# Physicochemical Characterization of Kaffir Lime Leaves (*Citrus hystrix* D.C.) Powder with Oxidized Starch Encapsulation

**Niken Widya Palupi*, Mira Adilia Salsabillah, Jay Jayus**

Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Jember, Jl. Kalimantan Tegalboto No. 37, Krajan Timur, Sumbersari, Jember, East Java 68121, Indonesia
*Corresponding author: Niken Widya Palupi, Email: niken.ftp@unej.ac.id

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**ABSTRACT**

Kaffir lime leaves (*Citrus hystrix* D.C.) contain various bioactive compounds, including phenols, terpenoids, alkaloids, and flavonoids. These bioactive constituents are highly susceptible to degradation. Therefore, encapsulation is necessary to enhance their stability. The use of maltodextrin as the sole coating material often results in particle agglomeration and weak microcapsule structures. This research evaluated how the ratios and concentrations of coating materials affect the physicochemical properties of kaffir lime powder. This research involved the preparation of starch using the photooxidation method, the extraction of kaffir lime leaves, the production of kaffir lime leaves powder, and the analysis of kaffir lime leaves powder. The parameters evaluated included moisture content, hygroscopicity, solubility, and Fourier Transform Infrared (FTIR). Data were analyzed using the Analysis of Variance (ANOVA) test with a significance level of ≤ 0.05. The results showed that the combination of photooxidized starch and maltodextrin as an encapsulation material, at a total concentration of 60% had a moisture content of 9.18%, hygroscopicity 5.71%, solubility 61.91%, polyphenol content 5.33 mg GAE/g, and polyphenol damage 10.06%.

**Keywords**: Encapsulation; kaffir lime; maltodextrin; photooxidized starch

## INTRODUCTION

Kaffir lime leaves (*Citrus hystrix D.C.*) are a widely recognized and frequently used part of kaffir lime plant due to various applications in daily life. These leaves are rich in bioactive compounds, including phenolic compounds, terpenoids, alkaloids, and flavonoids (Muzuka et al., 2018). These compounds contribute to the leaves' natural antioxidant and antibacterial activities. The presence of these bioactive compounds makes kaffir lime leaves valuable as food additives, enhancing the flavor, functional quality, and preservation of food products. Additionally, kaffir lime leaves are effective against foodborne pathogens, including *Salmonella typhi, Staphylococcus aureus,* and *Escherichia coli* (Abu Bakar et al., 2021). Despite their numerous benefits, the use of kaffir lime leaves in their fresh form is limited due to their short shelf life, thereby necessitating the application of post-harvest handling methods to extend their usability (Widyasanti et al., 2024). According to previous studies, encapsulation techniques have been demonstrated to ensure the stability of bioactive compounds, thus maintaining their functionality for extended applications (Agustin et al., 2021).

Encapsulation is a method used to encapsulate solid, liquid, or gaseous active ingredients (Core materials) in natural or synthetic macromolecular polymers (wall materials) (Zhang et al., 2022). The core material is referred to as the encapsulated substance, while the wall or coating material is called the encapsulant (Agustin

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et al., 2021). Encapsulation techniques preserve food ingredients by stabilizing them against oxidation and deterioration, enabling controlled release of additives, and improving sensory quality by masking or adsorbing unwanted tastes and odors (Xu et al., 2024).

Maltodextrin is commonly applied as a wall material in the encapsulation of bioactive compounds owing to its excellent water solubility (Xiao et al., 2022). However, maltodextrin is limited due to the relatively low emulsion stability, resulting in a reduced efficiency in encapsulating the active compound (Santoso et al., 2020). Each coating material possesses distinct strengths and limitations, which often necessitate combining to achieve optimal encapsulation outcomes (Sulistiyani et al., 2022). Another encapsulant that can be combined to improve capsule quality is oxidized starch. As reported by Palupi et al. (2020), oxidized starch exhibits low starch paste viscosity and a smaller particle size, resulting in an increased active surface area and binding capacity. This is in accordance with Chakraborty et al. (2022), oxidized starch is utilized in the food industry due to the outstanding functional characteristics, including low viscosity, high stability, clarity (optical transparency when dissolved or used in formulations), as well as the ability to form films and act as a binding agent.

