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Formation of chitosan from black soldier fly (hermetia illucens) pupae using microwaves radiation energy

Rachmawaty[1*)], Sahribulan[1], Suriati Eka Putri [2], Widya Faramitha Arisma[1]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar

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This study aims to determine the effect of microwave radiation energy on the formation of chitosan from Black soldier fly pupae (Hermetia illucens). Microwave radiation Chitin isolation from BSF pupae was demineralized (HCl 5%, 45 C) and deproteinated (NaOH 4%, 45 C), then the transformation of chitin into chitosan (deacetylation) was carried out using microwave radiation with different powers, namely 240 watts, 400 watts, 560 watts and 800 watts with a radiation time of 5 minutes. Determination of the degree of deacetylation is determined by observing the spectrum produced using the infrared spectrophotometer (FTIR). The results showed that the degree of chitosan deacetylation produced on average was 61% at 240 watts; 400 watts at 63%; 560 watts by 66%; and 800 watts by 72%. Based on the research results, it can be concluded that microwave radiation energy affects the formation of chitosan in this case indicating that the higher the microwave radiation energy, the higher the degree of deacetylation of chitosan produced.

Kata kunci:

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**) corresponding author*

Rachmawaty

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Jl. Daeng Tata Raya, Makassar 90222, Indonesia

Email: rachmawaty@unm.ac.id

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A B S T R A K

Penelitian ini bertujuan untuk mengetahui pengaruh energi radiasi gelombang mikro terhadap pembentukan kitosan dari pupa Black soldier fly (Hermetia illucens). Radiasi gelombang mikro Isolasi Kitin selongsongan pupa BSF demineralisasi (HCl 5%, 45 C) dan deproteinasi (NaOH 4%, 45 C), selanjutnya transformasi kitin menjadi kitosan (deasetilasi) dilakukan dengan menggunakan radiasi gelombang mikro dengan daya yang berbeda-beda yaitu 240 watt, 400 watt, 560 watt dan 800 watt dengan waktu radiasi selama 5 menit. Penentuan derajat deasetilasi ditentukan dengan mengamati spektrum yang dihasilkan dengan menggunakan spektrofotometer inframerah (FTIR). Hasil pengamatan menunjukkan bahwa derajat deasetilasi kitosan yang dihasilkan rata-rata yaitu pada daya 240-watt sebesar 61%; 400 watt sebesar 63%; 560 watt sebesar 66%; dan 800 watt sebesar 72%. Berdasarkan hasil penelitian, dapat disimpulkan bahwa energi radiasi gelombang mikro berpengaruh terhadap pembentukan kitosan dalam hal ini menunjukkan bahwa semakin tinggi energi radiasi gelombang mikro semakin tinggi pula derajat deasetilasi kitosan yang dihasilkan.

INTRODUCTION

The black soldier fly or *Black Soldier Fly* (BSF) has the scientific name *Hermetia illucens,* belonging to the family Stratiomyidae. *The Black Soldier Fly* is a fly that is somewhat unique because it is not a pest like ordinary house flies. On the other hand, BSF flies have potential as decomposers, capable of decomposing various types of organic waste and various types of household or factory waste. BSF flies are also insects that efficiently produce protein, fat and other important bioactive substances such as chitin and melanin, so that they become insects that can be used by humans for all phases of their life.

The phase before becoming an adult fly, the BSF larva will metamorphose into a pupa. The pupa will enter the prepupa phase where BSF flies in the prepupa stage contain 32% protein, 37% lipid, 19% minerals, and 9% chitin in dry weight extraction (Andari et al., 2021) . In the prepupa phase, the larva will harden so that it eventually forms a hard shell or sleeve. The fly will come out of the shell and then the pupa bag (sleeve) will be left just like that. Pupae shells that are allowed to accumulate will become waste produced by BSF flies. Utilization of Black soldier fly pupa shells can be used as a material for producing chitin and chitosan because they contain chitin, which is quite high. The average BSF fly contains about 23.2% chitin of its total mass and is deacetylated to its nitrogen group (Wardhana, 2016) .

