O óleo de semente de *Hymenaea stigonocarpa* e *Hymenaea courbaril* desempenhou papéis importantes no desenvolvimento de matrizes de biopolímeros e na atividade biológica especial sobre agentes fitopatológicos potenciais de frutas e grãos.

Palavras-chave: *Colletotrichum acutatum*, *Aspergillus niger*, atividade antioxidante, *Rhizopus stolonifer*.

1. INTRODUCTION

Synthetic packaging is widespread in any industrial segment and even in products of “green” origin, produced from petroleum processing, which presents serious and potential environmental problems due to the extended stay in the mainly marine environment (Nielsen et al., 2020). The degradation process of different types of plastics used to store agricultural, biological, and chemical products is long, exceeding 500 years in a favorable environment for this process. It is also worth mentioning that synthetic polymers used as packaging can be associated with food contamination (Kalpana et al., 2019; Santos et al., 2020).

In addition, the packaging industry increases its production by 8% a year, which negatively impacts accumulation, where 90% of all plastic produced is accumulated in the environment, and only 5% is recycled (Nor Adilah et al., 2018). The microplastics produced from the abrasion process can be inserted in both animal and human food, and studies carried out about the absorption of this material by humans generate an accumulation of 1 kg per year, which is toxic (Nor Adilah et al., 2018; Henry et al., 2019).

Several studies characterize synthetic packaging as a harmful promoter for man and the environment. Thus, the need arose to develop biodegradable packaging comparable to synthetic plastics. Green chemistry is the area that develops products that combine natural bases such as fats, oils, chitosan, and starches, among other biopolymers, as raw materials in the development of sustainable packaging (Gandini et al., 2018).

According to Tavassoli-Kafran et al. (2016) and Beikzadeh et al. (2020), their growing application is due to the advantages such as being environmentally friendly, preventing losses of moisture, aromas, colors, gas barrier attributes (O_2 , CO, CO_2 and C_2H_4), reduction enzymatic of spoilage, microbial contamination; consequently, they could extend the shelf life of main food products with no side effects by inhibiting the dehydration, browning, and oxidative rancidity.

Furthermore, incorporating substances such as fixed oil, essential oil, oil-resin, and plant extracts positively aggregate in a resistant polymeric matrix and have bioactive characteristics in inhibiting fungal and bacterial agents. Several vegetable oils incorporated in biopolymers have potential activity on many pathological and phytopathological fungicide species (Arruda et al., 2019; Aydogdu et al., 2020).

In agricultural production, mainly fruits and grains, the community constantly pressures producers to provide products free of fungal agents that present health problems for humans and animals. Several *Colletotrichum*, *Aspergillus*, and *Rhizopus* genera

produce adverse effects every year on the economy from natural products such as fruits, vegetables, and grains used in food. Several of these microorganisms during the metabolic rate can produce aflatoxins involved in the death of animals fed with contaminated feed. There are reports that these toxins may be associated with an increase in CD₄ cells in carriers of the acquired immunodeficiency virus (HIV) (Ikarugi et al., 2008; Souza et al., 2014; Li et al., 2017; Tahir et al., 2018).

The constant need for studies to evaluate natural products from plants as the main renewable source makes it necessary to evaluate species in the most varied biomes and Cerrado domain (Bueno et al., 2018). Thus, there is a need to study native plant species of this domain such as *Hymenaea stigonocarpa* and *Hymenaea courbaril* popularly known as “jatobá-do-Cerrado and jatobá-do-campo”, both belonging to the Fabaceae family that annually have large fruit harvests. Each *Hymenaea* fruit produces around 5-10 seeds which they present in their cotyledons compounds such as fatty acids (fixed oil) (Pereira et al., 2011; Silva, 2018).

This oil can be used in the incorporation of polymeric matrices where they can present important characteristics such as increased impermeability, antioxidant activity, average extended shelf life, visible and ultraviolet light transmission, elasticity, and water vapor transfer. It is also noteworthy that both species of *Hymenaea* still have a limited number of studies, especially for the use of seeds in different industrial processes (Menezes Filho et al., 2020).

In addition, eco-friendly bioactive packaging that features natural preservatives may be better options to overcome health concerns, and environmental issues and mitigate losses in agriculture. For that, arrowroot-based high-performance biodegradable packaging materials are considered a relatively easy and suitable method for developing edible emulsion materials and biodegradable packaging with antioxidant, antifungal, and antibacterial biological activities.

This study aimed to evaluate the incorporation of fixed oil from the seed of two species of *Hymenaea stigonocarpa* and *Hymenaea courbaril* and evaluate the characteristics of biofilms regarding structural antifungal characteristics on potential agricultural phytopathological agents.

2. MATERIAL AND METHODS

Six hundred grams (600 g) of *H. stigonocarpa* and *H. courbaril* seeds were

collected in two Cerrado areas in Rio Verde, Goiás state, Brazil (174717.6" S and 505755.6" W, 174258.6" S and 505328.6" W) respectively. The specimens were authenticated by M.Sc. Antonio Carlos Pereira de Menezes Filho, and are stored in the Herbarium in Vegetable Systematic Laboratory, Biology Department, Goiano Federal Institute. (HRV No. 14097 and 14098).

Seeds were sprayed with a mixer grinder and dried in an oven at 35 °C for 12 h. Fixed oil was obtained using a Soxhlet-type system as described by Elagbar et al. (2016) adapted. The extractant solvent used was *n*-Hexane. Fifteen grams (15 g) processed seeds were used in the extraction. The system was refluxed for 8 h. Then, the solvent was evaporated using a rotary evaporator. The oil was collected in amber-colored bottles and stored in the refrigerator until analysis at -12 °C.

Physicochemical properties of specific gravity (pycnometer 1 mL) and refractive index (digital refractometer) were determined according to Adegbe et al. (2016). The methodology used for Thin Layer Chromatography (TLC) of *Hymenaea* seed oils followed as proposed by Ferreira et al. (2021). The samples were analyzed and standards were placed on a silica chromatographic plate (Xtra SIL G/UV254). The standards used were oleic acid (Sigma-Aldrich), linoleic acid (Vetec), myristic acid, and palmitic acid (Sigma-Aldrich). The distance for the chromatographic run was 10 cm. A binary mixture of *n*-hexane and ethyl acetate (8:2) was used as the mobile phase. The mobile phase and the chromatographic plate were placed inside a glass vat and kept at rest for 20 mL for the chromatographic run. After this period, chromatoplates were developed with solid iodine vapor (I₂) and ferric chloride, and the *R_f*s compared between oil samples and standards.

Oil samples were evaluated using DPPH radical (2,2-Diphenyl-1-picryl-hydrazyl) for free radical scavenging activity using the method described by Elagbar et al. (2016). Solutions were freshly prepared. DPPH (0.006 g%), different concentrations of oil conc. (10-2000 µg mL⁻¹) was prepared in *n*-hexane, and Ascorbic acid (10-2000 µg mL⁻¹) was prepared in ethanolic solution (70%) (v/v). One mL of DPPH was mixed with either oil (1 mL) or Ascorbic acid (1 mL). These solutions were homogenized at 25 °C for 30 s and kept aside for 30 min in the dark room. Using *n*-hexane as blank at Abs 517 nm the instrument UV-Vis spectrophotometer was set at zero. The free radical scavenging activity of residual DPPH against the blank was determined at Abs 517 nm using the following Equation 1.

$$\text{DPPH scavenging activity (\%)} = (1 - \text{AbsC}/\text{AbsS}) \times 100 \quad (1)$$

Where, AbsC = Absorbance control; AbsS = Absorbance sample.

All the experiments were conducted in quadruplicate. The values of the calculated inhibition concentration (IC_{50}) $\mu\text{g mL}^{-1}$.

Biodegradable films were obtained by the “casting” method, agreeing with the methodology proposed by Valadares et al. (2020). To obtain biofilms, 5 g commercial arrowroot starch *M. arundinacea* was dissolved in 100 mL distilled water. The mixture was moderately agitated at room temperature (25 °C) for 5 min. Afterwards, this solution was heated at 70 °C under constant agitation for 30 minutes. After starch gelatinization, glycerol was added conc. 30% (w/v). This dispersion was then agitated for 5 minutes. When the filmogenic solution reached 30 °C, a previously prepared suspension of fixed oil from *H. stigonocarpa* or *H. courbaril* in Tween 40 conc. 0.25 (g/g fixed oil⁻¹) was incorporated into it under constant agitation for 15 min. Final concentrations of seed fixed oil were 0.25%, 0.50%, 0.75%, and 1% (v/v), besides a control treatment with no fixed oil. Filmogenic solutions made from arrowroot starch into which fixed oil was incorporated were poured on a polyethylene plate and dried in an oven with air circulation at 35 °C for about 48 h.

A digital caliper measured biofilm thickness. Measurements were carried out in ten spots on every biofilm, and the thickness mean was calculated according to Santos et al. (2021). The moisture content was obtained in an oven at 105 °C for 4 h. Four replicates per film treatment were used, in agreement with the methodology described by Rambabu et al. (2019). Measurement of water solubility was performed as described by Santos et al. (2020) and proposed by Jahed et al. (2017). Biofilms, which measured about 2 cm², were dried in an oven at 105 °C for 4 h and then weighed so that initial mass (M_i) could be determined. They were immersed in 50 mL distilled water and kept under constant agitation at 25 °C for 24 h. Afterwards, solutions with the films were filtered through filter paper which had been previously weighed. Sheets of filter papers with films were dried at 105 °C for 24 h and weighed so that the final mass (M_f). The analysis was performed in triplicate. Biofilm solubility (%) was calculated by Equation 2.

$$\text{Water solubility assay (\%)} = (M_i - M_f/M_i) \times 100 \quad (2)$$

Ultraviolet and visible light transmittance of biofilms was conducted by UV-Vis spectrophotometer. Biofilm samples were cut and placed in cuvettes to measure transmittance over a wavelength range between 850 and 200 nm (Hosseini et al., 2015).

The FT-IR data were obtained in the range of 600-4000 cm^{-1} , with 60 scans and a resolution of 4 cm^{-1} , equipped with a diamond attenuated total reflectance (ATR) accessory. Data were evaluated using Microsoft Excel. Analysis of biofilm color was carried out by a colorimeter. Parameters under evaluation were L^* (luminosity) and chromaticity parameters [(+60/-60) a^* and (+60/-60) b^*]. Measurements were conducted at five randomly selected film spots (Valadares et al., 2020).

The analysis of biodegradability was carried out according to Martucci and Ruseckaite (2009) adapted. Biofilm samples ($2 \times 2 \text{ cm}^2$) were dried up to constant weight so that initial mass (M_i). Samples were then placed in open high-density polyethylene packages to enable microorganisms and moisture to gain access to them. After that, they were buried in natural soil, which had been previously prepared, at constant moisture and room temperature (70% R.U and 25 °C). Five, ten, fifteen, twenty, twenty-five, and thirty days after the experiment installment, the packages with the samples were removed from the soil, washed with distilled water, and dried to constant weight (M_f). The percentage of biodegradability was calculated by Equation 3.

$$\text{Biodegradability (\%)} = (M_f - M_i / M_i) \times 100 \quad (3)$$

The morphology of the biofilms was evaluated under high-resolution optical microscopy. A ($2 \times 2 \text{ cm}^2$) film sample was adhered to a microscope slide and analyzed at different magnifications of 4, 10, 40, and 100x in an optical microscope with an attached camera. Micrographs of the biofilm surface area were analyzed in ImageJ software in 3D pixel stacking analysis.

The antifungal activity of biofilms was analyzed against the phytopathogenic fungi *Colletotrichum acutatum* (BW-101), *Colletotrichum gloeosporioides* (BW-102), *Aspergillus tubingensis* (N2A), *Aspergillus fumigatus* (V1G), *Aspergillus niger* (VIF) and *Rhizopus stolonifer* (BW-116) belonging to the mycological bank of the Technological Chemistry laboratory of the Goiano Federal Institute, Goiás State, Brazil, by a diffusion test on disk described by Ma et al. (2016). Petri dishes, half full of medium potato dextrose agar (PDA), were inoculated with 100 μL suspension with $1 \times 10^8 \text{ CFU mL}^{-1}$ with 0.5 McFarland scale in the UV-Vis spectrophotometer. Then, three samples of biofilms which had been cut in circles with about 7 mm in diameter were placed on every dish. Dishes were incubated at 26 °C for 10 days (Weir et al., 2012; Damm et al., 2012). Finally, the diameters of the zone of inhibition (mm) were measured with a digital caliper. As standard fungicide Frowncide 500 SC concentration 10 $\mu\text{L mL}^{-1}$ was used (*C. gloeosporioides* and *C. acutatum*), Amphotericin B 100 MCG (*A. tubingensis*, *A.*

fumigatus and *A. niger*) and Botector® 50 µL mL⁻¹ Westbridge.

Analyses were carried out in quadruplicate, and $\pm$ SD was calculated. The data was statistically analyzed by ANOVA and the means were compared by the *Duncan* multiple range test significance with the use of the IBM SPSS Statistics 26 software program. The *P* level of $< 5\%$ was supposed to be significant in determining the variations among mean values of biofilm aspects.

3. RESULTS AND DISCUSSION

The physicochemical and antioxidant properties of the analyzed *Hymenaea* oils presented for specific gravity g mL⁻¹ (20 °C) $0.9274 \pm 0.04a$ and $0.9280 \pm 0.06a$, refractive index (25 °C) $1.44718 \pm 0.01a$ and $1.46237 \pm 0.03b$, *H. stigonocarpa* and *H. courbaril*, respectively. According to Dias et al. (2013) the refractive index is mainly related to the saturation degree and the ratio of fatty acids *Cis* and *Trans* double bonds, in is influenced by oxidative processes. In the analyzed oils of *H. courbaril* by Dias et al. (2013), the refractive indices at 40 °C were 1.4653 for the pulp and 1.4655 for the seeds. In both oil samples, it was verified the presence of Rfs close to the standards for oleic, linoleic, and palmitic acid (*H. stigonocarpa* and *H. courbaril*), and myristic (*H. courbaril*).

The TLC method showed good separation results for the evaluated fatty acids. Several retention spots were observed on chromatoplates containing *Hymenaea* oil on I2 vapor and UV₃₆₅ nm light, suggesting the presence of other fatty acid groups. Ferric chloride developers revealed a blue spot at 33 mm which suggests the presence of the hydrolyzable or gallic tannin group in *H. courbaril*. This result corroborates the study by Dias et al. (2013) where they evaluated total phenolic compounds in *H. courbaril* seed oil with a result of 3.43 mg GAE 100 g⁻¹.

Antioxidant activity IC₅₀ = $2.19 \pm 0.06a$ µg mL⁻¹ (Ascorbic acid), IC₅₀ = $398.17 \pm 1.08c$ and IC₅₀ = $211.30 \pm 1.96b$ µg mL⁻¹, *H. stigonocarpa* and *H. courbaril*, respectively. Observed that there is no significant difference by the Duncan ($p < 5\%$) for specific gravity, however, there is a statistical difference for the refractive index assay and in the DPPH free radical reduction for both samples and the standard antioxidant. Fixed oils are extracted not only from seeds, but they also have potential antioxidant agents. Dias et al. (2013) found for the pulp and seeds of *H. courbaril* moderate DPPH free radical reduction efficiency of 22.19% (IC₅₀ = 49.04 g/g) and 83.49% (IC₅₀ = 48.56 g/g) respectively. This

anti-free radical activity has potential activity incorporated in polymeric matrices capable of reducing the deleterious effects on food (Santos et al., 2020).

The incorporation of fixed oils of *H. stigonocarpa* and *H. courbaril* into the arrowroot biopolymer matrix showed significant effects when compared to the control on thickness, moisture, and solubility (Table 1). These effects are due to the high concentration of amylose found in arrowroot starch (Thakur et al., 2019; Santos et al., 2020). Thickness analysis showed that the biofilms varied in terms of thickness according to Duncan's test ($p < 5\%$) noting that the biofilm incorporated with 1% of *H. stigonocarpa* oil had a higher thickness than the others. Aydogdu et al. (2020) found thinner thicknesses between 0.11-0.18 mm for guar gum as a polymer matrix incorporated with 1% and 2% orange oil, emphasizing that the polymer and the oil concentration considerably influence this characteristic. According to Hussein et al. (2015) biofilms are a crucial parameter on mechanical properties and water vapor permeability values. These results indicated that the *Hymenaea* oils addition altered the thickness and microstructure of the films.

In the study of Kadzińska et al. (2020) the moisture content of the researched biofilms ranked from 15.49-19.18% for the biofilms with coconut oil and rapeseed oil, respectively, with sodium alginate polymer matrix. In the study by Niknam et al. (2019) the researchers obtained a thickness of biodegradable films inferior to that of this study, ranging from 0.12-0.19 mm incorporated in different oilseed oils (olive, canola, and maize). It is noteworthy that the polymer matrix differs from this study, where each biopolymer has a unique behavior.

According to Peres-Mateos et al. (2009) and Niknam et al. (2019) addition of fixed oil to the biofilm matrix may lead to the replacement of strong polymer-polymer interactions with weak polymer-oil interactions and thus increase the biofilm volume which in turn causes increasing of the biofilm thickness. As for the moisture content, biofilms are directly dependent on the oil concentration in both *Hymenaea* species.

Although there are no standards for moisture content in biofilms in organ regulatory bodies of food products, the packages obtained in this study had similar moisture percentages observed in other studies (Niknam et al., 2019) and were considered low. Biodegradable packaging with starch-based polymers, gelatine, gum, and chitosan have different moisture content, this is observed in the study by Niknam et al. (2019) where researchers found moisture content ranging from 22.46-15.05%. Galus et al. (2016) obtained higher moisture content than in this study evaluating whey protein biofilms incorporated with rapeseed oil between 16.8-17.9%.