Higher concentrations of encapsulants enable more effective coating of the core material (Sumarni et al., 2018). In this research, maltodextrin and oxidized starch are used as encapsulants. These materials are expected to improve the stability and functional properties of the powder while preserving the bioactive compounds. Additionally, this research aims to evaluate how the ratios and concentrations of coating materials affect the physicochemical properties of kaffir lime powder.

## METHODS

### Materials

The materials used for the production of kaffir lime extract powder were kaffir lime leaves, corn starch, maltodextrin, 30% hydrogen peroxide ($H_2O_2$), distilled water, and aluminum foil. For analysis, the reagents used were Folin-Ciocalteu reagent, gallic acid, aquadest, $Na_2CO_3$, and NaCl.

The equipment used for the production of kaffir lime extract powder was a set of photooxidation apparatus with UV-C lamps (Alinco), a pump (Interbit model DB-108 HATTEN), an analytical balance, a drying cabinet, and a spray dryer. Analytical equipment included a centrifuge (Thermo Scientific Sorvall ST 16R), moisture analyzer (Ohaus MB 120), spectrophotometer UV-VIS:U-2900 (Hitachi 2J1-0004), and Fourier Transform Infrared (FTIR) Shimadzu IRPrestige 21.

Table 1. Formulation for kaffir lime leaves extract powder

| Ratio of coating materials oxidized starch: maltodextrin | Volume of coating solvent (%) | |
|---|---|---|
| | 50 (V1) | 60 (V2) |
| 0: 100 | P0 V1 | P0 V2 |
| 50: 50 | P50 V1 | P50 V2 |
| 100 : 0 | P100 V1 | P100 V2 |

### Research Implementation

This research was conducted using a Completely Randomized Design (CRD) with two factors. The first treatment factor was the ratio of coating materials, specifically oxidized starch to maltodextrin (P), which consisted of three levels, including 0:100 (P0), 50:50 (P50), and 100:0 (P100). The second treatment factor was the volume of the coating solvent (V), comprised of two levels: 50% (V1) and 60% (V2). Each treatment combination was repeated three times. The specific combinations for the production kaffir lime leaves extract powder are presented in Table 1.

### Preparation of Oxidized Starch

The preparation of oxidized starch was carried out based on a modification of the method described by Palupi et al. (2020). The photooxidation of starch was initiated by preparing a 4000 mL mixture consisting of 200 g of starch (5% w/v), 133.33 mL of 30% (v/v) hydrogen peroxide ($H_2O_2$ 1%), and 3866.67 mL of Aquadest. These materials were then mixed and transferred to the sample container tube in a photooxidation device equipped with a stirrer inside. Photooxidation was performed at room temperature using a circulation system, with exposure achieved by pressing the UV-C lamp power button on the apparatus, and the photooxidation process lasted for 2 hours. The resulting suspension was then removed from the sample container through the output valve and poured onto a tray for drying in a drying cabinet at 50 °C for 24 hours. The dried starch was then processed to break down aggregates, resulting in fine granules of oxidized starch.

### Extraction of Kaffir Lime Leaves

The extraction of kaffir lime leaves was performed following the method described by Dayanti (2016) with modifications. The extraction procedure involved selecting fresh, clean kaffir lime leaves and wilting them. Forty-five grams of wilted kaffir lime leaves were chopped and then extracted using a leaves: water ratio of 1:5 (w/v). The

extraction was carried out by soaking for 24 hours at room temperature, followed by blending and filtration to separate the residue and obtain kaffir lime leaves extract.

