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Research Article

Synthesis of Carboxylated Chitosan Amide Using Some Cyclic Anhydride and Their Activities as Antifungal

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Abstract

Chitosan is a natural polymer that has antifungal activity. It is necessary to modify chitosan into its derivatives to increase its activity. One modification of chitosan that has the potential to be developed as an antifungal is carboxylated chitosan amide because this chitosan derivative contains a carboxylic group and is more hydrophilic. This research aims to synthesize chitosan amide carboxylate using several cyclic anhydride compounds and test its antifungal activity against *Aspergillus flavus*. The cyclic anhydrides used in this research are maleic anhydride and phthalic anhydride. In the initial stage of chitosan amide carboxylate synthesis, reaction optimization was carried out at varying temperatures of 25, 50, and 72°C for 7 hours. Compound characterization was carried out using FTIR and UV-Vis spectrophotometry. The disc diffusion method tested the chitosan amide carboxylate product for its antifungal activity against *Aspergillus flavus*. The optimal MCA (Maleoyl Chitosan Amide) product is (MCA_50), synthesized at a reaction temperature of 50°C. Under these optimal reaction conditions, PCA_50 (Pthaloyl Chitosan Amide) was successfully synthesized to produce a brownish-yellow solid with a yield of 46.1% (w/w) and a degree of substitution (DS) of 41.93%. The diameter of the inhibition zone against *Aspergillus flavus* for PCA_50 was 30 mm at the 12th hour of observation. The product (PCA_50) has better antifungal activity than chitosan and MCA_50.

Keywords: antifungal, *Aspergillus flavus*, Chitosan, phthaloyl-chitosan-amide

1. INTRODUCTION

Food hygiene is something that must be considered because it has an impact on health. Food not kept clean is easily exposed to microorganisms, including mold. Mold can grow on food due to direct contact between the food and spores in the air. Several factors that can influence fungal growth are water content, temperature, oxygen, pH, substrate or media, and inhibitory components [1].

To maintain food hygiene, mold growth on food must be prevented and its growth inhibited. One way to inhibit fungal growth is to use antifungal compounds. Antifungal compounds can interfere with the development and activity of fungi, especially fungi that are dangerous to humans. One of the exciting compounds to be developed as an antifungal compound is chitosan. Chitosan is a natural polymer with branched chains and the general formula $(C_6H_{11}NO_4)n$ [2]. Chitosan has unique characteristics such as biocompatibility [3] and biodegradability, it is easily decomposed by microorganisms [4]. Chitosan can be an antifungal material due to the nature of the interaction between the positive ammonium groups in chitosan with negatively charged components on the surface of fungal cells through electrostatic interactions, thereby disrupting the shape of the fungal cell wall and the permeability of the cell membrane [5].

Apart from that, chitosan has a free $-NH_2$ group bound to C atom number 2, where the free $-NH_2$ group has high reactivity and can be branched or modified [6]. One of the basics of chitosan modification is the reaction between the free amine

in chitosan and the acyl group to form an amide compound [7]. Amides are organic compounds with a carbonyl group (>C=O) attached to a nitrogen atom [8]. Chitosan is reacted with several cyclic anhydrides, producing a carboxylated chitosan amide derivative [9]. In this reaction, the amine or hydroxyl in chitosan attacks the cyclic anhydride carbonyl site to produce an amide or ester whose chain end contains a carboxylate group [10]. This carboxylated chitosan amide derivative has the potential to be a better antifungal compound. To test antifungal activity, you can use the fungus *Aspergillus flavus* [11].

Chitosan modification in this research was done by reacting chitosan with cyclic anhydride compounds, namely maleic anhydride and phthalic anhydride. This synthesis produces two types of carboxylated chitosan amide compounds. The product resulting from this synthesis has a carboxylic group with different styles and numbers of carbon chains. These structural differences result in characteristic differences in reactions and activities. On the other hand, this difference causes its antifungal properties to change, potentially even having better antifungal activity [12]. The results of synthesizing this chitosan carboxylate amide derivative were tested for antifungal activity against the fungus *Aspergillus flavus* [11].

2. RESEARCH METHODS

Material and Tools

The materials used in this study were chitosan (CV.Chimultiguna), glacial acetic acid (CH_3COOH) p.a. *(Merck)*, NaCl, distilled water, maleic anhydride *(Merck)*, phthalic anhydride *(Merck)*, technical acetone, sodium bicarbonate ($NaHCO_3$) *(Merck)*, ethanol (C_2H_5OH) technical, potato dextrose broth (PDB) *(Himedia)*, agar pack *(Swallow)*, chloramphenicol *(Novapharin)*, peptone water *(Merck)*, pH universal indicator, cotton, gauze, yarn, Whatman filter paper.

The tools used in this study were glassware, Ubbelohde viscometer, analytical balance, hot plate magnetic stirrer, Buchner funnel set, reflux apparatus set, vacuum pump, oven, Langmuir Air Flow (LAF), autoclave, incubator, orbital shaker, 10-100 L micropipette, tweezers, loop needle, spreader, Genesys 10S UV-Vis spectrophotometer, Fourier Transform InfraRed (FTIR) Perkin Elmer Frontier Type.

Experimental Procedure

Determination of the Molecular Weight of Chitosan

Molecular weight determination was determined using the Ubbelohde viscometer and the Mark-Houwink equation.

The Mark-Houwink equation is as follows:

$$[\eta] = K.M^{\alpha} \quad (1)$$

Description:

- $[\eta]$ = Intrinsic viscosity (mL/g)
- K = $1{,}81 \times 10^{-3}$
- M = Molecular weight (Da)
- A = 0,93 (Constant for chitosan polymer and acetic acid solvent)

Chitosan solution was prepared by making 1% acetic acid and 0.2 M NaCl in a ratio of 3:1 (v/v). Preparation is done by dissolving chitosan 0.01 g/mL into acetic acid and NaCl (3:1). Then variations in the concentration of chitosan solution as much as 0.001 g/mL; 0.002 g/mL; 0.003 g/mL; 0.004 g/mL; and 0.005 g/mL. Concentration variations were carried out to obtain different flow times from x to y, while time measurements were performed 5 times to get constant results.