Solubility is an important property of edible biofilms as they are used as protective layers on food. In its various uses, potential applications may require water insolubility for harmonic interactions between product integrity and water resistance (Hosseini et al., 2015), with this, it is observed that a variation between solubility results between the control biofilm and other concentrations of both oils. Biofilms incorporated with a higher concentration of 1% oil did not show a significant difference according to statistical analysis, although it is observed that FOHs and FOHc 1% had the lowest solubility. Similar results were obtained in the study by Galus et al. (2016) evaluating whey protein biofilms incorporated with rapeseed oil between 37.4-42.4%.

Table 1. Thickness, moisture, and solubility of arrowroot biofilms incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*.

Biofilm	Thickness (mm)	Moisture (%)	Solubility (%)
Control	0.23 ± 0.01f	15.01 ± 0.59a	51.07 ± 2.26a
FOHs 0.25%	0.25 ± 0.01ef	12.35 ± 0.92c	49.35 ± 1.57ab
FOHs 0.50%	0.28 ± 0.01d	10.55 ± 0.56d	46.88 ± 1.29b
FOHs 0.75%	0.40 ± 0.02b	8.21 ± 0.91e	39.34 ± 1.84c
FOHs 1%	0.43 ± 0.01a	5.14 ± 0.60f	35.35 ± 2.10d
FOHc 0.25%	0.24 ± 0.01f	13.73 ± 0.50b	49.93 ± 1.92a
FOHc 0.50%	0.26 ± 0.01e	11.65 ± 0.49c	46.86 ± 1.76b
FOHc 0.75%	0.29 ± 0.02d	8.84 ± 0.32e	40.74 ± 1.40c
FOHc 1%	0.32 ± 0.03c	5.69 ± 0.43f	34.10 ± 2.18d

Note. Different letters in a column show significant differences ($p < 5\%$) in Duncan's test.

FOHs: Fixed oil *Hymenaea stigonocarpa*. FOHc: Fixed oil *Hymenaea courbaril*.

Regarding the colors of developing biofilms (Figure 1, A-B), there is a decrease in the light transmittance rates of biofilms that incorporate different doses of fixed oil of *H. stigonocarpa* and *H. courbaril* in the visible region (850 at 250nm). Light transmission rates were higher at lower concentrations when compared to the control. Concentrations between 0.75-1% showed low transparency rates, especially for biofilms incorporated with the oil of *H. stigonocarpa* due to the opacity promoted by the emulsion incorporated with large volumes of oil. According to Pereda et al. (2012) and Galus and Kadzińska (2016) the transparency of emulsion-based biofilms is related to their internal structure, which is affected by the fixed and essential oil and droplet size distribution in biofilm-forming emulsions and its rearrangement during drying. Galos and Kadzińska (2016) also emphasize the solvent thermal evaporation during drying induces changes in the emulsion

structure by destabilization phenomena such as creaming and aggregation, which have an important role in the visual and optical properties of emulsion-based biofilms.

According to Santos et al. (2020), Rambabu et al. (2019), and Romani et al. (2018), color is an important parameter to be evaluated, as it directly influences the acceptance of the product by consumers. Biofilms used as packaging generally have a high transparency rate so that the product can be seen by the consumer as fruits, vegetables, and legumes. However, opaque, or colored biofilms offer potential protection, especially to foods exposed to visible and UV light, especially foods with high-fat content, such as meat products, thus preventing them from suffering oxidative degradation of fats and proteins.

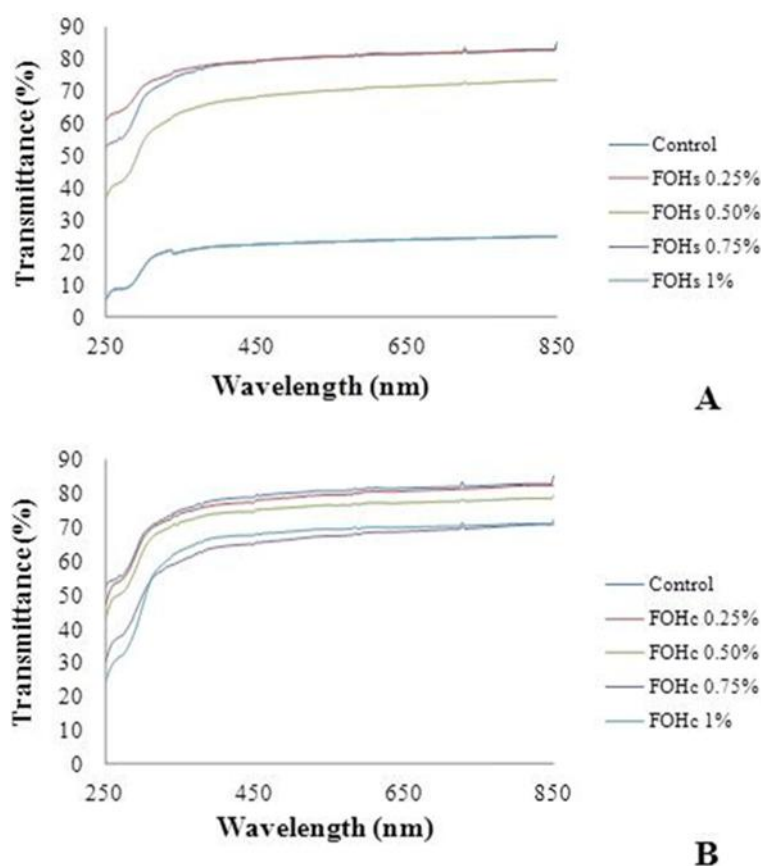


Figure 1. UV-*Vis* light transmittance rate in arrowroot biofilms incorporating different doses of fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B). Source: Authors, 2022.

Biofilms were analyzed in agreement with color parameters L^* , a^* and b^* , where L^* is the biofilm luminosity (100 light/0 dark), a^* is the red/green coordinate (+/-); and b^* is the yellow/blue coordinate (+/-) (Table 2). The values of L^* in this study showed statistical differences in all samples, demonstrating that the arrowroot starch-based

polymer has low luminosity. This was also verified by Santos et al. (2020) where they developed arrowroot starch-based biofilms obtaining a maximum for $L^* = 15.51$. Compared with other natural polymers, Galos and Kadzińska (2016) found L^* between 89.5-90.8 for whey protein biofilms incorporated with rap seed oil. The study by Kadzińska et al. (2020) describes pure sodium alginate biofilm with high transparency with a high L^* value of 93.69.

For a^* chromaticity, all packages showed a tendency towards red, and for b^* a tendency towards blue was verified, except for FOHs 1% and FOHc 1% with a tendency to yellow. As discussed, polymers have different colorations, although what is incorporated into the matrix influences this color characteristic considerably. Thus, this ability to incorporate substances in different matrices is observed, as in the study by Galos and Kadzińska (2016) where the researchers found values of a^* with a tendency to green (0.81 to 0.95) and b^* to blue (-0.1 to -0.7).

Table 2. Measurements of biofilm colors incorporating fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*.

Biofilm	L^*	a^*	b^*
Control	$14.59 \pm 0.24h$	$-0.71 \pm 0.09e$	$-0.91 \pm 0.05e$
FOHs 0.25%	$16.43 \pm 0.07g$	$-0.70 \pm 0.05e$	$-0.93 \pm 0.02e$
FOHs 0.50%	$18.21 \pm 0.48e$	$-0.71 \pm 0.05e$	$-1.26 \pm 0.08f$
FOHs 0.75%	$17.65 \pm 0.16f$	$-0.57 \pm 0.03d$	$-0.96 \pm 0.04e$
FOHs 1%	$30.29 \pm 0.12b$	$-0.54 \pm 0.01d$	$0.40 \pm 0.01a$
FOHc 0.25%	$26.13 \pm 0.55c$	$-0.24 \pm 0.01a$	$-0.50 \pm 0.04d$
FOHc 0.50%	$31.35 \pm 0.03a$	$-0.32 \pm 0.01b$	$-0.23 \pm 0.10c$
FOHc 0.75%	$22.89 \pm 0.01d$	$-0.39 \pm 0.04b$	$-0.56 \pm 0.05d$
FOHc 1%	$30.24 \pm 0.01b$	$-0.47 \pm 0.02c$	$0.23 \pm 0.06b$

Note. Different letters in a column show significant differences ($p < 5\%$) in Duncan's test. Parameters CIELab of color L^* (luminosity), a^* , and b^* (chromaticity). ($\pm$) mean standard deviation. FOHs: Fixed oil of *Hymenaea stigonocarpa*. FOHc: Fixed oil of *Hymenaea courbaril*.

Biodegradation can be designated as degradation occurring in a biological environment, where microorganisms (fungi and bacterial), moisture (RU%), and enzymes are responsible for degrading biopolymers (Fernandes et al., 2020). The biodegradability test (Figure 2) evaluates how much in percentage the biopolymers incorporated with varying concentrations of *H. stigonocarpa* and *H. courbaril* oil influence over time in the

soil. It is observed that the lowest oil concentrations (0.50%) for both species statistically presented biodegradability results close to the standard. Concentrations between 0.75 and 1% for *H. stigonocarpa* showed statistically similar degradation effects. All concentrations except 1% had a biodegradability rate greater than 90% for this study.

After the 25-day analysis, the hollow polyethylene packages that contained arrowroot starch films were removed from the soil. Thus, the conclusion may be the fact that the incorporation of fixed oil into films does not decrease the biodegradability of arrowroot starch, which may be considered a promising material for biodegradable packaging (Santos et al., 2020). Several studies show biodegradability time longer than 30 days (Seligra et al., 2016), Fernandes et al. (2020) obtained a time greater than 40 days in the soil biodegradation test for chia mucilage biofilm incorporated with chia oil. Arancibia et al. (2014) evaluated the biodegradability in topsoil for biofilms of a mixture of soy protein lignin with citronella essential oil, where they obtained a rate of reduction in the mass of biopolymer over six months. It is suggested that the degradation period is influenced by the type of polymer, incorporation, moisture, and degree of crosslinking, this is also suggested by Arancibia et al. (2014) and González et al. (2011). Where a low degree of crosslinking makes biopolymers more degradable in a shorter period.

As reported by Pantini and Sorrentino (2013), denser and more crystalline structures are expected to have a slower degradation rate compared to amorphous structures once it affects the water diffusion into de biofilm structure. The rapid degradation of the films in this study demonstrates the thesis proposed by the researchers, suggesting that the crystalline structure of arrowroot starch biofilms is less dense and easier to degrade in an environment with uncontrolled climatic effects.

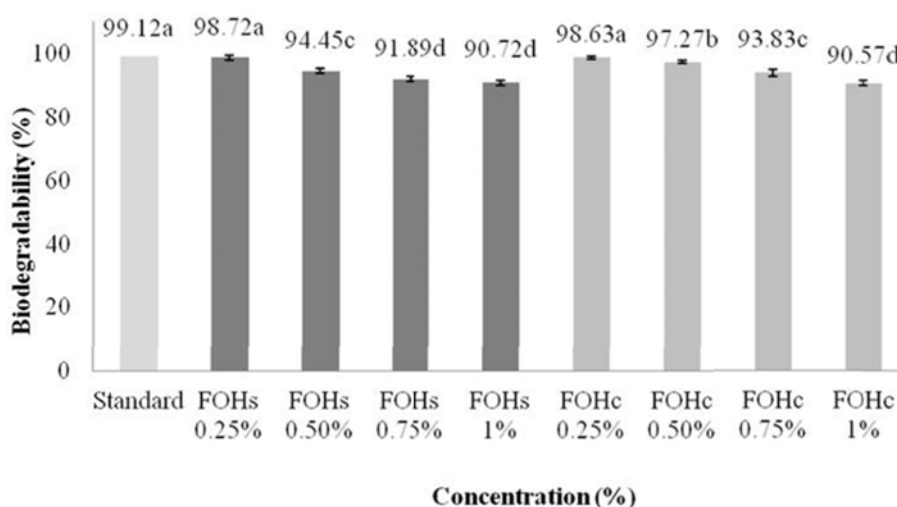


Figure 2. Biodegradability assay of arrowroot biofilms incorporated with fixed oil of

Hymenaea stigonocarpa and *Hymenaea courbaril*. Different letters in the bars show significant differences ($p < 5\%$) in Duncan's test. Source: Authors, 2022.

Arrowroot starch biofilms incorporated with oils of *H. stigonocarpa* and *H. courbaril* at concentrations of 0.25-0.50% showed homogeneous surface area (Figure 3, B-C, K-L) 3D imaging through pixel staking provided a color image over the surface area and its relief, where the red color represents a homogeneous plateau (Figure 3, G-H, P-Q). And green presents a shapeless sinuous plateau. It is suggested that this characteristic may be involved with the drying process, emulsion, and molecular conjugation between the biopolymer and fixed oil.

Embedded biofilms with concentrations of 0.75-1% showed bubbles and small cracks for both fixed lobes of *Hymenaea* (Figure 3, D-E, M-N). As for the 3D imaging, a deep heterogeneous plateau can be seen in (Figure 3, I), and the same is observed in (Figure 3, R). Arrowroot polymer showed homogeneity in all samples when compared to concentrations (Figure 3, A-F). 3D imaging presents a satisfactory attribute in the morphological analysis of biofilms, as also stated by Menezes Filho et al. (2019) who evaluated this imaging technique in watermelon biofilm.

This means that most formulations were successful in forming biofilms that were not too sticky or brittle (Figure 3). Guar gum biofilms incorporated with orange oil and glycerol as plasticizers also showed similar characteristics to the treatments in this study, although Aydogdu et al. (2020) have not reported the formation of bubbles. Guar gum biofilms incorporated with orange oil and glycerol as plasticizers also showed similar characteristics to the treatments in this study, although Aydogdu et al. (2020) have not reported the formation of bubbles. This study suggests that mechanical stirring by Vortex at the time of oil addition may be the cause of bubbles, however, future studies should be carried out evaluating an alternative to substitute for concentrations above 0.75%.

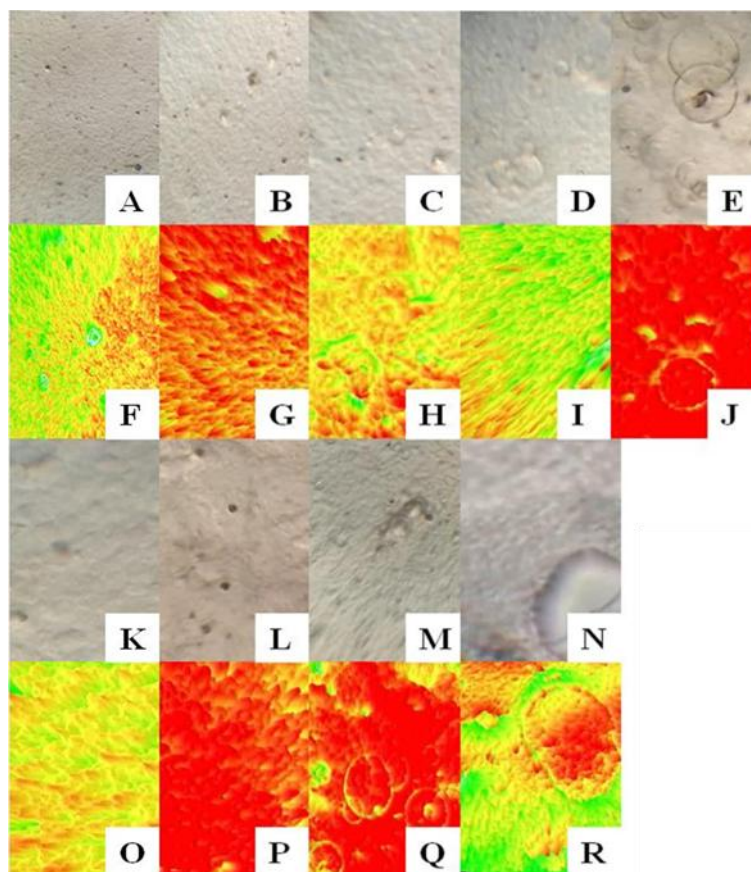


Figure 3. Micrographs and 3-D surface area imaging analysis of arrowroot starch biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril*. (A-F) Control biofilm. (B-G) FOHs 0.25%. (C-H) FOHs 0.50%. (D-I) FOHs 0.75%. (E-J) FOHs 1%. (K-O) FOHc 0.25%. (L-P) FOHc 0.50%. (M-Q) FOHc 0.75%. (N-R) FOHc 1%. Source: Authors, 2022.

According to Aydogdu et al. (2020), FTIR analyses permit the investigation of interactions between functional groups of the chains and can be recognized as shifts in the chains and vibration IR bands. FTIR spectra of biofilms are given in (Figure 4).

The spectra of the control and biofilms fixed oil concentration showed similar behavior with elongation bands of OH and N–H flexion at 3329 and 3315 cm^{-1} , C=O stretching at 1662 and 1650 cm^{-1} (amide I), C–N and N–H vibration at 1377 and 1369 cm^{-1} (amide III), and C–H stretching band at 2925 and 2924 cm^{-1} . The bands located between the 800 and 1150 cm^{-1} region are related to C–C bonds (864 and 858 , and 998 and 996 cm^{-1}), and C–O (at 1050 and 1151 cm^{-1} corresponds to the C–O bond at C1 and C3 and 1111 and 1114 cm^{-1} is the C – O bond in C2) of glycerol, respectively of biofilms incorporated FOHs and FOHc. At 2362 cm^{-1} , in Figure 3 (A), a strong and narrow band

is observed, suggesting the presence of (alkyne) $C\equiv C$ for the biofilm with 1% FOHs (Aydogdu et al., 2020; Khah et al., 2021; Hejazi et al., 2021).

There were no significant changes in the characteristic peaks of oils in the biofilm matrix. In addition, the reduction in the intensity of the characteristic bands of glycerol and a slight displacement of the bands at 1050 and 1110 cm^{-1} suggest an interaction between glycerol and biopolymer (Aydogdu et al., 2020).

