BÁRBARA LEMES OUTEIRO ARAÚJO

MORPHOLOGY, CHEMICAL COMPOSITION AND STORAGE OF GRAINS OF *Moringa oleifera* Lam.

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação *Strictu-Sensu* em Engenharia Agrícola, na área de concentração em Processamento de Produtos Agrícolas, para a obtenção do título de Doutor.

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MORFOLOGIA, COMPOSIÇÃO QUÍMICA E ARMAZENAMENTO DOS GRÃOS DE *Moringa oleifera* Lam.

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APROVADA em 24 de junho de 2024.

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2025

À minha mãe, Sandra, por todo seu
amor, cuidado e doação.
Dedico!

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RESUMO

A Moringa oleifera tem despertado interesse como cultura agrícola devido à sua versatilidade funcional. Contudo, a carência de estudos abrangentes sobre as características físicas, químicas, processamento e armazenamento dos grãos é notória, o que é crucial para aprimorar os processos e a cadeia produtiva. Assim, com o objetivo de compreender as propriedades dos grãos de Moringa e sua interação com os processos pós-colheita, este estudo teve como objetivo realizar a caracterização física e química dos grãos, bem como analisar a qualidade após o armazenamento, considerando a variação do teor de água. A pesquisa foi conduzida no Departamento de Engenharia Agrícola da Universidade Federal de Lavras, utilizando grãos provenientes do estado do Maranhão com teores de água de 13%, 10% e 7% (b.s). Os grãos foram analisados quanto às suas características físicas, ultraestruturais e químicas. Ainda, foi avaliado o rendimento de extração mecânica do óleo e a qualidade deste para todas as amostras, em função do teor de água. Os grãos foram armazenados em silos bolsa de polietileno por 8 meses. Os resultados demonstraram a ausência de diferenças significativas nos valores morfométricos e nas características químicas do óleo, nos diferentes teores de água. Os grãos com teores de água de 0,10 e 0,13 b.s. apresentaram maior atividade de água, o que significa que havia mais água disponível para interagir com outras substâncias presentes nos grãos. Isso levou a uma maior dificuldade de separação entre a fase aquosa e a fase lipídica, resultando em menor eficiência de extração do óleo durante o processo de extração mecânica. Além disso, após o armazenamento, observou-se uma degradação mais significativa do óleo nos grãos com teores de água inicial mais altos, sugerindo menor estabilidade do óleo em grãos com elevados teores de água.

Palavras-chave: Teor de água; Pós-colheita; Ácidos graxos.

ABSTRACT

Moringa oleifera has attracted interest as an agricultural crop due to its functional characteristics. However, the lack of comprehensive studies on the physical, chemical characteristics, processing and storage of grains is notable, which is crucial to improving processes and the production chain. Thus, with the aim of understanding the properties of Moringa grains and their interaction with post-harvest processes, this study aimed to carry out the physical and chemical characterization of the grains, as well as analyze the quality after storage, considering the variation in water content. Research was conducted at the Department of Agricultural Engineering at the Federal University of Lavras, using sources from the state of Maranhão with water contents of 13%, 10% and 7% (b.s). The grains were analyzed for their physical, ultrastructural and chemical characteristics. Even so, the mechanical extraction yield of the oil and its quality were evaluated for all samples, depending on the water content. The grains were stored in polyethylene silos for 8 months. The results demonstrated the absence of significant differences in the morphometric values and chemical characteristics of the oil, at different water contents. Grains with water contents of 0.10 and 0.13 b.s. showed greater water activity, which means that there was more water available to interact with other substances present in the grains. This led to greater difficulty in separating the wet phase and the lipid phase, resulting in lower oil removal efficiency during the mechanical exhaust process. Furthermore, after storage, a more significant manipulation of the oil was observed in grains with higher initial water contents, demonstrating lower oil stability in grains with high water contents.

Keywords: Water activity; Post-harvest; Fatty acids.

INDICADORES DE IMPACTO

Os impactos sociais, tecnológicos, econômicos e culturais decorrentes dos estudos sobre o processamento e armazenamento de grãos de Moringa oleifera são amplos e relevantes, especialmente para regiões agrícolas do Brasil, como o estado do Maranhão, onde as culturas são conduzidas. O trabalho demonstra um potencial significativo de transformação em setores agrícolas e industriais, com ênfase na otimização da extração de óleo vegetal, o que pode promover avanços tecnológicos e melhorias no setor produtivo. Ao investigar a relação entre o teor de água nos grãos e a eficiência da extração mecânica de óleo, os estudos proporcionam subsídios para o desenvolvimento de técnicas mais eficazes de pós-colheita e armazenamento, impactando diretamente os produtores locais e empresas de processamento de óleos vegetais. Além disso, a pesquisa revela a importância do controle de qualidade durante o armazenamento para preservar as propriedades físicas e químicas dos grãos, o que resulta em ganhos econômicos para os produtores ao evitar perdas devido à deterioração, e a mitigação de riscos de contaminação por fungos como o *Aspergillus flavus*. Esses resultados podem incentivar práticas agrícolas mais sustentáveis e a aplicação de novas tecnologias na conservação de grãos, fomentando o crescimento econômico e a inclusão de pequenos produtores no mercado global de óleos vegetais, alinhando-se aos Objetivos de Desenvolvimento Sustentável da ONU, como o ODS 2 (Fome Zero e Agricultura Sustentável), ODS 8 (Trabalho Decente e Crescimento Econômico), ODS 9 (Indústria, Inovação e Infraestrutura), e ODS 12 (Consumo e Produção Responsáveis). Além disso, o trabalho reforça o impacto cultural ao valorizar uma cultura agrícola tradicional, promovendo sua adaptação tecnológica e inserção em cadeias produtivas mais modernas. Portanto, a pesquisa tem potencial de beneficiar diretamente comunidades agrícolas no Maranhão, proporcionando-lhes novas oportunidades econômicas e aprimorando práticas de cultivo e pós-colheita que podem ser replicadas em outras regiões, ampliando o impacto social e tecnológico.

IMPACT INDICATORS

The social, technological, economic, and cultural impacts resulting from studies on the processing and storage of Moringa oleifera grains are extensive and significant, particularly for agricultural regions in Brazil, such as the state of Maranhão, where these crops are cultivated. The work demonstrates a considerable potential for transformation in agricultural and industrial sectors, with a focus on optimizing vegetable oil extraction, which can promote technological advancements and improvements in the production sector. By investigating the relationship between the water content of the grains and the efficiency of mechanical oil extraction, the studies provide insights for the development of more effective post-harvest and storage techniques, directly impacting local producers and vegetable oil processing companies. Additionally, the research highlights the importance of quality control during storage to preserve the physical and chemical properties of the grains, leading to economic gains for producers by preventing losses due to deterioration and reducing the risk of contamination by fungi such as *Aspergillus flavus*. These findings may encourage more sustainable agricultural practices and the application of new technologies in grain conservation, fostering economic growth and the inclusion of small producers in the global vegetable oil market, aligning with the United Nations' Sustainable Development Goals (SDGs), such as SDG 2 (Zero Hunger and Sustainable Agriculture), SDG 8 (Decent Work and Economic Growth), SDG 9 (Industry, Innovation, and Infrastructure), and SDG 12 (Responsible Consumption and Production). Furthermore, the work underscores the cultural impact by valuing a traditional agricultural crop, promoting its technological adaptation, and integrating it into more modern production chains. Therefore, the research has the potential to directly benefit agricultural communities in Maranhão, offering them new economic opportunities and enhancing cultivation and post-harvest practices that can be replicated in other regions, expanding the social and technological impact.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A moringa oleífera (*Moringa oleifera* Lam.) é uma árvore de porte médio, originária da Índia, que tem despertado o interesse de pesquisadores e agricultores em todo o mundo devido às suas propriedades químicas e à facilidade de cultivo. Suas propriedades químicas incluem a presença de diversos compostos bioativos, como vitaminas, minerais, aminoácidos, antioxidantes e polifenóis, que conferem à planta vários benefícios. Pertencente à família Moringaceae, a moringa é uma angiosperma semelhante às leguminosas, por produzir frutos parecidos com vagens; contudo, estes frutos fazem parte da categoria de plantas de fruto capsular, com abertura entre lóculos.

É uma espécie que consegue se adaptar em diferentes tipos de solos e condições climáticas, sendo resistente a períodos de estiagem, devido ao papel fundamental desempenhado por suas raízes pivotantes e tuberosas na retenção de água e nutrientes. Pode atingir até 12 metros de altura, com produção anual de grãos de 9 toneladas por hectare.

Esta é uma planta com múltiplas possibilidades de utilização. Isso por suas folhas possuírem quantidades significativas de bioativos e nutrientes como flavonoides, proteínas e minerais essenciais, e seus grãos apresentarem alta concentração de proteínas e lipídios, favorecendo diversas aplicações industriais. O óleo extraído dos grãos é rico em ácido oleico e possui excelente estabilidade oxidativa, podendo ser empregado na indústria alimentícia, de biocombustíveis e farmacológica. Além disso, os grãos têm demonstrado ser um ótimo coagulante natural no tratamento de águas, apresentando eficácia comparável a compostos convencionais.

Apesar da vasta aplicabilidade comercial da moringa e do aumento do seu cultivo em diversos estados do Brasil e ao redor do mundo, há uma escassez de estudos sobre o seu manejo pós-colheita. O conhecimento das propriedades físicas e químicas dos grãos são fundamentais para otimização do processamento e armazenamento, garantindo assim a eficácia e a sustentabilidade ao longo da cadeia produtiva. Informações referentes as características físicas dos grãos, como tamanho, forma e massa específica, são cruciais no aprimoramento de equipamentos existentes e na implementação da eficiência nas operações de pós-colheita.

Outro fator de suma importância que influencia diretamente no valor do produto, é a manutenção da qualidade durante o armazenamento, por meio de práticas eficazes. Durante esse período, os grãos estão sujeitos a diversos fatores ambientais, como temperatura, umidade, oxigênio e presença de microrganismos, os quais podem afetar suas propriedades físicas e químicas. O teor de água emerge como um dos principais determinantes da qualidade dos grãos, influenciando sua densidade, capacidade de fluxo e processamento.

Dentro de uma faixa específica de atividade de água, ocorre um ambiente propício para reações químicas e enzimáticas nos grãos, potencializando processos de decomposição e favorecendo o crescimento de microrganismos, como fungos e bactérias. Portanto, compreender e controlar esses parâmetros é essencial para garantir a qualidade e segurança dos produtos durante o armazenamento e transporte. Além disso, a interação química da água com os componentes dos grãos exerce influência sobre sua estabilidade e durabilidade durante a armazenagem.

Neste contexto, com o objetivo de compreender as propriedades físicas e químicas dos grãos de moringa e sua interação com os processos pós-colheita, este estudo teve como objetivo realizar a caracterização dos grãos de moringa e analisar a qualidade destes após o armazenamento, considerando a variação do teor de água.

2 REFERENCIAL TEÓRICO

2.1 *Moringa oleifera* Lam.

A moringa, denominada cientificamente como *Moringa oleifera* Lamarck, é uma angiosperma arbórea da ordem Brassicales e pertencente à família Moringaceae. Popularmente chamada de Acácia-branca, é nativa da Índia e foi introduzida no Brasil na década de 50 como planta ornamental (Liu *et al.*, 2022). Nos últimos anos, tem sido considerada uma importante cultura agrícola, devido ao interesse comercial em suas folhas e grãos, os quais podem ser empregados em diversos setores industriais (Meireles *et al.*, 2020; Islam *et al.*, 2021; Abdel-Latif *et al.*, 2022).

A espécie é uma planta alógama, caracterizada por sua fácil disseminação e ampla adaptabilidade às condições edafoclimáticas. Consegue se desenvolver em diversos tipos de solos, com valor de pH entre 5 a 8, em climas tropicais e secos, podendo atingir até 12 metros de altura (Gualberto *et al.*, 2015; Noronha *et al.*, 2018; Lopes *et al.*, 2023). Suas raízes pivotantes e tuberosas desenvolvem-se rapidamente, surgindo poucos dias após a semeadura, e desempenham um papel crucial na acumulação de água e nutrientes em seus tubérculos, conferindo à planta a capacidade de resistir a períodos de estiagem (Ramos *et al.*, 2010; Castro, 2022).

A copa da árvore de moringa apresenta uma configuração aberta semelhante a uma sombrinha, com folhas bipinadas e flores de tonalidade próxima ao branco. Os frutos, que se assemelham a vagens, são, na verdade, cápsulas loculicidas, com três valvas que se abrem ao longo das nervuras medianas. Estes têm coloração esverdeada durante o desenvolvimento dos grãos, tornam-se castanhos quando amadurecem, contendo aproximadamente de 15 a 20 sementes cada. A produção anual de grãos, quando a árvore atinge a fase adulta, pode chegar a 9 toneladas por hectare (Ramos *et al.*, 2010; Pereira, 2015; Castro, 2022; Lopes *et al.*, 2023).

A moringa oleífera é uma espécie que tem se destacado por sua multiplicidade funcional, podendo ser utilizada desde suas folhas aos frutos. As folhas são ricas em bioativos, contendo flavonoides, taninos, cumarinas e alcaloides, exibindo potenciais atividades anti-inflamatórias, hepatoprotetoras, antioxidantes e antimicrobianas (Saraiva *et al.*, 2018). Ademais, apresentam um teor substancial de proteínas (superior a 25%), carboidratos, e minerais essenciais como cálcio e potássio (Yaméogo *et al.*, 2011). Ao analisar o conteúdo nutricional e vitamínico nas folhas de moringa em comparação com outros alimentos, Seguimoto (2013) constatou que elas apresentam uma concentração de cálcio quase 3 vezes maior que o leite e potássio que a banana, mais de 7 vezes a quantidade de vitamina C que a laranja, destacando-se como um produto de alto valor nutricional.

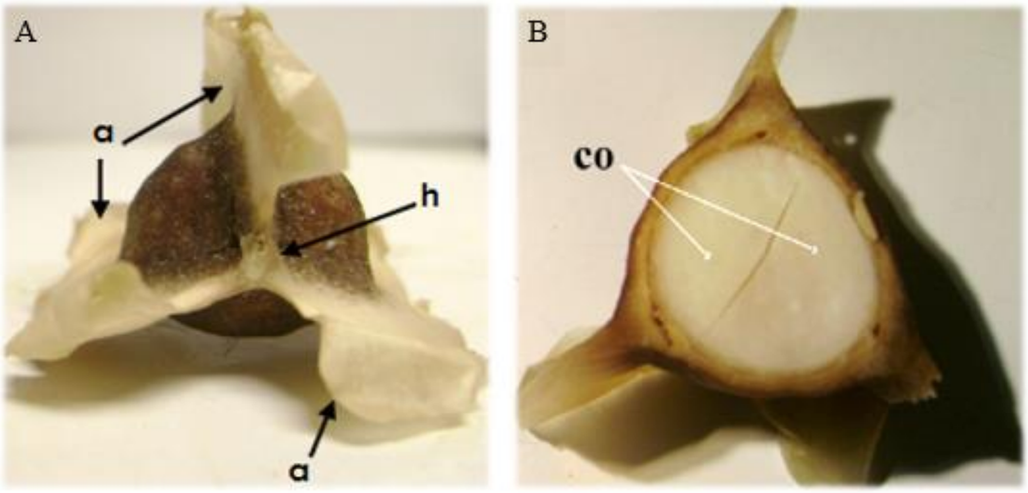
Os grãos de moringa, por sua vez, são ricos em proteínas (35%) e lipídios (40%), sendo o óleo composto por alto teor de ácido oleico e baixo teor de ácido linoleico, tornando-o uma opção promissora como substituto do azeite, bem como uma matéria-prima valiosa na elaboração de cosméticos, biodiesel e lubrificantes (Araújo *et al.*, 2020; Omonhinmin *et al.*, 2020; Barbosa *et al.*, 2021; Cárdenas; Serrano, 2021). Devido à composição química do óleo e sua excelente estabilidade oxidativa, atribuída à presença de δ-tocoferol, destaca-se como uma alternativa viável em meio à crescente busca por óleos vegetais, sendo aplicado nas indústrias alimentícia, de biocombustíveis e farmacológica (Magalhães *et al.*, 2020). Além disso, a proteína presente nos grãos de moringa destaca-se como coagulante natural no tratamento de águas, demonstrando notável potencial na redução da carga bacteriana, com eficácia comparável à do sulfato de alumínio. Essa abordagem de baixo custo favorece uma purificação ecologicamente sustentável (Mourão *et al.*, 2020; Ribeiro, 2021; Figueiredo *et al.*, 2022).