Determination of the Deacetylation Degree of Chitosan

$$\%DD = 100 - [\left(\frac{A1655}{A3450}\right) x \frac{100}{1{,}33}] \quad (2)$$

Description:

- DD : Deacetylation degree
- A_{1655} : Absorbance at wavenumber 1655 cm^{-1} which shows absorption in the amide band in the *N*-Acetyl group
- A_{3450} : Absorbance at a wave number of 3450 cm^{-1} which indicates hydroxyl absorption and is used as an internal standard
- 1,33 : Factors indicating the value of the ratio A_{1655}/A_{3450} for chitin that has not been deacetylated

The degree of deacetylation (%DD) of chitosan was determined using FTIR spectrum analysis with the baseline method, at a wavenumber of 4000–400 cm^{-1}. The calculation of the degree of deacetylation can be done by comparing the absorbance at a wavenumber of 1650 cm^{-1} for an acetyl group and a hydroxyl group (OH) at a wavenumber of 3450 cm^{-1}, with an absorbance value of 1.33 on a complete deacetylation degree process [13].

Synthesis of Maleoyl Chitosan Amide (MCA) with Variation of Reaction Temperature

The synthesis of maleoyl chitosan amide (MCA) with various reaction temperatures was conducted to find the optimal conditions for synthesizing carboxylated chitosan amide derivatives. A magnetic stirrer dissolved one (1 g) of chitosan in 25 mL of 1% acetic acid (CH_3COOH) solvent in a beaker using a magnetic stirrer. The chitosan solution was poured into a round bottom flask to which 0.445 g (2.269×10^{-2} mol) of maleic anhydride was added. The reaction mixture was stirred at reaction temperatures of 25, 50, and 72^oC for 7 hours using a magnetic stirrer. The product formed from the reaction was precipitated by adding acetone and filtered using a Buchner funnel to remove the precipitated residue formed. The precipitate residue was added with 25 ml of 3% $NaHCO_3$ to salt the acetic acid. The solution was filtered using a Buchner funnel, washed using distilled water to pH 7, rinsed with ethanol, and dried in an oven. The synthesized powder was characterized using a Genesys 10S UV-Vis spectrophotometer and Perkin Elmer's Fourier Transform InfraRed (FTIR) Frontier Type [14].

Synthesis of Carboxylated Chitosan Amide

The synthesis of Carboxylated Chitosan Amide was carried out using the optimal conditions that have been carried out in the above reaction (MCA). Five (5 g) of chitosan were dissolved in 125 mL of 1% acetic acid (CH_3COOH) solvent in a beaker using a magnetic stirrer. The chitosan solution was poured into a round bottom flask which was then added 2.225 g (2.269 x 10-2 mol) maleic anhydride for the synthesis of MCA_50 (addition of 3.361 g (2.269 x 10-2 mol) phthalic anhydride for the synthesis of PCA_50), which had been dissolved in 30 mL ethanol at a temperature 30^oC. During the reflux reaction process, stirring was carried out at a temperature of 50oC for 7 hours using a magnetic stirrer. The product formed from the reaction was precipitated by adding acetone and filtered using a Buchner funnel to remove the precipitated residue formed. A Solution of 3% $NaHCO_3$ 125 ml was added to the precipitate to salt the acetic acid. The answer was filtered using a Buchner funnel, washed using distilled water to pH 7, rinsed with ethanol, and dried in an oven. The product was characterized using a Genesys 10S UV-Vis spectrophotometer and Perkin Elmer Frontier Type Fourier Transform InfraRed (FTIR) [14].

Antifungal Activity Test

The antifungal activity test on the synthetic product was carried out using the disc diffusion method against the fungus *Aspergillus flavus*. The mushroom stock was obtained from the Biochemistry Laboratory of the Faculty of Science and Mathematics, Diponegoro University.

Preparation of Fungal Inoculation on Oblique Media

The fungus *Aspergillus flavus* inoculation is prepared by making the media oblique. 0.5 g of PDB (Potato Dextrose Broth) and 1 g of agar were dissolved in 50 mL of distilled water. The test tube, loop needle, and media solution were sterilized using an autoclave for 45 minutes. The media that has been fixed is put into 2 test tubes as much as 5 mL, the media in the test tube is tilted to a flat plane and allowed to solidify in Lamin Air Flow so that the press remains sterile. Fungal colonies were taken aseptically with a loop needle and then inoculated on inclined media. Incubation at 37^oC with an incubator for 72 hours [15].

Preparation of Fungal Inoculation on Liquid Media (Suspension)

The preparation of liquid media (suspension) was prepared by dissolving 0.75 g of peptone water in an Erlenmeyer using 50 mL of distilled water. The test media and needles were sterilized by autoclaving for 45 minutes. Inoculation was done by scraping the fungus on an inclined medium using an oseptic needle aseptically inserted into a suspension of liquid media. The injection of bacteria in liquid media was carried out in Lamin Air Flow to keep it sterile and then incubated in an incubator shaker until the suspension became cloudy for 1 day. To obtain the same turbidity was carried out using 0.5 standard Mc. Farland has an absorbance of 0.08-0.1 and a wavelength of 625 nm [16]. McFarland is an equalization of fungal concentrations in liquid media which has a density of $0.5 - 2.5x10^3$ spores/mL for mushrooms [17]. If the suspension has reached the standard value of Mc. Farland was then tested for antifungal activity [18].