Gelatin-pectin biofilms incorporated with virgin olive oil and grape seed oil analyzed by Khah et al. (2021) presented similar results for most functional groups characteristic of natural polymers incorporated with fatty acids obtained from vegetables. Aydogdu et al. (2020) also did not observe changes in functional groups in guar gum biofilms incorporated with orange oil, like this study. The researchers add that the low concentration of oil used may be related to this characteristic, where no extra band is due to the lack of covalent bonds between the oil of *Hymenaea*, with the matrix and the plasticizer glycerol.

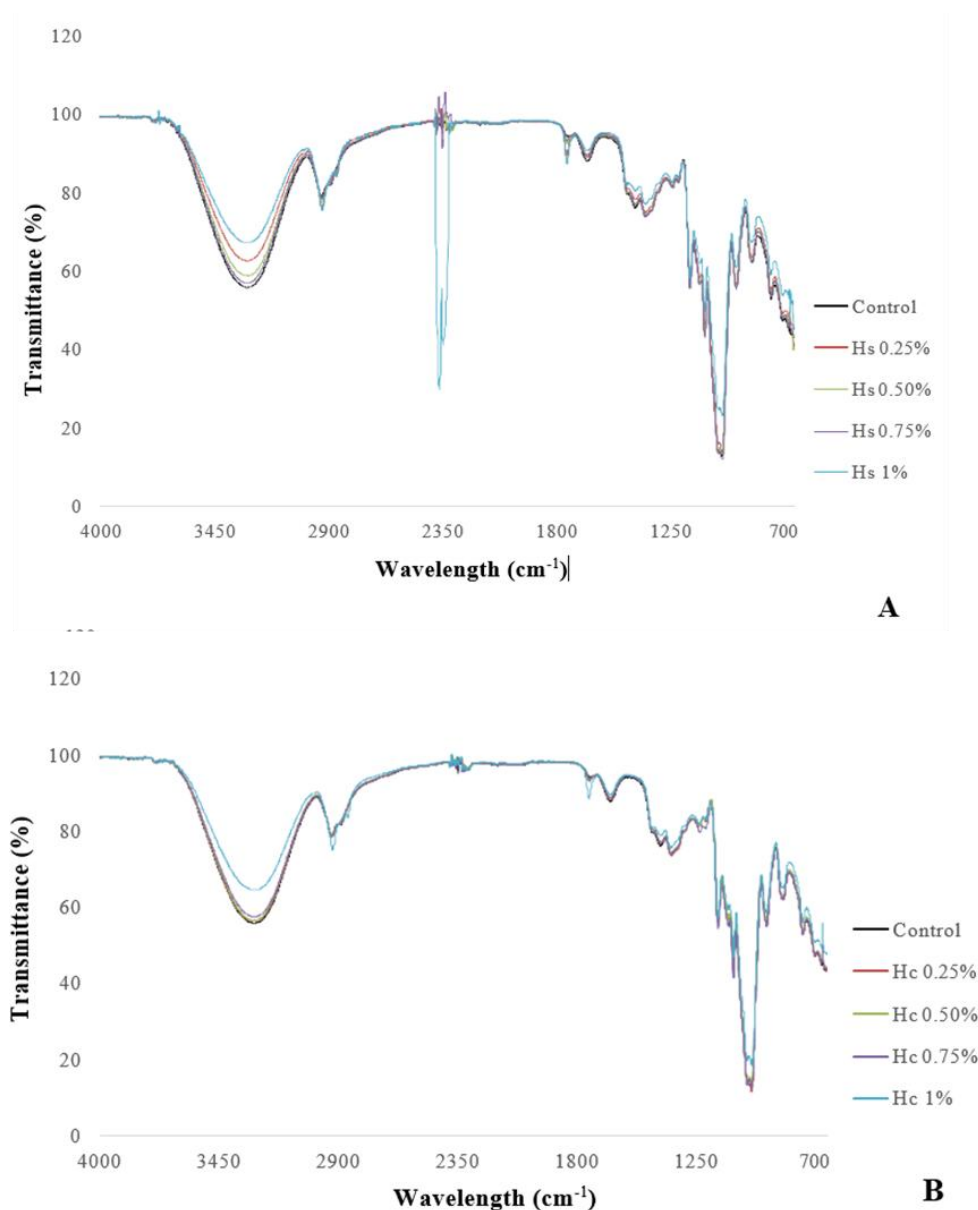


Figure 4. FTIR-ATR spectra of the arrowroot biofilms incorporated with fixed oil *Hymenaea stigonocarpa* (A) and *Hymenaea courbaril* (B). Source: Authors, 2022.

Biofilms incorporated with concentrations above 0.50% showed antifungal activity on all evaluated phytopathogens. However, the 1% concentration proved to be more effective in inhibiting the development of fungi, especially for *C. acutatum*, *C. gloeosporioides*, *A. niger*, and *R. stolonifer* (Table 3) for FOHs and FOHc. When compared to standard antifungals, the *Duncan* test ($p < 5\%$) demonstrates a statistical difference between concentrations and standards. It is noteworthy that the antifungals Frowncide 500 SC and Amphotericin B are synthetic molecules and the Botector is a

conjugation of microorganisms, which is a biological antifungal.

Control biofilm exhibited no antimicrobial activity, as expected. It is suggested that the different inhibition activities of the evaluated fungi are involved in the structural characteristics of the cell wall. Arruda et al. (2019) also suggest such a statement because the complex structure of this wall is constituted by polysaccharides, linked or not to proteins or lipids, polyphosphates, and inorganic ions. The results of this study demonstrate that the use of both oils from *Hymenaea* species is promising for the alternative control of these phytopathogens when used in biodegradable packaging.

The study of fixed oils incorporated in biopolymeric matrices is scarce in the literature. Although there are studies evaluating the lipid fraction extracted from numerous vegetables on different forms of pathogenic and phytopathogenic fungi. Aydogdu et al. (2020) found potential antibacterial activity against *Escherichia coli* and lower inhibition activity against *Bacillus subtilis* in conc. 1%, and higher activity in conc. 2%.

Arruda et al. (2019) did not find antifungal activity on *Rhizoctonia solani* and *Sclerotium rolfsii* evaluating the fixed oil of *Jatropha curcas* seed. Ali et al. (2017) developed nanoemulsions incorporated with neem and citronella oils where they found important phytopathogenic activity on *R. solani* and *S. rolfsii*. Passos et al. (2002) report in a study using *Caryocar brasiliense* seed and almond oil high antifungal action on *Cryptococcus neoformans* in conc. $15.6 \mu\text{g mL}^{-1}$, where the seed oil showed 21.1% inhibition, and $62.5 \mu\text{g mL}^{-1}$ with 10.5% for the almond oil.

Table 3. Antifungal activity of biofilms incorporated with fixed oil of *Hymenaea stigonocarpa* and *Hymenaea courbaril* at different concentrations on phytopathological microorganisms.

Microorganisms	Inhibition Zone (mm) Concentrations (%) FOHs					Standard antifungal
	Control	0.25%	0.50%	0.75%	1%	
<i>C. acutatum</i>	0.00±0.00d	0.00±0.00d	0.00±0.00d	6.34±0.09c	8.17±0.08b	100±0.00a
<i>C. gloeosporioides</i>	0.00±0.00d	0.00±0.00d	0.00±0.00d	8.53±0.04c	10.85±0.09b	100±0.00a
<i>A. tubingensis</i>	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	6.57±0.43b	100±0.00a
<i>A. fumigatus</i>	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	6.22±0.29b	100±0.00a
<i>A. niger</i>	0.00±0.00d	0.00±0.00d	0.00±0.00d	7.89±0.98c	13.28±0.99b	100±0.00a
<i>R. Stolonifer</i>	0.00±0.00e	0.00±0.00e	0.00±0.00d	9.39±0.68d	14.42±0.86b	100±0.00a
Microorganisms	Inhibition Zone (mm) Concentration (%) FOHc					Standard

	Control	0.25%	0.50%	0.75%	1%	antifungal
<i>C. acutatum</i>	0.00±0.00d	0.00±0.00d	0.00±0.00d	6.15±0.08d	10.10±0.07b	100±0.00a
<i>C. gloeosporioides</i>	0.00±0.00c	0.00±0.00c	0.00±0.00d	4.63±0.09c	5.47±0.07b	100±0.00a
<i>A. tubingensis</i>	0.00±0.00b	0.00±0.00b	0.00±0.00d	0.00±0.00b	0.00±0.00b	100±0.00a
<i>A. fumigatus</i>	0.00±0.00c	0.00±0.00c	0.00±0.00d	0.00±0.00c	7.41±0.08b	100±0.00a
<i>A. niger</i>	0.00±0.00c	0.00±0.00c	0.00±0.00d	0.00±0.00c	9.77±0.04b	100±0.00a
<i>R. stolonifer</i>	0.00±0.00d	0.00±0.00d	6.15±0.07d	7.42±0.00c	11.07±0.09b	100±0.00a

Note. Different letters in a line show significant differences ($p < 5\%$) in *Duncan's* test.

4. CONCLUSIONS

This study revealed that fixed oil from seed of *Hymenaea stigonocarpa* and *Hymenaea courbaril* has great potential for application in the manufacture of bioactive packaging. Both oils have antioxidant activity. The presence of oleic, linoleic, palmitic, and myristic acids was observed in the samples. Physicochemical observations related to the incorporation of the fixed oil of *H. stigonocarpa* and *H. courbaril* into arrowroot biofilms led to an increase in thickness, decrease in moisture, solubility transparency, and change in color as its concentrations increased.

Biofilms also showed variation according to the increase in oil concentration over the percentage of biodegradability, in the structural morphology of the surface area with bubbles at concentrations above 0.75%, functional groups are below expectations, and antifungal activity was also observed on *Colletotrichum*, *Aspergillus*, and *Rhizopus*.

5. REFERENCES

- Adegbe, A. A., Larayetan, R. A., & Omojuwa, T. J. (2016). Proximate analysis, physicochemical properties and chemical constituents characterization of *Moringa oleifera* (Moringaceae) seed oil using GC-MS analysis. *American Journal of Chemistry*, 6, 23-28. 10.5923/j.chemistry.20160602.01
- Ali, E. O. M., Shakil, N. A., Rana, V. S., Sarkar, D. J., Majumder, S., Kaushik, P., Brushan, B. B., & Kumar, J. (2017). Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. *Industrial Crops & Products*, 108, 379-387. <https://doi.org/10.1016/j.indcrop.2017.06.061>

- Arancibia, M. Y., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2014). Release of volatile compounds and biodegradability of active soy protein ligning blend films with added citronella essential oil. *Food Control*, 44, 7-15. <https://doi.org/10.1016/j.foodcont.2014.03.025>
- Arruda, E. L., Lopes, M. B. S., Júnior, A. F. C., Fidelis, R. R., & Santos, M. M. (2019). Atividade antifúngica de negramina, eucalipto e pinhão manso sobre os fitopatógenos. *Applied Research & Agrotechnology*, 12, 47-58. <https://doi.org/10.5935/PAeT.V12.N2.04>
- Aydogdu, A., Radke, C. J., Bezci, S., & Kirtil, E. (2020). Characterization of curcumin incorporated guar gum/orange oil antimicrobial emulsion films. *International Journal of Biological Macromolecules*, 148, 110-120. <https://doi.org/10.1016/j.ijbiomac.2019.12.255>
- Beikzadeh, S., Khezerlou, A., Jafari, S. M., Pilevar, Z., & Mortazavian, A. M. (2020). Seed mucilages as the functional ingredients for biodegradable films and edible coatings in the food industry. *Advances in Colloid and Interface Science*, 280. <https://doi.org/10.1016/j.cis.2020.102164>
- Bueno, M. L., Dexter, K. G., Pennington, R. T., Pontara, V., Neves, D. M., Ratter, J. A., & Oliveira-Filho, A. T. (2018). The environmental triangle of the Cerrado domain: Ecological factors driving shifts in tree species composition between forests and savannas. *Journal of Ecology*, 106, 2109-2120. <https://doi.org/10.1111/1365-2745.12969>
- Damm, U., Cannon, P. F., Woudenberg, J. H. C., & Crous, P. W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology*, 73, 37-113. <https://doi.org/10.3114/sim0010>
- Elagbar, Z. A., Naik, R. R., Shakya, A. K., & Bardaweel, S. K. (2016). Fatty acids analysis, antioxidant and biological activity of fixed oil of *Annona muricata* L. seeds. *Journal of Chemistry*, 2016, 1-6. <https://doi.org/10.1155/2016/6948098>

Fernandes, S. S., Romani, V. P., Filipini, G. S., & Martins V. G. (2020). Chia seeds to develop new biodegradable polymers for food packaging: Properties and biodegradability. *Polymer Engineering & Science*, 60, 2214-2223. <https://doi.org/10.1002/pen.25464>

Ferreira, C. J. R., Kisukuri, T. C., Fluminhan, A., & Grigoli, A. A. (2015). Análises de parâmetros físico-químicos do óleo extraído de espécies vegetais utilizadas para a produção do biodiesel. *Colloquium Exactarum*, 7, 130-141. <https://doi.org/10.5747/ce.2015.v07.n2.e121>

Galus, S., & Kadzińska, J. (2016). Moisture sensitivity, optical, mechanical and structural properties of whey protein-based edible films incorporated with rapeseed oil. *Food Technology and Biotechnology*, 54, 78-89. <https://doi.org/10.17113/ftb.54.01.16.3889>

Gandini, A., Carvalho, A. J. F., Trovatti, E., Kramer, R. K., & Lacerda, T. M. (2018). Macromolecular material based on the application of the diels-alder reaction to natural polymers and plant oils. *European Journal of Lipid Science and Technology*, 120. <https://doi.org/10.1002/ejlt.201700091>

González, A., Strumia, M. C., & Alvarez Igarzabal, C. I. (2011). Cross-linked soy protein as material for biodegradable films: synthesis, characterization and biodegradation. *Journal of Food Engineering*, 106, 331-338. <https://doi.org/10.1016/j.jfoodeng.2011.05.030>

Hejazi, M., Zareshahrabadi, Z., Ashayeri, S., Saharkhiz, M. J., Iraji, A., Alishahi, M., & Zomorodian, K. (2021). Characterization and physical and biological properties of tissue conditioner incorporated with *Carum copticum* L. *BioMed Research International*, 2021, 1-10. <https://doi.org/10.1155/2021/5577760>

Henry, B., Laitala, K., & Klepp, I. G. (2019). Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. *Science of the Total Environment*, 652, 483-494. <https://doi.org/10.1016/j.scitotenv.2018.10.166>

- Hosseini, S. F., Rezaei, M., Zandi, M., & Farahmandghavi, F. (2015). Fabrication of bio-nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles. *Food Hydrocolloids*, 44, 172-183. <https://doi.org/10.1016/j.foodhyd.2014.09.004>
- Ikarugi, M., Celoto, B., Papa, M. F. S., Sacramento, L. V. S., & Celoto, F. J. (2008). Atividade antifúngica de extratos de plantas a *Colletotrichum gloeosporioides*. *Acta Scientiarum Agronomy*, 30, 1-5. <https://doi.org/10.4025/actasciagron.v30i1.1104>
- Kadzińska, J., Bryś, J., Ostrowska-Ligęza, E., Estéve, M., & Janowicz, M. (2020). Influence of vegetable oils addition on the selected physical properties of Apple-sodium alginate edible films. *Polymer Bulletin*, 77, 883-900. <https://doi.org/10.1007/s00289-019-02777-0>
- Kalpana, S., Priyadarshini, S. R., Maria Leena, M., Moses, J. A., & Anandharamakrishnan, C. (2019). Intelligent packaging: Trends and applications in food systems. *Trends in Food Science & Technology*, 93, 145-157. <http://doi.org/10.1016/j.tifs.2019.09.008>
- Khah, M. D., Ghanbarzadeh, B., Nezhad, L. R., & Ostadrahimi, A. (2021). Effect of virgin olive oil and grape seed oil on physicochemical and antimicrobial properties of pectin-gelatin blend emulsified films. *International Journal of Biological Macromolecules*, 171, 262-274. <https://doi.org/10.1016/j.ijbiomac.2021.01.020>
- Li, Q., Li, C., Li, P., Zhang, H., Zhang, X., Zheng, X., ... Sun, Y. (2017). The biocontrol effect of *Sporidiobolous pararoseus* Y16 against postharvest diseases in table grapes caused by *Aspergillus niger* and the possible mechanisms involved. *Biological Control*, 113, 18-25. <https://doi.org/10.1016/j.biocontrol.2017.06.009>
- Martucci, J. F., & Ruseckaite, R. A. (2009). Tensile properties, barrier properties, and biodegradation in soil of compression-molded gelatin-dialdehyde starch films. *Journal of Applied Polymer Science*, 112, 2166-2178. <https://doi.org/10.1002/app.29695>
- Menezes Filho, A. C. P., Oliveira Filho, J. G., & Castro, C. F. S. (2020). Avaliações antioxidante e antifúngica dos óleos essenciais de *Hymenaea stigonocarpa* Mart. ex

Hayne e *Hymenaea courbaril* L. *Journal of Biotechnology and Biodiversity*, 8, 104-114. <https://doi.org/10.20873/jbb.uft.cemaf.v8n2.menezes>

Menezes Filho, A. C. P., Souza, J. C. P., & Castro, C. F. S. (2019). Avaliação das características poliméricas do biofilme do resíduo de melancia. *Scientia Plena*, 15. <https://doi.org/10.14808/sci.plena.2019.080202>

Nielsen, T. D., Hasselbalch, J., Holmberg, K., & Stripple, J. (2020). Politics and the plastic crisis: A review throughout the plastic life cycle. *WIREs Energy and Environment*, 9. <https://doi.org/10.1002/wene.360>

Nor Adilah, A., Jamilah, B., Noraniza, M. A., & Nur Hanani, Z. A. (2018). Utilization of mango peel extracts on the biodegradable films for active packaging. *Food Packaging and Shelf Life*, 16, 1-7. <https://doi.org/10.1016/j.fpsl.2018.01.006>