The chitin content in BSF flies also depends on the type of food. The results of the study showed that the BSF chitin content was 57 gr/Kg dry matter if the feed was leaf or vegetable waste. If given feed in the form of household waste, the resulting chitin weighs 67 gr/Kg. Chitin is the main constituent component found in the cell walls of fungi, invertebrate exoskeletons, crustaceans and insects where this component functions as a supporting and protective component. While chitosan is a natural polymer which is very abundant in nature so that it becomes a material that has high economic value and can be utilized for human life. (Dompeipen EJ, 2017)

Chitin is a stable compound against chemical reactions, due to its low chemical reactivity, it is also non-toxic and biodegradable. Naturally chitin is in the shell bound with polysaccharides, fats and inorganic salts. Chitin is insoluble in water (hydrophobic), in alcohol and insoluble in dilute acids or alkalis. Chitin can be dissolved by a degradation process using concentrated mineral acids in formic acid. (Suptijah, 2004)

Chitosan produced from chitin has the same chemical structure as chitin, consisting of long molecular chains and high molecular weights. The difference between chitin and chitosan in each ring of the chitin molecule is that there is an acetyl group (-CH3-CO) on the second carbon atom, whereas In chitosan there is an amine group (-NH). Chitosan can be produced from chitin through a deacetylation process, namely by reacting it with high concentrations of alkali for a relatively long time and at high temperatures. Chitosan is also called biopolymer because it is unique, namely in an acidic solution, chitosan has cationic characteristics and is positively charged, while in an alkaline solution, chitosan will precipitate.

Chitin and chitosan have very wide uses in everyday life as adsorbents for heavy metal waste and dyes, preservatives, anti-fungal, cosmetics, pharmaceuticals, flocculants, anti-cancer and anti-bacterial. Because chitin and chitosan are non-toxic and biocompatible. Chitin isolation was obtained through several process steps, namely demineralization, deproteination, and deacetylation of BSF shells or shells.

The results of the chitin isolation produced were used to isolate chitosan through the deacetylation process. Chitin is deacetylated through an alkaline hydrolysis process using a strong and concentrated base to obtain chitosan. The deacetylation process aims to break the covalent bond between the acetyl group and the nitrogen in the chitin acetamide group to produce deacetylated amine groups. The removal of a large acetyl group in the acetamide group of chitin is known as the degree of deacetylation. During the process of forming chitosan it is very possible for changes in both physical and chemical properties and parameters to cause differences in the properties of chitin and chitosan. These differences in properties and parameters may include differences in solubility, hygroscopicity, critical point and decomposition, average molecular weight and polymer decomposition. (Kahar et al., 2020) .

Chitosan has several uses according to the field of utilization, in the pharmaceutical, biochemical, biotechnology, biomedical, food, nutrition, health, membrane, agriculture, textile and paper industries (Bahri et al., 2015). used as a substitute for chemicals that are harmful to the environment, in the field of water treatment chitosan can be used as a raw material for making ultrafiltrates, and so on (Kusumawati, 2009) .

One of the important parameters that determine the quality of chitosan is the degree of deacetylation. The free amine group charge of the degree of deacetylation of chitosan determines the application of chitosan (Kahar et al., 2020) . The degree of deacetylation is generally determined volumetrically or using infrared spectroscopy (FTIR / Fourier Transform Infrared) instruments. The minimum degree of chitosan deacetylation standards for the food industry is 70%, the cosmetics industry is 80% and the biomedical industry is 90% (Setyawati, Kartini, & Pranowo , 2016). Factors that affect the degree of chitosan deacetylation include the concentration of NaOH in chitosan isolation, temperature and time of the chitosan deacetylation process (Fahmi et al., 2007)

Determination of the value of the degree of deacetylation of chitosan also influences the method of synthesizing chitosan. In recent years the use of microwave or microwave radiation to synthesize chitosan has attracted a lot of attention from researchers and has become very popular in accelerating chemical reaction processes that are safe and fast. In addition, the spectroscopic method has the advantage of easy-to-find equipment in every laboratory and easy measurement procedures, but it takes time to make a calibration curve. Infrared spectroscopy is a fast analytical method, but signals from impurities will also affect the analysis results (Andari et al., 2021)