**Production of Kaffir Lime Leaves Powder**

The process of producing kaffir lime leaves powder was carried out following the method described by Lia Umi et al. (2015). The production of kaffir lime leaves powder began by heating aquadest in a beaker to 75 °C. After that, the encapsulating agents (oxidized starch and maltodextrin) were used at ratios of 0:100, 50:50, and 100:0. Encapsulant concentrations were prepared at 50% (w/v) and 60% (w/v), and the mixtures were homogenized for 30 minutes at 75 °C. The homogenized suspensions were allowed to stand for 10 minutes at room temperature. Maltodextrin was then added and homogenized for 15 minutes and followed by the addition of oxidized starch, which was homogenized for 15 minutes. Subsequently, the encapsulant mixer was combined with 200 mL of kaffir lime leaves extract. The mixture was homogenized for 15 minutes at room temperature and then filtered through a 100 mesh sieve. The mixture of encapsulant and kaffir lime leaves extract was then dried using a spray dryer with an inlet temperature of 120 °C and an outlet temperature of 60 °C.

**Moisture Content Analysis**

Moisture content in this research was analysed according to a method described by Nurhidayati (2021) using a moisture analyzer. One gram sample was placed in an aluminum sample container, and the instrument was set to 130 °C. Once the measurement was complete, the final moisture content was automatically displayed on the instrument's screen.

**Hygroscopicity Analysis**

The hygroscopicity analysis method was analysed according to a method described by Hasan et al. (2022). One gram of the sample was placed in a desiccator without silica gel at room temperature for one week. A saturated NaCl solution was placed inside the desiccator to control the relative humidity(RH). Hygroscopicity was calculated using Equation 1 and expressed as the weight of absorbed water vapor per 100 g of dry material.

$$\text{Hygroscopicity (\%)}:\left(\frac{\text{final sample weight} - \text{initial sample weight}}{\text{initial sample weight}}\right) \times 100\% \quad (1)$$

**Solubility in Water**

The solubility analysis method was analysed according to a method described by Hasan et al. (2022). Water solubility was measured by dissolving 1 g of the sample in 10 mL of Aquadest. The mixture was centrifuged at 2000 rpm for 15 minutes. After that, 2 mL of the supernatant was transferred to a pre-weighed, oven-dried weighing bottle. The weighing bottle containing the supernatant was dried in an oven at 105 °C. The weighing process was repeated until a constant weight was achieved. Solubility in water was calculated using Equation 2.

$$\text{Solubility in water (\%)} = \left(\frac{\text{weight of dry supernatant}}{\text{weight of the sample}}\right) \times 100\% \quad (2)$$

**Polyphenol Content Analysis**

Polyphenol content was analyzed using the Folin-Ciocalteu method described by Sujana et al. (2022). A 0.1 mL sample solution was pipetted into a test tube and diluted with Aquadest to a volume of 5 mL. The solution was mixed with 0.5 mL Folin-Ciocalteu reagent, vortexed, and left to stand for 5 minutes. A total of 1 mL of 7% $Na_2CO_3$ solution was added, then vortexed, and allowed to stand for 60 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. The total polyphenol content was determined based on a gallic acid standard curve and expressed in mg/gram of sample.

**Encapsulation Efficiency Analysis**

The total polyphenol content from the analysis was used to determine encapsulation efficiency. The encapsulation efficiency analysis in this research was adapted from Osvaldt Rosales et al. (2021). Encapsulation efficiency of polyphenols was a measure of the percentage of core material encapsulated in the shell, and was calculated using Equation 3.

$$\text{Encapsulation efficiency (EE)(\%)} = \frac{\text{Polyphenol content encapsulated}}{\text{Polyphenol content before encapsulation}} \times 100\% \quad (3)$$

**Loading Capacity Analysis**

Loading capacity analysis in this research followed the method described by Osvaldt Rosales et al. (2021). Loading capacity refers to the amount of material retained within a given quantity of capsules after the drying process. It was calculated using Equation 4, where the encapsulated weight (*Weo*) (g) was divided by the weight of the sample capsules obtained after drying (*Wmp*) (g).

$$\text{Loading capacity (\%)} = \frac{\text{Weo}}{\text{Wmp}} \times 100\% \quad (4)$$

**FTIR Analysis of Microcapsule Functional Groups**

FTIR spectroscopic analysis in this study followed the method described by (Damto et al., 2023). FTIR

was utilized to identify the functional groups present in the microcapsules based on the wavelength ranges and characteristic peaks of specific functional groups. Each component of the microcapsules had distinct functional groups. The sample was analyzed in the wavelength range of 400 - 4000 $cm^{-1}$ (in the mid-infrared range).