Pantani, R., & Sorrentino, A. (2013). Influence of crystallinity on the biodegradation rate of injection-molded poly(lactic acid) samples in controlled composting conditions. *Polymer Degradation and Stability*, 98, 1089-1096. <https://doi.org/10.1016/j.polymdegradstab.2013.01.005>

Passos, X. S., Santos, S. C., Ferri, P. H., Fernandes, O. F. L., Paula, T. F., Garcia, A. C. F., & Silva, M. R. R. (2002). Atividade antifúngica de *Caryocar brasiliensis* (Caryocaraceae) sobre *Cryptococcus neoformans*. *Revista da Sociedade Brasileira de Medicina Tropical*, 35, 623-627. <https://doi.org/10.1590/S0037-86822002000600013>

Pereda, M., Amica, G., & Marcovich, N. E. (2012). Development and characterization of edible chitosan/olive oil emulsion films. *Carbohydrates Polymers*, 87, 1318-1325. <https://doi.org/10.1016/j.carbpol.2011.09.019>

Pereira, S. R., Giraldelli, G. R., Laura, V. A., & Souza, A. L. T. (2011). Tamanho de frutos e de sementes e sua influência na germinação de jatobá-do-cerrado (*Hymenaea stigonocarpa* var. *stigonocarpa* Mart. ex Hayne, Leguminosae-Caesalpinoideae). *Revista Brasileira de Sementes*, 33, 141-148. <https://doi.org/10.1590/S0101-31222011000100016>

Santos, L. S., Fernandes, C. C., Santos, L. S., Deus, I. P. B., Sousa, T. L., & Miranda, M. L. D. (2020). Ethanolic extract from *Capsicum chinense* Jacq. Ripe fruits: phenolic compounds, antioxidant activity and development of biodegradable films. *Food Science and Technology*, 41, 497-504. <https://doi.org/10.1590/fst.08220>

Seligra, P. G., Jaramillo, C. M., Famá, L., & Goyanes, S. (2016). Biodegradable and non-retrogradable eco-films based on starch-glycerol with citric acid as crosslinking agent. *Carbohydrate Polymers*, 138, 66-74. <https://doi.org/10.1016/j.carbpol.2015.11.041>

Silva, P. O. (2018). Fenologia reprodutiva de *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae). *Acta Biológica Catarinense*, 5, 89-97. <http://186.237.248.25/index.php/ABC/article/view/304>

Souza, G. F., Mossini, S. A. G., Arroteia, C. C., Kemmelmeier, C., & Junior, M. M. (2014). Evaluation of the mycoflora and aflatoxins from the pré-harvest to storage of peanuts: a case study. *Acta Scientiarum Agronomy*, 36, 27-33. <https://doi.org/10.4025/actasciagron.v36i1.16972>

Souza, K. C., Correa, L. G., Silva, T. B. V., Oliveira, A. O., Sakanaka, L. S., Dias, M. I., Shirai, M. A. (2020). Soy protein isolate films incorporated with pinhão (*Araucaria angustifolia* (Bertol.) Kuntze) extract for potential use as edible oil active packaging. *Food and Bioprocess Technology*, 13, 998-1008. <https://doi.org/10.1007/s11947-020-02454-5>

Tahir, N. I., Hussain, S., Javed, M., Rehman, H., Shahzady, T. G., Parveen, B., & Ali, K. G. (2018). Nature of aflatoxins: Their extraction, analysis, and control. *Journal of Food Safety*, 38. <https://doi.org/10.1111/jfs.12561>

Tavassoli-Kafrani, E., Shekarchizadeh, H., & Masoudpour-Behabadi, M. (2016). Development of edible films and coatings from alginates and carrageenans. *Carbohydrate Polymers*, 137, 360-374. <https://doi.org/10.1016/j.carbpol.2015.10.074>

Verdi, A. G., Souza, A. G., Rocha, D. B., Oliveira, S. A., Alves, R. M. V., & Rosa, D. S. (2021). Biodegradable films functionalized with *Moringa oleifera* applied in food packaging. *Iranian Polymer Journal*, 30, 235-246. <https://doi.org/10.1007/s13726-020-00885-7>

Weir, B. S., Johnston, P. R., & Damm, U. (2012). The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, 73, 115-180. <https://doi.org/10.3114/sim0011>

CHAPTER II – ACTIVE AND INTELLIGENT BIODEGRADABLE PACKAGING WITH *Hymenaea stigonocarpa*, *H. courbaril* AND *Annona crassiflora* OIL AND *Syzygium cumini* EXTRACT FOR CHEESE

(Article submitted for publication in the Food Science and Technology)

Abstract: This study aimed to develop biodegradable packaging materials composed of oils from *Hymenaea stigonocarpa*, *H. courbaril*, *Annona crassiflora*, and an extract from *Syzygium cumini*, applied as a coating on mozzarella cheese. The materials were evaluated for morphological, physicochemical, mechanical, and antimicrobial parameters. Oils and extracts were extracted, and the packaging materials were subsequently produced. The extract was evaluated for its antibacterial and antioxidant activities. The packaging materials and coated mozzarella cheese were analyzed for thickness, moisture content, solubility, biodegradability, total titratable acidity, color, and microbiological parameters. The anthocyanin content was measured at 349.90 mg L⁻¹. Packaging with increasing concentrations of oil and extract showed greater thickness, lower moisture content, and reduced solubility. Luminosity and chromaticity values (a* and b*) varied according to the incorporated material. Packaging composed of the extract demonstrated intelligent responses under different pH conditions. Neither color nor titratable acidity showed significant changes after 15–25 days of storage. The biodegradability of coated mozzarella cheese indicated degradation times of less than 35 days. Cheese coated with active and intelligent packaging showed no microbiological activity. Active packaging containing oils from *Hymenaea stigonocarpa*, *H. courbaril*, and *Annona crassiflora*, along with intelligent packaging with *Syzygium cumini* extract, demonstrated promising potential for food applications.

Keywords: mozzarella cheese, antioxidant activity, storage, smart packaging, antibacterial activity.

CAPÍTULO II – EMBALAGENS BIODEGRADÁVEIS ATIVAS E INTELIGENTES COM ÓLEO DE *Hymenaea stigonocarpa*, *H. courbaril* E *Annona crassiflora* E EXTRATO DE *Syzygium cumini* PARA QUEIJO

(Artigo submetido para publicação na Food Science and Technology)

Resumo: Este estudo teve por objetivo, desenvolver embalagens biodegradáveis compostas com óleos de *Hymenaea stigonocarpa*, *Hymenaea courbaril*, *Annona crassiflora*, e extrato de *Syzygium cumini* sobre revestimento de queijo tipo Muçarela avaliando parâmetros morfológicos, físico-químicos, mecânicos e antimicrobianos. Óleos e extrato foram extraídos e embalagens produzidas. O extrato foi avaliado quanto a atividade antibacteriana e antioxidante. Embalagens e queijo muçarela revestidos foram avaliados quanto a espessura, umidade, solubilidade, biodegradabilidade, acidez titulável total, cor e parâmetros microbiológicos. Teor de antocianinas foi de 349,90 mg L⁻¹, embalagens em concentração crescente de óleo e extrato apresentaram maiores espessuras, e menor umidade e solubilidade. Luminosidade, cromas (a* e b*) apresentaram variações conforme material incorporado. Embalagens compostas por extrato apresentaram ação inteligente em diferentes pHs. Cor e acidez titulável não apresentaram grandes variações com 15-25 dias de armazenamento. A biodegradabilidade de queijos muçarela revestidos apresentaram tempo de biodegradação inferior a 35 dias. Queijos revestidos com ativos e inteligentes não demonstraram atividade microbiológica. Embalagens ativas compostas com óleos de *Hymenaea stigonocarpa*, *H. courbaril* e *Annona crassiflora* e inteligentes com extrato de *Syzygium cumini* demonstraram potencial para uso em alimentos.

Palavras-chave: queijo muçarela, atividade antioxidante, armazenamento, embalagem inteligente, atividade antibacteriana.

1. INTRODUCTION

The future of packaging is approaching faster than we expected. Consumers with diverse demands for “green” labeled products are becoming increasingly demanding. This is due to the first packaging materials being produced from petroleum processing, which has resulted in a wide variety of packaging types being unsuitable for commercialization due to their long environmental degradation time. Particularly, the food industry, which requires tons of packaging for its products, has been developing new biodegradable packaging that can break down in a short period after controlled disposal (Filipini et al., 2020; Shaikh et al., 2021).

Several natural biopolymers are employed in the development of new bioplastics that do not alter product quality when used, while also minimizing the environmental impact caused by synthetic plastics, which interfere with the Earth's marine fauna and flora (Yong et al., 2019). Various edible polymeric bases are utilized, with chitosan, polysaccharides, lipids, proteins, gelatin, starch, and flour standing out as the most studied materials in bioplastic development due to their easy manipulation during the thermoplastic process (Abdillah; Charles, 2021).

According to Jiang et al. (2020), starch-based polymers are among the most promising commercial hydrocolloids for developing biodegradable packaging due to their low cost, shorter biodegradability period, easy production, good bioplastic formation properties, and environmental friendliness. However, a disadvantage of using starch-based packaging lies in its sensitivity to moisture, which reduces its mechanical properties, such as tensile strength, and increases water vapor permeability (Jiang et al., 2020; Wu et al., 2024).

Arrowroot starch (*Maranta arundinacea*), from the Marantaceae f., is a tuberous plant that produces high-quality starch due to its easy digestibility. It has medicinal properties and is successfully used in composite starches (Charles et al., 2016), serving as the polymeric base in this study. Arrowroot starch exhibits excellent absorption properties and compatibility when incorporating essential oils, particularly fixed oils and plant extracts (Tarique et al., 2022).

Several species of flora in the Cerrado domain produce fixed oil in their seeds, such as *Hymenaea stigonocarpa* Mart. ex Hayne and *Hymenaea courbaril* L., from the Fabaceae family and Caesalpinioideae subfamily, commonly known as “jatobás,” and *Annona crassiflora*, known as “araticum,” from the Annonaceae family. *H. stigonocarpa* is a late-secondary arboreal legume that grows in the Brazilian Cerrado on dry, nutrient-

poor soils, though it prefers well-drained ones. It can reach up to 20 meters in height and annually produces many fruits containing seeds. *H. courbaril* is a climax, semi-deciduous, heliophytic species that grows between 20 and 40 meters tall (Luz et al., 2023). *A. crassiflora* is a medium-sized arboreal species, reaching 4–8 meters in height, with edible fruits of pleasant taste (Arruda et al., 2023).

Regarding the use of plant extracts, various fruits contain anthocyanin compounds, such as the "jamun or jambolão" (*Syzygium cumini*), a species native to India that is widely distributed in Brazil, where it has adapted well to the country's diverse regions. *S. cumini* is a large tree species that can reach up to 15 meters in height and produces an abundant quantity of edible fruits rich in anthocyanins, flavonoids, and tannins. Additionally, it serves as a natural acid-base indicator (Filipini et al., 2020).

Special metabolites such as oils and extracts exhibit biological activities, including antioxidant, antidiabetic, anti-inflammatory, pro-apoptotic, and antiproliferative effects against various types of cancer. They are also excellent antifungal and antibacterial agents (Leite et al., 2020; Filipini et al., 2020; Menezes Filho et al., 2022; Almeida et al., 2024). In this context, the use of biodegradable packaging incorporating fixed oils from plant species demonstrates active functionality, while plant extracts with acid-base indicator properties exhibit intelligent functionality by changing color due to enzymatic activity, particularly in foods such as cheese.

This study aimed to evaluate the application of fixed oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora* as active packaging, and the extract of *Syzygium cumini* as intelligent packaging for Mozzarella cheese, using arrowroot starch as the biopolymer.

2. MATERIALS AND METHODS

2.1 Fixed oil extraction

Seeds (500 g) of *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* were collected from a reserve area in the municipality of Rio Verde, Goiás, Brazil, with coordinates (17°47'18.5''S and 50°57'56,3''W). The first author identified the species, and three specimens were deposited in the Herbarium of IF Goiano, Rio Verde, with voucher codes (HRV 13.2768; 13.2769, and 14.621), respectively.

The seeds were immersed in boiling water for 2 h and then cut using a stainless-steel knife. The cut seeds were dried in an oven at 65 °C for 3 h and subsequently

processed in a knife mill. The processed material was dried at 35 °C for 24 h, after which the oil was extracted using a Soxhlet-type system with *n*-hexane as the extraction solvent for 6 h. Following extraction, the oil was recovered using a rotary evaporator, and its mass was determined and expressed as a percentage of extraction (%). The obtained oil was stored refrigerated at -12 °C until analysis.

2.2 Production of jambolan extract

Fresh skin and fruit pulp (350 g) were obtained from *S. cumini* fruits collected in a preservation area in the municipality of Rio Verde, Goiás, Brazil, with coordinates (17°43'14.2''S and 50°53'04.7''W). A specimen was deposited in the Herbarium of IF Goiano with voucher code (HRV 4529).

To prepare the crude extract, 50 g of the pulp with skin was blended in a manual mixer with 100 mL of a 70% hydroethanolic solution (v/v). The mixture was kept in an amber flask at 8 °C for 48 h. After this period, the extract was filtered and centrifuged at 2500 rpm for 15 min, then stored under refrigeration at 8 °C until analysis.

2.2.1 Anthocyanins content

The anthocyanin content was quantified using the pH differential method according to Giusti and Wrolstad (2001). Briefly, two dilutions of *S. cumini* extract were prepared with potassium chloride buffer (pH 1) or sodium acetate buffer (pH 4.5) to achieve absorbance between 0.2 and 0.8 at 520 nm ($\lambda_{vis-max}$) in a UV-Vis spectrophotometer to determine the dilution factor (DF). The absorbances of the solutions were measured at $\lambda_{vis-max}$ and 700 nm against a quartz cuvette blank containing distilled water. The anthocyanin content was calculated according to Equation (1).

$$ANT \text{ (mg L}^{-1}\text{)} = (A \times MW \times DF \times 1000) / (\epsilon \times 1) \quad (1)$$

Where A was calculated as $A = (A_{\lambda_{vis-max}} - A_{700 \text{ nm}})_{pH 1} - (A_{\lambda_{vis-max}} - A_{700 \text{ nm}})_{pH 4.5}$, being $A_{700 \text{ nm}}$ the absorbance values at 520 and 700 nm, respectively, at pH 1 and pH 4.5; MW is the molecular mass of the predominant anthocyanin in the sample (449.2 g mol⁻¹); DF is the dilution factor of the extract; ϵ (26.900 L mol cm⁻¹).

2.3 Production of biodegradable packaging

The biodegradable packaging was produced using a casting technique, following the methodology described by Menezes Filho et al. (2022). To create the packaging, 5 g of commercial arrowroot starch was dissolved in 100 mL of deionized water. The solution was then stirred moderately at room temperature. Next, it was heated to 70 °C with constant stirring for 30 min.

After the starch gelatinization, glycerol was added as a plasticizer (30% w/w, equivalent to 1.5 g), and the mixture was stirred for 5 min. When the film-forming solution cooled to 30 °C, a previously prepared suspension of fixed oil from *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* in Tween 40 at concentrations of 0.25%, 0.50%, 0.75%, and 1% (g/g fixed oil) was incorporated under constant stirring for 15 min. The extract from *S. cumini* was prepared using the same methodology as the oils.

The final concentrations of fixed oil from the seeds were 0.25%, 0.50%, 0.75%, and 1% (v/v), along with a control treatment without fixed oil or extract. Film-forming solutions made from arrowroot starch, which incorporated the fixed oil or extract, were maintained at 30 °C and then used to coat the cheese.

2.4 Application of biodegradable packaging in cheese

Eighteen pieces of Mozzarella cheese were purchased from a local market (artisanal cheese). Ingredients: Pasteurized milk, calcium chloride, rennet, sodium chloride, and lactic ferments (*Lactococcus lactis* and *Lactococcus cremoris*). The production process followed these steps: pasteurization at 65 °C for 30 min, preparation of the milk for coagulation, treatment of the curd, stirring and cooking of the curd, whey drainage, molding, cooling, salting, packaging (vacuum packaging in plastic (Cryovac), and storage. The cheeses were stored in a BOD incubator at 10 °C for the curing process for 5 days and kept in Starpack packaging.

The cheeses were cut into 50 g squares and packaged in transparent Starpack (PP 04) 1000 mL containers, lined with transparent PVC film and biodegradable packaging containing oil, extract, or a control. Fourteen treatments were evaluated for the cheese type analyzed, as follows: T1 - transparent PVC packaging; T2 - biodegradable control packaging (without any oil or extract); T3 to T6 - varying concentrations of *H. stigonocarpa* oil (0.25%, 0.50%, 0.75%, and 1%, respectively); T7 to T10 - concentrations of *H. courbaril* oil (0.25%, 0.50%, 0.75%, and 1%); T11 to T14 - *A. crassiflora* oil (0.25%, 0.50%, 0.75%, and 1%); and T15 to T18 - *S. cumini* extract

concentrations (0.25%, 0.50%, 0.75%, and 1%). The experiment was performed in quadruplicate.

2.5 Physicochemical analysis

2.5.1 Biofilms

The thickness of the biofilms was measured using a digital caliper, with results expressed in millimeters (mm) (Santos et al., 2021). The biofilms pH was evaluated using a digital pH meter (Brasil, 2006). A 1 g biofilm sample was added to a beaker containing 50 mL of distilled water. The mixture was homogenized for 1 min and allowed to rest for 5 min. After this time, the pH was measured.