Preparation of Test Sample Solution

Chitosan, the result of the synthesis of maleoyl Chitosan amide (MCA_50) and phthaloyl Chitosan amide (PCA_50), each of 0.005 g was dissolved in 10 mL of 1% acetic acid to obtain a concentration of 500 ppm. A total of 0.005 g of chloramphenicol dissolved in 10 mL of distilled water to get a concentration of 500 ppm was used

as a positive control [19]. In contrast, 1% acetic acid was used as a negative control.

Preparation of Media Test

Test media was prepared by dissolving 1 g of PDB (Potato Dextrose Broth) and 2 g of agar in 100 mL of distilled water. The media solution and petri dish were sterilized using an autoclave for 45 minutes. The media was poured into 2 Petri dishes of 20 mL each, and then the media was allowed to solidify in Lamin Air Flow to keep it sterile. A total of 100 µL of fungal suspension, whose absorbance had been measured, was inoculated on the test medium and could be used to test the antifungal activity.

Antifungal Activity Test

It poured 100 µL of Aspergillus Flavus mushroom suspension into a petri dish. Even distribution of the mushroom suspension using a cotton bud until evenly distributed. Next, drip 20 µL of the test solution on a 0.5 cm diameter paper disc into a solution of chitosan compound, MCA_50, PCA_50, and chloramphenicol. Wet disc paper was placed on nutrient agar media on a petri dish previously inoculated with *Aspergillus flavus* fungus. Furthermore, incubation and observations were carried out at 6, 12, 18, and 24 hours at 37ºC. The formation of a bright zone around the paper disc is measured in diameter [20].

RESULTS AND DISCUSSION

Chitosan characteristics

Chitosan characteristics determined in this research include molecular number and weight DD (degree of deacetylation). Determining this character is essential for the modification process and its anti-fungal properties. The Molecular weight was determined using an Ubbelohde viscometer, while the DD value was determined using the IR spectrum.

Based on calculations using the Ubbelohde viscometer, it can be determined that the molecular weight of the chitosan used in this study is 121173.14 Daltons. It can be estimated that the number of monomers in chitosan is about 748.

Based on **Figure 1.**, which shows the FTIR spectra of chitosan, absorption appears at wavenumber 3380 cm^{-1}, which shows overlapping of –OH and $–NH_2$ groups. The absorption of wavenumber 2879 cm^{-1} indicates a stretching in the aliphatic C_{sp}^3–H group. Absorption at wavenumber 1651 cm^{-1} shows the >C=O group from the acetyl group in chitosan [21].

The results of calculating the degree of deacetylation according to the baseline method for chitosan were 66%. It is estimated that the number of monomers containing amine groups (-NH2) in the chitosan structure used in this study is 494 monomers.

3.2 Synthesis of carboxylated chitosan Amide derivative

Generally, the synthesis of carboxylated chitosan amide derivatives (MCA, PCA) is an addition reaction. This product occurs due to the reaction between the amine in chitosan and cyclic anhydride. The mechanism of this amidation reaction is shown in **Figure 2** [22].

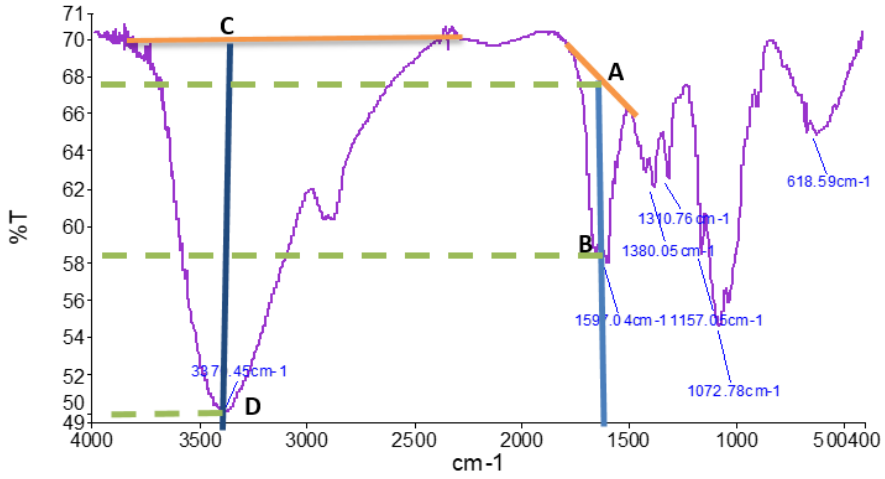

Figure 1. FTIR Spectra of Chitosan

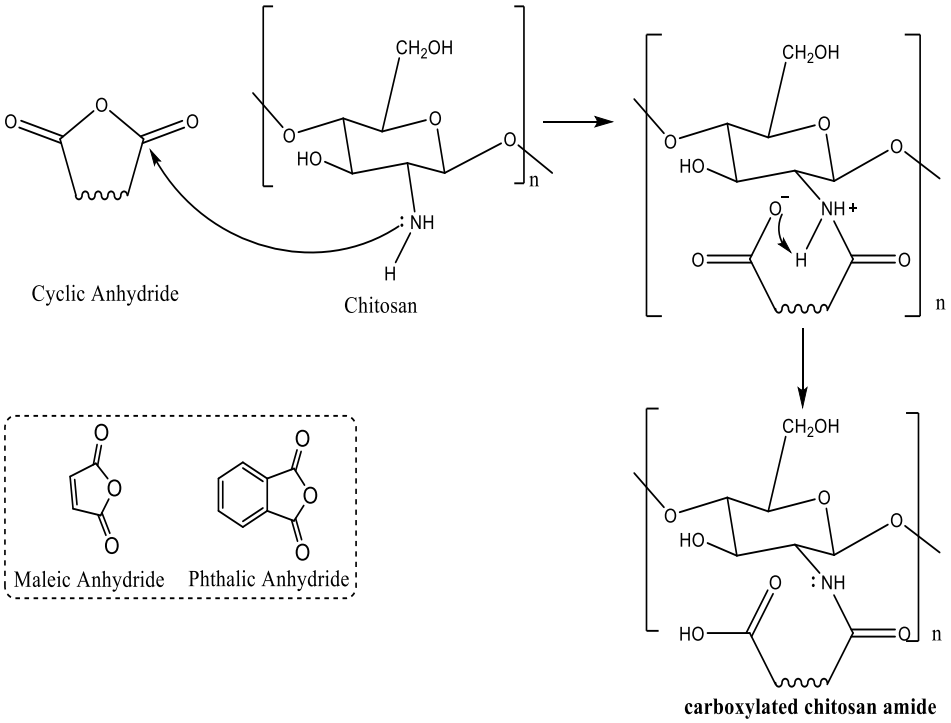

Figure 2. Reaction Mechanism of carboxylated chitosan amide synthesis

Table 1. Synthesis of carboxylated chitosan Amide derivative and product characterization