2.1.1 Morfologia e composição química de sementes de moringa

A morfologia de sementes refere-se ao estudo da forma, tamanho, cor, textura e outras características físicas externas destas, desempenhando um papel crucial em diversas áreas do setor agrícola (Santos *et al.*, 2018; Silva *et al.*, 2018). As informações acerca das características morfológicas contribuem em vários aspectos, tais como: na identificação de plantas, auxiliando na taxonomia e no entendimento de suas adaptações evolutivas; no manejo agrícola eficiente, ao fornecer informações sobre a qualidade, viabilidade e potencial de germinação; e na compreensão das interações entre a planta e o ambiente (Lopes *et al.*, 2019).

As sementes de moringa são classificadas como ortodoxas e dicotiledôneas, e sua morfologia é caracterizada por uma forma global, onde três alas equidistantes de coloração clara estão inseridas em um tegumento de tonalidade castanho médio, envolvendo o endosperma oleaginoso. Essas alas fundem-se em uma das extremidades e, na outra, protegem o hilo, que é pequeno, linear e saliente, de onde ocorre a protrusão da radícula e da alça cotiledonar (Ramos *et al.*, 2010).

Figura 1 – Semente de moringa oleifera, evidenciando (A) as alas (a) e o hilo (h), e com corte transversal (B), demonstrando o endosperma e dois cotilédones (co).



Fonte: Ramos *et al.* (2010).

As dimensões médias das sementes podem variar conforme o ambiente de cultivo, tipo de solo, estágio de desenvolvimento, e outros fatores. Aguiar (2017), ao investigar o impacto da adubação nas características físicas dos grãos de moringa, observou variações no comprimento (8,39 a 23,10 mm), largura (7,16 a 13,34 mm), espessura (8,13 a 16,15 mm) e peso unitário (0,10 a 0,37 g).

Além das características externas inerentes das sementes de moringa, estas possuem propriedades químicas que propiciam diversas aplicações funcionais. Os grãos são ricos em óleo, podendo variar entre 26% e 40%, e proteína, de 24% e 37% (Oliveira *et al.*, 2012; Garza *et al.*, 2017; Dantas, 2018; Jesus, 2018; Araújo *et al.*, 2020).

O óleo extraído dos grãos de moringa é composto majoritariamente de ácidos graxos monoinsaturados, sendo o principal o oleico, com aproximadamente 70%. Entre os ácidos graxos saturados, os mais comumente encontrados são o esteárico (C18:0), araquídico (C:20:0) e o behênico (C22:0), com percentuais próximos à 5% (Boukandoul *et al.*, 2018; Araújo *et al.*, 2020; Chaves *et al.*, 2023).

O alto teor oleico contribui para resistência térmica e estabilidade oxidativa do óleo de moringa, por ter apenas uma insaturação na cadeia carbônica, diferindo dos ácidos graxos poli-insaturados. Essa característica faz com que seja mais estável quando exposto ao calor e menos propenso a reações de oxidação. Além disso, a presença de antioxidantes naturais como tocoferóis (vitamina E) e compostos fenólicos no óleo, contribuem para sua resistência a oxidação (Boukandoul *et al.*, 2018; Dinesha *et al.*, 2018). Duarte *et al.* (2018) avaliaram a estabilidade oxidativa e térmica do óleo de moringa em comparação com os óleos de canola e azeite de oliva. Os resultados indicaram que tanto o de moringa quanto o azeite foram mais estáveis em relação à oxidação. Entretanto, o óleo de moringa apresentou maior estabilidade térmica que os demais. Além disso, os óleos de canola e azeite de oliva sofreram isomerização, conforme evidenciado pela absorbância de ácidos graxos com duplas ligações trans no espectro infravermelho (Duarte *et al.*, 2018).

Considerando as propriedades físicas dos grãos, é importante mencionar que as propriedades físico-químicas do óleo de moringa podem ser alteradas de acordo com o local de cultivo, práticas de manejo e o tipo de processamento pós-colheita Segundo alguns pesquisadores, os índices de acidez e peróxido do óleo de moringa podem variar de 0,2 a 9,9 mg NaOH.g^{-1} e 1,9 a 28,6 meq.kg^{-1}, respectivamente (Oliveira *et al.*, 2012; Pereira *et al.*, 2016; Chaves *et al.*, 2018; Carvalho, 2019; Araújo *et al.*, 2020). Tais alterações são influenciadas por fatores como a localização de produção, o método de secagem e extração do óleo dos grãos, além da condição deste, se bruto ou purificado (Pereira *et al.*, 2016; Chaves *et al.*, 2018; Araújo *et al.*, 2020).

2.2 Processamento e armazenamento de grãos

No ciclo produtivo de grãos, as etapas de processamento e armazenamento na pós-colheita são fundamentais, podendo influenciar significativamente a qualidade e a disponibilidade final dos produtos. Estas desempenham um papel essencial na preservação e manutenção das características dos grãos, afetando diretamente sua utilidade e comercialização (Amaral *et al.*, 2018, Haeberlin *et al.*, 2021).

O conhecimento das propriedades físicas das sementes é indispensável para a otimização de equipamentos existentes, visando aprimorar a eficiência nas operações de processamento e sistemas de armazenamento (Sirisomboon *et al.*, 2007; Milman *et al.*, 2014). Informações sobre o tamanho, como área superficial, área projetada e volume, assim como a forma, expressa por características como circularidade e esfericidade, são elementares para estabelecer os limites inferiores no dimensionamento de equipamentos de transporte, como esteiras, elevadores de canecas e transportadores helicoidais. Outro aspecto físico de grande importância é a massa específica aparente e unitária, para projetos de armazenamento, cálculos de transportadores, desenvolvimento de separadores e classificadores de sementes (Sirisomboon *et al.*, 2007; Gomes *et al.*, 2018; Silva *et al.*, 2018; Morais *et al.*, 2018).

Dentre os processamentos pós-colheita, a secagem é uma das principais etapas para manutenção da qualidade durante o período de armazenamento. Esta consiste em um processo complexo que envolve a transferência simultânea de calor e massa entre o produto e o ar de secagem, reduzindo a quantidade de água dos grãos, por meio da evaporação (Brooker *et al.*, 1992). A preservação das propriedade físico-químicas dos grãos pela secagem baseia-se no fundamento de que com a diminuição da atividade de água, ocorra a redução da velocidade das reações químicas, bioquímicas e do desenvolvimento de microrganismos, durante a armazenagem (Prado *et al.*, 2020; Haeberlin *et al.*, 2021).

O teor de água dos grãos além de influir na qualidade dos produtos no armazenamento, pode afetar o rendimento de extração mecânica de óleo. Pesquisas indicam que níveis mais elevados de teores de água estão associados a uma diminuição na eficácia do processo de extração, por dificultar a ruptura do produto e aumentar a emulsificação e plasticidade da massa, comprometendo a remoção do óleo (Singh *et al.*, 2002; Beerens, 2007; Pighinelli *et al.*, 2009).

2.2.1 Armazenamento e qualidade

A manutenção da qualidade dos grãos ao longo do período de armazenamento é imprescindível para a comercialização, por exercer impacto direto no valor do produto. Durante a armazenagem, a massa de grãos está constantemente exposta a uma variedade de fatores externos, que podem ser físicos, como temperatura e umidade; químicos, incluindo o fornecimento de oxigênio; e biológicos, representados por bactérias, fungos, insetos e roedores (Brooker *et al.*, 1992; Haeberlin *et al.*, 2021; Silva *et al.*, 2022). Essa complexidade destaca a importância de estratégias eficazes para preservar as propriedades químicas e físicas destes, garantindo sua integridade, evitando perdas qualitativas e quantitativas no processos pós-colheita (Amaro *et al.*, 2019; Ferreira *et al.*, 2021).

O teor de água emerge como o fator preponderante que impacta diretamente a longevidade das sementes durante o armazenamento. Isso porque pode provocar um aumento na taxa respiratória e no consumo das reservas energéticas destas (Ullmann *et al.*, 2018; Wang *et al.*, 2018).

Devido às suas propriedades higroscópicas, os grãos tendem a alcançar um equilíbrio com as condições ambientais, especialmente em relação à temperatura e à umidade relativa do ar, resultando em um comportamento específico nas isotermas de sorção (Isquierdo *et al.*, 2020). A composição química das sementes influencia diretamente esse comportamento, onde espécies ricas em lipídios exibem teores de água mais baixos quando comparadas às ricas em amido, sob condições ambientais semelhantes. Esse fenômeno ocorre devido à natureza hidrofóbica dos lipídios, que adsorvem menos água, impactando diretamente seu equilíbrio higroscópico (Isquierdo *et al.*, 2020; Bessa *et al.*, 2021).

A atividade de água está intrinsicamente ligada ao teor de água do produto, representada por isotermas de sorção, de forma gráfica ou analítica, relacionando o teor de água de equilíbrio com a atividade de água, em temperatura constante. Essa relação é fundamental para compreender a estabilidade dos alimentos, quanto aos processos microbiológicos, enzimáticos e químicos (Arslan-Tontul, 2020). Embora o teor de água seja indicativo de propensão à deterioração ou contaminação, isoladamente não é suficiente para predizer a perecibilidade, por não considerar a interação da água com outros componentes dos grãos (Rosa *et al.*, 2021; silva *et al.*, 2021).

De acordo com as isotermas de sorção, determinadas por alguns pesquisadores, observa-se que grãos com o mesmo teor de água, mas com alto teor de amido e baixo teor de lipídios, tendem a ter uma atividade de água menor em comparação aos oleaginosos (Resende *et al.*, 2006; Smaniotto *et al.*, 2012; Bessa *et al.*, 2021; Santos, 2022). Estudos presentes na literatura indicam que sementes de milho e feijão, com 13% de teor de água a 30ºC, possuem atividade de água em torno de 0,50, enquanto as de soja e cártamo apresentam aproximadamente 0,70 (Resende *et al.*, 2006; Smaniotto *et al.*, 2012; Bessa *et al.*, 2021; Santos, 2022). O mesmo padrão é observado em grãos com teor de água de 10%, onde os de feijão têm uma atividade de água de 0,26, ao passo que os de soja e cártamo possuem cerca de 0,52 (Resende *et al.*, 2006; Bessa *et al.*, 2021; Santos, 2022).

Como os grãos de moringa são ricos em lipídios, sua atividade de água tende a seguir o padrão dos oleaginosos, devido à natureza hidrofóbica dos lipídios, que adsorvem menos água, impactando diretamente o equilíbrio higroscópico (Isquierdo *et al.*, 2020; Bessa *et al.*, 2021). Essas variações refletem diferenças na estabilidade dos grãos em relação à atividade de água, destacando a importância desse parâmetro na qualidade e conservação dos alimentos (Kooshki *et al.*, 2018).

2.2.2 Impacto do teor de água na preservação da qualidade

O teor de água interfere diretamente na quantidade de água livre presente nos grãos, impactando na preservação da qualidade durante o armazenamento (Htwe *et al.*, 2018). Em uma faixa de Aw entre 0,4 e 0,8, cria-se um ambiente propício para reações químicas e enzimáticas, impulsionadas pelo aumento das concentrações dos substratos orgânicos (Van Den Berg *et al.*, 1981; Damodaran; Parkin, 2019). Quando a atividade de água atinge um valor de aproximadamente 0,6, o processo de decomposição tende a aumentar, como a oxidação de lipídeos, potencializado pela atividade enzimática (Damodaran; Parkin, 2019; Önder *et al.*, 2020). O crescimento de microrganismos, como fungos e bactérias, ocorre normalmente a partir de valores de Aw de 0,70 e 0,80, respectivamente, intensificando a degradação química dos grãos (Damodaran; Parkin, 2019).

A quantidade de água presente nas sementes modula a ocorrência dos processos bioquímicos e fisiológicos, impactando na atividade mitocondrial e nas reações oxidativas, contribuindo para perdas de vigor e qualidade ao longo do tempo (Chhabra *et al.*, 2019; Önder *et al.*, 2020). As espécies reativas de oxigênio, responsáveis pelo estresse oxidativo e pela deterioração dos grãos, têm origem principalmente em reações não enzimáticas, como a oxidação de ácidos graxos poli-insaturados, e nas reações de Maillard e Fenton (Fotouo *et al.*, 2020; Zhang *et al.*, 2021). As plantas oleaginosas, devido ao seu elevado teor de lipídeos, são particularmente vulneráveis aos impactos dessas espécies reativas de oxigênio (Whitehouse *et al.*, 2020).

Os fungos pertencentes aos gêneros *Aspergillus* e *Penicillum* são frequentemente identificados em instalações de armazenamento, originados diretamente do campo e presentes em sementes recém-colhidas, geralmente em proporções reduzidas (Prestes *et al.*, 2019). Estes são considerados xerofílicos e osmofílicos, uma vez que são capazes de crescer e se reproduzir em ambientes com baixa umidade relativa e atividade de água, sendo limitados apenas em atividades de água menores que 0,62 (Barbosa-Cánovas *et al.*, 2007; Damodaran; Parkin, 2019).

A presença de fungos no armazenamento de grãos é prejudicial devido à produção de enzimas e geração de compostos tóxicos, como micotoxinas (Abd-allah *et al.*, 2018; Ramirez *et al.*, 2018). Isso acarreta alterações na composição química e estrutural das sementes, resultando na redução da qualidade destas e gerando prejuízos financeiros (Abd-allah *et al.*, 2018; Lamichaney *et al.*, 2019).

Haeberlin *et al.* (2021) avaliaram os efeitos do teor de água na qualidade de grãos de canola, com 8%, 10%, 12% e 14%, durante um período de armazenamento de 180 dias, em diferentes temperaturas. Os resultados demonstraram que os grãos com teores de água de 12% e 14%, em temperaturas de 17°C e 27°C, apresentaram perda de matéria seca e desenvolvimento de fungos.