The water content was determined from a 1 g aliquot of dehydrated biofilm in an oven with forced air circulation at 105 °C for 24 h (AOAC, 1997). The solubility determination of the biofilms in water was performed according to the methodology described by Bertuzzi et al. (2007), adapted. The biofilms were cut into squares (2x2 cm), dried in an oven at 45 °C for 24 h, and the initial mass was recorded (M1). The samples were then immersed in 50 mL of distilled water and maintained under agitation at 25 °C for 24 h. The biofilm was removed and left at 45 °C for 24 h to dry (M2), and its mass was measured to calculate (Equation 2) the amount of insoluble biofilm expressed as a percentage.

$$\text{Sol}(\%) = (M1 - M2)/(M1) \times 100 \quad (2)$$

Where: Sol is solubility; M1 is the initial mass, and M2 is the final mass.

The color parameters were analyzed using a Hunter Lab color spectrophotometer, model Color Flex EZ. Objective color quantification was performed using a tristimulus colorimeter, with direct reflectance readings of the chromaticity coordinates “L” (lightness), “a” (green shades -60 to red +60), and “b” (blue shades -60 to yellow +60), employing the Hunter-Lab scale (Valadares et al., 2020). Ultraviolet and visible light transmittance of biofilms was conducted using a UV-Vis spectrophotometer. Biofilm samples were cut and placed in cuvettes to measure transmittance over a wavelength range between 850 and 250 nm (Hosseini et al., 2015). Pieces measuring (2 x 2 cm) were used to assess the biofilms' qualitative color change. Acidic solutions with pH 2 and 4, neutral solutions with pH 6, and alkaline solutions with pH 8 and 12 were prepared in *Petri* dishes. After 15 min, the biofilm color change was evaluated (Silva et al., 2020).

The biodegradability was carried out by the methodology described by Martucci; Ruseckaite, (2009) and modified by Santos et al. (2021). Biodegradable film samples (2 x 2 cm) were dried up to constant weight so that initial mass (M_i) could be determined. Samples were then placed in open tea packages to enable microorganisms and moisture to gain access to them. After that, they were buried in (*in natura*) organic soil, which had been previously prepared, at constant moisture and room temperature 25 °C. 30 days after the experiment was installed, the sachets containing biofilm samples were removed from the soil, brushed, and dried until constant weight (M_f). Biodegradability expressed in (%) was calculated by equation (3):

$$\text{Biodegradability (\%)} = [(M_f - M_i)/M_i] \times 100 \quad (3)$$

Biodegradable assay of the biofilms was carried out as described by Alves-Silva et al. (2022), with modification, in beach sand with seawater. The method of biodegradability in beach sand followed the methodology for biodegradability in soil *in natura*. All analyses were performed in quadruplicate, except for the thickness assay, which was conducted in decuplicate, and the UV-Vis transmittance and qualitative visual analysis, which were performed only once.

2.5.2 Antioxidant assay of the extract

The antioxidant activity test was performed using the DPPH method. The IC_{50} was calculated based on the equation obtained from the standard curve (Pratiwi et al., 2021). The ferric-reducing antioxidant power (FRAP) assay was performed as described by Ruan et al. (2008). Briefly, 3 mL of freshly prepared FRAP reagent was mixed with 0.1 mL of the extracted sample, or methanol (for the blank reagent). The FRAP reagent was prepared by dissolving 2.5 mL of 10 mM TPTZ in 40 mM HCl (w/v), 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 0.3 M acetate buffer (pH 3.6) (w/v). The absorbance of the reaction mixture was measured using a UV-Vis spectrophotometer at a wavelength of 593 nm after incubation at 25 °C for 10 min. All solutions were prepared on the day of analysis. The FRAP values were expressed in mmol equivalents of ascorbic acid (AAE) g of dry sample mass⁻¹, derived from a standard curve with $R^2 = 0.9990$.

The hydroxyl radical scavenging activity of the *S. cumini* extract was determined by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+} /ascorbate/EDTA/ H_2O_2 system. Different concentrations of the extract (1-25 $\mu\text{g mL}^{-1}$) were added to the reaction mixture containing 0.1 mL of 3.0 mM (w/v) deoxyribose, 0.5 mL of $FeCl_3$ (0.1 mM) (w/v), 0.5 mL of EDTA (0.1 mM) (w/v),

0.5 mL of Ascorbic acid (0.1 mM) (w/v), 0.5 mL of H₂O₂ (1 mM), and 0.8 mL of phosphate buffer (20 mM, pH 7.4) (w/v), making up a final volume of 3.0 mL. The reaction mixture was incubated at 37 °C for 1 h. An aliquot of 1 mL from the incubated mixture was then mixed with 1 mL of 10% (w/v) trichloroacetic acid and 1.0 mL of 0.5% (w/v) thiobarbituric acid. The solution was read using a UV-Vis spectrophotometer at a wavelength of 532 nm. The hydroxyl radical scavenging capacity was calculated and expressed as the percentage of inhibition of deoxyribose degradation. The IC₅₀ values (concentration of the sample required to scavenge 50% of the free radicals) were calculated using the regression equation derived from different extract concentrations.

The nitric oxide radical scavenging assay for the *S. cumini* extract was performed as described by Eshwarappa et al. (2014). Different concentrations of the *S. cumini* extract (1-25 µg mL⁻¹) were prepared and mixed with 2 mL of sodium nitroprusside (10 mM) (w/v) in standard phosphate-buffered saline solution (50 mM, pH 7.4) (w/v) and then incubated at room temperature for 3 h. After this incubation period, the samples were diluted with 0.5 mL of Griess reagents. The absorbance was then determined using a UV-Vis spectrophotometer at a wavelength of 550 nm. Ascorbic acid was used as the standard. The nitric oxide radical scavenging capacity was calculated and expressed as the percentage of inhibition, and the results were expressed in terms of the inhibition concentration (IC₅₀) (the concentration of the sample required to scavenge 50% of the free radicals), calculated using the regression equation derived from different extract concentrations.

2.5.3 Antibacterial activity

The antibacterial assay was conducted using the disk diffusion method as described by Mohamed et al. (2013). Sterile *Petri* dishes containing nutrient agar were prepared for the bacterial strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Salmonella enteritidis* (ATCC 90877), *Salmonella typhimurium* (ATCC 14028), *Bacillus subtilis* (ATCC 6051-U), and *Listeria monocytogenes* (ATCC 13932). Each strain was inoculated with 1 mL in the nutrient medium and spread evenly under aseptic conditions using a *Drigalski* loop.

Filter paper discs (7 mm diameter, Whatman no. 1) were prepared and sterilized. Aliquots of 5 mL of *S. cumini* extract (500 mg/mL⁻¹) and 5 mL of

Tetracycline/Fosfomycin/Erythromycin (2 mg/mL⁻¹; used as a positive control) were added to each disc. The sterile discs, impregnated with the extract, were then placed on the surface of the nutrient agar using flamed forceps. Filter paper discs soaked in 70% hydroethanolic solvent (v/v) served as a negative control. The antibacterial activity of the crude *S. cumini* extract was determined by measuring the mean diameter of the inhibition zone (in mm) produced after the incubation period.

2.5.3 Cheese coated with biofilm

The pH of the cheese samples with and without coating was obtained using a digital pH meter. A 1 g of aliquot sample for each treatment was collected and transferred to a beaker containing 25 mL of distilled water. The mixture was homogenized, and the reading was taken after 5 min of resting, as Brasil (2006) described. Total titratable acidity with and without coating was performed using the titrimetric method, employing 10 g of sample and a 0.1 N NaOH (w/v) solution, as described by the International Dairy Federation (1993) adapted.

The moisture content of the cheese with and without coating was obtained using the gravimetric method adapted in an oven at 105 °C for 24 h, as described by AOAC (1997). The color parameters for cheeses with and without coating were obtained using a Hunter Lab color spectrophotometer, model Color Flex EZ. The objective quantification of color was performed using a tristimulus colorimeter, with direct reflectance readings of the chromaticity coordinates “L” (lightness), “a” (green tones -60 to red +60), and “b” (blue tones -60 to yellow +60), employing the Hunter-Lab scale (Valadares et al., 2020) adapted.

The biodegradability of cheese coated with biofilms containing oil and/or extract was evaluated in natural dystrophic red soil. Samples of cheese coated in squares (2 x 2 cm) were placed in polyester fiber pouches and buried at a depth of 5 cm. The soil was moistened daily for 35 days. The integrity and rate of biodegradability were monitored over 35 days using an analytical balance, and the results were expressed as a percentage (%).

Regarding the microbiological quality of Mozzarella cheese, tests were conducted to detect *Listeria* spp., *Enterobacteria* (ETB), Coliforms (CF), and *Staphylococcus aureus* using Compact Dry[®] kits (PRLabor, Brazil). After 30 days of testing, a 1 g cheese sample was processed in distilled and sterilized water. 5 mL of the solution was

transferred to the plates and incubated for 24 h at 36 °C. After this time, the presence or absence of colonies was observed. All analyses were performed at 15 and 30 days in quadruplicate.

2.6 Statistical analysis

The means obtained in all analyses presented results with $\pm$ SD. The data were statistically analyzed by ANOVA and the means were compared by Duncan's multiple range significance test with $P < 5\%$ level using IBM SPSS Statistics 26 software.

3. RESULTS AND DISCUSSION

3.1 Anthocyanins content

The anthocyanins content in the extracted sample used in this study was 349.90 mg of anthocyanins L⁻¹, higher than the values obtained by Jampani et al. (2014), 123.07 mg L⁻¹, and Filipini et al. (2020), 236.3 mg of anthocyanins L⁻¹. Anthocyanins have a high capacity to reduce oxidizing agents that can interfere with biomolecules. Free radicals such as singlet oxygen–reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) are easily oxidized by anthocyanins. Additionally, anthocyanins can alter the pH of microorganisms, such as fungi and bacteria, inhibiting their antimicrobial effects (Tena et al., 2020).

3.2 Physicochemical parameters of the packaging

In our study, all packaging materials displayed a continuous structure without fractures or bubbles. The effects of the concentrations of *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* oils and *S. cumini* extract were evaluated regarding the physicochemical properties of all packaging at different concentrations. Thickness, moisture, and solubility are essential properties in the development of new food bioplastics. According to Filipini et al. (2020), different types of biodegradable packaging require materials with specific properties to ensure mechanical, sensory, and quality guarantees from production, storage, and distribution to the end consumer.

Table 1 shows the effects of different oils and extract concentrations on the properties of arrowroot starch-based packaging films. The oils and extract incorporation significantly affected ($p < 0.05$) thickness, moisture, and solubility as concentrations increased compared to the control. Packages containing 1% *H. stigonocarpa* and *A.*

crassiflora oils showed the greatest thickness among the other samples, measuring 0.40 and 0.41 mm, respectively, although no statistical difference was observed between them. Regarding moisture content in the control, it showed a distinction between samples with oils and extract.

Increasing oil concentrations decreases the moisture content. This can be easily explained by the hydrophobic interaction between water and oil (Chandler, 2002; Subbiahdoss & Reimhult, 2020). This effect was also observed by Menezes Filho et al. (2022) in arrowroot-based packaging composed of *H. stigonocarpa* and *H. courbaril* oils. However, packaging with extract behaved differently, as higher extract concentrations resulted in increased moisture content.

Samples containing *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* oils statistically showed lower solubility compared to the control. This is due to the hydrophobic effect exerted by the oil on water, limiting the matrix's solubility. Control samples and those with 0.25% extract exhibited higher solubility, although no significant difference was observed between them. This same effect was observed by Filipini et al. (2020) in methylcellulose-based packaging containing *S. cumini* extract, which proved to be completely water-soluble. This is attributed to the type of polymeric matrix used, as cellulose exhibits greater solubility compared to arrowroot starch. However, in our results, solubility decreased at concentrations above 0.75%. Similar results were also described by Santos et al. (2021), using arrowroot starch as the matrix and *Capsicum chinense* extract. This decrease in solubility was also reported by Adilah et al. (2018) in gelatin films containing mango peel extract, as hydrogen bonds formed between the extract and gelatin molecules, preventing hydrogen bonding with water.

Although it was observed low solubility, Filipini et al. (2020) discussed that the extract likely promotes intermolecular bonding between the packaging components, increasing water resistance. Further studies are needed to better understand this phenomenon. Koosha and Hamed (2019) noted that anthocyanin groups form hydrogen bonding interactions between their OH groups and the structure of the polymeric matrix.

Evaluating the application of highly soluble packaging, this characteristic is not advantageous for certain foods, such as meat or dairy products. However, it is beneficial for the production of soluble sachets for small portions of items like salt, sugar, powdered juice, coffee, or effervescent medications (Filipini et al., 2020).

Table 1. Thickness, humidity, and solubility parameters of arrowroot biofilms composed of *Hymenaea stigonocarpa*, *Hymenaea courbaril* and *Annona crassiflora* oil, and *Syzygium cumini* extract.

Biofilms	Thickness (mm)	Moisture (%)	Solubility (%)
Control	0.22 ± 0.01i	14.96 ± 0.46a	53.36 ± 2.06a
Hs 0.25%	0.25 ± 0.01gh	13.79 ± 0.22ab	48.13 ± 1.84abc
Hs 0.50%	0.28 ± 0.01fg	11.39 ± 0.59cd	46.84 ± 1.80abcd
Hs 0.75%	0.38 ± 0.04b	8.81 ± 0.40fg	44.13 ± 3.09cde
Hs 1%	0.40 ± 0.42a	6.15 ± 0.35ij	39.17 ± 3.14cde
Hc 0.25%	0.24 ± 0.01hi	13.64 ± 0.42b	48.11 ± 1.89abc
Hc 0.50%	0.26 ± 0.01gh	12.04 ± 0.67c	45.35 ± 3.22abcd
Hc 0.75%	0.30 ± 0.02de	9.86 ± 0.60ef	40.03 ± 4.41cde
Hc 1%	0.33 ± 0.02c	6.87 ± 0.34hi	33.50 ± 4.44ef
Ac 0.25%	0.28 ± 0.02fg	10.80 ± 0.33de	43.04 ± 4.84bcd
Ac 0.50%	0.29 ± 0.01ef	7.84 ± 0.64gh	36.53 ± 3.08def
Ac 0.75%	0.32 ± 0.01cd	6.76 ± 0.55hi	29.54 ± 1.58f
Ac 1%	0.41 ± 0.02a	5.08 ± 0.63j	20.17 ± 4.92g
Ext 0.25%	0.27 ± 0.01fg	6.94 ± 0.10hi	52.80 ± 2.76a
Ext 0.50%	0.29 ± 0.01ef	9.22 ± 0.33f	49.71 ± 4.10ab
Ext 0.75%	0.30 ± 0.01de	10.79 ± 0.41de	42.87 ± 3.67bcd
Ext 1%	0.33 ± 0.02cd	13.29 ± 0.34b	32.41 ± 6.09ef

Note: Hs = *Hymenaea stigonocarpa*. Hc = *Hymenaea courbaril*. Ac = *Annona crassiflora*. Ext = *Syzygium cumini* extract. Different letters in the same column indicate significant differences ($p < 0.05$). Source: Authors, 2024.

The optical properties of biodegradable packaging made with *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* oils, and *S. cumini* extract in an arrowroot starch matrix, on both the matte and glossy sides, are described in (Table 2). The packaging was analyzed according to the color parameters L*, a*, and b*. L* represents the brightness of the packaging (light/dark), a* is the red/green coordinate (+/-), and b* is the yellow/blue coordinate (+/-). The results showed that the oils and extracts incorporation affected the color of the packaging when compared to the control. For L*, the packaging incorporated with 0.50% *A. crassiflora* showed higher brightness compared to the control, which had

no oil or extract added. Regarding brightness in the other packaging with oils, no drastic changes were observed for L^* according to statistical analysis. For chroma (a^*), packaging with oil exhibited a tendency toward green ($-a^*$), and for chroma (b^*), a tendency toward blue. Packaging with *S. cumini* extract showed the lowest L^* values due to the color of the anthocyanins.

For L^* , the extract demonstrated greater brightness at all concentrations. For chroma (a^*), the packaging showed a tendency toward red, and for chroma (b^*), toward blue. Similar results were described by Filipini et al. (2020) in methylcellulose-based packaging containing different concentrations of *S. cumini* extract. With the increase in extract concentration, the packaging had a negative impact on brightness, as well as a decrease in blue (b^*) and an increase in red (a^*).

Several extracts are suitable for composing edible biodegradable packaging. Kontogianni et al. (2023) evaluated a whey protein packaging composed of spirulina, where they found that increasing concentrations of this algae extract negatively affected the L^* value when compared to the control. Chroma (a^*) showed a tendency toward green, and (b^*) toward yellow, except at the highest concentration (4%), where it tended toward blue.

Table 2. Color parameters in biofilms composed of oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril* and *Annona crassiflora*, and *Syzygium cumini* extract.

Films	L	a^*	b^*
Control	$18.03 \pm 0.13k$	$-0.50 \pm 0.03fg$	$-2.05 \pm 0.05g$
Hs 0.25%	$19.75 \pm 0.02h$	$-0.37 \pm 0.03defg$	$-2.30 \pm 0.02g$
Hs 0.50%	$21.42 \pm 0.01f$	$-0.46 \pm 0.01defg$	$-1.90 \pm 0.01fg$
Hs 0.75%	$20.22 \pm 0.03g$	$-0.27 \pm 0.03de$	$-1.37 \pm 0.02de$
Hs 1%	$22.38 \pm 0.03e$	$-0.52 \pm 0.05g$	$-1.90 \pm 0.10fg$
Hc 0.25%	$25.08 \pm 0.02b$	$-0.49 \pm 0.02efg$	$-1.08 \pm 0.03abcd$
Hc 0.50%	$27.13 \pm 0.06a$	$-0.34 \pm 0.05defg$	$-1.05 \pm 0.02abcd$
Hc 0.75%	$19.40 \pm 0.02h$	$-0.31 \pm 0.02def$	$-1.21 \pm 0.05bcde$
Hc 1%	$22.52 \pm 0.03e$	$-0.29 \pm 0.06de$	$-1.15 \pm 0.06bcde$
Ac 0.25%	$18.33 \pm -0.4jk$	$-0.41 \pm 0.07defg$	$-1.54 \pm 0.08ef$
Ac 0.50%	$18.56 \pm 0.05ij$	$-0.41 \pm 0.08defg$	$-0.94 \pm 0.05abc$
Ac 0.75%	$18.93 \pm 0.02i$	$-0.33 \pm 0.06defg$	$-0.87 \pm 0.07ab$

Ac 1%	23.54 ± 0.11d	-0.51 ± 0.05g	-2.06 ± 0.03g
Ext 0.25%	13.68 ± 0.41n	2.94 ± 0.20a	-1.04 ± 0.07abcd
Ext 0.50%	14.91 ± 0.18m	2.97 ± 0.03a	-1.21 ± 0.02bcde
Ext 0.75%	15.67 ± 0.03l	0.67 ± 0.05c	-1.28 ± 0.02cde
Ext 1%	24.07 ± 0.05c	1.04 ± 0.00b	-0.69 ± 0.52a

Note: Hs = *Hymenaea stigonocarpa*. Hc = *Hymenaea courbaril*. Ac = *Annona crassiflora*. Ext = *Syzygium cumini* extract. Different letters in the same column indicate significant differences ($p < 0.05$). Source: Authors, 2024.