No	Sample	Yield (%)	*(Based on peak changes in IR spectra)*					DS (%)
			Csp^3-H	=C-H, aromatic	>C=C<	>C=O	-NH-	
1	MCA_25	9.70	Observed	-	Observed	Observed	-	48.33
2	MCA_50	35.30	Observed	-	Observed	Observed	-	20.75
3	MCA_72	27.00	Observed	-	Observed	Observed	-	18.75
4**	MCA_50	23.50	-	-	Observed	Observed	Observed	45.40
5**	PCA_50	46.10	-	Observed	-	Observed	Observed	41.93
6	Chitosan	-	-	-	-	Observed	Observed	-

(MCA_25) = Product of Synthesis Maleoyl chitosan Amide at (25ºC); (MCA_50) = Product of Synthesis Maleoyl chitosan Amide at (50ºC); (MCA_72) = Product of Synthesis Maleoyl chitosan Amide at (72ºC); (MCA_50) = Product of Synthesis Phthaloyl chitosan Amide at (50ºC);

DS = Degree of Substitution (%);

Reaction on entry 1,2,3 used Chitosan (1g) and Maleic Anhydride (0.445 g);

****Reaction on entry 4,5 used Chitosan (5 g), Maleic Anhydride (2.225 g), and phthalic Anhydride (3.361g).***

A series of experiments were carried out to obtain optimal reaction conditions for the synthesis of carboxylated chitosan amide, the results of which are presented in Table 1. In **Table 1**, from entries 1-3, the higher the reaction temperature (25ºC to 72ºC), the lower the degree of substitution obtained (MCA). MCA products formed at higher reaction temperatures and in an acidic environment will more easily undergo hydrolysis reactions. This hydrolysis reaction will produce free chitosan and dicarboxylic acid. This hydrolysis process plays a role in reducing the resulting DS value.

On the other hand, the higher the reaction temperature, the higher the yield obtained. The higher the reaction temperature, the smaller the DS obtained. The relatively small DS value causes the solubility of the MCA product in a water solvent to decrease. As in the experimental procedure, the MCA product isolation process involves filtration and purification by using water. This process plays a role in reducing the yield value of the resulting MCA product. It can be seen that the reaction temperature of 50ºC is the optimal reaction temperature. The highest results of maleoyl chitosan amide (MCA) were obtained in this condition. The resulting MCA_50 product is a yellow solid, with a yield of 35.30% and DS (20.75%).

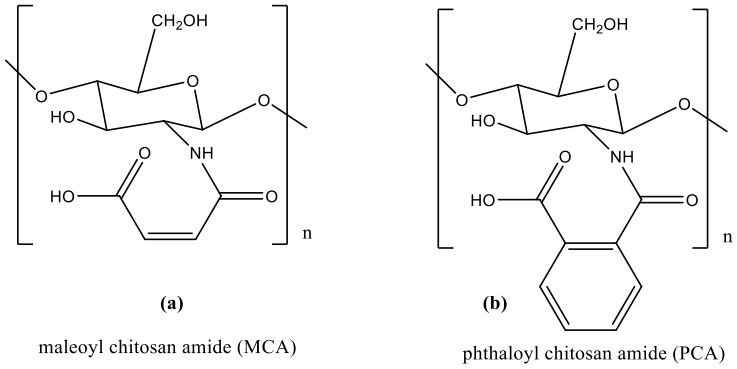

Figure 3. Maleoyl chitosan amide **(a)** and Phthaloyl chitosan amide **(b)**

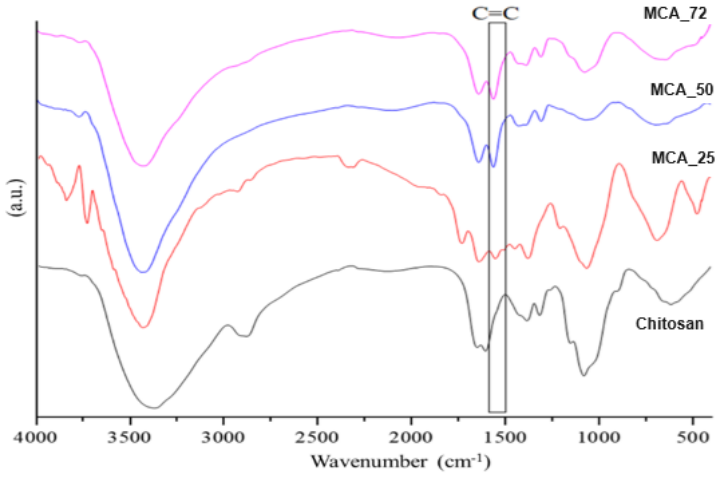

Figure 4. FTIR Spectra of Chitosan and Maleoyl Chitosan Amide (**MCA**) With Variation of Reaction Temperature

The synthesis of phthaloyl chitosan amide (PCA) is carried out using optimal conditions for MCA synthesis (Table 1, in entries 4-5). In this synthesis, an amidation reaction occurs between chitosan and phthalic anhydride to produce phthaloyl chitosan amide (PCA). The resulting PCA_50 product is a brownish-yellow solid. The yield obtained in PCA_50 synthesis (46%) was greater than that of MCA (23.5%). This is because the PCA_50 structure has a more extended conjugation system than MCA_50, so the PCA_50 structure is a more stable product. It is for this reason that the PCA_50 products produced have greater yields.