Based on the reviews explained above, the aim of the study was "Formation of Chitosan from Pupae of *Black Soldier Fly* (Hermetia illucens*)* using microwaves", because in this study a synthesis of chitosan was carried out using microwave radiation and studied several parameters that influence such as power, temperature and radiation time. Thus this research can provide solutions to the community regarding the management of BSF shell or shell waste into chitosan, by providing an introduction to a fast and safer method of chitosan synthesis using the principle of using *microwave waves*.

METHOD

Materials

The materials used in this study were samples of BSF maggot casings or pupae obtained from Balla Insecta Celebes located in Panaikang Village, Pattalassang District, Gowa Regency, South Sulawesi, aquadest, 5% HCl, 4% NaOH, 60% NaOH, paper filter, vaseline, aluminum foil, label paper.

Tools

The tools used in this research were oven, blender, measuring cup, beaker glass, stir bar, magnetic stirrer, filter, analytical balance, microwave, Soxhlet, Hotplate, Stirrer, and FTIR (Fourier Transform Infra-red).

Sample Preparation

In the preparation stage, samples of the BSF Pupa sleeves were washed thoroughly, then air-dried for several days to dry, after drying they were crushed using a blender and sieved using a 100-mesh sieve or sieve. The next step is that the BSF pupa powder is dried again in the oven at 45 °C for 24 hours.

Chitin isolation

Chitin was obtained through three stages of the isolation process, namely demineralization to remove minerals, deproteination to remove protein, and deacetylation to remove acetyl groups. The demineralization process is carried out by mixing 50 g of BSF pupa powder which has been baked into a beaker glass with 500 ml of 5% HCl solution. The mixture was stirred using a magnetic *stirrer* for 3 hours on the stirrer, then the BSF pupa powder was filtered, washed and then dried again in the oven at 45oC for 24 hours. Furthermore, the deproteination process was carried out by reacting 16 g of the demineralized product into a beaker glass with 160 ml of 4% (w/v) NaOH solution. This mixture is then soxhleted for ± 1 hour. The deproteinated solid was filtered, washed, and dried in an oven at 45 °C for 24 hours. The deproteinase results in the form of chitin powder were then analyzed using the FTIR tool.

Conventional synthesis of chitosan

The conventional chitosan isolation process without heating or deacetylation of chitin is carried out by reacting 2 g of chitin in a beaker with a mixture of 20 ml of 40% w/v NaOH solution. This mixture was soaked for ± 1 hour. The result of this deacetylation is in the form of a powder which is then washed, filtered, and dried in an oven at 45oC for 24 hours. The results of deacetylation which will later become chitosan will be characterized by FTIR.

Chitosan synthesis with microwaves

Testing the effect of differences in microwave radiation power on the degree of deacetylation. It was carried out by reacting 1 g of chitin transferred into a closed Teflon then added 10 mL of 40% (w/v) NaOH solution. Stir until evenly distributed, after a homogeneous solution is transferred in a microwave oven with optimum time, then heated with several power variations, namely 240, 400, 560 and 800 Watt. The resulting product is washed until neutral, filtered and dried in an oven at 45oC for 24 hours. Chitosan powder was then analyzed using FTIR.

Determination of Degree of Deacetylation of Chitosan

Test the degree of deacetylation of chitosan with FTIR (Fourier Transform-Infra Red). The degree of chitosan deacetylation was determined by Fourier transform infrared spectroscopy (FTIR) with a wavelength of 4000 cm1 to 600 cm-1. The degree of deacetylation was determined by the *base line method* formulated by Baxter. The degree of deacetylation was calculated from the ratio between the absorbance at 1655 cm-1 and the absorbance at 3450 cm-1. Measurement of the degree of deacetylation is based on the curve illustrated by

spectrophotometer. The highest peak (Po) and lowest peak (P) are recorded and measured with the selected baseline. The absorbance ratio is calculated by the formula: A = $\text{Log}\frac{P0}{P}$