Regarding the transmittance of biodegradable films composed of *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* oils and *Z. cumini* extract (Figure 1), it is possible to observe that films containing oils exhibited higher light transmission rates in the visible range between 250-850 nm. Biodegradable films with increasing concentrations of *C. cumini* extract showed a decline in light transmission, with these results being lower compared to the biodegradable films with oils. Filipini et al. (2020) applied different concentrations of *S. cumini* extract in methylcellulose-based films, varying the extract concentrations from 10 to 50%, and described transparency rates ranging from 58.8 to 20.4%, respectively. Our study obtained higher transparency rates, although with a decline, which was expected. This characteristic was also described by Santos et al. (2021) with *Capsicum chinense* extracts, where increasing extract concentrations influenced light transmission.

According to Romani et al. (2018) and Rambabu et al. (2019), biodegradable films composed of different sources of plant extracts exhibit unique behaviors. Furthermore, color is an important quality parameter, influencing consumer acceptance. Colored and opaque biodegradable films serve an “intelligent” function, as they protect foods exposed to UV-visible light, particularly high-fat foods such as meats, by preventing oxidative degradation.

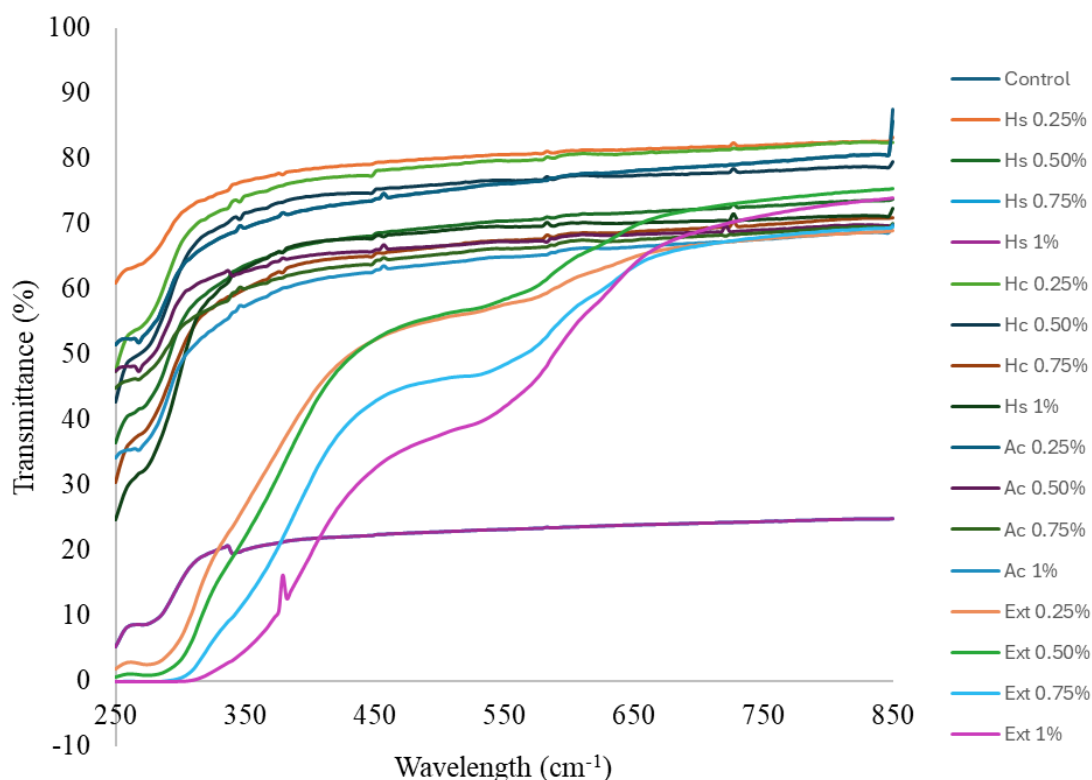


Figure 1. UV-Vis light transmission rate in biodegradable arrowroot films composed of different concentrations of *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora* oils, and *Syzygium cumini* extract. Source: Authors, 2024.

In Figure 2, biodegradable films composed of *Z. cumini* extract at different concentrations are presented, soaked in various solutions with pH values ranging from 2 to 12. The *Z. cumini* extract exhibits pH indicator dye activity: in acidic solutions (pH 2-4), the films displayed a reddish color; in neutral solution (pH 6), the films retained the natural purple color of the extract; and in alkaline solutions, shades from greenish yellow to ochre were observed.

Intelligent films can change their colorimetric patterns based on the enzymatic activity occurring in the product, particularly in cheeses when not produced according to good manufacturing practices. Thus, if, for example, enzymatic activity promoted by bacteria or fungi occurs, the environment will become acidic, and the natural indicator dye will change color, revealing potential manufacturing issues. Along these lines, Choi et al. (2017) developed biodegradable films composed of anthocyanins and a purple sweet potato polymer matrix, which were used to coat pork. In this study, the authors demonstrated the smart activity of the films through color change due to pH variation, shifting from red to green.

Choi et al. (2017) found that at pH 2, a reddish hue is more characteristic, at pH 6 it appears slightly purple, and at pH 10 it turns green. The color variations between our findings and those of Choi and collaborators may be possibly linked to different anthocyanin molecules. According to Wang et al. (2024), the anthocyanins are natural pigments responsible for the red, purple, and blue colors in many fruits, flowers, and vegetables. They belong to the flavonoid family and can be classified into several types, according to their chemical structure and modifications in the chemical groups that compose them. The main types of anthocyanins include:

Cyanidin – It has an intense red color and is found in various fruits such as strawberries, cherries, and grapes. Delphinidin – It presents a purple color and is found in fruits such as blackberries, blueberries, and some varieties of grapes. Pelargonidin – It is responsible for an orange-red color and is present in strawberries, raspberries, and tomatoes. Malvidin – It appears in shades of blue and purple and is common in grapes, wines, and blueberries. Peonidin – It has a red or violet color and is found in fruits such as cherries and blackberries. Petunidin – It also gives purple shades and is found in some types of grapes and flowers. Cyanidin-3-glucoside – A common form of anthocyanin found in many red fruits, such as strawberries and raspberries.

These pigments can vary in color depending on the medium pH in which they are found, changing from red in more acidic environments to blue or purple in more alkaline ones. Additionally, anthocyanins have several health benefits, such as antioxidant and anti-inflammatory properties.

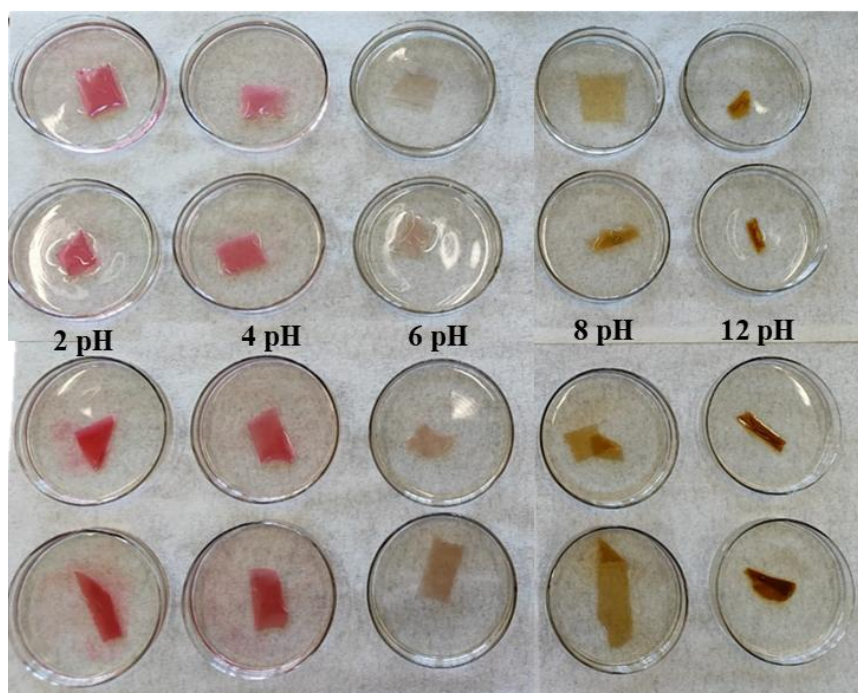


Figure 2. The behavior of biodegradable packaging composed of *Syzygium cumini* extract in different pH levels. Concentrations of 0.25%, 0.50%, 0.75%, and 1%, respectively from top to bottom. Source: Authors, 2024.

Biodegradable films composed of *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora* oils demonstrated soil biodegradability capacity (Figure 3). The highest concentration (1%) showed low biodegradability, possibly due to the long-chain fatty acids that take longer to be degraded by the microbial flora, which absorbs carbon as a food source. Regarding the *S. cumini* extract, good degradation was observed within 19 days. The 1% concentration achieved a maximum biodegradation rate of 85%, and this low rate is possibly related to the action of anthocyanins, which can manipulate the pH of microorganisms, reducing or inhibiting their functions.

Filipini et al. (2020) conducted a study with *S. cumini* extract in a methylcellulose polymer matrix found near-total biodegradation within less than 15 days, compared to our study. According to Carissimi et al. (2018), biodegradable films composed of extracts exhibit high solubility, leading to rapid biodegradation, as their water sensitivity helps make the components of the film structure available to be metabolized by microorganisms. This same statement does not apply to films composed of oils, where in our study, nearly 100% biodegradation was achieved around 35 days. This longer period

is possibly due to the action of oils, which have hydrophobic properties and/or long carbon chains.

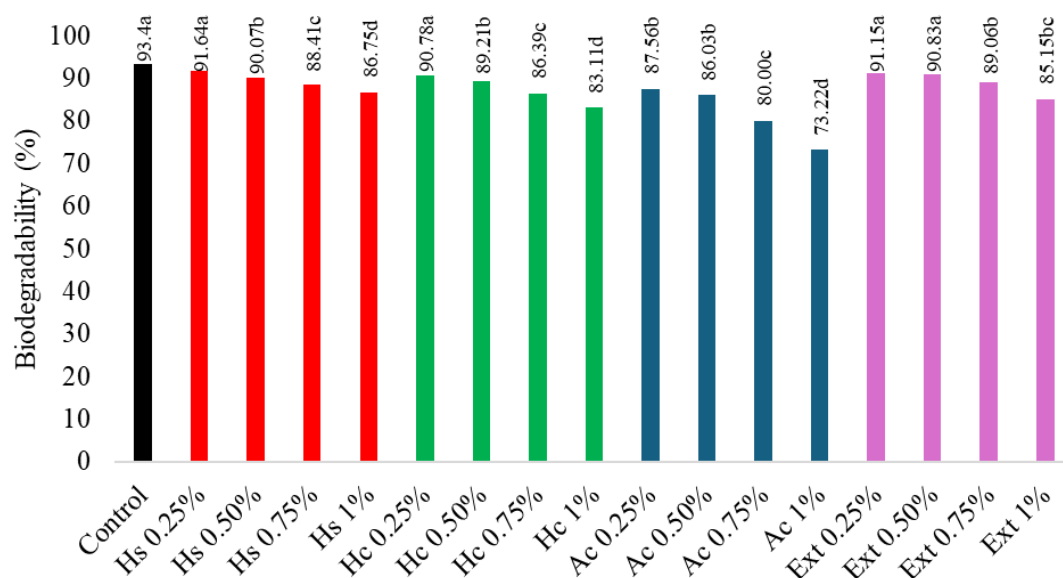


Figure 3. Biodegradability of biodegradable films composed by oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, and extract from *Syzygium cumini* in natural soil. Statistical analysis evaluated each oil or extract as an individual component compared to the control. Different letters between control and oil or extract separately indicate significant differences ($p < 0.05$). Source: Authors, 2024.

Biodegradation is defined as the loss of mechanical properties, fragmentation, or chemical modifications due to the action of microorganisms and enzymes present in nature, both in soil and in marine environments (Filipini et al., 2020). In beach environments, about 10% of various types of petroleum-based synthetic polymers have long decomposition periods, contaminating beaches, seas, and oceans, as well as causing marine biological imbalances (Avio et al., 2017). In this regard, our study demonstrated that microorganisms in sand and seawater showed good biodegradation results. Our biodegradable film samples took 20-33 days to degrade (Figure 4). Again, films composed of oils showed lower degradation rates at higher concentrations of 0.75% and 1%. Films with *A. crassiflora* oil showed the lowest rate, with 55% degradation at 1% oil, which suggests a potential for further studies. As for the effect of *S. cumini* extract, the 1% extract showed only 53% degradation.

Filipini et al. (2020) found degradation rates lower than our findings for methylcellulose films composed with *S. cumini* extract after two days of study. This

exceptional result is due to the physicochemical characteristics of the study, as the films were completely solubilized.

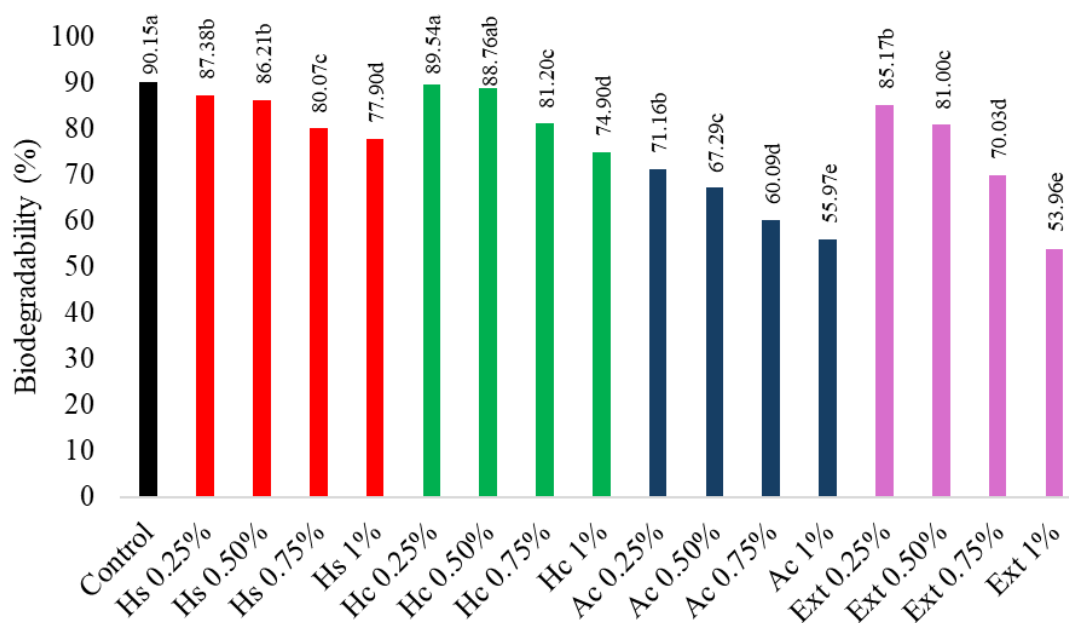


Figure 4. Biodegradability of biodegradable films composed of oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, and extract from *Syzygium cumini* in sand and seawater. Statistical analysis evaluated each oil or extract as an individual component compared to the control. Different letters between control and oil or extract separately indicate significant differences ($p < 0.05$). Source: Authors, 2024.

Regarding antioxidant activity in reducing DPPH, FRAP, HRSA, and NOSA, the *S. cumini* extract proved to be a viable and potential option for reducing different free oxidizing agents (Table 3). When compared, the extract showed a difference when compared to the standard ascorbic acid with an IC_{50} of $32 \mu\text{g mL}^{-1}$. For HRSA, the extract showed a greater reduction than the standard with an IC_{50} of $12 \mu\text{g mL}^{-1}$, and for NOSA, the standard ascorbic acid showed a difference when compared to the extract with an IC_{50} of $65 \mu\text{g mL}^{-1}$.

Filipini et al. (2020) found that the crude *S. cumini* extract had a reduction capacity for DPPH and FRAP greater than 80%, and for film samples composed of different concentrations of this extract, with responses above 40%. This ability to eliminate free oxidizing agents is attributed to the presence of anthocyanins, which are a special group with antioxidant activity. Moreover, Castañeda-Ovando et al. (2009) support our results,

adding that anthocyanins contain a group of phenolic hydroxyls in their structures, capable of capturing free radicals by donating phenolic hydrogen atoms.

Table 3. Antioxidant activities of *Syzygium cumini* extract.

Sample	DPPH (IC ₅₀ µg mL ⁻¹)	FRAP (mmol AAE g ⁻¹)	HRSA (IC ₅₀ µg mL ⁻¹)	NOSA (IC ₅₀ µg mL ⁻¹)
<i>S. cumini</i> extract	32.41 ± 0.56b	9.57 ± 0.08	12.56 ± 0.18a	65.45 ± 0.68b
Ascorbic acid	4.95 ± 0.44a	-	21.47 ± 0.32b	37.41 ± 0.84 ^a

Note: FRAP values are expressed in mmol ascorbic acid equivalent g⁻¹ sample in dry weight. HRSA hydroxyl radical scavenging assay. NOSA nitric oxide scavenging assay. Different letters in the same column indicate significant differences ($p < 0.05$). Source: Authors, 2024.