### Data Analysis

The results were analyzed using the Analysis of Variance (ANOVA) test followed by Duncan's Multiple Range Test (DMRT) to determine the effect of the measured parameters at a significance level of ≤ 0.05. All statistical analyses were carried out using Microsoft Excel and SPSS software, and the results were then presented in the form of diagrams.

## RESULTS AND DISCUSSION

### Moisture Content

Moisture content is a critical parameter in determining the quality and stability of products. The ANOVA results (α ≤ 0.05) showed that the ratio of coating materials and the water volume significantly affects the moisture content of kaffir lime leaves extract powder. The observed moisture content ranged from 8.27% to 9.44%.

Figure 1 shows that all samples of kaffir lime leaves extract powder produced in this research meet the SNI (Indonesian National Standard) for powdered spices, which specifies a maximum moisture content of 12%. Additionally, the figure shows that kaffir lime leaves extract powder using oxidized starch with a 60% coating concentration had a higher moisture content of 9.28% compared to 8.27% with maltodextrin. According to Kusuma et al. (2023), products made with the addition of maltodextrin tend to have lower moisture content because maltodextrin can bind free water in the material. The free water is bound by maltodextrin through the formation of hydrogen bonds, which are easily evaporated during the drying process.

An increase in coating concentration results in a thicker suspension. The higher viscosity, caused by the reduced proportion of solvent, hinders moisture removal and makes the product more difficult to dry during the spray-drying process. According to Permanadewi et al. (2022), the increased viscosity leads to the formation of larger particles, which tend to retain more moisture, thereby reducing the efficiency of the drying process. A thicker suspension makes it more difficult to fully evaporate moisture, resulting in higher moisture content in the final product.

### Hygroscopicity

Hygroscopicity is an important indicator that affects product stability. The hygroscopicity evaluation process of kaffir lime leaf extract powder was carried out using spray drying techniques and under saturated humidity conditions. The higher the hygroscopic value, the more water the powder absorbs from its environment. The results of ANOVA analysis at a significance level of (α ≤ 0.05) showed that the ratio of coating materials and the volume of water had a significant effect on the hygroscopicity of kaffir lime leaves extract powder. The hygroscopicity values obtained ranged between 4.90% - 6.16%.

Figure 2 shows that the hygroscopicity of kaffir lime leaves extract powder was lower when oxidized

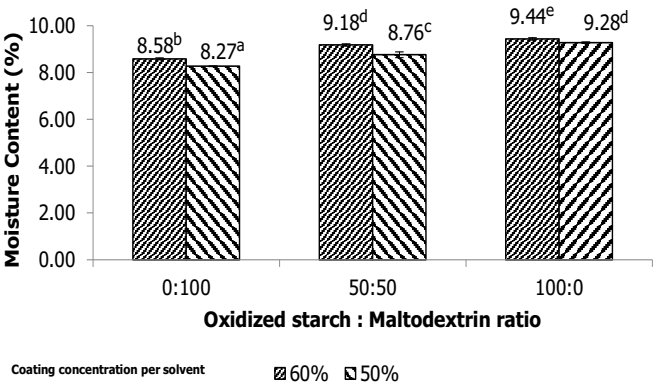



Figure 1. Moisture content of kaffir lime leaves extract powder with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences (p < 0.05).

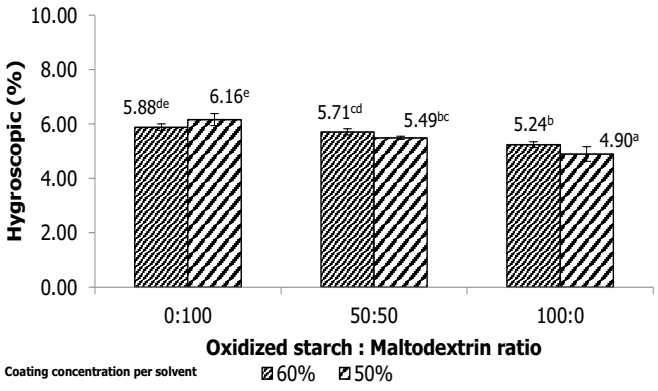



Figure 2. Hygroscopicity of kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences (p < 0.05).