Description: A = Absorbance
P_0 = % transmittance at baseline
P = % transmittance at minimum peak

$$DD = 1 - \frac{A1655}{A3435} \text{x} \frac{1}{1,33} \text{x } 100\%$$

RESULTS AND DISCUSSION

Chitin Isolation

Isolation of chitin from BSF pupa shells was carried out in several stages. The first stage is the demineralization process which aims to remove minerals and some inorganic compounds contained in the sample. The solution used in the demineralization process in this study was HCl. The purpose of using an acid solution is to support the decomposition process of several minerals such as calcium carbonate, sodium carbonate, and calcium phosphate contained in the BSF pupa shells into their chloride salts and carbon dioxide, based on the reaction equation (1) (Gómez-Ríos et al., 2017; Merami et al., 2020).

$$\begin{aligned} &CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2 \\ &Na_2CO_3 + 2HCl \rightarrow 2NaCl + H_2O + CO_2 \\ &Ca_3(PO_4)_2 + 6HCl \rightarrow 3CaCl_2 + 2H_3PO_4 \end{aligned} \quad (1)$$

The next stage is deproteination to remove protein. In this study using 4% NaOH. One of the advantages of using NaOH is the possibility of partial deacetylation of chitin and hydrolysis of biopolymers (Merami et al., 2020). The chemical reactions that may occur during the deproteination stage are shown in the reaction equation (2) (Gómez-Ríos et al., 2017; Merami et al., 2020).

$$\begin{aligned} &C_6H_{12}N_2O_3 + 2NaOH \rightarrow 2C_3H_6NNaO_2 + H_2O \\ &C_{10}H_{16}N_2O_7 + 2NaOH \rightarrow 2C_5H_8NNaO_4 + H_2O \\ &C_{18}H_{20}N_2O_3 + 2NaOH \rightarrow 2C_9H_{10}NNaO_2 + H_2O \end{aligned} \quad (2)$$

Chitin is produced in the form of light brown powder. The chitin obtained was analyzed using FTIR to determine its functional groups and degree of deacetylation. The results of the characterization of chitin and its functional group absorption are shown in Figure 1 and the interpretation results are shown in Table 1.

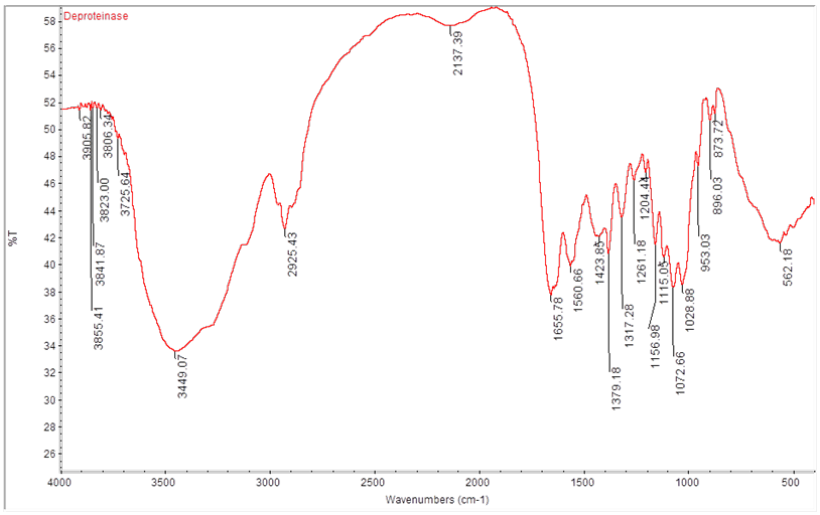


Figure 1. Chitin infrared spectrum from BSF pupae

Table 1. Interpretation of chitin vibrational absorption bands from BSF pupae

Wavenumber cm-1		Functional groups
Chitin	Frequency Area	
3855.41	3000-3850	(vb) Amides
3449.07	3300-3500	(vb) OH overlap
2925,43	2850-2970	(vs) CH aliphatic
2137,39	2100-2250	(vs) CH aromatic
1655.78	1630-1690	(v) NH2 [Secondary amine]
1560.66	1400-1600	(v) CN [Amine]
1379,18	1300-1400	(ώ)C–O
1072.66	1000-1200	(v) NH [Primary amine]
953.03	900-1350	(v) COCs
896.03	800-955	(ώ) β-1,4-glycosidic