MCA characteristics using IR

MCA characterization was carried out using FTIR. These aim to ensure double bonds >C=C< formation in MCA on various reaction temperatures. Analysis of maleoyl chitosan amide using FTIR spectrophotometer on reaction temperatures of 25, 50, and 72ºC and chitosan as a comparison gave the spectra shown in **Figure 4.**

The results of FTIR spectra on the synthesis of **MCA** with variations in reaction temperatures of 25, 50, and 72ºC showed an absorption peak at wavenumber 3380-3450 cm^{-1}, which was the stretching vibrational absorption of the hydroxyl group (-OH) [21]. This absorption overlapped with the stretching vibrational absorption -NH_2.

MCA characteristics

The bending vibration of –NH_2 appears at wavenumber 1597 cm^{-1} in the chitosan spectrum [21]. This vibration does not appear in the carboxylation spectrum of maleoyl chitosan amide (MCA). The band's disappearance at 1597 cm^{-1} indicates that the amine in chitosan has been successfully converted into amide at the temperature variations used. The appearance of a band at wavenumbers 1590-1650 cm^{-1} indicates the emergence of stretching vibrations in the amide group >C=O. The band at wavenumbers 1550-1590 cm^{-1} represents the strain absorption >C=C< (alkene) [22]. The aliphatic bending vibration absorption of $C_{sp}{}^3$-H appears at wavenumbers around 1310-1380 cm^{-1}.

Table 2. Data Interpretation of FTIR absorption of MCA products

No.	Wavenumber (cm^{-1})				Functional Group Vibration
	C	MCA_25	MCA_50	MCA_72	
1	3380	3443	3434	3432	Stretching O-H dan N-H
2	1651	1643	1641	1640	Stretching C=O
3	1597	-	-	-	Bending $–NH_2$
4	-	1588	1560	1559	Stretching >C=C< (alkena)
5	1379	1383	1320	1319	Bending C_{sp}^3-H aliphatic

Note: C (Chitosan) dan MCA (*maleoyl chitosan amide*) on reaction temperature at (25, 50, dan 72ºC).

MCA characteristics using UV-Vis

The amidation reaction product (MCA) resulting from the reaction between chitosan and maleic anhydride is also analyzed using a UV-Vis spectrophotometer.

Figure 5. compares the spectra of chitosan, maleic anhydride, and MCA. Chitosan compounds produced two absorptions in the 227 nm region (peak I), which was the transition of π→π* carbonyl group (>C=O) and 268 nm (peak II), which was the transition of n→π* carbonyl group (>C=O) [21]. Maleic anhydride produces an absorption in the 235 nm region, which is the π→π* transition of the >C=C< conjugate and >C=O bond.

The MCA compound produced at various reaction temperatures (25, 50, and 72oC) has UV-Vis absorption at 227-230 nm. This peak represents the π→π* electronic transition of the >C=C< bond conjugated with the >C=O group [23]. The interpretation data of wavelength absorption of the MCA compound with three variations of reaction temperature (25, 50, and 72ºC) are shown in **Table 3.**

PCA characteristics

PCA characteristics using IR

The characterization of PCA_50 was carried out using FTIR, which aims to confirm the presence of >C=C< groups from the aromatic side of PCA_50 and C_{sp}^2-H compounds in MCA_50. Analysis by FTIR of carboxylated acyl chitosan and chitosan as a comparison gave the spectra shown in **Figure 6**. The results of FTIR spectra on MCA_50 and PCA_50 showed absorption peaks at wavenumber 3436 and 3420 cm^{-1}, which were the stretching vibrational absorption of the hydroxyl group (-OH) which overlapped with the stretching vibrational absorption $-NH_2$. Wavenumber at 2930 and 2891 cm^{-1}, which shows aliphatic C_{sp}^3-H symmetric stretching vibration absorption, at wavenumber 1642 and 1648 cm^{-1} the appearance of Stretching >C=O amide vibrations.

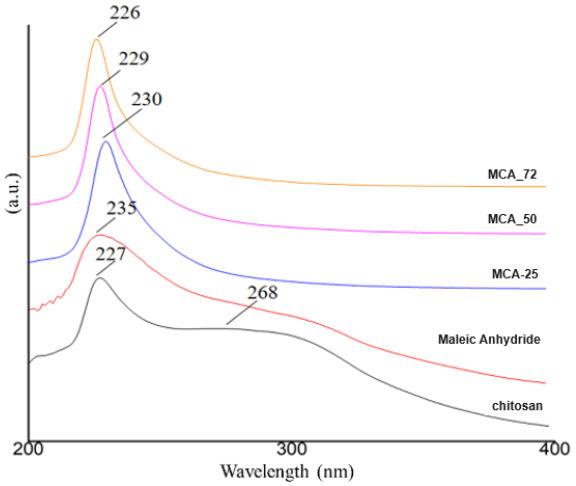

Figure 5. Comparison of UV-Vis Spectra of Chitosan, Maleic Anhydride, and MCA

Table 3. Interpretation data of MCA UV-Vis spectra

Sample	Wavelength (nm)			
	Peak I (nm)	Electronic Transition	Peak II (nm)	Electronic Transition
C	227	π→π* carbonyl group	268	n→π* carbonyl group
MA	235	π→π* >C=C< conjugates with >C=O bonds	-	-
MCA_25	230	π→π* >C=C< conjugates with >C=O bonds	-	-
MCA_50	228	π→π* >C=C< conjugates with >C=O bonds	-	-
MCA_72	227	π→π* >C=C< conjugates with >C=O bonds	-	-