Tripathi e Kumar (2020) investigaram o impacto da infestação por fungos nas características físico-químicas de sementes de *Salvadora oleoides* D. e *Salvadora persica* L. O estudo concluiu que diferentes níveis de infestação, em comparação com sementes saudáveis, induziram à biodeterioração, resultando na diminuição dos teores de lipídeos, proteínas e carboidratos, além do aumento de ácidos graxos livres no óleo. Em um estudo semelhante, Abd-Allah *et al.* (2018), ao analisarem a deterioração por fungos na qualidade de grãos armazenados de algumas leguminosas, verificaram um aumento significativo nos índices de acidez e peróxido no óleo de soja, demonstrando o efeito da deterioração fúngica na composição dos grãos.

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SEGUNDA PARTE - ARTIGOS

MORPHOLOGICAL AND CHEMICAL CHARACTERIZATION OF *Moringa oleifera* LAM. GRAINS

ABSTRACT

Moringa oleifera stands out as an agricultural crop due to its functional versatility. However, comprehensive studies on its physical characteristics and postharvest processing, which are crucial for process optimization and the production chain, are lacking. Thus, the aim of this study was to morphologically and chemically characterize M. oleifera Lam. grains as a function of water content. *M. oleifera* Lam. grains freshly harvested from the second half of the 2022 crop from the state of Maranhão were used. Morphological and chemical characterization of the grains with different water contents (13%, 10% and 7% (db)) was performed. The characteristic dimensions, unit mass, projected area, apparent specific gravity, unit specific mass, porosity, sphericity, circularity, water content and water activity of the grains were determined and microstructural analysis was performed. Moreover, the mechanical oil extraction efficiency and oil quality of all samples were also evaluated. The results showed that there were no significant differences in the morphometric values or chemical characteristics of the oils. However, a direct relationship was observed between the water content and the amount of free water, and there was an inverse relationship between water content and oil extraction efficiency. According to the ultrastructural analyses, the grains with a water content of 0.07 db presented more accessible oil droplets, resulting in more efficient oil extraction.

Keywords: *Moringa oleífera*; Postharvest; Biometrics; Electron microscopy.

1 INTRODUCTION

Moringa oleifera Lam. is an angiosperm tree of the order Brassicales and belonging to the family Moringaceae; it is native to India and was introduced in Brazil in the 1950s as an ornamental plant (Liu *et al.*, 2022). This plant species is allogamous and characterized by its easy dissemination and wide adaptability to soil and climate conditions. It can grow in various soil types with pH values between 5 and 8 and in tropical and dry climates and can reach up to 12 meters in height (Gualberto *et al.*, 2015; Noronha *et al.*, 2018; Lopes *et al.*, 2023).

Moringa fruits are pod-like; they are composed of loculicidal capsules with three valves that open along the midribs and may contain 15 to 20 seeds (Ramos *et al.*, 2010). When the tree reaches adulthood, its annual grain production can reach up to 9 tons per hectare (Ramos *et al.*, 2010; Pereira, 2015; Castro, 2022; Lopes *et al.*, 2023).

M. oleifera has become an important agricultural crop due to the growing commercial interest in its leaves and grains, which have been applied in various industrial sectors (Meireles *et al.*, 2020; Islam *et al.*, 2021; Abdel-latif *et al.*, 2022). The leaves are bipinnate and rich in bioactive compounds, proteins (more than 25%), carbohydrates and essential minerals such as calcium and potassium (Yaméogo *et al.*, 2011; Saraiva *et al.*, 2018). The seeds, classified as orthodox or dicotyledonous, have an overall morphology with three equidistant light-colored wings surrounded by a medium brown integument that includes the oilseed endosperm (Ramos *et al*., 2010). These products have high oil contents, ranging from 26% to 40%, and a protein content ranging from 24% to 37% (Oliveira *et al.*, 2012; Araújo *et al.*, 2017; Garza *et al.*, 2017; Dantas, 2018; Jesus, 2018; Araújo *et al.*, 2020).

Despite the wide functional multiplicity of *M. oleifera*, there is a lack of studies on its physical characteristics, postharvest processing, and fundamental elements process and production chain optimization (Silva *et al.*, 2020). Detailed knowledge of the physical properties of seeds is essential for improving existing equipment and increasing efficiency in terms of processing and storage operations (Sirisomboon *et al.*, 2007; Milman *et al.*, 2014). Information related to seed size, such as area and volume, as well as seed shape, indicated by characteristics such as circularity and sphericity, is essential for establishing lower limits when designing transportation equipment, such as conveyors and elevators. The specific gravity is also of paramount importance in the preparation of storage projects, conveyor calculations, and the development of separators, precleaning machines and seed classifiers (Sirisomboon *et al.*, 2007; Gomes *et al.*, 2018; MORAIS et al., 2018; Silva *et al.*, 2018).

Moreover, the physical and chemical characteristics of grains should also be studied, which may be affected by the water content. The water content is directly related to the density and compactness of the product, which can change its physical dimensions and, consequently, its flow, packaging and processing capacity (Morais *et al.*, 2018). In agreement with this, studies have indicated that a higher water content can decrease the efficiency of mechanical oil extraction, hinder the crushing of grains, increase the emulsification and malleability of the mass, and compromise the removal of lipids (Pighinelli *et al.*, 2009; Mendonça *et al.*, 2020).

Given the importance of the physical properties of grains during postharvest processing, the aim of this study was to morphologically and chemically characterize the grains of *M. oleifera* Lam. as a function of water content.

2 MATERIALS AND METHODS

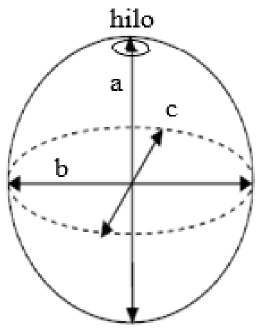
This research was conducted at the Department of Agricultural Engineering of the Federal University of Lavras, and the experiments were conducted at the Laboratory of Agricultural Product Processing, Laboratory of Oilseed Plants, Oils, Fat and Biodiesel and Laboratory of Electron Microscopy and Ultrastructural Analysis of this institution.

Freshly harvested *M. oleifera* Lam. grains from the second half of the 2022 crop from the state of Maranhão were used. The manual harvest was made of fruits with a dark brown color, indicating maturity.

Morphological and chemical characterization of grains with different initial water contents of 13% (ds) and those subjected to drying until the initial water content reached 10 and 7% (ds) were conducted. Drying of a thin layer of grain was performed artificially using a mechanical laboratory dryer with forced convection at an air speed of 0.33 $m.s^{-1}$ and a controlled drying air temperature of 40°C. The water content was determined before and after drying following the recommendations of the Rules for Seed Analysis (Brasil, 2009) using the oven method of heating to 105 ± 1°C for 24 hours.

Physical characterization of the grains was based on the length (a), width (b) and thickness (c) of the grains, as shown in Figure 1. A digital caliper with a resolution of 0.01 mm was used following the methodology established by Mohsenin (1986). There were 30 units for each water content, and samples were selected randomly.

Figure 1 – Schematic of the moringa grain and its characteristic dimensions.



Source: Author (2024).

wherein:

a - length, mm;

b - width, mm; and

c - thickness, mm.

According to the measurements obtained from the orthogonal axes, the projected area, sphericity and circularity of the grains were determined using equations 1, 2 and 3, respectively, as suggested by Mohsenin (1986):

$$\mathrm{Ap} = \frac{\pi ab}{4} \tag{1}$$

$$\mathrm{Es} = \left[\frac{(abc)^{\frac{1}{3}}}{a}\right] x\, 100 \tag{2}$$

$$\mathrm{Cr} = \frac{b}{a}\, x\, 100 \tag{3}$$

wherein:

A_p - projected area, mm ^{2};

E_s – sphericity, %; and

C_r – circularity, %.

To determine the real or unit density (equation 5), the grain mass (g) was determined with a scale with a resolution of 0.001 g, and the volume (equation 4) for each water content was determined according to Mohsenin (1986):

$$\mathrm{Vg} = \frac{\pi(abc)}{6} \tag{4}$$

$$\rho u = \frac{mg}{Vg} \tag{5}$$

wherein:

V_g – grain volume, mm^3;

m_g – unit mass of the grain, g; and

ρ_u – unit specific mass, kg.m^{-3}.

The apparent specific gravity was determined using hectoliter weight scale with a resolution of 0.01 g (Gehaka - BK 4001) and a container with a capacity of 1 L. Subsequently, the porosity of the grain mass was calculated by determining the ratio of the unit and apparent specific gravity, as presented in equation 6, according to Araújo *et al.* (2014):

$$\varepsilon = \left[1 - \frac{\rho ap}{\rho u}\right] x\ 100 \tag{6}$$

wherein:

ε – porosity, %; and

ρ_{ap} – apparent specific gravity, kg.m^{-3}.

Water activity analysis was rapidly performed by measuring the dew point using Decagon Devices Aqualab series 3TE equipment, at a temperature of 25 ± 2°C.

The moringa oil from each sample was extracted by a mechanical expeller press with a radial tubular extraction system, model ERT 50, to determine its physicochemical parameters. The lipid content was quantified after chemical extraction with organic solvent (hexane) via the direct Soxhlet method (IAL, 2008). The efficiency of mechanical extraction was calculated based on the lipid content determined by chemical extraction of the grain and cake, as shown in equation 7.

$$\mathrm{Ef} = \frac{(\mathrm{Tg} - \mathrm{Tt})\ \mathrm{x}\ 100}{\mathrm{Tg}} \tag{7}$$

wherein:

Ef – extraction efficiency, by the mechanical method, %;

Tg – grain oil content, determined by the chemical method, %; and

Tt – oil content of the cake, calculated by the chemical method, %.

The acid, unsaturated fatty acid and oleic fatty acid contents in the crude moringa oil was analyzed in triplicate according to the methodology of the Instituto Adolfo Lutz (2008). The acid content is expressed as the number of milligrams of potassium hydroxide (KOH) required to neutralize 1 g of oil, using equation 8. The percentages of unsaturated fatty acids and oleic fatty acids were determined by means of gas chromatography of the ester methyl groups (IAL, 2008).

$$\mathrm{IA} = \frac{\mathrm{v\ x\ f\ x\ 5{,}61}}{\mathrm{P}} \tag{8}$$

wherein:

IA – acid value, mg $KOH.g^{-1}$;

v – volume of KOH solution (0.1 M) used in the titration, ml;

f – correction factor of the KOH solution; and

P – mass of the oil sample, g.

Microstructural analysis was performed by scanning electron microscopy to explore the internal conformation of moringa grains with different water contents. The methodology used was that described by Saath *et al.* (2010), where one grain from each treatment was randomly selected for analysis. The samples were immersed in a modified Karnovsky (1965) fixative solution composed of 2.5% glutaraldehyde, 2.0% paraformaldehyde and 0.05 M cacodylate buffer, pH 7.2, and then transferred to a cryoprotectant (30% glycerol) for 30 minutes. Subsequently, the grains were cut longitudinally under nitrogen and transferred to a solution of 1% osmium tetroxide and distilled water for one hour. Then, the samples were washed three times with distilled water, dehydrated in a series of acetone solutions (25%, 50%, 75%, 90% and 100% three times), transferred to a Balzers CPD 030 critical point apparatus to replace the acetone with CO_2, and dried. Finally, the sections were mounted on aluminum stubs, coated with gold and observed under an LEO EVO 40 XVP field emission scanning electron microscope. The operating conditions were maintained at 20 kV and a distance of 7 to 15 mm, and the images were acquired and processed using PhotoPaint software from the CorelDraw 12 package.

The data obtained from the physical and chemical characterization of the moringa beans were subjected to analysis of variance, and the F test was applied at the 5% significance level. Then, linear regression graphs were prepared for the different levels of water content in the grains, reflecting the relationship between the variables analyzed (Ferreira, 2011).

3 RESULTS AND DISCUSSION

The mean values of the characteristic dimensions and the analysis of variance of the physical properties of moringa oleifera grains, with different water contents, are presented in Table 1.

Table 1 - Dimensions (a, b, c), unit mass (m_g), projected area (A_p), apparent specific mass (ρ_{ap}), unit specific mass (ρ_u), porosity (ε), sphericity (E_s) and circularity (C_r) of *M. oleifera* grains with different moisture contents.

Water content (db)	Orthogonal axes (mm)			m_g (g)	A_p (mm^2)	ρ_{ap} ($kg.m^{-3}$)	ρ_u ($kg.m^{-3}$)	ε (%)	E_s (%)	C_r (%)
	a	b	c							
0.07	12.49	11.49	10.89	0.32	112.97	167.36	398.41	56.81	93.26	88.31
0.10	12.28	11.60	10.97	0.31	112.30	168.36	373.07	54.97	95.02	90.56
0.13	12.64	11.40	10.70	0.34	113.30	176.71	429.97	58.29	92.03	87.63
Mean	12,47	11.50	10.86	0.32	112.86	170.81	400.48	56.68	93.44	88.83
MS	1.00 ^{ns}	0.29 ^{ns}	0.58 ^{ns}	0.0064 ^{ns}	7.90 ^{ns}	222.85 ^{ns}	4877.13 ^{ns}	16.53 ^{ns}	67.71 ^{ns}	52.00 ns
CV (%)	11.35	7.61	8.60	19.22	15.13	6.55	14.83	13.20	8.21	7.80

MS: Mean squares. CV: Coefficient of variation. ns: not significant according to the F test.

Source: Author (2024).

The average characteristic dimensions of the grains (length, width and thickness) as a function of water content were similar, with a variation of less than 0.36 mm along the orthogonal axis “a” and 0.20 mm along axes “b” and “c”. Cavalcante *et al.* (2017) determined the morphometric values for M. oleifera grains with a water content of 0.07 (db), where the average length was 10.92 mm and the thickness was 10.41 mm, differing from the results of this study. Vasconcelos *et al.* (2018) characterized grains with an 8% water content and obtained average length and width values of 10.01 mm and 9.68 mm, respectively. Such differences in the average dimensions of seeds occur particularly due to abiotic factors in the plant's cultivation environment (Chaves *et al.*, 2023).

The apparent specific mass showed an increase of 5.6% in the range of water content variation 0.07 to 0.13 (db), but it was not statistically significant. Despite the tendency for grain dimensions to increase with increasing water content, due to their biological nature, they exhibit natural and complex variations in their physical characteristics, not limited to a specific behavior (Vale; Monteiro, 2021). This was corroborated by the study by Gomes *et al.* (2018), who investigated the relationship between the size and shape of cowpea grains and water content. In this study, the major axis, the projected surface area and the grain volume increased in the water content range of 0.12 to 0.20 but decreased when the water content reached 0.23 (db).