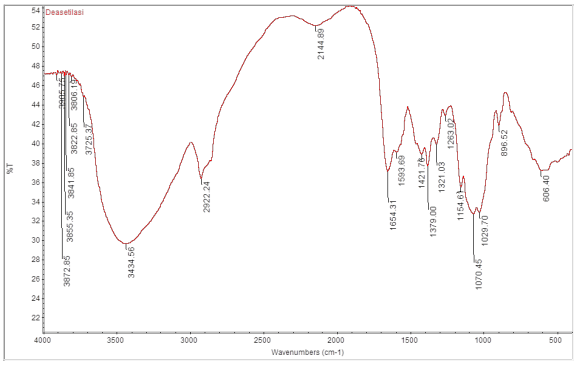
Description
v = stretching vibration
vs = symmetrical stretching vibration
b = bending vibration
ώ = stretching vibration

Based on the FTIR spectrum in Figure 1, a typical chitin absorption band has appeared at 1655.78 cm $^{-1}$ which shows C=O stretching in the -NHCOCH $_{3\ bond}$. In addition, at 1560 cm $^{-1}$ it is suspected that there is absorption of CN on the amine for b-chitin (Dompeipen EJ, 2017) . The stretching vibration at absorption 1379 cm $^{-1}$ is suspected of the presence of CO groups and at 953 cm $^{-1}$ there is stretching of the COC groups. Another characteristic found in the FTIR spectrum is the CH deformation of β-1,4-glycosidic at an absorption of 896 cm-1. The extracted chitin obtained in this study did not show any splits in the absorption bands at 1620 and 1650 cm $^{-1}$, so it can be concluded that the chitin obtained was of the b-chitin type. The results obtained are in line with the results of previous studies which succeeded in extracting chitin from tiger shrimp shells (Dompeipen EJ, 2017) . However, different things were obtained from chitin which was also extracted from BSF, where there were broken absorptions in the 1620 and 1650 cm $^{-1\ regions}$ indicating the type of chitin obtained was a-chitin (Triunfo et al., 2022). This difference is thought to be due to the species used coming from different regions so that the mineral content in the samples will also be different.

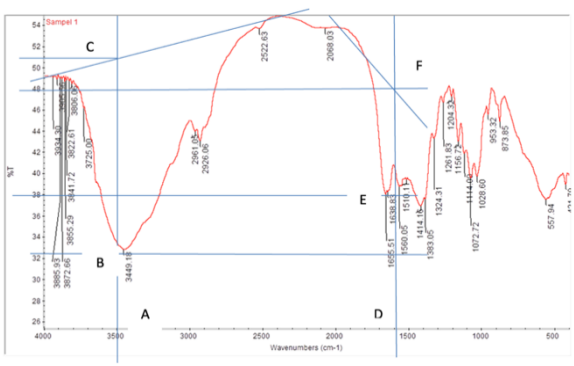
Chitosan Isolation

The reaction of forming chitosan from chitin is a hydrolysis reaction of an amide by a base. Chitin acts as the amide and NaOH as the base. In this study, the synthesis of chitosan was carried out in two ways, namely by conventional means by heating and by means of microwave radiation. The change of chitin to chitosan was evaluated using FTIR to determine its structure and then the degree of deacetylation was calculated. The characterization of the FTIR or infrared spectrum used to determine the degree of deacetylation of the chitosan formed from the synthesis results is shown in Figure 2 and Table 2.