The crude extract of *S. cumini* demonstrated potential antibacterial activity against all strains evaluated, except for *S. Enteritidis* and *S. Typhimurium*, which did not show inhibition activity. However, the synthetic antibacterial standards exhibited a larger inhibition zone compared to the crude extract (Table 4). Several bacteria used in our study are linked to contamination in various types of cheeses. Contaminations are directly related to poor preparation and storage conditions.

Regarding antibacterial effects, the strains of *E. coli*, *E. faecalis*, and *B. subtilis* showed greater sensitivity in the extract presence, with inhibition values of 13, 13, and 10 mm, respectively. Nasution et al. (2024) verified potential antibacterial activity in PLA-PCL (chitosan) packaging composed with *S. cumini* seed extract against *S. aureus* and *E. coli*. Tambe et al. (2021) found potential antibacterial activity against *S. Enteritidis*, *S. Typhi*, *Salmonella Paratyphi A* and *B*, *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus* using different concentrations of methanolic and aqueous leaf extract of *S. cumini*.

In the study by Haque et al. (2017), the researchers found that the fruit extract of *S. cumini* is selective for bacteria, showing strong bactericidal activity against *S. Typhimurium*, *Shigella flexneri*, and *S. aureus*. However, its selective activity did not affect the growth of *Lactobacillus acidophilus* and *L. bulgaricus*. Anupam et al. (2017) observed that the extract derived from *S. cumini* seeds showed promising results against *B. subtilis*, *S. aureus*, and *Bacillus cereus* from the Gram-positive group, as well as *S. Typhimurium*, *S. Enteric*, and *E. coli* from the Gram-negative group. Prasad and Swamy

(2013) found that *S. cumini* bark extract doped with silver nanoparticles exhibited strong antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Azotobacter chroococcum*, and *Bacillus licheniformis*.

Antibacterial activity in *Syzygium* species is described and discussed due to various phytochemical groups of medical interest, such as alkaloids, flavonoids, steroids, saponins, phenols, tannins, and terpenoids (Aiyelaagbe; Osamudiamen, 2009).

Table 4. Antibacterial activity of *Syzygium cumini* extract.

Bacteria strain	Zone of inhibition (mm)	
	Extract <i>S. cumini</i>	Standard antibacterial agent
<i>E. coli</i>	13.68 ± 0.91 ^b	32.67 ± 0.94 ^a
<i>P. aeruginosa</i>	9.56 ± 1.06 ^b	28.54 ± 1.05 ^a
<i>S. aureus</i>	5.07 ± 0.39 ^b	30.78 ± 0.77 ^a
<i>E. faecalis</i>	13.13 ± 0.31 ^b	31.88 ± 1.07 ^a
<i>S. Enteritidis</i>	0.00 ± 0.00 ^b	28.36 ± 0.91 ^b
<i>S. Typhimurium</i>	0.00 ± 0.00 ^b	29.10 ± 1.32 ^b
<i>B. subtilis</i>	10.55 ± 1.04 ^b	30.09 ± 1.26 ^a
<i>L. monocytogenes</i>	6.64 ± 0.99 ^b	28.56 ± 0.68 ^c

Note: ^aTetracycline, ^bFosfomycin, and ^cErythromycin standard. Each value is expressed as mean ± SD. Different letters in the same line indicate significant differences ($p < 0.05$).

3.3 Physicochemical parameters of coated cheese

In this study, our results for the physicochemical parameter of titratable acidity in Mozzarella cheese samples stored for 15 and 25 days at 10 °C showed significant differences when compared to the control group with packaging without the addition of oils and extract, expressed as % lactic acid (Table 5). The addition of oil and extract concentrations influenced the increase in acidity, although the 25-day period exhibited the highest results compared to the earlier analysis period. The highest total acidity index was observed in cheese samples coated with 1% *A. crassiflora* oil, with values of 0.07% and 0.08% expressed as lactic acid for 15 and 25 days of storage, respectively.

Increased hardness was noted during the slicing of samples stored for 25 days. This observation was also reported by Ghasemian et al. (2024) for white cheese coated with chitosan polymer and *Rosmarinus officinalis* extract. However, contrary to our findings, they observed that cheeses without adding extract exhibited easier slicing and

lower acidity when stored at 4 °C for 45 days. At the end of the storage period, the *R. officinalis* extract incorporated into the biodegradable polymer matrix demonstrated activity by inhibiting acidity compared to the control, which had 1.79 g of lactic acid. In the study by Amjadi et al. (2019), evaluating gelatin polymer combined with ZnO for coating "Egyptian soft white cheese," the researchers observed that acidity increased at ZnO concentrations above 8%, where the pH significantly decreased, extending the cheese's shelf life from 1 to 30 days. Azhdari and Moradi (2022) observed that the storage time of Mozzarella cheese coated with carboxymethyl cellulose combined with Natamycin resulted in more acidic samples starting from the fourth day. The experiment was monitored for up to eight days, showing borderline results.

Table 5. Total titratable acidity of refrigerated cheeses coated with biodegradable packaging containing oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, *Annona crassiflora*, and *Syzygium cumini* extract, expressed as % lactic acid.

Sample	0 day	15 days	25 days
Control	0.02 ± 0.00C	0.02 ± 0.00efB	0.03 ± 0.00efghA
Hs 0.25%	-	0.02 ± 0.00ef	0.03 ± 0.00efgh
Hs 0.50%	-	0.02 ± 0.00ef	0.03 ± 0.00efgh
Hs 0.75%	-	0.03 ± 0.00def	0.03 ± 0.00efgh
Hs 1%	-	0.03 ± 0.00de	0.04 ± 0.00de
Hc 0.25%	-	0.02 ± 0.00def	0.03 ± 0.00fgh
Hc 0.50%	-	0.03 ± 0.00ef	0.03 ± 0.00efgh
Hc 0.75%	-	0.03 ± 0.00abde	0.04 ± 0.01def
Hc 1%	-	0.04 ± 0.00bcd	0.04 ± 0.00de
Ac 0.25%	-	0.02 ± 0.00ef	0.02 ± 0.00gh
Ac 0.50%	-	0.02 ± 0.00f	0.03 ± 0.00gh
Ac 0.75%	-	0.05 ± 0.01abc	0.05 ± 0.01cd
Ac 1%	-	0.07 ± 0.00a	0.08 ± 0.01a
Ext 0.25%	-	0.04 ± 0.00cd	0.04 ± 0.00efg
Ext 0.50%	-	0.05 ± 0.00b	0.06 ± 0.01bc
Ext 0.75%	-	0.06 ± 0.00ab	0.06 ± 0.01bc
Ext 1%	-	0.06 ± 0.01b	0.07 ± 0.00b

Note: (-) no determined. Results expressed as % lactic acid. Different letters in the same column indicate significant differences ($p < 0.05$). Source: Authors, 2024.

The color of the packaging incorporated with cheese plays an important role in its quality. Biodegradable packaging composed of oils or extracts should provide protective (active) effects against UV light; therefore, optical properties such as UV-Vis absorption/transmission, color, and transparency of the natural polymers (polymeric matrix) used in the packaging are strategically vital.

When it comes to cheese, the packaging must protect the product from UV light exposure while preserving the cheese's nutrients, minimizing lipid oxidation, and limiting discoloration, ensuring it does not negatively affect the flavors. Additionally, the color of the biodegradable packaging determines consumer acceptance, which is a critical attribute that impacts its application (Jafarzadeh et al., 2017, 2021).

In our study, Mozzarella cheese coated and stored for 15 days with *H. stigonocarpa* oil (0.50%), *H. courbaril* oil (0.50%), and *A. crassiflora* oil (0.25%) showed higher L* values compared to the control and other samples. However, no significant differences were observed between them (Table 6). Regarding the films, all concentrations showed significant differences ($p < 0.05$), with the highest L* values found in the lowest concentration of 0.25%.

For chroma (a*), all samples showed a tendency toward red, except for *A. crassiflora* 0.50% and 0.75%, which tended toward green. For chroma (b*), all samples showed a tendency toward blue. After 25 days of storage, L* exhibited slight changes, with higher brightness compared to the 15-day storage period for packaging with oils. However, packaging with *S. cumini* extract showed lower L* compared to the previous analysis period. For chromas a* and b*, the samples showed patterns with a decrease in red and yellow tones, respectively.

Several studies have shown promising results regarding the coating with active and intelligent biodegradable packaging, incorporating extracts, oils, and other natural compounds, with potential effects on various types of cheese studied. Salem et al. (2021) incorporated *Lepidium sativum* seed extract into a fish gelatin polymer matrix, where they found that L* values were significantly different from the control. However, brightness declined at concentrations above $5 \mu\text{g mL}^{-1}$ of extract. For chroma (a*), the packaging exhibited a greenish tone (-a*), and for chroma (b*), a yellowish tone (+a*). The loss of L*, a*, and b* tonalities was also reported in this study up to the sixth day of storage for Ricotta cheese. In another study proposed by Kontogianni et al. (2023), using whey protein polymer combined with spirulina algae extract, the researchers found that the

packaging for "Kefalotyri" cheese coating at different concentrations, stored at 4°C, did not show any color change, although it broke down, leaving the cheese samples uncovered after seven days of storage.

Table 6. Color parameters in refrigerated Mozzarella-type cheese coated with oils of *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, and *Syzygium cumini* extract.

Sample	15 days			25 days		
	L*	a*	b*	L*	a*	b*
Control	63.02 ± 0.01d	0.74 ± 0.01fg	27.75 ± 0.02d	62.18 ± 0.01i	0.76 ± 0.01k	27.60 ± 0.01d
Hs 0.25%	64.15 ± 0.00bc	0.55 ± 0.01h	27.33 ± 0.01e	66.00 ± 0.04g	0.71 ± 0.00k	23.67 ± 0.05k
Hs 0.50%	65.74 ± 1.37a	0.77 ± 0.20fg	27.19 ± 0.14f	67.92 ± 0.00b	1.27 ± 0.01e	25.05 ± 0.01j
Hs 0.75%	63.03 ± 0.01d	0.54 ± 0.01h	25.29 ± 0.02j	66.97 ± 0.01d	0.84 ± 0.01j	25.34 ± 0.01i
Hs 1%	64.08 ± 0.02c	0.50 ± 0.00h	25.25 ± 0.02j	65.83 ± 0.00g	1.05 ± 0.00h	25.45 ± 0.02h
Hc 0.25%	65.14 ± 0.00ab	1.16 ± 0.01e	29.50 ± 0.01a	65.15 ± 0.00h	1.16 ± 0.01g	29.50 ± 0.01a
Hc 0.50%	65.27 ± 0.01a	0.87 ± 0.01f	27.74 ± 0.03d	65.27 ± 0.00h	0.87 ± 0.01ij	27.74 ± 0.03d
Hc 0.75%	65.45 ± 0.04a	0.62 ± 0.01gh	27.92 ± 0.03c	66.01 ± 0.00g	1.21 ± 0.00f	23.61 ± 0.03k
Hc 1%	64.80 ± 0.02abc	0.48 ± 0.01h	26.77 ± 0.01g	67.19 ± 0.00c	1.04 ± 0.00h	25.71 ± 0.00g
Ac 0.25%	65.48 ± 0.29a	0.59 ± 0.01h	28.33 ± 0.00b	66.49 ± 0.02e	0.62 ± 0.01l	28.95 ± 0.03c
Ac 0.50%	60.81 ± 0.02e	-0.06 ± 0.00i	23.42 ± 0.02k	68.06 ± 0.00b	1.20 ± 0.00fg	27.08 ± 0.03e
Ac 0.75%	64.82 ± 0.01abc	-0.06 ± 0.00i	26.22 ± 0.02h	68.88 ± 0.01a	0.88 ± 0.00ij	29.23 ± 0.01b
Ac 1%	62.01 ± 0.03d	-0.06 ± 0.02i	25.54 ± 0.04i	66.25 ± 0.00f	0.90 ± 0.01i	26.18 ± 0.01f
Ext 0.25%	54.06 ± 0.10f	3.14 ± 0.01d	22.38 ± 0.03l	54.27 ± 0.03j	3.17 ± 0.03d	17.83 ± 0.04l
Ext 0.50%	40.90 ± 0.01h	6.22 ± 0.01b	12.46 ± 0.04n	35.48 ± 0.29l	7.44 ± 0.04a	7.85 ± 0.05n
Ext 0.75%	46.76 ± 0.02g	5.11 ± 0.01c	16.91 ± 0.03m	43.02 ± 0.01l	5.33 ± 0.01c	13.18 ± 0.04m
Ext 1%	38.78 ± 0.03i	7.08 ± 0.01a	10.97 ± 0.01o	35.55 ± 0.01k	6.90 ± 0.02 b	6.84 ± 0.00o

Different letters in the same column indicate significant differences ($p < 0.05$). Source: Authors, 2024.

Cheese samples containing oils of *H. stigonocarpa*, *H. courbaril*, *A. crassiflora*, and *S. cumini* extract showed a biodegradation rate exceeding 50% within 35 days (Figure 5). Cheeses coated with *A. crassiflora* oil demonstrated a lower biodegradability rate, suggesting further studies on the chemical profile of this oil, which may contain compounds with antifungal or antibacterial activity. Silva et al. (2020) evaluated "prato" cheese with biodegradable films using a cassava starch polymer matrix derived from jabuticaba fruit peel extract, observed complete biodegradation in composted soil. Rosa et al. (2001) found similar behavior when analyzing the biodegradation of starch-based

polymer solutions, showing a higher degradation rate, which supports our study on the feasibility of producing low-cost biodegradable polymers.

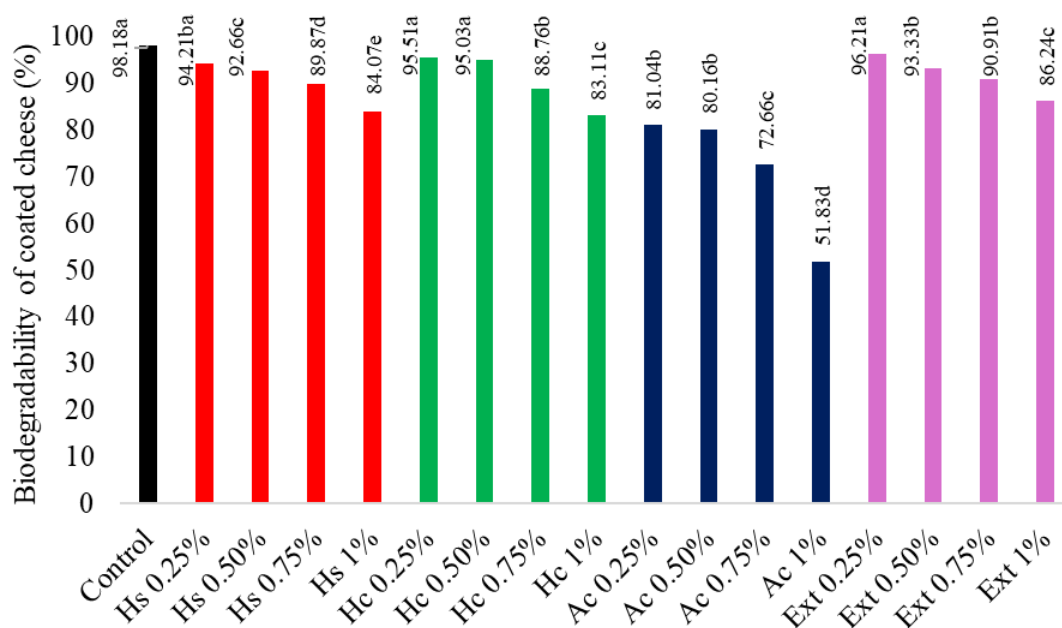


Figure 5. Biodegradability of cheeses coated with oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, and extract from *Syzygium cumini* in natural soil. Statistical analysis evaluated each oil or extract as an individual component compared to the control. Different letters between control and oil or extract separately indicate significant differences ($p < 0.05$). Source: Authors, 2024.

3.4 Microbiological parameters of coated cheese

Samples of raw cheese, control, and those coated with oil from *H. stigonocarpa*, *H. courbaril*, and *A. crassiflora*, as well as extract of *S. cumini*, showed no positive response for microbiological contamination throughout the entire experiment. Biodegradable films used in the coating of different types of cheeses, incorporated with antimicrobial agents, have sparked interest in the dairy industry.

The antimicrobial agents can be natural extracts, fixed oils, resin oils, essential oils, and/or synthetic fine chemicals. Cuenca and Albani (2024) found that cheeses coated with biopolymers (amylose and starch) without the addition of the antimicrobial agent natamycin, stored for 30 days under refrigeration at 4 °C and 92% relative humidity (RH), showed a statistically significant difference in mold and yeast counts when compared to cheeses coated with biopolymers supplemented with the antimicrobial agent natamycin.

Salem et al. (2021) found that fish gelatin-based biodegradable films incorporated with *Lepidium sativum* extract showed lower counts of mesophilic and psychrophilic bacteria when compared to uncoated cheese samples, where a higher concentration of colonies from these bacterial groups was identified.

These authors further complement that fish gelatin-based biodegradable films composed of plant extract reduced the diffusion of oxygen in the samples when exposed to the external environment, thereby limiting the growth of microorganisms. Pirsá et al. (2020) also describe the potential antibacterial activity of biodegradable packaging made from chitosan polymer matrices, composed of *Punica granatum* (pomegranate) extract and *Melissa officinalis* essential oils, against *B. cereus* and *E. coli*.