starch was used as a coating material compared to maltodextrin. The lowest hygroscopicity was found in the sample with oxidized starch and a 50% coating concentration, with a value of 4.9%. In this research, low hygroscopicity indicates that the powder absorbs less moisture from the environment, which helps maintain the stability, prevents clumping, and extends its shelf life. In contrast, high hygroscopicity can lead to various problems, including changes in the product's physical and chemical properties and reduced stability during storage (Ng et al., 2022a).

The addition of oxidized starch reduced the hygroscopicity of kaffir lime leaves extract powder. A material that clumps easily indicates high hygroscopicity. The powder with added oxidized starch did not clump easily. Oxidized starch has larger carboxyl groups than hydroxyl groups (Sumardiono et al., 2021). The presence of large carboxyl groups in oxidized starch creates steric hindrance, which limits the ability of starch molecules to align or aggregate closely. This disruption decreases the tendency of starch to retrograde (Gałkowska et al., 2023).

The type of coating material significantly influences the hygroscopicity of the final product, primarily due to the inherent properties of the coating itself, which can lead the powder to absorb substantial amounts of moisture. Maltodextrin, as a coating agent, has hygroscopic characteristics, indicating that it readily absorbs moisture from the surrounding environment. Consequently, it is essential to minimize prolonged exposure to open air, as this can lead to hardening and diminish the material's functionality (Luliana et al., 2023). A higher concentration of coating material impacts hygroscopicity, resulting in lower moisture absorption due to reduced moisture content. This decrease in moisture content lessens the moisture gradient between the product and its environment, thereby curtailing the product's propensity to absorb water. In formulations containing maltodextrin, a greater moisture gradient encourages increased moisture absorption, thereby intensifying hygroscopic behavior as the material seeks equilibrium with the surrounding humidity (Luliana et al., 2023).

## Solubility

The solubility of kaffir lime leaves extract powder was evaluated at room temperature, and the solubility of the product determines its usability. Higher solubility values indicate that the active compounds in the product will be released more quickly during use. The results of ANOVA at a significance level of ($\alpha \leq 0.05$) showed that the ratio of coating materials had a significant effect on solubility. According to findings, solubility values obtained ranged between 48.37% - 76.73%.

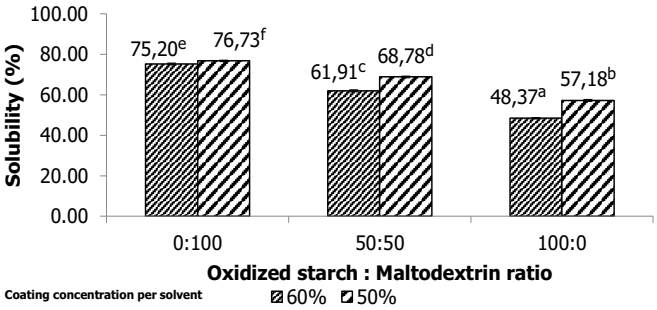



Figure 3. Solubility of kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences ($p < 0.05$).

Figure 3 shows that the more starch added, the lower the solubility, as the amount of maltodextrin decreases. Maltodextrin has good water solubility (Xiao et al., 2022), which can accelerate the dissolution of the encapsulation when applied to food. The properties of coating material used in the encapsulation process significantly influence the final product's physical and chemical attributes, as variations in emulsion properties and encapsulation techniques can lead to differences in product stability, release behavior, and sensory characteristics (Baysan et al., 2021)

Higher coating concentrations result in thicker mixtures, which lead to higher moisture content in the product. High moisture content in the material makes it difficult to disperse in water because the material tends to stick together, preventing pore formation, which reduces the material's ability to absorb water. Materials with high moisture content can cause fine powders to clump together, forming a sticky surface. This stickiness promotes particle agglomeration and consequently reduces the available surface area for proper wetting and dispersion (Ng et al., 2022b).