A



B



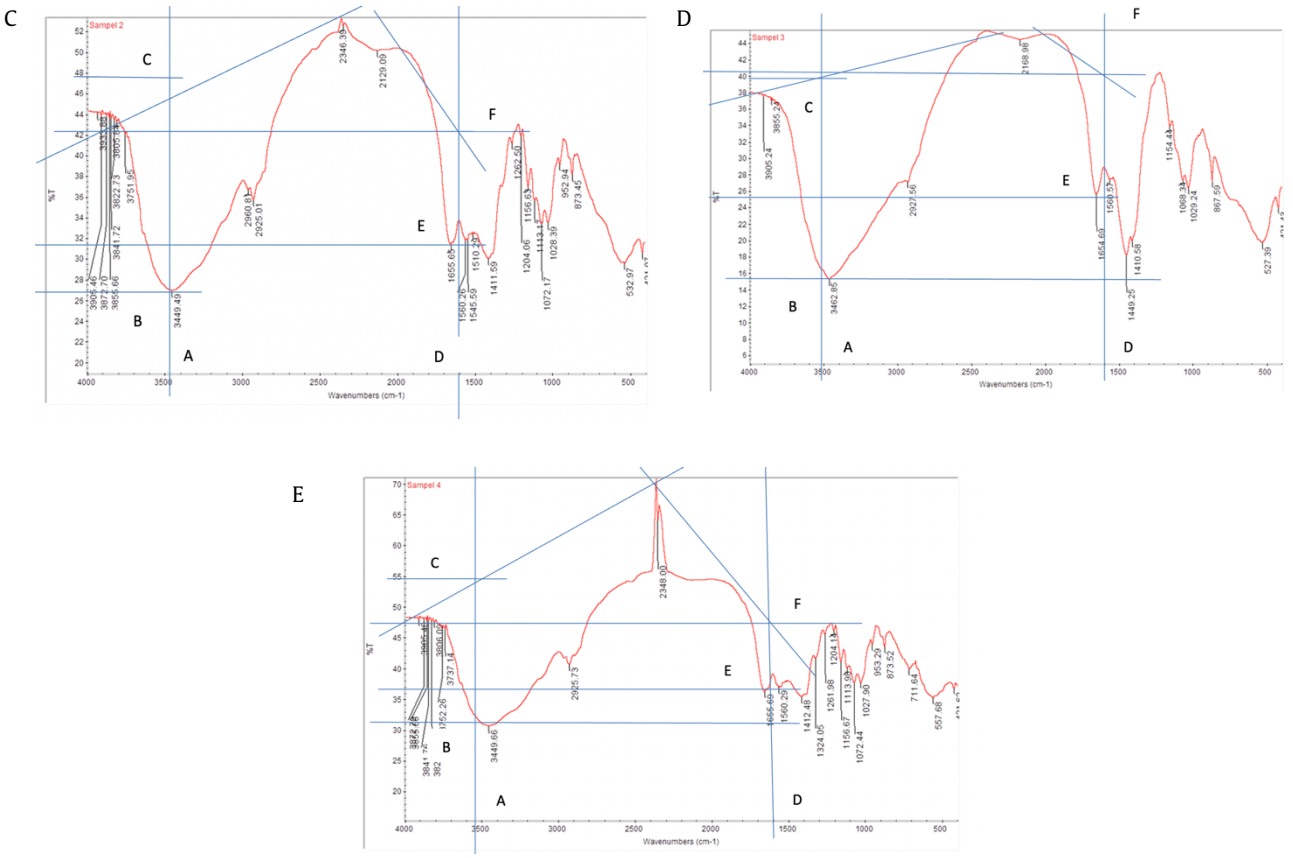


Figure 2. FTIR characterization of standard chitosan (A), 240 watt chitosan (B), 400 watt chitosan (C), 560 watt chitosan (D), and 800 watt chitosan (E).