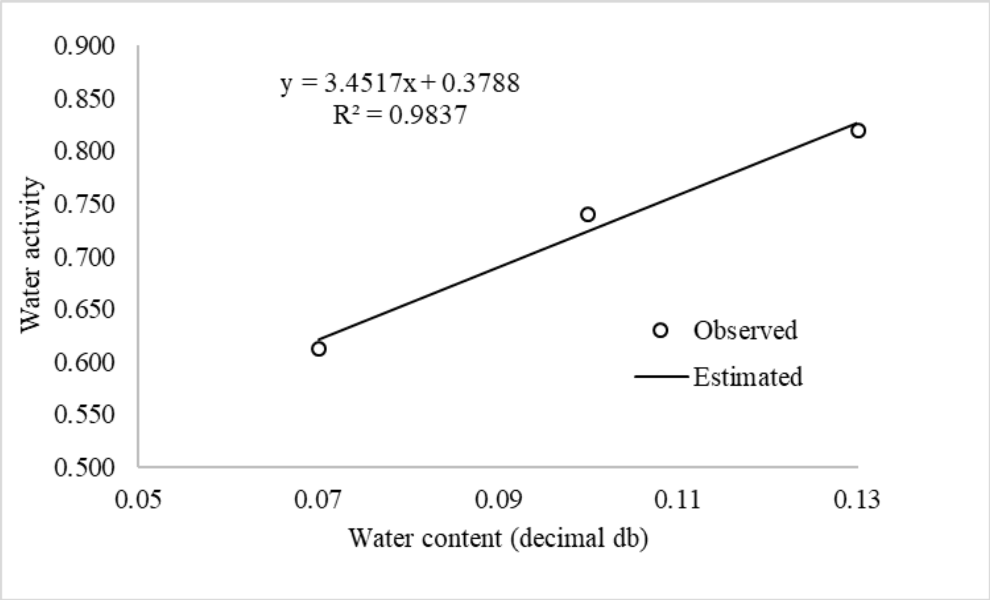
According to the data in Table 1, when analyzing the samples with water contents of 0.10 and 0.13, it was observed that the projected area, unit specific mass, porosity, sphericity and circularity did not present a specific relationship with the water contente. This is possibly due to the fact that these physical properties were obtained through data on orthogonal axes and specific mass, which did not show a significant difference. A study conducted by Morais *et al.* (2018) reporting the characteristics related to the physical properties of soybean seeds with different moisture contents noted that an increase in the dimension of the orthogonal axis of a cultivar from a moisture content of 11% to 13% resulted in an increase in surface area of 16.6%, a result similar to that found for the projected areas of grains with water contents of 0.10 and 0.13 (db) in this study.

Thus, based on the analysis of variance presented, there was no significant difference between the physical characteristics of the grains in relation to the water content. The high coefficient of variation indicates high variability in grain size, which is attributable to the biological composition of the grain, even in the case of a homogeneous sample. Similarly, Cavalcante *et al.* (2017) and Vasconcelos *et al.* (2018) reported variations of 10.2 to 11.7 mm and 6.8 to 14.2 mm in the length of moringa grains, respectively, within the same sample.

The grain dimensions may vary depending on the moisture content, as well as the cultivar, the environmental conditions of the process, the geometry and the specific composition of the product (Lopes *et al.*, 2019). In both adsorption and desorption processes, the interactions between water and the chemical components of the grains, such as carbohydrates and proteins, result in distinct changes to the molecular structure, such as volumetric contraction or expansion (Borém *et al.*, 2013; Gomes *et al.*, 2018; Rodrigues *et al.*, 2020).

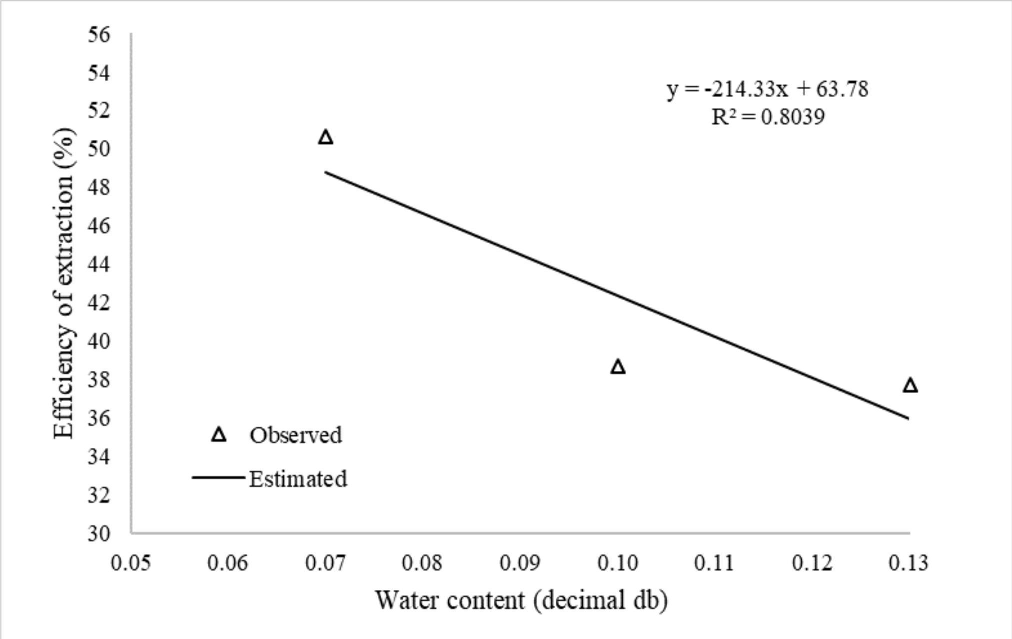
Figures 2 to 7 show the regression analyses for water activity, lipid content of the grains, oil extraction efficiency and oil characteristics, such as acidity index and percentage of unsaturated fatty acids, including oleic acid.

Figure 2 – Water activity of *M. oleifera* grains as a function of water content.



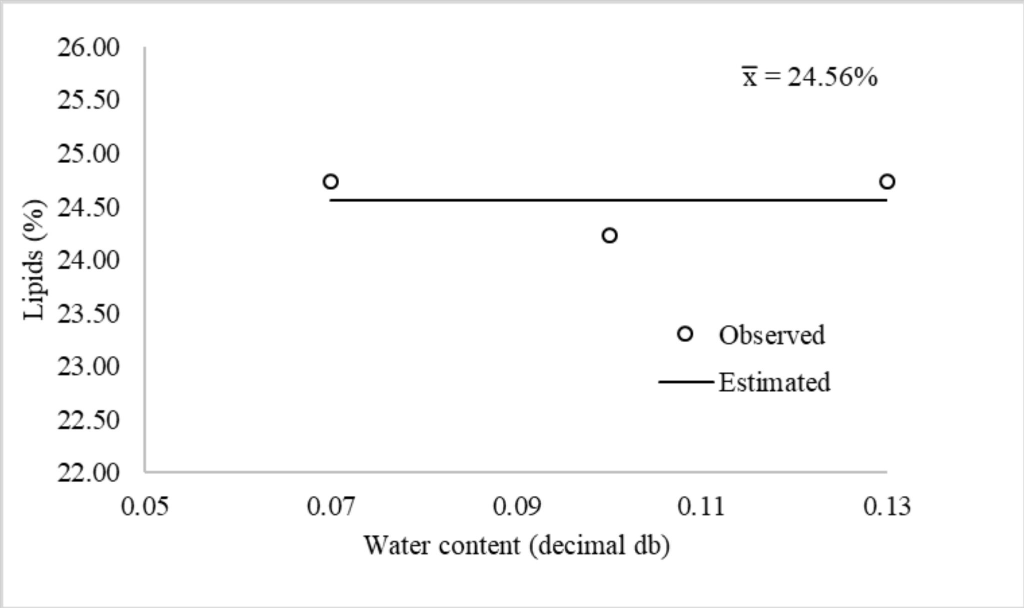
Source: Author (2024).

Figure 3 – Efficiency of mechanical extraction of oil from *M. oleifera* grains with different water contents.



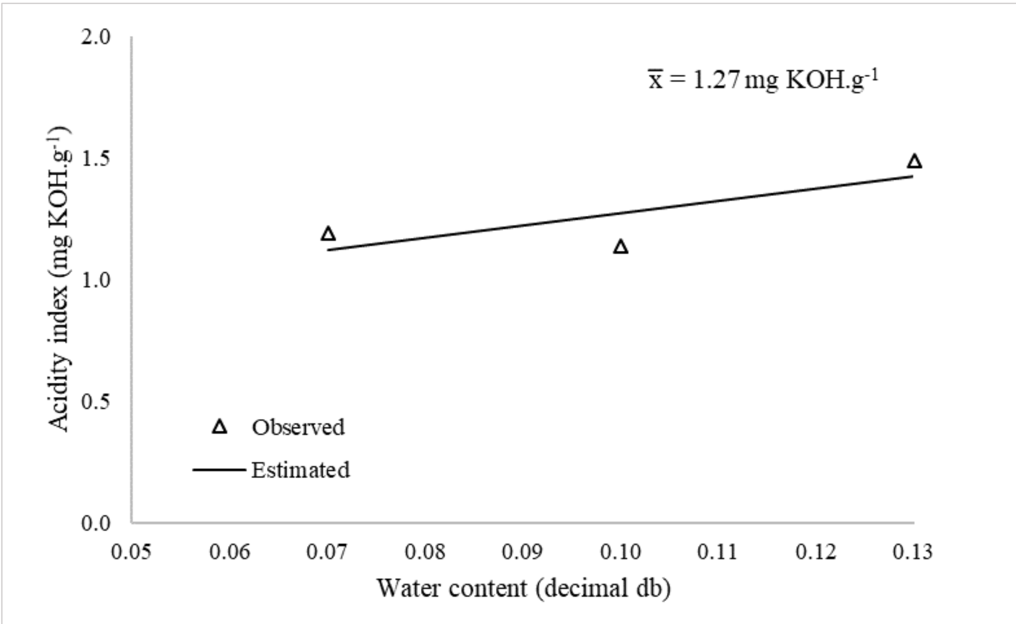
Source: Author (2024).

Figure 4 – Lipid content of *M. oleifera* grains as a function of water content.



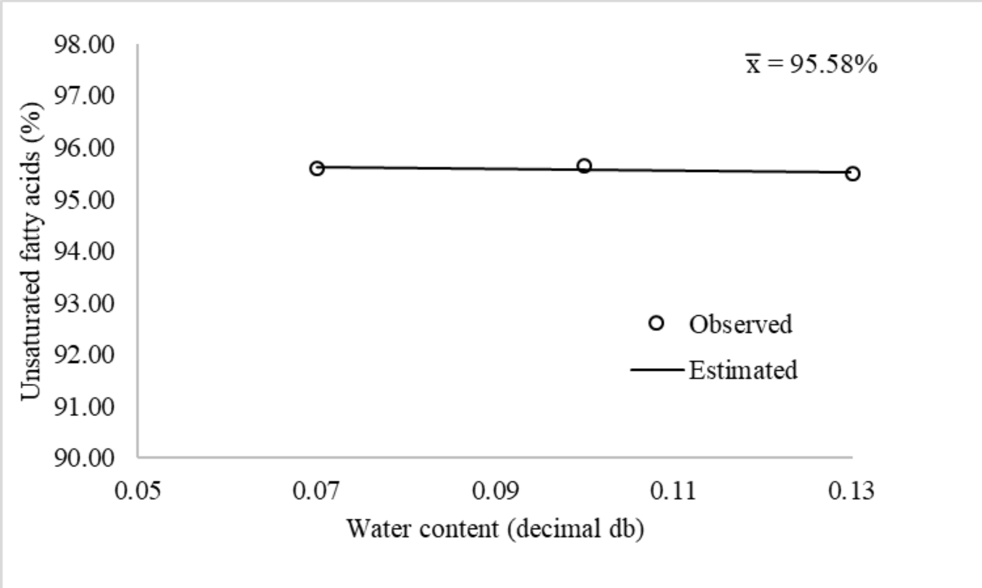
Source: Author (2024).

Figure 5 – Acidity index of the oil from *M. oleifera* grains as a function of water content.



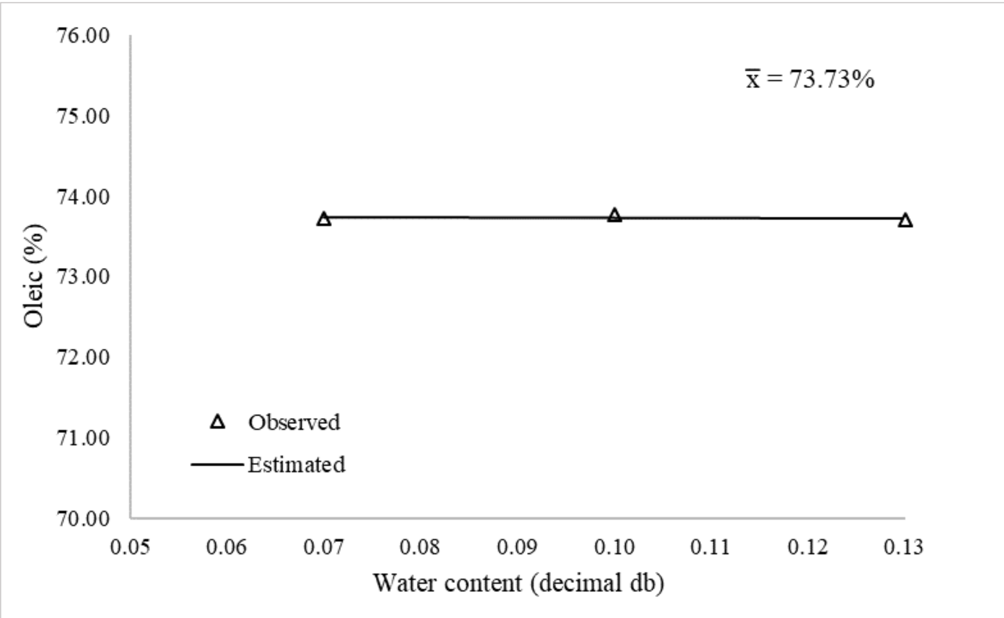
Source: Author (2024).

Figure 6 – Unsaturated fatty acid content of the oil from moringa oleifera grains as a function of water content.



Source: Author (2024).

Figure 7 – Oleic acid content of the oil from *M. oleifera* grains as a function of water content.



Source: Author (2024).

As identified by the regression analysis presented in Figures 2 and 3, the water content of the grains significantly influenced both the water activity of the grains and the efficiency of mechanical oil extraction. In contrast, the parameters related to the quantification and qualification of moringa oil, shown in Figures 4, 5, 6 and 7, did not demonstrate sensitivity to the variations in water content tested. Among the samples with water contents of 0.07, 0.10 and

0.13, the variations in the percentages of lipids (0.5%), unsaturated fatty acids and oleic acid (less than 0.14%), and in the acidity index of the oil (0.35 mg $KOH.g^{-1}$) were not significant. Pohndorf (2012) analyzed the quality of soybean oil with water content of 16, 14 and 12% and also did not notice major changes in these parameters.

In Figure 2, it is possible to observe a significant increase in the amount of free water in the grains as their total water content increases. Water activity, unlike total water content, which includes all the water present in the grains, measures the fraction of water effectively available to participate in biological and chemical processes (Arslan-tontul, 2020).