4. CONCLUSIONS

Biodegradable packaging composed of oils from *Hymenaea stigonocarpa*, *Hymenaea courbaril*, and *Annona crassiflora*, as well as *Syzygium cumini* extract, demonstrated active and intelligent functions within arrowroot polymer matrices. The oils and extract addition showed positive effects on the morphology, physicochemical properties, biodegradability, and microbiological characteristics of the packaging, both with and without coatings on Mozzarella cheese. Packaging with oils exhibited active properties, while those with the extract demonstrated intelligent functionality at different pH levels during a storage period of up to 25 days at 10 °C. Cheese coated with arrowroot polymer containing oils and extract showed potential for the development of new food storage products.

5. REFERENCES

AACC -American Association of Cereal Chemists. Approved Methods of the American Association of Cereal Chemists. 10. ed. Saint Paul: AACC, 2006.

Abdillah, A., Charles, A. L. (2021). Characterization of natural biodegradable edible film obtained from arrowroot starch and iota-carrageenan and application in food packaging. International Journal of Biological Macromolecules, 191, 618-626. <https://doi.org/10.1016/j.ijbiomac.2021.09.141>

Adilah, A. N., Jamilah, B., Noranizan, M. A., Hanani, Z. A. N. (2018). Utilization of mango peel extracts on the biodegradable films for active packaging. *Food Packaging and Shelf Life*, 16, 1-7. <http://dx.doi.org/10.1016/j.fpsl.2018.01.006>

Aiyelaagbe, O. O., Osamudiamen, P. M. (2009). Phytochemical screening for active compounds in *Mangifera indica*. *Plant Science Research*, 2(1), 11-13.

Almeida, R. F., Moreno, I. F., Machado, A. P. O., Meireles, M. A. A., Silva, L. K. F., Batista, E. A. C. Araticum (*Annona crassiflora* Mart.): A critical review for the food industry. *Food Research International*, 184, 114241. <https://doi.org/10.1016/j.foodres.2024.114241>

Alves-Silva, G., Romani, V. P., Martins, V. G. Jatobá (*Hymenaea stigonocarpa*) pulp films: Properties, antioxidant potential and biodegradability. *Food Packaging and Shelf Life*, 34, 100923. <https://doi.org/10.1016/j.fpsl.2022.100923>

Amjadi, S., Emaminia, S., Nazari, M. (2019). Application of reinforced ZnO nanoparticle-incorporated gelatin bionanocomposite film with chitosan nanofiber for packaging of chicken fillet and cheese as food models. *Food and Bioprocess Technology*, 12, 1205-1219. <https://doi.org/10.1007/s11947-019-02286-y>

Anupam, K., Anu., Anand, M., Raj, S. N., Hasibur, R. Antibacterial, antioxidant analysis of phytochemical extracts derived from seeds of *Syzygium cumini* L. against pathogenic bacteria. *Research Journal of Pharmacy and Technology*, 10(8), 2707-2712. <http://dx.doi.org/10.5958/0974-360X.2017.00481.4>

Arruda, H. S., Borsoi, F. T., Andrade, A. C., Pastore, G. M., Júnior, M. R. M. Scientific advances in the last decade on the recovery, characterization, and functionality of bioactive compounds from the araticum fruit (*Annona crassiflora* Mart.). *Plants*, 12(7), 1536. <https://doi.org/10.3390/plants12071536>

Avio, C. G., Gorbi, S., Regoli, F. (2017). Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. *Marine Environmental Research*, 128, 2-11. <https://doi.org/10.1016/j.marenvres.2016.05.012>

Azhdari, S., Moradi, M. (2022). Application of antimicrobial coating based on carboxymethyl cellulose and natamycin in active packaging of cheese. *International Journal of Biological Macromolecules*, 209, 2042-2049. <https://doi.org/10.1016/j.ijbiomac.2022.04.185>

Castañeda-Ovando, A., Pacheco-Hernandez, M. de L., Paez-Hernandez, M. E., Rodríguez, J. A., & Galan-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113(4), 859-871. <https://doi.org/10.1016/j.foodchem.2008.09.001>

Chandler, D. (2002). Hydrophobicity: Two faces of water. *Nature*, 417. <https://www.nature.com/articles/417491a>

Charles, A.L ., Cato, K., Huang, T. C., Chang, Y. H., Ciou, J. Y., Chang, J. S., Lin, H. H. (2016). Functional properties of arrowroot starch in cassava and sweet potato composite starches. *Food Hydrocollody*, 53, 187-191. <https://doi.org/10.1016/j.foodhyd.2015.01.024>

Choi, I.; Lee, J. Y.; Lacroix, M.; Han, J. (2017). Intelligent pH indicator film composed of agar/potato starch and anthocyanin extracts from purple sweet potato. *Food Chemistry*, 218, 122-128. <https://doi.org/10.1016/j.foodchem.2016.09.050>

Cuenca, P., Albani, O. Biodegradable active films to improve the preservation of regional cheese during refrigerated storage. *Food and Bioprocess Technology*, 17, 217-230. <https://doi.org/10.1007/s11947-023-03131-z>

Eshwarappa, R. S. B., Iyer, R. S., Subbaramaiah, S. R., Richard, S. A., Dhananjaya, B. L. Antioxidant activity of *Syzygium cumini* leaf gall extracts. *Bioimpacts*, 20(4), 101-107. <https://doi.org/10.5681/bi.2014.018>

Filipini, G. S., Romani, V. P., Martins, V. G. (2020). Biodegradable and active-intelligent films based on methylcellulose and jambolão (*Syzygium cumini*) skins extract for food packaging. *Food Hydrocolloids*, 109, 106139.

Ghasemian, S. O., Ahmadi-Dastgerdi, A., Abdollahi, A., Tirtashi, F. E., Zokaei, M., Fallah, N., NajafAbadi, P. I., Dolatyari, F. (2024). The effect of active packaging film based on chitosan containing Rosemary (*Rosmarinus officinalis* L.) extract on cheese shelf life. *Journal of Food Biochemistry*, 2024(1), 2108707.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current Protocols in Food Analytical Chemistry*, (1). <https://doi.org/10.1002/0471142913.faf0102s00>

Haque, R., Sumiya, M. K., Sakib, N., Sarkar, O. S., Siddique, T. T. I., Hossain, S., Islam, A., Parvez, A. K., Talukder, A. A., Dey, S. K. Antimicrobial activity of jambul (*Syzygium cumini*) fruit extract on enteric pathogenic bacteria. *Advances in Microbiology*, 7, 195-204. DOI: 10.4236/aim.2017.73016

International Dairy Federation. (1993). 21B: 1993: milk cream and evaporated milk: determination of total solids content (reference method). Brussels, 2 f.

Jafarzadeh, S., Salehabadi, A., Mohammadi, N., Oladzadabbasabadi, N., Jafari, S. M. (2021). Cheese packaging by edible coatings and biodegradable nanocomposite; improvement in shelf life, physicochemical and sensory properties. *Trends in Food Science & Technology*, 116, 218-231.

Jafarzadeh, S., Alias, A. K., Ariffin, F. (2017). Fabrication and characterization of novel semolina-based antimicrobial films derived from the combination of ZnO nanorods and nanokaolin. *Journal of Food Science & Technology*, 54, 105-113. <https://doi.org/10.1007/s13197-016-2441-3>

Jampani, C., Naik, A., Raghavarao, K. S. M. S. (2014). Purification of anthocyanins from jamun (*Syzygium cumini* L.) employing adsorption. *Separation and Purification Technology*, 125, 170-178. <https://doi.org/10.1016/j.seppur.2014.01.047>.

Jana, A. H., Talgalpallewar, G. P. (2017). Functional properties of Mozzarella cheese for end use application. *Journal of Food Science and Technology*, 54(12), 3766-3778. <https://doi.org/10.1007/s13197-017-2886-z>

Jiang, T., Duan, Q., Zhu, J., Liu, H., Yu, L. (2020). Starch-based biodegradable materials: challenges and opportunities, *Advanced Industrial and Engineering Polymer Research*, 3(1), 8-18, <https://doi.org/10.1016/j.aiepr.2019.11.003>

Kontogianni, V. G., Kosma, I., Mataragas, M., Pappa, E., Badeka, A. V., Bosnea, L. (2023). Innovative intelligent cheese packaging with whey protein-based edible films containing spirulina. *Sustainability*, 15(18), 13909. <https://doi.org/10.3390/su151813909>

Koosha, M., Hamed, S. (2019). Intelligent Chitosan/PVA nanocomposite films containing black carrot anthocyanin and bentonite nanoclays with improved mechanical, thermal and antibacterial properties. *Progress in Organic Coatings*, 127, 338-347. <https://doi.org/10.1016/j.porgcoat.2018.11.028>

Leite, D. O. D., Nonato, C. F. A., Camilo, C. J., Carvalho, N. K. G., Nobrega, M. G. L. A., Pereira, R. C., Costa, J. G. M. *Annona* genus: Traditional uses, phytochemistry and biological activities. *Current Pharmaceutical Design*, 26(3), 4056-4091. <https://doi.org/10.2174/1381612826666200325094422>

Luz, L. M., Alves, E. C., Vilhena, N. Q., Oliveira, T. B., Silva, Z. G. B., Freitas, J. M. N., Neto, C. F. O., Costa, R. C. L., Costa, L. C. (2023). Distinct physiological mechanisms underpin growth and rehydration of *Hymenaea courbaril* and *Hymenaea stigonocarpa* upon short-term exposure to drought stress. *Journal of Forest Research*, 34, 113-123. <https://doi.org/10.1007/s11676-022-01558-2>

Menezes Filho, A. C. P., Ventura, M. V. A., Favareto, R., Castro, C. F. S., Teixeira, M. B., Soares, F. A. L. (2022). Incorporation of oil from *Hymenaea stigonocarpa* and *Hymenaea courbaril* into biofilms made from arrowroot starch: physicochemical, biodegradability and antifungal activity. *Journal of Agricultural Science*, 14(4), 156-169.

Mohamed, A. A., Ali, S. I., El-Baz, F. K. Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. PLOS One, 8(4), e60269. <https://doi.org/10.1371/journal.pone.0060269>

Nasution, H., Harahap, H., Julianti, E., Safitri, A., Jaafar, M. (2024). Properties of active packaging of PLA-PCL film integrated with chitosan as an antibacterial agent and *Syzygium cumini* seed extract as an antioxidant agent. Heliyon, 10, e23952.

Pirsa, S., Sani, I. K., Khalil, M., Erfani, A. Smart film based on chitosan/Melissa officinalis essences/pomegranate peel extract to detect cream cheeses spoilage. Food Additives & Contaminants: Part A, 37(4), 634-648. <https://doi.org/10.1080/19440049.2020.1716079>

Prasad, R., Swamy, V. S. Antibacterial activity of silver nanoparticles synthesized by bark extract of *Syzygium cumini*. Journal of Nanoparticles, 1-6. <https://doi.org/10.1155/2013/431218>

Pratiwi, I., Susilowati, A., Pangastuti, A. (2021). Incorporation of purslane extract (*Portulaca oleracea*) to chitosan edible film as a packaging material to prevent damage of mozzarella cheese during storage. IOP Conf. Series: Earth and Environmental Science, 828. doi:10.1088/1755-1315/828/1/012026

Rambabu, K., Bharath, G., Banat, F., Show, P. L., Cocolletzi, H. H. (2019). Mango leaf extract incorporated chitosan antioxidant film for active food packaging. International Journal of Biological Macromolecules, 126, 1234-1243. <http://dx.doi.org/10.1016/j.ijbiomac.2018.12.196>

Romani, V. P., Hernández, C. P., & Martins, V. G. (2018). Pink pepper phenolic compounds incorporation in starch/protein blends and its potential to inhibit apple browning. Food Packaging and Shelf Life, 15, 151-158. <http://dx.doi.org/10.1016/j.fpsl.2018.01.003>

Rosa, M. D. S.; Franco, B. L. M.; Calil, M. R. (2001). Biodegradabilidade e propriedades mecânicas de novas misturas poliméricas. Polímeros: Ciência e Tecnologia, 11(2), 82-88.

Ruan, Z. P., Zhang, L. L., Lin, Y. M. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules*, 13(10), 2525-2556. <https://doi.org/10.3390/molecules13102545>

Salem, A., Jridi, M., Abdlhedi, O., Fakhfakh, N., Nasri, M., Debeaufort, F., Zouari, N. Development and characterization of fish gelatin-based biodegradable film enriched with *Lepidium sativum* extract as active packaging for cheese preservation. *Heliyon*, 7(10), 08099.

Santos, L. S., Fernandes, C. C., Santos, L. S., Deus, I. P. B., Sousa, T. L., Miranda, M. L. D. (2021). Ethanolic extract from *Capsicum chinense* Jacq. ripe fruits: phenolic compounds, antioxidant activity and development of biodegradable films. *Food Science and Technology*, 41(2), 497-504. <https://doi.org/10.1590/fst.08220>

Shaikh, S., Yaqoob, M., Aggarwal, P. (2021). Na overview of biodegradable packaging in food industry. *Current Research in Food Science*, 4, 503-520. <https://doi.org/10.1016/j.crfs.2021.07.005>

Silva, N. M., Sandre, M. F. B., Souza, D. G., Ferreira, A. V., Gonçalves e Silva, J. A., Mesquita, A. A., Egea, M. B., & Pereira da Silva, M. A. (2020). Adição de extrato de casca de jaboticaba no desenvolvimento de embalagem inteligente para queijo prato. *Brazilian Journal of Development*, 6(4), 19512-19532. <https://doi.org/10.34117/bjdv6n4-207>

Subbiahdoss, G., Reimhult, E. (2020). Biofilm formation at oil-water interfaces is not a simple function of bacterial hydrophobicity. *Colloids and Surfaces B: Biointerfaces*, 194, 111163. <https://doi.org/10.1016/j.colsurfb.2020.111163>

Tarique, J., Zainudin, E. S., Sapuan, S. M., Ilyas, R. A., Khalina, A. (2022). Physical, mechanical, and morphological performances of arrowroot (*Maranta arundinacea*) fiber reinforced arrowroot starch biopolymer composites. *Polymers*, 14(3), 388. <https://doi.org/10.3390/polym14030388>

Tena, N., Martín, J., Asuero, A. G. State of the art of anthocyanins: Antioxidant activity, sources, bioavailability, and therapeutic effect in human health. *Antioxidants*, 9(5), 451. <https://doi.org/10.3390/antiox9050451>

Wang, J., Zhao, Y., Sun, B., Wang, S., Feng, Z., Li, J. The structure of anthocyanins and the copigmentation by common micromolecular copigments: A review. *Food Research International*, 176, 113837. <https://doi.org/10.1016/j.foodres.2023.113837>

Wu, X., Yan, X., Zhang, J., Wu, X., Luan, M., Zhang, Q. (2024). Preparation and characterization of pH-sensitive intelligent packaging films based on cassava starch/polyvinyl alcohol matrices containing *Aronia melanocarpa* anthocyanins. *LWT*, 194, 115818. <https://doi.org/10.1016/j.lwt.2024.115818>

Yong, H., Wang, X., Zhang, X., Liu, Y., Qin, Y., & Liu, J. (2019). Effects of anthocyanin-rich purple and black eggplant extracts on the physical, antioxidant and pH-sensitive properties of chitosan film. *Food Hydrocolloids*, 94, 93-104. <https://doi.org/10.1016/j.foodhyd.2019.03.012>

CONCLUSÃO GERAL

Os óleos fixos de *Hymenaea stigonocarpa*, *Hymenaea courbaril* e *Annona crassiflora* demonstram potencial aplicação em embalagens bioativas em matriz polimérica de araruta.

O uso de óleos fixos apresentou maior espessura, diminuição da umidade, transparência e solubilidade.

A biodegradabilidade também foi influenciada conforme a adição crescente de óleos na matriz polimérica de araruta.

Embalagens com concentrações superiores a 0.75% houve a formação de bolhas na morfologia estrutural.

Os principais grupos vibracionais no espectro do infravermelho próximo (grupos funcionais) foram descritos em todas as amostras.

As embalagens biodegradáveis compostas com óleos de *H. stigonocarpa*, *H. courbaril* e *A. crassiflora* desempenharam função antifúngica sobre *Colletotrichum*, *Aspergillus* e *Rhizopus*.

Embalagens de araruta incorporadas com óleos de *H. stigonocarpa*, *H. courbaril* e *A. crassiflora* e extrato de *Syzygium cumini*, apresentaram maior espessura, menor teor de umidade e solubilidade em concentrações superiores a 0,75%, exceto para o extrato de *S. cumini* que apresentou maior teor de umidade.

A cor demonstrou maior luminosidade para embalagens compostas por óleos fixos de *H. stigonocarpa*, *H. courbaril* e *A. crassiflora*. Embalagens compostas por extrato de *S. cumini* apresentaram menor luminosidade.

Embalagens compostas por óleo fixo foram mais transparentes que embalagens compostas com extrato, apresentando maior transmissão de luz UV-Vis.

Embalagens compostas por óleos e extrato apresentaram maior biodegradabilidade em solo e inferior biodegradação em areia + água de mar.

O extrato de *S. cumini* desempenhou potencial capacidade de inibição dos radicais livres DPPH, FRAP, HRSA e NOSA, e na inibição bacteriana, principalmente para *Escherichia coli*, *Enterococcus faecalis* e *Bacillus subtilis*.

Embalagens compostas por extrato de *S. cumini* em diferentes pHs demonstraram ação inteligente na mudança de coloração em pHs ácido, neutro e alcali.

Embalagens compostas por óleos e extrato em queijo tipo Muçarela revestidos apresentaram baixa taxa de acidez titulável total, até 25 dias de armazenamento a 10°C.

A cor das embalagens compostas por óleos e extrato em queijo tipo Muçarela revestidos não apresentaram alteração drástica de coloração mantendo padrões até o fim do experimento com 25 dias a 10°C.

Amostras de queijo tipo Muçarela revestidos com embalagens compostas por extrato apresentaram maior biodegradabilidade em solo *in natura*.

Queijos tipo Muçarela revestidos com embalagens compostas por óleos e extrato não apresentaram contaminação bacteriana, fúngica ou por bolores durante o armazenamento.