Note: C = chitosan, MA = maleic anhydride, MCA_25 to MCA_72

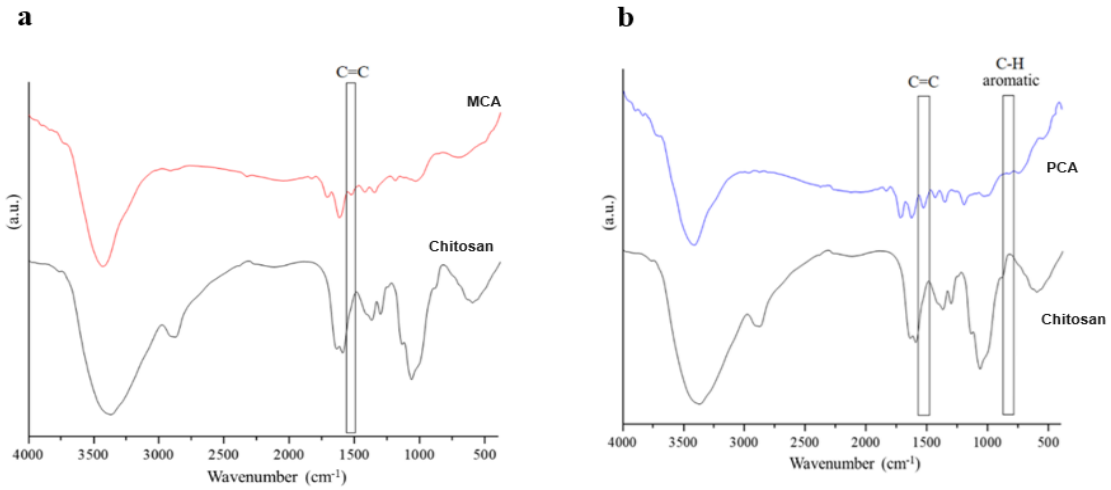

Figure 6. FTIR Spectra of Chitosan and MCA_50 **(a); s**pectra of Chitosan and PCA_50 **(b)**

Table 4. Data Interpretation of FTIR Absorption of Carboxylated Chitosan Amide

No	Wavenumber (cm^{-1})			Functional Group Vibration
	C	MCA	PCA	
1	3380	3436	3420	Stretching O-H and N-H
2	2885	2930	2891	Stretching C_{sp}^3-H aliphatic
3	1651	1642	1648	Stretching C=O
4	1597	-	-	Bending $–NH_2$
5	-	1558	1550	Stretching C=C
6	1384	1383	1375	Bending C_{sp}^3-H aliphatic
7	-	-	769	C-H aromatic ring

Note: C (Chitosan), MCA (maleoyl chitosan amide), dan PCA (phthaloyl chitosan amide).

In the IR spectrum, **figure 6.** (**a** dan **b**) shows that the bending vibration of $–NH_2$ at the wavenumber 1597 cm-1 has disappeared. This illustrates that the amide formation reaction (MCA_50 and PCA_50) has been successful. The successful formation of carboxylated chitosan amide (MCA_50 and PCA_50) was also proven by the appearance of a peak at wavenumber 1558 cm^{-1}. This peak shows the stretching vibration absorption >C=C< in the two chitosan carboxylate amides that were successfully synthesized. Spectra at wavenumber 1383 and 1375 cm^{-1} show aliphatic C_{sp}^3-H bending vibrations. In the spectrum of PCA_50, a peak at a wavenumber of about 769 cm^{-1} appears. This peak represents an aromatic ring C-H absorption [24].

PCA characteristics using UV-Vis

Figure 7. (a) compares UV-Vis spectra between chitosan, maleic anhydride, and MCA. Chitosan compounds produced two absorptions in the 227 nm region (peak I), which was the transition of π→π* carbonyl group (>C=O), and 268 nm (peak II), which was the transition of n→π* carbonyl group (>C=O). Maleic anhydride produces an absorption in the 235 nm region, which is the π→π* transition of >C=O conjugated >C=C< bonds. The MCA compound produces absorption in the 228 nm region, which is the π→π* transition of the >C=C< conjugated >C=O bond system [23].

Figure 7. (b) compares UV-Vis spectra between chitosan, phthalic anhydride, and PCA. The PCA compound produces two absorptions in the 225 nm region (peak I), which is an electron transition from the π→π* conjugated system (>C=O), and 279 nm (peak II), which is an n→π* electron transition in the carbonyl system (> C=O) which is conjugated to a benzene ring. Phthalic anhydride has two UV-Vis absorptions in the 231 nm (peak I) region, which is the π→π* electron transition of the C=C conjugated C=O bond, and 280 nm (peak II), which is the n→π* electron transition in the system carbonyl group (>C=O-) of conjugated benzene. The interpretation of the absorption wavelength of carboxylated chitosan amide data is shown in **Table 5.**

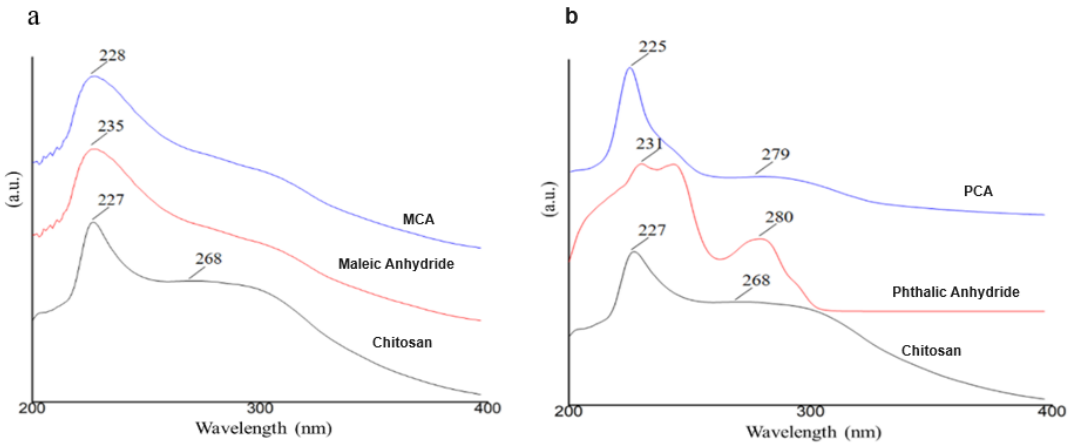

Figure 7. Comparison of UV-Vis spectra: (a) chitosan, maleic anhydride, and MCA; (b) chitosan, phthalic anhydride, and PCA.