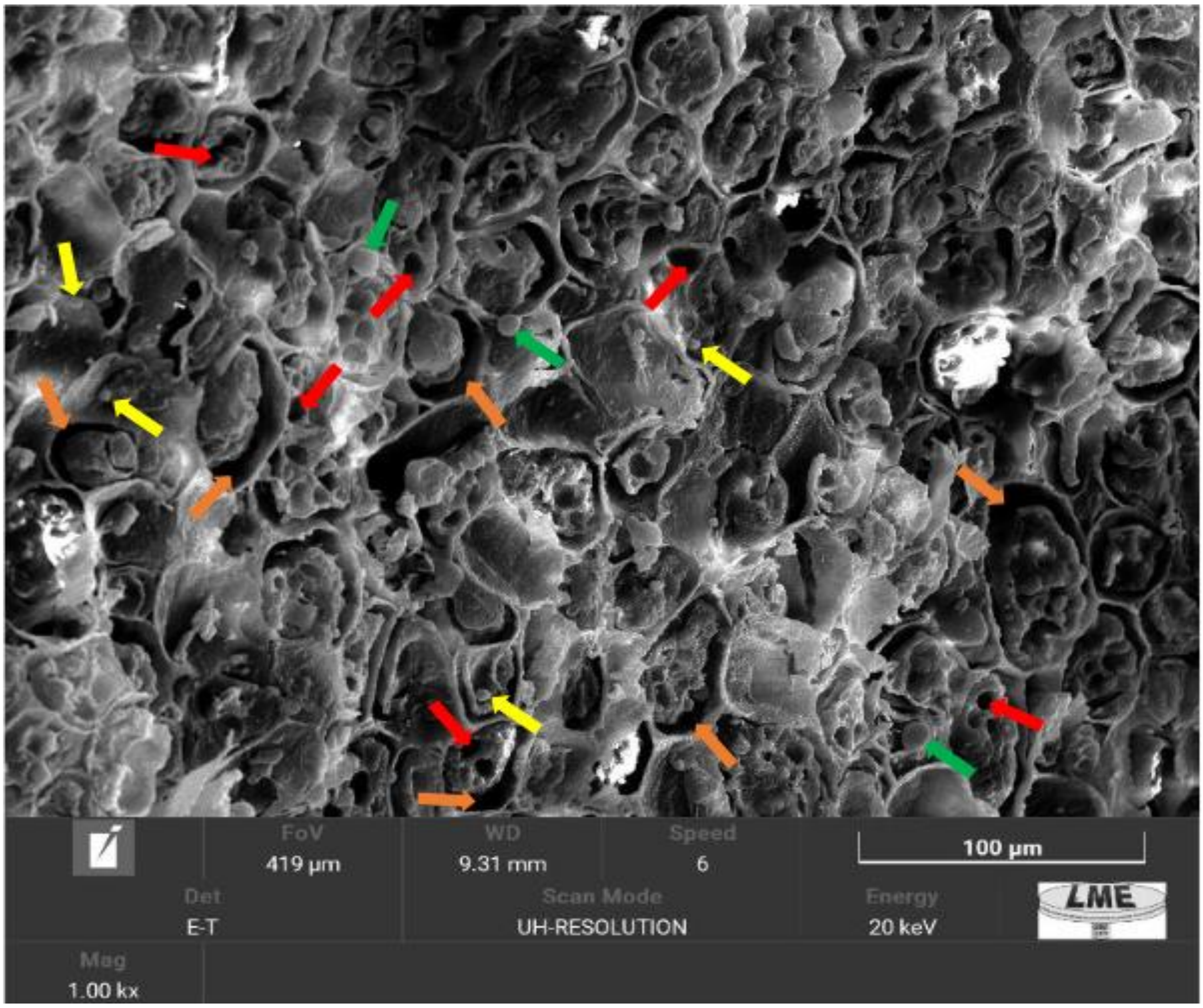
Silveira *et al.* (2019) evaluated the physical properties of broad beans with different water contents and observed that the dimension of the perpendicular axis and water activity decreased with decreasing water content. Such a decrease in free water in the medium resulted in an increase in osmotic pressure, creating an environment less conducive to the proliferation of microorganisms and affecting enzymatic activity. This, in turn, contributed to a reduction in the speed of biochemical reactions and better conservation of the product (Damodaran; Parkin, 2018; Prado *et al.*, 2020).

In addition to preserving the quality of the agricultural product, with the reduction of the water, it can be seen in Figure 3 that moringa grains with a water content of 7% had higher performance in mechanical oil extraction, compared to the others. Mendonça *et al.* (2020) extracted oil from the seeds of two andiroba species with different water contents after drying at different air temperatures and reported that both the temperature and the water content in the grain can significantly influence the efficiency of this process.

In general, for most species, the lower the water content, the greater the hardness and lower the elasticity of the product, which gives the product less resistance to rupture, favoring the mechanical extraction of oil (Singh *et al.*, 2002; Beerens, 2007; Pighinelli *et al.*, 2009, Araújo *et al.*, 2020).

To further analyze the structures of the endosperm cell membranes of moringa grains in detail, Figures 8, 9 and 10 show images obtained by scanning electron microscopy. These images correspond to grains with water contents of 0.07, 0.10 and 0.13 db, respectively.

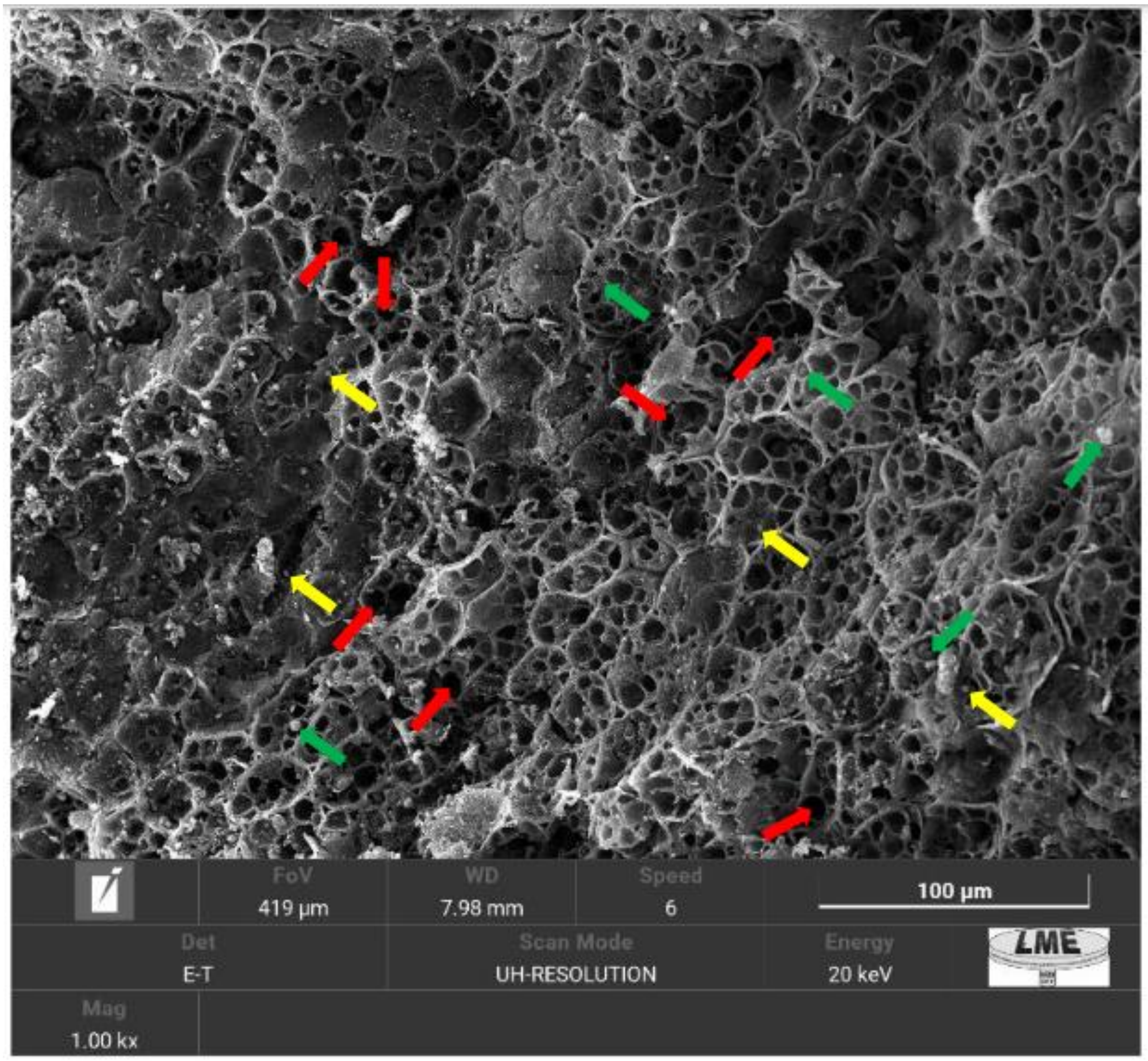
Figure 8 – Scanning electron microscopy images of the endosperms of moringa grains with a water content of 0.07 (dry basis) in a field of view (FoV) of 419 µm.



*The orange arrows indicate the spaces between cells, the red arrows indicate the cell lumina, the green arrows indicate the protein bodies, and the yellow arrows indicate oil droplets.

Source: Author (2024).

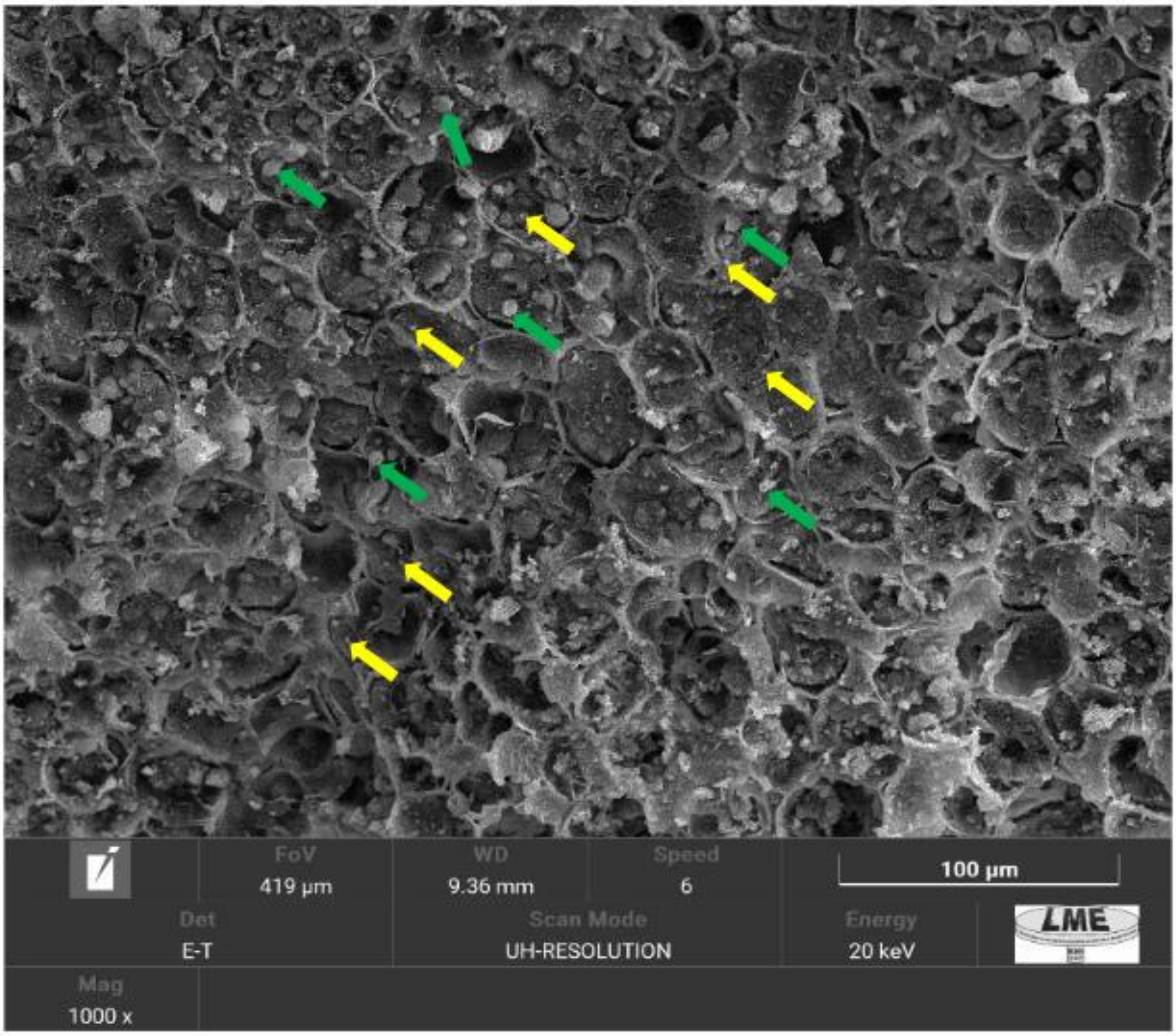
Figure 9 – Scanning electron microscopy images of the endosperms of moringa grains with a water content of 0.10 (dry basis) in a field of view (FoV) of 419 µm.



*The red arrows indicate the cell lumina, the green arrows indicate the protein bodies, and the yellow arrows indicate oil droplets.

Source: Author (2024).

Figure 10 – Scanning electron microscopy images of the endosperms of moringa grains with a water content of 0.13 (dry basis) in a field of view (FoV) of 419 µm.



*The green arrows indicate the protein bodies and the yellow arrows indicate oil droplets.

Source: Author (2024).

Microtructural analysis of the endosperms of moringa plants with different water contents revealed significant changes in the cellular structures. Figure 8 shows that the driest grains with a water content of 0.07 db presented with intercellular spaces and voids in the cell lumina and signs of cell extravasation. These changes make the protein bodies and oil droplets more accessible due to the reduction in the water content on the internal and external surfaces of the macromolecules present in the cellular structure, which may contribute to the reduction of emulsification and plasticity of the mass during the oil extraction process (Mendonça *et al.*, 2020). As a result, the extraction efficiency was higher, as evidenced in Figure 3.

When analyzing the grains with a water content of 0.10 db, there were decreases in the spaces between the cells and in the availability of the lumina due to the higher water contente. This is documented in Figure 9, where the protein bodies and oil droplets are not as abundant as those in Figure 8. In addition, certain compaction of the grains is observed, which is in agreement with the results in Table 1, characterized by a smaller projected area and greater circularity and sphericity compared to grains with a water content of 0.13. These results are in agreement with the study by Morais *et al.* (2018).

With cell contraction resulting from water loss, the structure and composition of the cell wall can change. This results in greater compression of the wall fibers, making them more dense and rigid, and this rigidity hinders access to cell organelles during extraction processes (Rovaris, 2011; Botelho *et al.*, 2018).

Figure 6 shows that the cells with a high water content (0.13 db) are more expanded, and the intercellular spaces and lumen are filled. The turbidity of the cells in the image indicates the presence of water surrounding the protein bodies and oil droplets, making them less accessible during the extraction process (Zhang *et al.*, 2018). The grains with water contents of 10% and 13% showed similar oil yield efficiencies although the lipid droplets in the sample with 10% water content appeared to be more readily available for removal. The cell contraction observed in the latter sample, as evidenced by ultrastructural analysis (Figure 9), possibly influenced cell wall disruption, consequently affecting oil removal (Araújo *et al.*, 2020).