## Polyphenol Content

Polyphenol content in this research refers to polyphenol compounds present in kaffir lime leaves extract powder. The encapsulation of kaffir lime leaves extract powder aims to protect the active compounds from environmental conditions. The results of ANOVA at a significance level of $\alpha \leq 0.05$ showed that the ratio of encapsulating materials (starch and maltodextrin) and the volume of water used to dissolve coating materials significantly affected polyphenol content in the resulting powder. The average value of polyphenol content was approximately 3.74 mg GAE/g – 5.53 mg GAE/g.

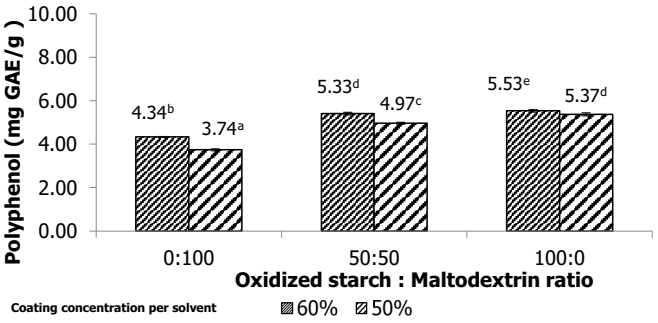



Figure 4. Polyphenol content of kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences ($p < 0.05$).

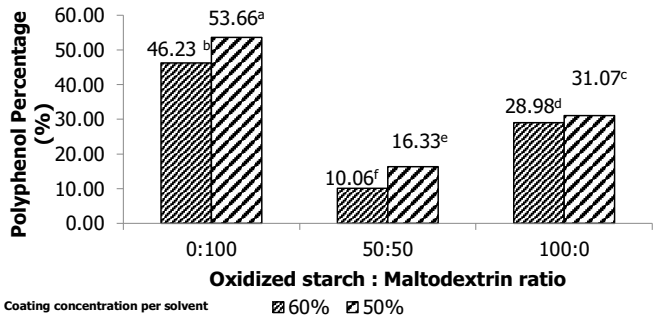



Figure 5. Polyphenol loss in kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences ($p < 0.05$).

Figure 4 shows that the highest polyphenol content was in the powder produced with oxidized starch and a 60% coating concentration, with a value of 5.53 mg GAE/g. This indicates that polyphenol content is influenced by the type of coating material used.

Figure 5 shows that the lowest polyphenol loss occurred with the use of two coating materials with a 60% coating concentration. The spray drying technique, which uses high temperatures, can reduce the amount of core material during the encapsulation process.

## Encapsulation Efficiency

The encapsulation efficiency value is calculated based on the comparison between encapsulated polyphenol content and polyphenol content before encapsulation. Encapsulation efficiency reflects the effectiveness of the encapsulation process, with higher values indicating greater retention of polyphenol

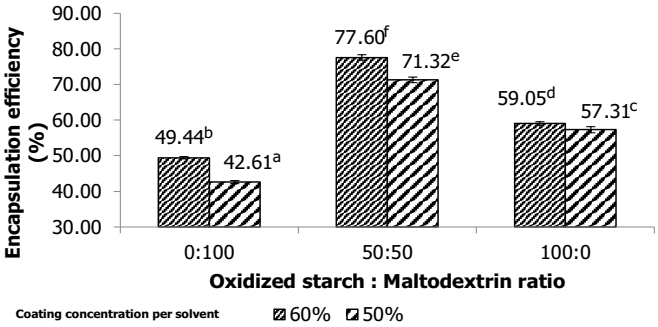



Figure 6. Encapsulation efficiency of kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences ($p < 0.05$).

content (active compounds) in the capsules. The results of ANOVA at a significance level of $\alpha \leq 0.05$ showed that the ratio of coating materials significantly affected encapsulation efficiency. According to findings, the encapsulation efficiency values obtained ranged between 42.61 – 77.6%.