Table 5. Interpretation of UV-Vis data for structure analysis

Sample	wavelength (nm)			
	Peak I	Electronic Transition	Peak II	Electronic Transition
C	228	π→π* amide group	268	n→π* amide group
MA	235	π→π* *conj*. >C=C with C=O bond		
MCA_50	228	π→π* *conj*. >C=C with C=O bond		
PA	231	π→π* *conj*. >C=C with C=O bond	280	n→π* *conj*. carboxyl group
PCA_50	225	π→π* *conj*. >C=C with C=O bond	279	n→π* *conj*. carboxyl group

Note: C=chitosan, MA=maleic anhydride, MCA= carboxylated maleoyl chitosan amide, PA=phthalic anhydride, PCA= carboxylated phthaloyl chitosan amide and (*conj*=Conjugated).

Table 6. Antifungal Inhibition Zone Data

No	Sample	(ppm)	Inhibition Zone (mm)			
			6 hours	12 hours	18 hours	24 hours
1	Chitosan	500	0	26	22	16
2	Maleoyl Chitosan Amide (MCA-50)	500	0	22	15	12
3	Phthaloyl Chitosan Amide (PCA-50)	500	0	30	21	17
4	Cloramphenicol ((+) Control)	500	0	35	35	35
5	Acetic Acid 1% ((-) Control)	1000	0	11	0	0

Antifungal Activity Test

Test the antifungal activity of several acyl chitosan amides using the disc diffusion method [18]. The disc diffusion method is a method for measuring the diameter of the inhibition zone of the fungus *Aspergillus flavus*. Antifungal tests were carried out on samples of chitosan, maleoyl chitosan amide (MCA_50), and phthaloyl chitosan amide (PCA_50). Chloramphenicol was used as a positive control for this antifungal test, while a 1% acetic acid solution was used as a negative control [19].

Table 6. shows the inhibitory zone data on the antifungal activity against the fungus *Aspergillus flavus* and that chitosan, MCA-50, and PCA-50 have antifungal activity. This activity is due to the interaction between the partial positive group (–NH) on chitosan, MCA-50, and PCA-50 with the negative group on the surface of fungal cells. This electrostatic interaction affects the shape

of the fungal cell wall and the permeability of the cell membrane [25], thereby allowing leakage of intracellular materials into the medium [26]. The growth of *Aspergillus flavus* fungus can also be inhibited by inserting chitosan into the fungal cells so that the chitosan can absorb important nutrients in the fungal cells. This nutrient absorption process causes the slow process of protein synthesis in fungi [27].

The data in **Table 6.** shows that MCA-50 has a lower inhibitory effect on fungal growth than chitosan. This happens because PCA-50 has a more significant steric effect than chitosan. This more excellent steric effect reduces the ability to enter fungal cells. The diameter of the PCA-50 inhibition zone has a more excellent value than chitosan. This happens because the (-NH) group on PCA-50 is more positive than the (-NH) group on chitosan. Structurally, PCA-50 has a benzoate group, which is more acidic than the acid group in MCA-50. The presence of this stronger acid group causes the amino group (-NH) to accept protons to produce more ammonium ions quickly. Structurally, PCA-50 has a stronger partial positive side when compared to MCA-50 and chitosan.

The antifungal activity test increased with increasing the positive charge of the (–NH) group on the material. The more positive the (-NH) group on the material, the stronger the electrostatic interaction, causing the negative charge component on the fungal cell surface to change its permeability. This reason allows the leakage of intracellular materials into the medium.

4. CONCLUSIONS

Based on the research results, it can be concluded that the optimal temperature for the synthesis of sulfonated chitosan amide derivatives is 50ºC. For the synthesis of phthaloyl chitosan amide (PCA) under optimal conditions, it produces a brownish-yellow solid with a yield of 46.1% (w/w) and a degree of substitution of 9.1%. The antifungal activity of a derivative of chitosan amide carboxylate, namely PCA-50, is better against *Aspergillus flavus* than MCA-50 or chitosan. PCA-50 has antifungal activity against *Aspergillus flavus* with an inhibition zone diameter of 30 mm at 12 hours, 21 mm at 18 hours, and 17 mm at 24 hours. Phthaloyl chitosan amide (PCA) can be an active anti-fungal agent in food packaging.

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REFERENCES

1. Pitt JI, Hocking AD. *Fungi and Food Spoilage*. Springer US; 2009. doi:10.1007/978-0-387-92207-2

2. Akman F. Prediction of Chemical Reactivity of Cellulose and Chitosan Based on Density Functional Theory. *Cellulose Chemistry and Technology*. 2016;51:253-262.

3. Pokhrel S, Yadav PN. Functionalization of chitosan polymer and their applications. *Journal of Macromolecular Science, Part A*. 2019;56(5):450-475. doi:10.1080/10601325.2019.1581576

4. Champagne LM. The Synthesis of Water Soluble N-Acyl Chitosan Derivatives for Characterization As Antibacterial Agents. *Water (Basel)*. Published online 2008.