4 CONCLUSION

This study provides a comprehensive analysis of the morphological and chemical properties of *M. oleifera* grains with different water contents. The results presented here highlight the absence of significant differences in morphometric values and chemical characteristics, such as lipid content, acidity index, and percentages of unsaturated fatty acids and oleic acid, in response to variations in product moisture. However, there was a significant increase in the amount of free water in the grains as the moisture content increased, which influenced the yield of mechanically extracted moringa oil.

The ultrastructural analyses revealed that the driest grains with a water content of 0.07 db had intercellular spaces and voids in the cell lumina along with signs of cell extravasation. These characteristics made the protein bodies and oil droplets more accessible, resulting more efficient oil extraction.

Despite the stability of the morphological and chemical properties of *M. oleifera* grains with different water contents, the moisture content clearly played a crucial role in the oil extraction process, indicating the importance of controlling this parameter during grain processing.

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STORAGE OF MORINGA OLEIFERA GRAINS AND ITS EFFECTS ON QUALITY

ABSTRACT

Moringa oleifera Lam. is an angiosperm tree that produces seeds with high lipid and protein contents. The oil extracted from these grains has emerged as a promising alternative to meet the growing demand for vegetable oils. Despite the wide applicability of this technique, there is a lack of research related to the storage of *M. oleifera* grains and the preservation of their physicochemical properties. Therefore, this study aimed to evaluate the effect of water content on the quality of stored *M. oleifera* Lam. grains. Freshly harvested grains from the second half of the 2022 crop in the state of Maranhão, with water contents of 13%, 10% and 7% (db), were used. The seeds were stored in polyethylene bag silos for 8 months. The physical, biological and chemical parameters for quality evaluation were measured in triplicate before storage and after 240 days, with the exception of water content and water activity, which were also analyzed after 120 days. The results showed considerable variation in the water content and water activity of the grains during the first 120 days of storage, as well as a high incidence of *Aspergillus flavus* fungi in the newly harvested grains, which decreased from 100% to 10%, 30% and 1% in samples T7, T10 and T13, respectively, at the end of storage. There was also a reduction in the ether extract and crude protein content, which was potentiated in samples T7 and T10, suggesting a process of grain degradation due to water activity and the action of colonizing fungi. In the analysis of the oil quality, the intensification of the lipid degradation process was observed in the grains stored with water contents of 0.10 and 0.13 db.

Keywords: Moringa; Water activity; Postharvest; Fatty acids.

1 INTRODUCTION

Moringa oleifera Lam., popularly known as white wattle, is a tree angiosperm of the order Brassicales that belongs to the family Moringaceae, is native to India and was first identified in Brazil more than 70 years ago as an ornamental plant (Liu *et al.*, 2022). This species is an allogamous plant characterized by its easy dissemination, wide adaptability, and ability to develop in different types of soils and climates (Gualberto *et al.*, 2015; Noronha *et al.*, 2018; Lopes *et al.*, 2023).

The components of moringa, such as its leaves and seeds, have high nutritional and photochemical value, allowing a variety of applications in the industrial sector (Islam *et al.*, 2021; Meireles *et al.*, 2020; Abdel-latif *et al.*, 2022). Leaves contain a significant amount of proteins, carbohydrates and minerals, in addition to bioactive substances such as flavonoids, tannins, coumarins and alkaloids (Yaméogo *et al.*, 2011; Saraiva *et al.*, 2018). The seeds have a high oil content, ranging from 26% to 40%, and a protein content ranging from 24% to 37% (Oliveira *et al.*, 2012; Garza *et al.*, 2017; Dantas, 2018; Jesus, 2018; Araújo *et al.*, 2020). Due to their protein content, moringa grains have been used as a natural coagulant in water treatment, with an efficacy comparable to that of aluminum sulfate (Mourão *et al.*, 2020; Ribeiro, 2021; Figueiredo *et al.*, 2022).

The oil from moringa seeds has emerged as a promising alternative to meet the growing demand for vegetable oils. Predominantly composed of monounsaturated fatty acids, such as oleic acid, which represents approximately 70% of the composition, moringa oil also contains natural antioxidants, such as tocopherols (vitamin E) and phenolic compounds (Boukandoul *et al.*, 2018; Dinesha *et al.*, 2018; Chaves *et al.*, 2023). In addition, this oil has notable thermal resistance and oxidative stability compared to those of canola oil and olive oil (Boukandoul *et al.*, 2018; Duarte *et al.*, 2018).

The preservation of chemical and physical properties during grain storage is of paramount importance because it directly influences the value of the product. During the storage period, the grain mass is constantly exposed to several external factors, including physical factors, such as temperature and humidity; chemical factors, such as the supply of oxygen; and biological factors, such as bacteria, fungi, insects and rodents (Brooker *et al.*, 1992; Haeberlin *et al.*, 2021; Silva *et al.*, 2022). In this context, the water content is a major factor that impacts the quality of seeds by influencing their respiratory rate and consumption of their energy reserves, which can generate qualitative and quantitative losses of the product (Ullmann *et al.*, 2018; Wang *et al.*, 2018; Amaro et al., 2019). In addition, the water content directly interferes with the amount of free water present in grains, favoring the occurrence of chemical reactions and deterioration of biological processes (Htwe *et al.*, 2018; Isquierdo *et al.*, 2020).

According to some researchers, when the water activity is between 0.4 and 0.8, there is a favorable environment for chemical and enzymatic reactions in the grains, and when the water activity reaches approximately 0.6, the decomposition process, including lipid oxidation, is potentiated (Van Den Berg *et al.*, 1981; Damodaran; Parkin, 2019; Önder *et al.*, 2020). The growth of microorganisms, such as fungi and bacteria, usually starts at water activity values of 0.70 and 0.80, respectively, intensifying the chemical degradation of the grains (Damodaran; Parkin, 2019). However, fungi of the genera Aspergillus and Penicillium, common in storage facilities, are able to grow and reproduce in environments with low relative humidity and water activity and are limited only to water activities below 0.62 (Barbosa-Cánovas *et al.*, 2007; Damodaran; Parkin, 2019; Prestes *et al.*, 2019).

Although the amount of water present in grains modulates the occurrence of biochemical and physiological processes, which is indicative of the propensity for deterioration and contamination, it is not sufficient to predict the perishability of a product (Rosa *et al.*, 2021; Silva *et al.*, 2021). The chemical composition of the grains also needs to be considered, as it interferes with their hygroscopic properties, resulting in specific behaviors in the sorption isotherms (Isquierdo *et al.*, 2020). According to some studies, when comparing grains with the same water content at similar temperatures, it has been observed that starch-rich species tend to have lower water activity than lipids (Resende *et al.*, 2006; Smaniotto *et al.*, 2006; Bessa *et al.*, 2021; Santos, 2022).

Considering the scarcity of studies related to the postharvest life of *M. oleifera* and the importance of preserving its physicochemical properties, this study aimed to evaluate the effect of water content on the quality of stored *M. oleifera* Lam.

2 MATERIALS AND METHODS

This research was conducted at the Department of Agricultural Engineering of the Federal University of Lavras, and the experiments were conducted at the Laboratory of Agricultural Product Processing, Laboratory of Seed Pathology and Laboratory of Oilseed Plants, Oils, Fat and Biodiesel of this institution.

Freshly harvested M. oleifera Lam. grains from the second half of the 2022 crop, from the state of Maranhão, with 13%, 10% and 7% water content (dm) were used. For grain storage, semi-hermetic silos were made of polyethylene bags, with thermohygrometers coupled to measure the temperature and relative humidity inside the grain mass. As the temperature and relative humidity of the environment are very unstable, the data provided by the National Institute of Meteorology (INMET, 2023) were used.

The samples were stored in duplicate in the Experimental Area of Process Engineering of the Department of Agricultural Engineering (UFLA) containing 1 kg of seeds in each bag silo for 240 days. The physical, biological and chemical parameters for quality evaluation were measured in triplicate before storage (initial) and after 8 months (final), with the exception of water content and water activity, which were also analyzed after 120 days.

The water content was determined by the oven method at 105 ± 3°C for 24 hours (Brasil, 2009). Water activity analysis was performed by the rapid method by measuring the dew point using the Decagon Devices Aqualab series 3TE equipment.

The apparent specific mass, expressed in kg.m-3, was obtained using an electronic hectoliter scale with 0.01 g resolution (Gehaka - BK 4001) and a 1 L container, according to the Rules of Analysis of Seeds (Brasil, 2009).

The identification and quantification of fungi in the grains were performed by the "blotter test" method (Brasil, 2009). A total of 100 seeds were individually placed on a layer of filter paper moistened with distilled water inside transparent acrylic containers (gerboxes), which were then capped. The boxes were distributed under a white fluorescent lamp at a distance of 30 to 40 cm in a BOD-type chamber with a controlled temperature of 20 ± 2°C and a 12-hour photoperiod for 7 days. After the incubation period, the individual seeds were evaluated with the aid of a stereomicroscope, with a resolution of 30 to 80×, from the occurrence of fruiting inherent to the process of fungal proliferation. The results are presented as percentages of fungal occurrence.

The chemical analyses of the grains were performed according to methods described by the Association of Official Analytical Chemists (AOAC) and the Adolfo Lutz Institute. The lipid content of the previously ground seeds was determined by chemical extraction with the organic solvent hexane using the direct Soxhlet method (IAL, 2008). The percentage of protein was determined based on the measurement of total nitrogen using the Kjeldahl method, in which the value was multiplied by a factor of 6.25; the results are expressed as crude protein (CP) content (AOAC, 2000). The dry matter (DM) was determined by subtracting the water content from the initial weight of the sample (AOAC, 2000).

To determine the organic matter (OM) and total carbohydrate (TC) contents, the mineral matter content was measured by incineration in a muffle furnace at 500°C (AOAC, 2000). Thus, the percentages of OM and total carbohydrates (TC) were calculated by equations 1 and 2, respectively, in accordance with the methodology of the Association of Official Analytical Chemists (AOAC, 2000).

$$\mathrm{OM} = 100 - (\mathrm{DM} + \mathrm{MM}) \tag{1}$$

where:

OM – organic matter, %;

DM – dry matter, %;

MM – mineral matter, %.

$$TC = 100 - (CP + EE + MM) \tag{2}$$

where:

TC – total carbohydrates, %;

CP – crude protein, %;

EE – ether extract, %.

The extraction of moringa oil for physicochemical parameter determination was performed by a mechanical method using an expeller press with a radial tubular extraction system (model ERT 50). The acidity, peroxide content and fatty acid composition of the crude oil were analyzed (IAL, 2008).

The acid value is expressed as the number of milligrams of potassium hydroxide (KOH) required to neutralize 1 g of oil and was calculated using equation 3.

$$IA = \frac{v \text{ x } f \text{ x } 5{,}61}{P} \tag{3}$$

where:

IA – acid value, mg $KOH.g^{-1}$;

v – volume of KOH solution (0.1 M) used in the titration, ml;

f – correction factor of the KOH solution;

P – mass of the oil sample, g.

By the peroxide value, all substances that oxidize potassium iodide under the test conditions were determined in terms of milliequivalents of peroxide per 1 kg of sample.

$$IP = \frac{(A - B) \text{ x } N \text{ x } f \text{ x } 1000}{P} \tag{4}$$

where:

IP – peroxide index, $meq.kg^{-1}$;

A – volume of sodium thiosulfate solution (0.01 M) used in sample titration, mL;

B – volume of sodium thiosulfate solution (0.01 M) used in the blank titration, mL;

N – normality of the sodium thiosulfate solution;

f – correction factor for sodium thiosulfate solution;

P – mass of the oil sample, g.

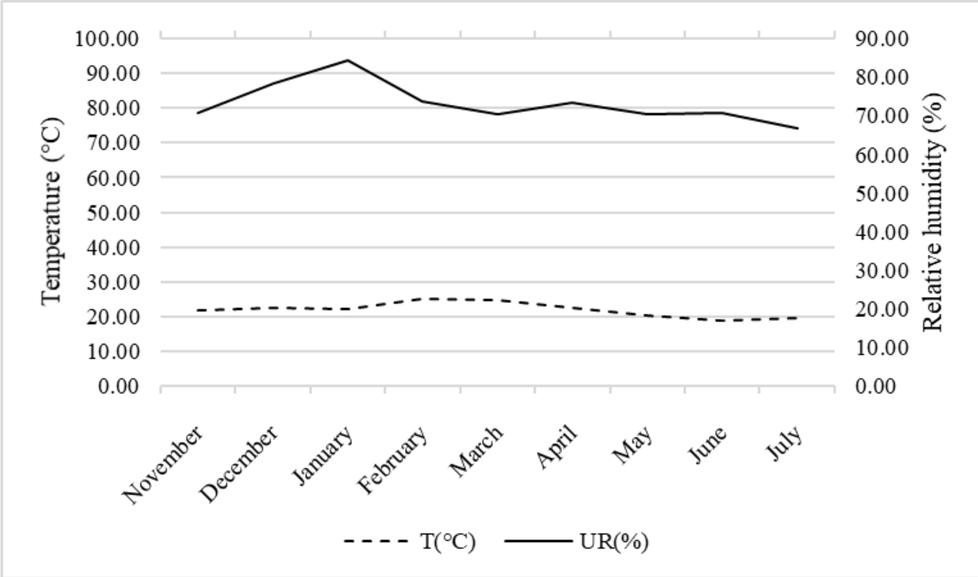
The fatty acid profile was obtained by gas chromatography of methyl esters (IAL, 2008). For this analysis, the oil samples were esterified, and subsequently, 10 µL aliquots were removed and diluted in 1 mL of hexane and analyzed in a SHIMADZU GC2010 gas chromatograph equipped with a flame ionization detector (FID) and one SP 2560 column (100 m × 0.25 mm × 0.20 µm). The initial oven temperature was 150°C, and the temperature was maintained for 5 min. The temperature was then increased to 240°C, with a heating rate of 15°C per min, and the temperature was maintained at this temperature for another 5 min (IAL, 2008). The reference standard used to identify and quantify the fatty acids in the sample was the fatty acid methyl ester (FAME) 37 standard. The catalytic tests were performed at the Center for Chemical Analysis and Prospection of the Department of Chemistry of the Federal University of Lavras.

The data obtained from the physical and chemical analyzes of the grains and moringa oil were subjected to analysis of variance, and the means were compared using the Scott-Knott test ($p < 0.05$), using the Sisvar program, version 5.5 (Ferreira, 2011).

3 RESULTS AND DISCUSSION

Characterization of the environmental conditions during the experiment was performed using the climatic data of temperature and relative humidity obtained from the conventional meteorological station of the UFLA, as shown in Figure 1.

Figure 1 – Temperature and relative humidity of the environment during the months of storage.



Source: INMET (2023).