Figure 6 shows that the highest encapsulation efficiency was obtained with the use of two coating materials at a 60% coating concentration, achieving 77.60%. The addition of starch demonstrated that modified starch enhances the encapsulation efficiency of polyphenols. Modified starch is frequently used in encapsulation processes because of its ability to form dense interfacial coatings. These coatings effectively protect and bind the encapsulated compounds, improving both encapsulation efficiency and the stability of bioactive ingredients during storage (Zabot et al., 2022).

High-viscosity mixtures tend to form larger droplets, which can improve the binding and encapsulation of active compounds, ultimately enhancing efficiency. Robert et al. (2015) conducted research that used two different solvent volumes, 60% and 50%. The highest encapsulation efficiency was obtained with 60% of solvent and a combination of oxidized starch and maltodextrin, indicating that this combination had optimal viscosity, thereby improving encapsulation efficiency.

## Loading Capacity

Loading capacity refers to the amount of encapsulated components in a specific number of capsules after the drying process, indicating the efficiency and ability of coating materials to encapsulate bioactive compounds (such as polyphenols) within the microcapsule matrix. The results of statistical analysis using ANOVA at a significance level of $\alpha \leq 0.05$ showed

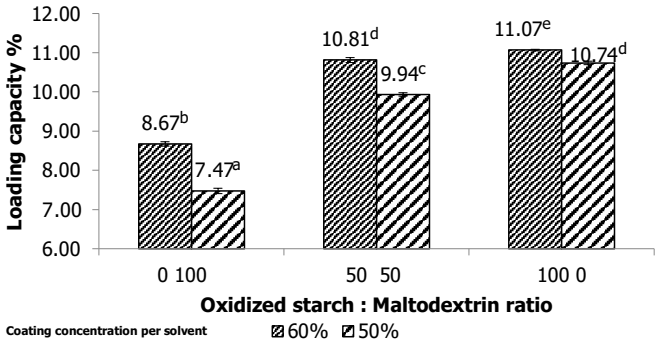
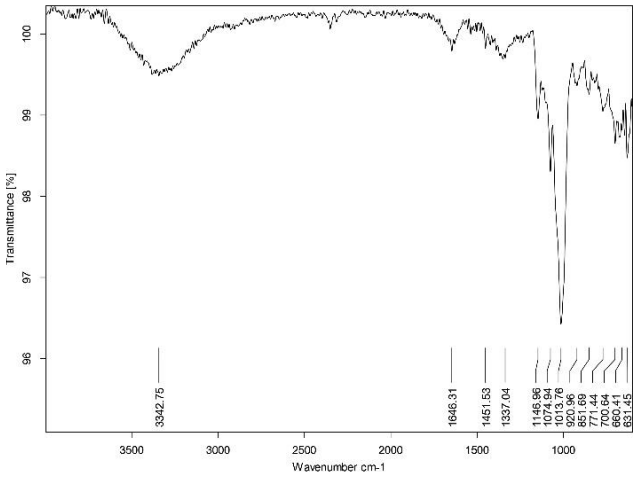


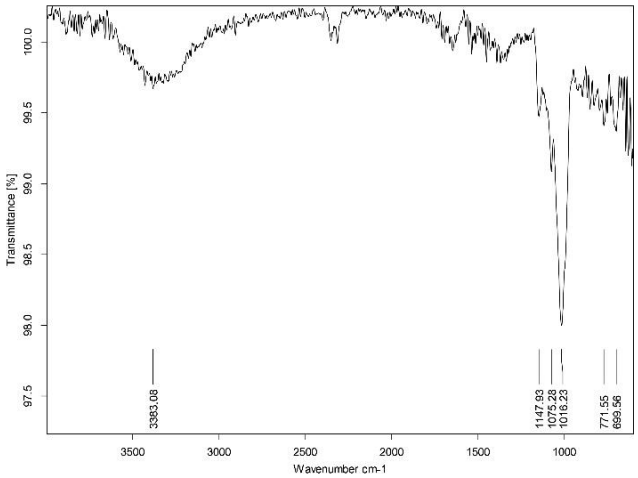

Figure 7. Loading capacity of kaffir lime leaves extract powder produced with different coating material ratios and coating concentrations. Values followed by different superscript letter(s) indicate statistically significant differences ($p < 0.05$).

that the choice of encapsulating materials (photo-oxidized starch and maltodextrin) in the production of kaffir lime leaves extract powder significantly affected the resulting loading capacity. According to findings, loading capacity values obtained ranged from 7.47% to 11.07%.