Table 2. Interpretation of chitosan vibration absorption bands at 240 watts, 400 watts, 560 watts, and 800 watts

chitosan	Wave Number cm $^{-1}$ Microwave Chitosan (Watts)				Area Frequency	Functional groups
	240	400	560	800		
3433.56	3449,18	3449,18	3462.85	3449,49	3000-3500	(vb) O – H overlap(vs) N – H
2922,24	2961.05	2961.05	2927,56	2935,40	2850-2970	(vb) C–H aliphatic
2922,20	2926.06	2926.06	2922.85	2925,73	2850-2970	(vs) C–H aliphatic
2144.89	2522.63	2522.63	2168.98	2348.00	2100-2550	(vs) C–H aromatic
1654,31	2068.03	2068.03	1654,69	1655,69	1630-1690	(v) C = O [Secondary amides]
1593,69	1655.51	1655.51	1560.57	1655,69	1630-1690	(v) C = O Protonation of secondary amides
1421.76	1560.05	1560.05	1449.25	1560,29	1300-1567	(v) C–H
1379.00	1383.05	1383.05	1410.58	1324.05	1000-1500	(vs) C–H
1154.61	1261.83	1261.83	1154.44	1156, 67	1000-1350	(vs) C–O
1070.45	1072,72	1072,72	1068,34	1072,44	1000-1350	v(COC)
1029,70	1028,60	1028,60	1029,24	1027,44	900-1350	v(COC)
896.52	873.85	873.85	867.59	873.52	800-955	ώ β-1,4 glycosidic

Description v = stretching vibration
vs = symmetrical stretching vibration
vb = bending vibration
ώ = stretching vibration

Based on the results of the analysis on several differences in microwave power, the wave number at 240 watt power is 1655.51 cm $^{-1}$, 400 watt power is 1655.65 cm $^{-1}$, 560 watt power is 1654.69 cm-1 and 800 watt power is 1655.69 cm $^{-1}$ indicates the presence of an amide group which is the absorption band of the C=O bond group, while for the wave number of the 240 watt power it is 3449.18 cm -1 ˙the 400 watt power is 3449.49 cm $^{-1}$, the 560 watt power is 3462.85 cm $^{-1}$, power 800 watts 3449.66 cm $^{-1}$ is the vibration of the OH group and the NH $_{2\ group}$. As according to (Dompeipen EJ, 2017) the results of the functional group analysis of the FTIR spectrum showed the presence of functional groups such as OH, NH, CH, C=O, CO, CN, COC, β-1,4- glycosidic. The wave number 1655.35 cm $^{-1}$ indicates the presence of an amide group which is the absorption band of the C=O bond group,

while the wave number 3440.67 cm-1 is the vibration of the OH group and the $NH_{2\ group}$.

The spectrum of one of the chitosan standards shows the characteristics of the standard chitosan according to the distinctive characteristics, namely the presence of amide and hydroxyl groups. The wave number of 2925.43 cm^{-1} indicates the presence of a symmetrical stretching vibration of the CH bond. The wave number of 1560.66 cm^{-1} indicates the presence of bond stretching vibrations between CN in chitin. The wave number of 896.03 cm^{-1} shows one of the characteristics of chitosan, namely the presence of β-1,4-glycosidic bonds. In addition, the successful formation of chitosan is indicated by the reduced absorption intensity of the amide groups (wave number around 1600 cm^{-1}) of chitosan compared to the absorption of chitin as shown in Figure 1. The appearance of absorption of amide groups indicates that the deacetylation process is not optimal so that only a few amine groups are formed. . This is in line with the results of previous studies which also used BSF pupae as a base material (Sulistyawati et al., 2022).

Degree of Deacetylation

The degree of deacetylation (DD) was performed to determine the formation of chitosan from chitin. The degree of chitosan deacetylation in this study was 61-72%, so that it corresponds to the standard degree of chitosan deacetylation, Yuliani (2011) stated that the degree of chitin deacetylation of chitosan usually ranges from 60-100% depending on its use. The DD of chitosan indicates the quality level of chitosan, the higher the DD, the more reactive the resulting chitosan will be because the greater the number of amine groups resulting from the deacetylation process. The results of calculating the degree of deacetylation are shown in Table 3.