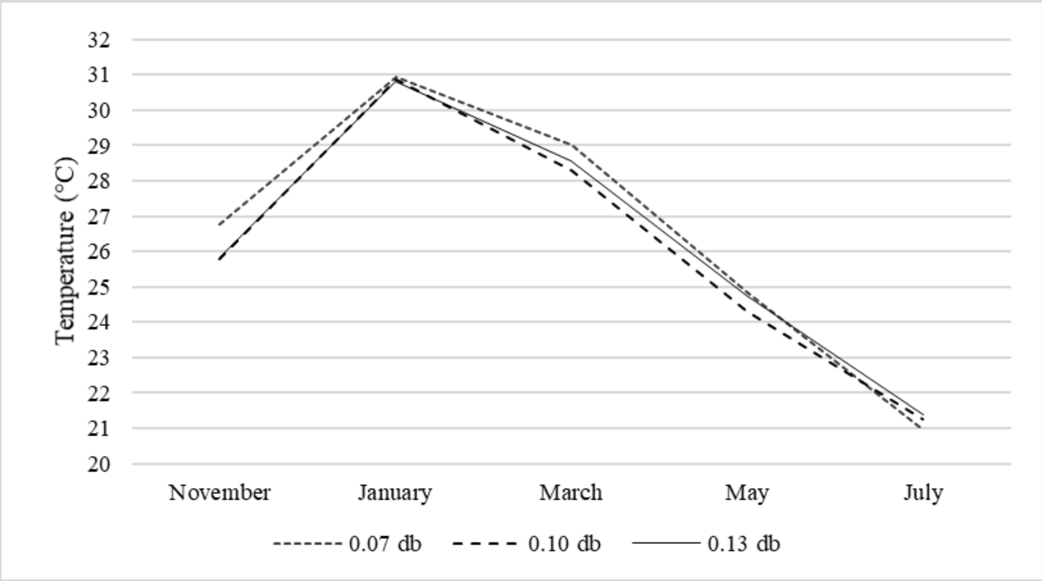
Based on the data collected from the storage site, the average temperature over the eight months of the experiment varied between 19 and 25°C. During the first five months (November to April), the temperatures were higher, exceeding 22°C, while June and July had the lowest temperatures, approximately 19°C.

The average relative humidity was in the range of 66 to 84% during the storage period. The relative humidity values were highest between December and January, ranging from 78 to 84%, and lowest in July, at 66%.

Considering that the storage system is semihermetic, which implies a limited exchange of air with the outside environment, monitoring the environmental conditions is essential. This is due to the small exchanges of water vapor between the external and internal storage environments, which can affect the water content and conservation of grains (Ziegler *et al.*, 2021; Silva *et al.*, 2022).

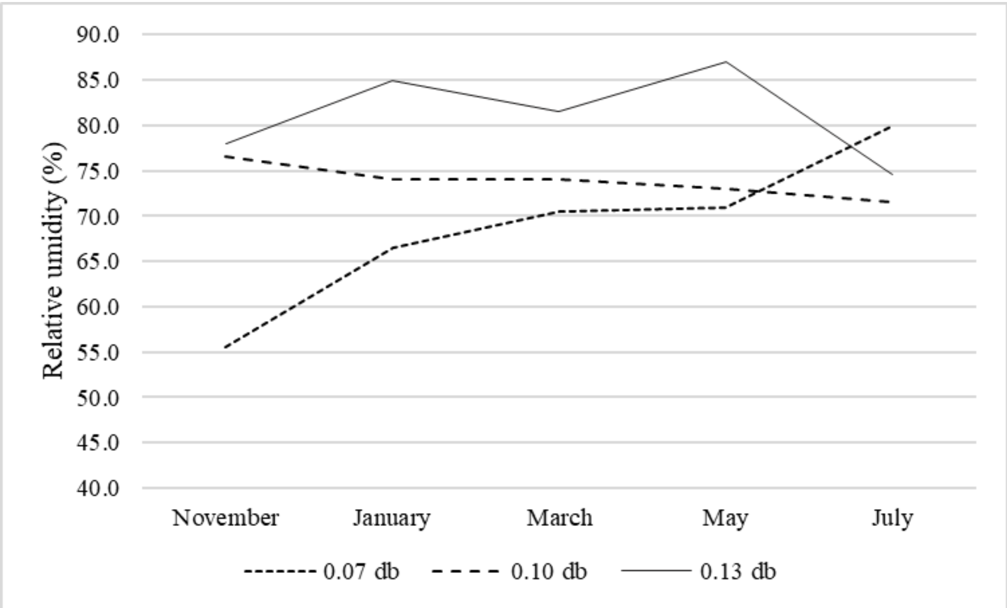
The temperature and relative humidity data inside the silos during the storage period are presented in Figures 2 and 3.

Figure 2 – Internal temperature of the silos where *M. oleifera* grains with different moisture contents were stored throughout the experimental months.



Source: Author (2024).

Figure 3 – Internal relative humidity of the silos where *M. oleifera* grains with different water contents were stored throughout the months of the experiment.



Source: Author (2024).

The analysis of temperatures within the grain mass, as illustrated in Figure 2, revealed a similar variation among all treatments. The highest temperatures, exceeding 28°C, were recorded in January and March.

Figure 3 shows that the internal relative humidity of the silos differed among the treatments; for example, for the sample with a 0.07 water content (T7), the relative humidity increased from 55% to 80%. For the sample with a 0.10 water content (T7), the water content (T10) remained practically constant, with a variation of less than 5%, while for the sample with a 0.13 water content (T13), the water content oscillated, with a minimum of 74% and a maximum of 87%. The greatest variations in the indoor relative humidity occurred from November to January, with increases of 10% and 7% for the T7 and T13 samples, respectively, and from May to July, with an increase of 9% for the T7 samples and a 13% decrease for T13 samples.

Fluctuations in the internal relative humidity of the silos demonstrate that during periods with greater climatic variation, external conditions favored the absorption and desorption of moisture by the samples, especially in those with lower water contente. These variations can be attributed to several factors, such as gas exchange with the environment, due to the semi-hermetic storage system, and possible differences in physical characteristics of the grains in each sample, which affect atmospheric humidity (Pinto *et al.*, 2021; Ziegler *et al.*, 2021).

Table 1 shows the statistical analyses of the means of the water content, water activity and apparent specific gravity of the moringa grains before and after 240 days of storage.

Table 1 - Water content, water activity and apparent specific gravity of moringa grains with different water contents before and after storage.

Tratament	Water content (decimal db)		Aw (dimensionless)		Specific mass ($kg.m^{-3}$)	
	Before	After	Before	After	Before	After
T7	0.070 aA	0.072 aA	0.61 aA	0.65 bC	167.36 aA	165.47 aA
T10	0.096 bB	0.069 aA	0.74 bB	0.64 aB	168.36 aA	165.26 aA
T13	0.13 bC	0.067 aA	0.82 bC	0.60 aA	176.70 aA	176.52 aA
CV (%)	1.89		1.19		6.32	

Values with the same lowercase letters in the row and capital letters in the column do not differ from each other at the 5% level of significance, using the Scott-Knott test. CV: Coefficient of variation; Aw: Water activity.

Source: Author (2024).

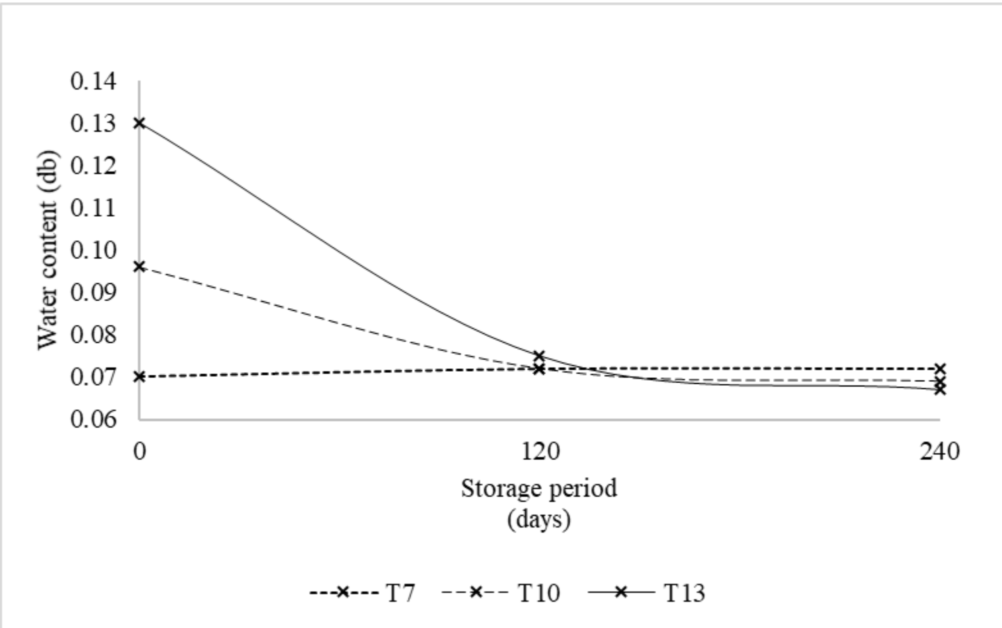
As shown in Table 1, the apparent specific mass of the grains was the only factor among those evaluated that did not present a statistically significant difference between the treatments and throughout the storage period. According to the literature, most Agricultural products tend to show an increase in apparent specific mass as the content of water decreases (Sangamithra *et al.*, 2016; Khan *et al.*, 2017).

Contrary to this trend, studies conducted by Araújo *et al.* (2014) and Martins *et al.* (2017) showed that, in peanut and safflower grains, respectively, the reduction of water content resulted in a decrease in specific gravity. Researchers attribute this effect to the greater contraction of the internal part of the cotyledons compared to the external dimensions, which contract less intensely, creating empty spaces within the grains during loss of water, resulting in a smaller reduction in volume compared to mass. In addition resistance of the grain coat to contraction can also influence the relationship between the content of water and specific mass, making the analysis complex and involving structural changes specific according to each product (Araújo *et al.*, 2014; Martins *et al.*, 2017).

At the end of the storage period, the water content and water activity significantly decreased in treatments T10 and T13. This can be attributed to the partial airtightness of the silos, which leads to the desorption of the grains to reach hygroscopic equilibrium (Silva *et al.*, 2022; Isquierdo *et al.*, 2020).

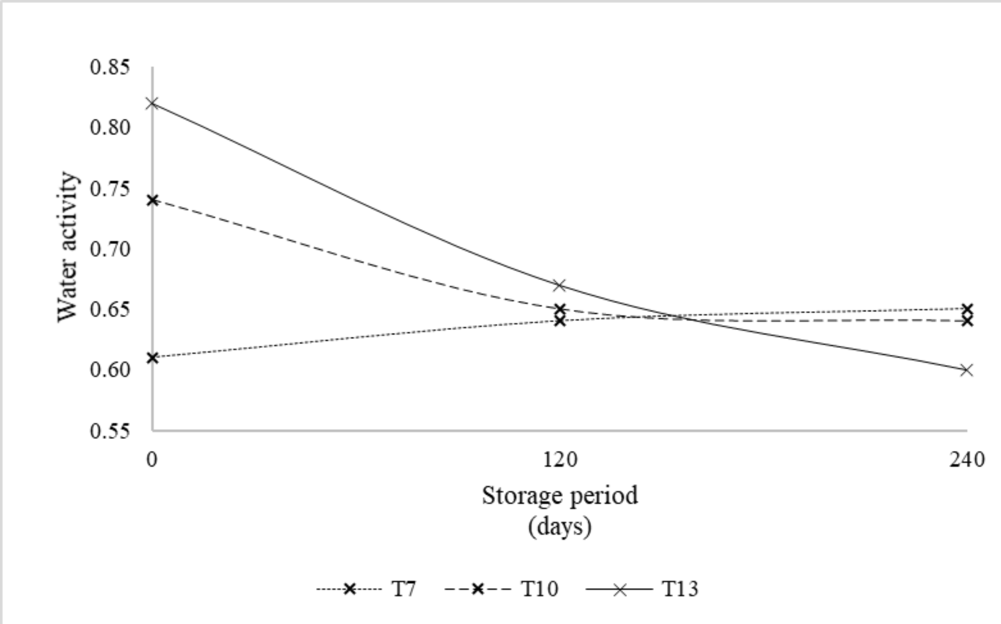
Figures 4 and 5 show the results of the water content and water activity, respectively, before and after the storage of moringa grains at 120 and 240 days.

Figure 4 – Moisture content of *M. oleifera* grains before, during (120 days) and after (240 days) storage.



Source: Author (2024).

Figure 5 – Water activity of M. oleifera grains before, during (120 days) and after (240 days) storage.



Source: Author (2024).

The greatest variation in the water content and water activity of the moringa grains occurred during the first 120 days of storage. During this period, the water content of the samples with 0.10 and 0.13 ds decreased to 0.07 ds, while the water activity decreased from 0.74 and 0.82 to 0.64 and 0.60, respectively, from November to March. As shown in Figures 2 and 3, this was also the period with the highest recorded temperatures and increased internal relative humidity in the T10 and T13 silos, indicating the loss of water from the grains to the environment. Despite the initial differences in the water contents of the grains, at the end of the storage period, the water content was similar between the samples, at approximately 0.07. This fact indicates a possible stabilization or equilibrium of storage conditions over time, resulting in the observed values.

The reduction in water content over the storage period can be attributed to the hygroscopic capacity of the seeds, which is their ability to absorb or release water to the surrounding environment (Hornke *et al.*, 2020). Thus, moisture variations inside the silo during storage can trigger changes in the water content of the grains (Capilheira *et al.*, 2019).

In a similar study conducted by Silva *et al.* (2022) on the storage of chickpea seeds in a conventional environment without temperature or relative humidity, a significant reduction in the water content of the grains was observed over 135 days. In hermetic containers, the water content decreased from 10.76% to 7.10%, while in permeable containers, it decreased to 6.53%, corroborating the phenomena observed in the present study.

Ziegler *et al.* (2016), when evaluating soybeans stored in semihermetic systems and subjected to different temperatures for 12 months, found that grains with higher initial moisture contents showed greater reductions in moisture over time. These reductions ranged from 16.26% to 12.04% and 11.91% and from 12.68% to 11.81% and 11.71% in grains stored at 15°C and 25°C, respectively, indicating an equilibrium water content close to 11.9% under the conditions studied.

Still in Table 1, it is observed that even though the final water content was the same for all samples, the water activity at the end of the storage period was lower for grains from T3, followed by T10 and T7.

The water content of the grains during storage is a crucial factor that directly influences the durability of the product, indicating its susceptibility to deterioration or contamination (Haeberlin *et al.*, 2021; Silva *et al.*, 2022). Thus, in Table 2, the results regarding the incidence of fungi before and after the storage period of moringa grains with different water contents are presented.

Table 2 – Incidence of fungi before and after 240 days of storage of moringa grains with different water contents.

Incidence of fungi (%)	Storage period	T7	T10 (%)	T13
Aspergillus flavus	Before	100.0 bA	100.0 bA	100.0 bA
	After	10.0 aB	30.0 aC	1.0 aA
Aspergillus ochraceus	Before	0.3 bA	0.3 bA	0.5 bB
	After	0.0 aA	0.0 aA	0.0 aA
Penicillium sp	Before	0.0 aA	0.0 aA	0.0 aA
	After	0.0 aA	0.0 aA	0.5 bB

Values with the same lowercase letters in the column and uppercase in the row do not differ from each other at the 5% significance level according to Scott-Knott's test.

Source: Author (2024).

According to the "blotter test" method, for identification and quantification of fungi, It is noted that only three species were found, *Aspergillus flavus*, *Aspergillus ochraceus* and *Penicillium* sp. This fact may be associated with the antimicrobial activity of some compounds present in seeds, such as benzyl isothiocyanate, acting directly on microorganisms, interrupting the synthesis of the cell membrane or inhibiting the synthesis of essential enzymes, making their growth unfeasible (Bukar *et al.*, 2010).