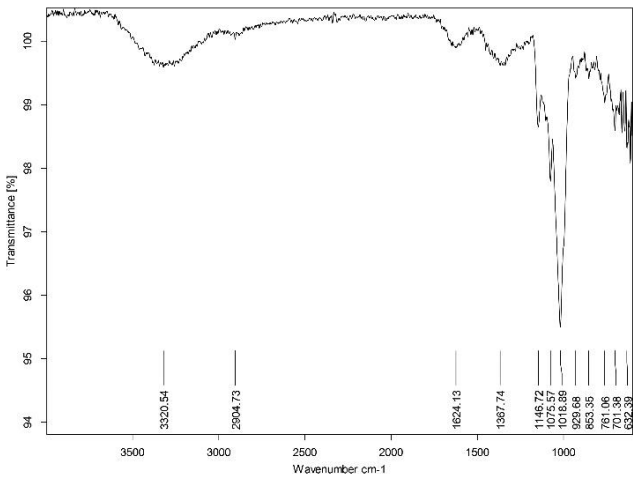
Figure 7 shows that the highest loading capacity was obtained with oxidized starch at a 60% coating concentration as a result of the high polyphenol content in the sample, which suggests that greater polyphenol content enhances the interaction between the active compounds (polyphenols) and coating material. To the same extent, an investigation on the encapsulation of curcumin demonstrated that an increased availability of active compounds leads to higher loading capacities, as more binding sites on the coating material are utilized (Chang et al., 2019).




(a)




(b)




(c)

Figure 8. FTIR spectra of photo-oxidized starch (a), maltodextrin (b), and kaffir lime leaves extract powder (prepared with capsule matrix of oxidized starch and maltodextrin with 60% coating concentration (c)

### FTIR Analysis

FTIR spectroscopy was used to identify the functional groups present in kaffir lime leaves extract powder. FTIR spectra presented in Figure 8 illustrate the presence of various functional groups across different samples. Figure 8a shows FTIR spectra of photo-oxidized starch, with a peak at 3342 $cm^{-1}$ indicating the presence of O–H stretching vibrations (Antunes et al., 2022). Additionally, peaks corresponding to carboxyl groups are observed at 1646 $cm^{-1}$ (Chen et al., 2022). The peak at 1013 $cm^{-1}$ corresponds to C–O stretching vibrations, which is characteristic of the chemical structure (Antunes et al., 2022).

Figure 8b shows FTIR spectra of maltodextrin, and the peak at 1147 $cm^{-1}$ is attributed to the C–O–C stretching mode of glycosidic linkages (Sekkal et al., 1995). Moreover, a peak at 1016 $cm^{-1}$ corresponds to C–O–H bending modes (Kačuráková et al., 1996). Figure 8c shows the FTIR spectra of kaffir lime leaves extract powder (P50V2). The hydroxyl group (O–H) is indicated at the peak of 3320 $cm^{-1}$. The same sample also showed aliphatic CH groups at 2904 $cm^{-1}$, aromatic C=C groups at 1624 $cm^{-1}$, and alkane C–H ($CH_3$) groups at 1367 $cm^{-1}$. These findings align with the FTIR spectrum of kaffir lime leaf extract reported in Malikhah et al. (2023).

## CONCLUSION

In conclusion, the addition of photo-oxidized starch in this research influenced the quality of kaffir lime leaves extract powder by increasing polyphenol content, while reducing hygroscopicity, solubility, and polyphenol loss. The increase in coating material concentration resulted in higher moisture content and polyphenol levels but reduced hygroscopicity and solubility. The use of photo-oxidized starch combined with maltodextrin at a 60% coating concentration effectively reduced polyphenol loss in kaffir lime leaves extract powder. Moreover, the formulation of kaffir lime leaves extract powder using photo-oxidized starch and maltodextrin with a 60% solvent volume yielded the highest encapsulation efficiency of 77.60%.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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