5. Dias AM, dos Santos Cabrera MP, Lima AMF, et al. Insights on the antifungal activity of amphiphilic derivatives of diethylaminoethyl chitosan against Aspergillus flavus. *Carbohydr Polym*. 2018;196:433-444. doi:10.1016/j.carbpol.2018.05.032

6. Miranda MES, Marcolla C, Rodrígues CA, et al. The role of N-carboxymethylation of chitosan in the thermal stability and dynamic mechanical properties of its films. *Polym Int*. 2006;55(8):961-969. doi:10.1002/pi.2060

7. Pasaribu SP, Kaban J, Ginting M, Silalahi J. Preparation of In Situ Cross-Linked N-Maleoyl Chitosan-Oxidized Sodium Alginate Hydrogels for Drug Delivery Applications. *Open Access Maced J Med Sci*. 2019;7(21):3546-3553. doi:10.3889/oamjms.2019.850

8. E. Budianto ASEMN. *Selektivitas Reaksi Pada Kitosan*. FMIPA UI; 2013.

9. Badawy MEI, Rabea EI. Synthesis and Characterization of N-(maleoyl) chitosan at Different Degrees of Substitution with Antibacterial Activity. *Journal of Polymer Materials*. 2017;34(1):249.

10. Skorik YA, Kritchenkov AS, Moskalenko YE, et al. Synthesis of N-succinyl- and N-glutaryl-chitosan derivatives and their antioxidant, antiplatelet, and anticoagulant activity. *Carbohydr Polym*. 2017;166:166-172. doi:10.1016/j.carbpol.2017.02.097

11. Guo Z, Xing R, Liu S, et al. Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydr Res*. 2007;342(10):1329-1332. doi:10.1016/j.carres.2007.04.006

12. Sahariah P, Másson M. Antimicrobial Chitosan and Chitosan Derivatives: A Review of the Structure–Activity Relationship. *Biomacromolecules*. 2017;18(11):3846-3868. doi:10.1021/acs.biomac.7b01058

13. Khan TA, Peh KK, Ch'ng HS. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. *J Pharm Pharm Sci*. 2002;5(3):205-212.

14. Golyshev AA, Moskalenko YuE, Skorik YuA. Comparison of the acylation of chitosan with succinic anhydride in aqueous suspension and in solution. *Russian Chemical Bulletin*. 2015;64(5):1168-1171. doi:10.1007/s11172-015-0994-3

15. Sahu S, Kundu M, Behari Sukla L. Bio-beneficiation of iron ore using heterotrophic microorganisms. *J Microbiol Biotechnol Res*. 2015;5(2):54-60.

16. Silvia, Savante A, Muhamad AW. Aktivitas Antimikroba Ekstrak Daun Soma (Ploiarium alternifolium Melch) Terhadap Jamur Malassezia furfur dan Bakteri Staphylococcus aureus. *Jkk*. 2015;4(3):84-93.

17. Thakur D, Sahani K. In Vitro Antimicrobial Activity and Mic of The Extracellular Ethyl Acetate Crude Extract of Endophytic Fungi Fusarium Sp. Isolated from tephrosia purpurea root. *Int J Pharm Pharm Sci*. Published online January 22, 2019:48-53. doi:10.22159/ijpps.2019v11i3.28230

18. Hasibuan HS, Erina E, TR TA. Daya Hambat Ekstrak Etanol Daun Sirsak (Annona muricata L.) Terhadap Pertumbuhan Jamur Aspergillus sp. *Jurnal Ilmiah Mahasiswa*. 2021;5(2):88-92.

19. Novita DA, Iswendi I, Iryani I. Uji Antimikroba Asap Cair Hasil Pirolisis Sabut Pinang (Areca Catechu L) Terhadap Pertumbuhan Aspergillus flavus dan Rhizopus stoloniferus. *Periodic*. 2012;1(2):6-8.

20. Dani IW, Nurtjahja K, Zuhra CF. Penghambatan Pertumbuhan Aspergillus Flavus Dan Fusarium Moniliforme Oleh Ekstrak Salam (Eugenia Polyantha) Dan Kunyit (Curcuma Domestica). *Saintia Biologi*. 2012;1(1):8-14.

21. Ismiyarto I, Saputri NW, Rahmatia LZ, et al. Synthesis of Mn(II) Complexes-Carboxymethyl Chitosan Schiff Base Salicylaldehyde and Antibacterial Activity. *Jurnal Kimia Valensi*. 2021;7(1):10-21. doi:10.15408/jkv.v7i1.19866

22. Rusu AG, Chiriac AP, Nita LE, Rosca I, Rusu D, Neamtu I. Self-Assembled Nanocarriers Based on Modified Chitosan for Biomedical Applications: Preparation and Characterization. *Polymers (Basel)*. 2020;12(11):2593. doi:10.3390/polym12112593

23. Chen Z, Zhang H, Song Z, Qian X. Preparation and Application of Maleic Anhydride-Acylated Chitosan for Wet Strength Improvement of Paper. *Bioresources*. 2013;8(3). doi:10.15376/biores.8.3.3901-3911

24. Ghiggi FF, Pollo LD, Cardozo NSM, Tessaro IC. Preparation and characterization of polyethersulfone/N-phthaloyl-chitosan ultrafiltration membrane with antifouling property. *Eur Polym J*. 2017;92:61-70. doi:10.1016/j.eurpolymj.2017.04.030

25. Abd El-Hack ME, El-Saadony MT, Shafi ME, et al. Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. *Int J Biol Macromol*. 2020;164:2726-2744. doi:10.1016/j.ijbiomac.2020.08.153

26. Meng D, Garba B, Ren Y, et al. Antifungal activity of chitosan against Aspergillus ochraceus and its possible mechanisms of action. *Int J Biol Macromol*. 2020;158:1063-1070. doi:10.1016/j.ijbiomac.2020.04.213

27. Dewi R, Nur RM. Antifungal Activity of Chitosan on Aspergillus spp Antifungal Activity of Chitosan on Aspergillus spp. *International Journal of Bioengineering & Biotechnology*. 2019;2:24-30.