Table 2 shows that the incidence of fungi was quite high in freshly harvested grains, possibly due to climate variations during grain transportation between the states of Maranhão and Minas Gerais. Initially, the percentage incidence of *A. flavus* was 100% in all treatments, decreasing to 10%, 30% and 1% in samples T7, T10 and T13, respectively. The fungi of the species *Aspergillus ochraceus* were identified only before storage, with percentages lower than 0.5%, and *Penicillium* sp. were identified only after storage in sample T13 but in a reduced amount of 0.5%.

Compared with those in the other treatments, the water activity in the *M. oleifera* grains in the treatment with an initial water content of 0.13 db decreased more at the end of the storage period, which may have contributed to a lower incidence of fungi.

According to Prestes *et al.* (2019), fungi belonging to the genera Aspergillus and Penicillium are often identified in storage facilities; they originate directly from the field and are present in newly harvested seeds. Because they are considered xerophilic, they can develop in environments with low relative humidity and water activity and are limited only by water activities lower than 0.62 (Eltariki *et al.*, 2018; Prestes *et al.*, 2019; Damodaran; Parkin, 2019).

The presence of fungi during grain storage is a considerable threat to the quality of the products, to food security and to the economy of agricultural production (Abd-allah *et al.*, 2018; Lamichaney *et al.*, 2019). This is due to the generation of toxic compounds, such as mycotoxins, which represent a serious risk to human and animal health, in addition to intensifying the deterioration of grains by producing enzymes that facilitate the decomposition of organic materials, including the components of seeds (Ramirez *et al.*, 2018; Alemayehu *et al.*, 2020; Tripathi; Kumar, 2020; Wang *et al.*, 2020). Table 3 shows the results of the analyses regarding the chemical composition of the moringa grains, including the percentage of dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), total carbohydrate (TC), and oil quality in terms of the acid index (IA) and peroxide index (IP), before and after storage.

Table 3 – Chemical composition of grains in dry matter and quality of moringa oil, before and after storage for 240 days.

Analysis	Storage period	T7	T10	T13	CV (%)
DM (%)	Before	93.50 aC	91.20 aB	88.59 aA	0.48
	After	92.64 aA	93.72 bB	94.26 bB	
OM (%)	Before	95.42 aA	95.39 aA	95.43 aA	0.22
	After	95.49 aA	95.49 aA	95.43 aA	
EE (%)	Before	24.73 bA	24.23 bA	24.73 aA	1.00
	After	23.47 aA	23.01 aA	25.15 aB	
CP (%)	Before	31.05 bA	30.80 bA	31.22 bA	2.06
	After	25.83 aA	25.62 aA	28.48 aB	
TC (%)	Before	39.64 aA	40.35 aA	39.50 aA	1.10
	After	46.19 bB	46.86 bB	41.81 bA	
IA (mg $KOH.g^{-1}$)	Before	1.19 aA	1.14 aA	1.49 aA	5.31
	After	2.63 bA	3.00 bB	3.01 bB	
IP ($meq.kg^{-1}$)	Before	7.05 aA	7.20 aA	7.15 aA	2.40
	After	9.01 bA	10.65 bB	13.9 bC	

Values with the same lowercase letters in the column and uppercase in the row do not differ from each other at the 5% significance level according to Scott-Knott's test. CV: coefficient of variation.

Source: Author (2024).

There was a significant increase in the percentage of dry matter in samples T10 and T13, resulting from the decrease in water content, as shown in Table 1, while OM content remained unchanged until the end of storage.

There was a reduction in the EE in samples T7 and T10, and although the CP content decreased in all samples, it was more pronounced in T7 and T10, suggesting a process of grain degradation due to water activity by colonizing fungi, corroborating the findings of Abd-Alla *et al.* (2018). The presence of these microorganisms at the beginning of storage may have metabolized the lipids present in the seeds as an energy source, resulting in a reduction in the oil content throughout the storage period. In addition, the enzymatic activity of fungi may also trigger the breakdown of proteins into amino acids (Abd-Alla *et al.*, 2018; Tripathi; Kumar, 2020).

When investigating the impact of fungal infestation on the chemical composition of seeds of two important oil-producing species, *S. oleoides* and *S. persica*, Tripathi and Kumar (2020) observed a reduction in the content of fatty oils and protein as the fungal incidence increased. They assigned three levels of infestation, up to 20% light, 30 to 50% intermediate and above 60% severe, and observed a decrease in oil and protein content in the seeds with an incidence from mild to severe, greater than 18% and 8%, respectively.

In a study on soybean storage in a semihermetic system performed by Ziegler *et al.* (2016), it was observed that grains stored at 15 and 25°C for 6 months did not show a significant reduction in oil content, but there were reductions above 2% between 6 and 12 months at both temperatures. The protein content decreased by approximately 2% after 1 year, but the difference was not significant, differing from the present study.

As shown in Table 3, the TC content increased in all the samples, which differs from the results of Tripathi and Kumar (2020), who reported that a significant decrease in TC content was correlated with fungal contamination. Specifically, samples T7 and T10 showed the most significant increase, with an increase of more than 6% after 8 months of storage. This increase is possibly associated with a significant decrease in the CP content and maintenance of the OM content, which is in agreement with the findings of Wang *et al.* (2019) and Prado *et al.* (2020), who reported a negative correlation between carbohydrates and grain proteins.

Abd-Alla *et al.* (2018) reported an increase in TC and crude fiber contents in fungus-infected soybeans, as well as a decrease in protein content. This suggests that fungal contamination may result in grain nutrient consumption, the production of fungal cellular material, the proliferation of fungal structures and the development of resistant tissues, contributing to the increase in the TC content observed in the samples (Abd-Alla *et al.*, 2018).

Oil quality analyzes revealed greater degradation in the grains stored with water contents of 0.10 and 0.13 db. It was observed that the acidity value and peroxide increased in all samples, however T13 and T10 had a greater increase significant. Previous studies, such as Abd-Alla *et al.* (2018) and Tripathi and Kumar (2020), corroborate these findings, highlighting the increase in the IA and IP in grains contaminated with fungi. This is attributed to the action of lipases produced by fungi, which hydrolyze the ester bonds of the oil, releasing free fatty acids and contributing to the degradation of the oil.

Furthermore, the water activity of moringa grains at the beginning of storage contributed to oil deterioration, which was more intensified in grains stored with Aw of 0.82 (T13) and 0.74 (T10). This fact is due to the amount of free water available, which enhances enzymatic activity and accelerates chemical reactions, including lipid oxidation (Damodaran; Parkin, 2019; Önder *et al.*, 2020). Thus, reducing water activity through the grain drying process is essential to reduce the speed of chemical and biochemical reactions, as well as the development of microorganisms during storage, preserving the quality of the oil (Prado *et al.*, 2020; Haeberlin *et al.*, 2021).

Pandorf (2012) conducted a study on soybeans stored at different moisture contents and temperatures and noted that the increase in peroxide formation is more intense in grains with relatively high moisture and temperature values. These changes demonstrate that the lipid oxidation rate of grains is influenced by several internal and external factors, such as fatty acid composition, presence of antioxidants, water content, temperature and atmospheric conditions.

Table 4 below shows the fatty acid profile of moringa oil before and after 8 months of storage.

Table 4 – Fatty acid profile of moringa oil extracted from the grains before and after storage for 240 days.

Fatty acid	Storage period	T7	T10 (%)	T13
Myristic Acid (C14:0)	Before	0.07 aA	0.07 aA	0.08 aA
	After	0.08 aA	0.08 aA	0.08 aA
Palmitoleic Acid (C16:1, Δ9 cis)	Before	4.40 aA	4.33 bA	4.32 bA
	After	4.10 bC	3.99 aB	3.87 aA
Heptadecanoic Acid (C17:0)	Before	1.09 bA	1.12 aA	1.12 aA
	After	2.60 aA	3.44 bB	3.41 bB
cis-10-Heptadecenoic acid (C17:1, Δ10 cis)	Before	1.41 bA	1.38 bA	1.38 aA
	After	0.82 aB	0.33 aA	1.07 aB
Stearic acid (C18:0)	Before	0.01 aA	0.00 aA	0.01 aA
	After	2.54 bA	3.88 bB	4.13 bC
Elaidic Acid (C18:1, Δ9 trans)	Before	5.78 bA	5.64 bA	5.65 bA
	After	5.31 aA	5.11 aA	5.10 aA
Oleic acid (C18:1, Δ9 cis)	Before	73.72 bA	73.77 bA	73.71 bA
	After	71.49 aB	70.72 aA	70.21 aA
Linoleic acid (C18:2, Δ9, 12 cis)	Before	0.82 aA	0.91 bA	0.93 bA
	After	0.92 aB	0.15 aA	0.26 aA
Archic acid (C20:0)	Before	3.21 aA	3.08 aA	3.23 aA
	After	4.21 bA	4.30 bA	4.38 bA
Gadoleic Acid (C20:1, Δ9 cis)	Before	2.32 bA	2.26 bA	2.34 bA
	After	1.95 aA	2.00 aA	1.90 aA
Erucic Acid (C22:1, Δ9 cis)	Before	5.93 bA	6.06 bA	5.81 bA
	After	4.85 aB	4.74 aA	4.45 aA
Arachidonic Acid (C20:4 Δ6)	Before	0.17 bA	0.15 bA	0.21 bA
	After	0.00 aA	0.00 aA	0.00 aA
Lignoceric Acid (C24:0)	Before	0.05 aA	0.10 aA	0.08 aA
	After	0.08 aA	0.10 aA	0.08 aA
Eicosapentaenoic Acid (C20:5, Δ3)	Before	1.09 bA	1.17 bA	1.16 bA
	After	0.01 aA	0.01 aA	0.00 aA
Eicosatrienoic Acid (C20:3, Δ3)	Before	0.02 aA	0.00 aA	0.01 aA
	After	1.06 bA	1.17 bA	1.09 bA
Saturated	Before	4.42 aA	4.36 aA	4.51 aA
	After	9.50 bA	11.80 bB	12.07 bB
Unsaturated	Before	95.59 bA	95.64 bA	95.50 bA
	After	90.50 aB	88.20 aA	87.93 aA

Values with the same lowercase letters in the column and uppercase in the row do not differ from each other at the 5% significance level according to Scott-Knott 's test.

Source: Author (2024).

Moringa oil is predominantly composed of unsaturated fatty acids, with oleic acid being the most abundant fatty acid, representing approximately 70% of the total fatty acid content (Faisal *et al.*, 2020). In this study, the most prevalent fatty acids in the oil were oleic, elaidic, erucic and palmitoleic acids. According to Table 4, a significant reduction was observed in the last two samples (C22:1, Δ9 cis e C16:1, Δ9 cis) in all samples after the storage period, with greater intensity in T10 and T13.

According to the results presented, there was a decrease of approximately 5% in the content of monounsaturated fatty acids in sample T7 and of 7% in samples T10 and T13 after 8 months of storage. The polyunsaturated fatty acid content decreased by approximately 5% in the T7 sample and by approximately 40.4% and 41.6% in the T10 and T13 samples, respectively.

Polyunsaturated fatty acids have two or more double bonds in their carbon chains, located at specific points and with a configuration that facilitates reaction with oxygen. These bonds are highly reactive because their electrons are more available and less stable, being more easily broken (Damodaran; Parkin, 2019; Önder *et al.*, 2020; Zhang *et al.*, 2021). Therefore, the higher water contents and amount of free water available in samples T10 and T13 contributed to a more enhanced double bonds of unsaturated fatty acids. There was a significant increase in eicosatrienoic acid, and a decrease in eicosapentaenoic and arachidonic in all samples. The increase in eicosatrienoic acid can be attributed to the degradation of eicosapentaenoic acid. This degradation is possible due to to the similar molecular structure of these fatty acids, with the difference that the acid eicosapentaenoic has an extra double bond in its carbon chain. During the storage, this additional double bond may have been broken, resulting in the formation of eicosatrienoic acid in a greater quantity. In addition, a reduction of more than 70% was observed for the linoleic acid content in samples T10 and T13.

Paraginski *et al.* (2015), when examining the fatty acid profile of corn kernel oil with 14% water content stored at temperatures ranging between 5°C and 35°C for 12 months, found a significant reduction only in linoleic acid and linolenic acid contents when the grains were kept at 35°C. Pandorf (2012) observed that increasing the water content and storage temperature of soybeans may increase the IA and IP of the oil, as well as reduce the linolenic acid content.

The significant decrease in unsaturated fatty acids, especially polyunsaturated fatty acids, in moringa grains stored at relatively high water contents (T10 and T13) suggested degradation of the lipid structure. This process can be attributed to the enzymatic action of lipases and phospholipases present in the grains or produced by the associated microflora, causing the rupture of the ester bonds of triglycerides, as well as the oxidation of the carbon chains of unsaturated fatty acids (Rocha *et al.*, 2017; Abd-Alla *et al.*, 2018; Tripathi; Kumar, 2020).

When analyzing the increase in the IA and IP of the oil, as shown in Table 3, together with the fatty acid profile (Table 4), more pronounced hydrolytic and oxidative degradation is observed in the samples stored with water contents of 0.10 and 0.13 db, respectively. Hydrolytic degradation involves breaking the ester bond in the presence of moisture, resulting in the release of fatty acids and a consequent increase in lipid acidity. Oxidative stress results from the auto-oxidation of triglycerides containing unsaturated fatty acids, which is catalyzed by oxygen, causing changes in the fatty acid profile (Oliveira *et al.*, 2021).

The most pronounced lipid oxidation in samples T13 and T10 due to the breakdown of unsaturated fatty acids, leading to the formation of saturated fatty acids and the reduction of oleic acid, the main component of moringa oil. According to Zhang *et al.* (2021), the oxidation of polyunsaturated acids is one of the main sources of reactive oxygen species in grains. These acids are particularly susceptible due to their multiple double bonds, which are preferred targets for reactions with oxygen free radicals. This results in the formation of several reactive products, such as lipid peroxides and aldehydes, which cause cellular damage and contribute to grain deterioration.

4 CONCLUSION

The results revealed considerable variation in the water content and water activity of the Moringa plants during the first 120 days of storage. There was a significant reduction in the water content of the samples with 0.10 and 0.13 db, reaching 0.07 db, while the water activity decreased from 0.74 and 0.82 to 0.64 and 0.60, respectively, between November and March.

In addition, a high incidence of fungi was observed in the freshly harvested grains, which decreased considerably throughout the storage period. The incidence of *A. flavus* decreased from 100% to 10%, 30% and 1% in samples T7, T10 and T13, respectively. There was also a reduction in the EE and CP content, especially in samples T7 and T10, suggesting a process of grain degradation due to water activity and the action of colonizing fungi.

The analysis of oil quality revealed greater degradation in grains stored with water contents of 0.10 and 0.13 db, with a more significant increase in the IP at T13, followed by T10. In addition, there were changes in fatty acid contents, with a decrease in the contents of polyunsaturated and monounsaturated fatty acids, such as oleic acid, and consequently an increase in the contents of saturated fatty acids, especially in samples T10 and T13, indicating lipid degradation.

Therefore, it is important to control the water content and water activity during the storage of *M. oleifera* grains not only to preserve their nutritional quality but also to prevent fungal proliferation and oil degradation.

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