Table 3. Calculation results for the degree of deacetylation

Microwave Power (Watt)	Time (Minute)	dd (%)
240	5	61
400	5	63
560	5	66
800	5	72

Based on the research, it shows that the degree of deacetylation of chitosan obtained is an average of 240 watts 61% successively. 400 watts 63%, 560 watts 66% and 800 watts 72%. In infrared spectrometry (FTIR) research it is used to view and calculate a rough estimate of the degree of deacetylation of chitosan and its derivatives. Calculation of DD values with good precision and good accuracy, using simple, readily available, inexpensive and reliable equipment for routine analytical methods. Showing that the difference in power affects the results of significant deacetylation is supported by the theory that the extraction results are said to be chitin if it has a degree of deacetylation <50% and it is said to be chitosan if it has a degree of deacetylation > 50% (Dompeipen EJ, 2017) . The isolated chitosan is chitosan with a low degree of deacetylation group, this is because in the deacetylation process, NaOH is used with a concentration lower than 50% and heating for less than 1 hour so that the degree of deacetylation produced is not high. It is known that the factors that affect the degree of chitosan deacetylation include the concentration of NaOH in chitosan isolation, temperature and time of the chitosan deacetylation process. The DD of chitosan produced was lower than the results of previous studies which also used BSF-based ingredients, with a DD of more than 90% because the basic materials used went through the bleaching stage before the deacetylation process (Triunfo et al., 2022). However, the DD produced in this study was much higher when compared to the results of research by Sulistyawati et al (2022) who also used BSF as a base material.

The degree of deacetylation of chitosan can generally be determined using infrared spectroscopy or FTIR instruments. The minimum degree of chitosan deacetylation standards for the food industry is 70%, the cosmetic industry is 80% and the biomedical industry is 90%. Chitosan is more widely used in various industrial fields such as a fat stabilizer and flavor stabilizer in the food industry, an addictive ingredient for shampoos and cosmetics, an antibacterial agent, immobilizing bacteria, an absorbent for removing heavy metals and water purification (Profession et al., 2015) .

The use of microwaves provides many advantages, including: relatively short heating time and efficiency, energy and process costs, easy and precise process control, selective heating, better quality of the final product and can improve the quality of dry matter. The use of microwaves shows that the application of wave energy from microwaves makes the reaction faster, the steps easier, the reaction rate increases and the separation process improves (Profession et al., 2015) . The deacetylation process in this study is by utilizing microwaves to accelerate the deacetylation reaction. Microwave radiation can accelerate the reaction rate 10-100 times compared to the use of conventional heaters. The results showed that the high power and time used would increase the degree of deacetylation.

The degree of chitosan deacetylation increased with increasing microwave irradiation time. The time used is just under 10 minutes to see the effectiveness of microwave technology in microwaves compared to conventional methods in previous studies. As the microwave irradiation time increases, the resulting chitosan yield decreases . This is because the longer the reaction time, the more microwave radiation emitted so that the radiation absorbed by the reaction components is greater. The orientation of the reaction component towards a large electromagnetic field will result in a large conversion of kinetic energy into heat energy, so that the temperature will rise.

An increase in temperature will cause an increase in the energy of the gas molecules, so that the evaporation speed of the compounds in the reaction mixture will be greater. While the hot plate or other heating principle of heating is by heating the environment first then the heat spreads to the sample. This allows the process to be slow and inefficient. Microwaves work by passing microwave radiation to water, fat or sugar molecules, which are often found in foodstuffs.

These molecules will absorb the electromagnetic energy. This energy absorption process is referred to as dielectric heating. The molecules in food are electrically dipole electric polished meaning they have a negative charge on one side and a positive charge on the other. As a result, the presence of a changing electric field induced through microwaves on each side will rotate to align themselves with each other. The movement of these molecules will create heat as friction arises between one molecule and another, the resulting heat energy by this event that serves as a heating agent for foodstuffs in the microwave.

CONCLUSION

The formation of chitosan by providing microwave radiation energy can produce chitosan more effectively,

saving energy with a more efficient time. The degree of deacetylation increases as the applied power increases. The maximum chitosan produced was at 800 watts of microwave radiation power with a deacetylation degree of 72% .

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