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Enhancing the Cholesterol-Binding Potential of Modified Chitosan

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ABSTRACT

Chitosan is a natural polysaccharide derived from the deacetylation of chitin, a major component of the exoskeletons of crustaceans such as crabs, shrimp, lobsters, and crawfish. It can be chemically modified to enhance its hydrophobicity, thereby reducing its affinity for water and increasing its enzymatic activity. Due to its hydrophobic nature, modified chitosan is believed to bind effectively to neutral lipids such as cholesterol. While cholesterol plays a vital role in human metabolism, elevated levels can pose significant health risks. This study investigates key parameters that influence the cholesterol-lowering potential of hydrophobically modified chitosan. The parameters examined include chitosan concentration, pH levels, and types of cooking oil. Additionally, the effect of gamma radiation on the samples is evaluated. Cholesterol entrapment is quantified using the entrapped oil method, while the characteristics of the formed oil droplets are observed using an MB1-1600X microscope. Sample analyses are conducted using UV-visible spectroscopy to measure absorbance, Fourier transform infrared spectroscopy (FTIR) to identify chemical bonds, and field emission scanning electron microscopy (FESEM) to examine sample morphology. The research concludes by assessing the interaction between chitosan and cooking oil under varying conditions and evaluating the impact of gamma radiation on these interactions. The findings of this study contribute to the development of chitosan-based materials with enhanced cholesterol-binding capacity, offering promising applications in functional foods, dietary supplements, and potential therapeutic agents for managing high cholesterol levels.

1. Introduction

Chitosan is a linear polysaccharide composed of randomly distributed β-(1, 4)- linked D-glucosamine and N-acetyl-D-glucosamine. It is a compound that can be extracted from the hard exoskeleton of crustaceans, including crabs, shrimps, lobsters, and krill. The process of producing chitosan involves deacetylating chitin, removing the acetyl group (-COCH3) and leaving a fully deacetylated amino group (-NH_2). Chitosan can be modified to become hydrophobic, thereby enhancing its enzyme activity. Hydrophobicity is the physical property of a molecule known as a hydrophobe that is repelled from a mass of water. Hydrophobic means "water-fearing," which

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describes the repulsive interaction between water and nonpolar substances. Through hydrophobic interactions, chitosan is reported to bind neutral lipids, such as fats and cholesterol [1].

Recently, chitosan has been widely used in biomedical, agricultural, biotechnology, and many other fields. Moreover, its applications have been extensively explored in all aspects of science. One of the uses of chitosan that has been researched is its ability as a bio-based material to lower cholesterol levels in the body. Cholesterol is essential to the human body, as liver-produced cholesterol helps build cells and membranes [2]. But remember, the excess of this waxy chemical compound has negative impacts, especially on the heart. Cholesterol in foods, such as animal products (meat, poultry, eggs, and dairy), is called dietary cholesterol. The body's cholesterol level, called blood cholesterol, can be measured by a blood test. Two types of cholesterol are low-density lipoprotein (LDL or bad cholesterol) and high-density lipoprotein (HDL or good cholesterol) ([3]. To maintain a healthy body, it is essential to control the intake of foods rich in saturated fats, which can raise LDL levels. These saturated fats, which raise bad cholesterol, are primarily found in butter, peanut butter, meat, fried foods, cheese, vegetable oil, and more.

Some previous studies have suggested that chitosan can prevent the absorption of fat into the bloodstream by binding to cholesterol and other lipids in the gastrointestinal tract [4]. The chitosan will act by entrapping the fat droplets in the stomach, as both chitosan and fat are consumed together. This condition inhibits lipid absorption through the intestinal wall, so the entrapped fat is excreted with the faeces. Therefore, it reduces the cholesterol level in the blood and, at the same time, can help someone control their weight.

Some studies have examined the effects of chitosan on cholesterol levels in animals, including rats, rabbits, chickens, and mice [5]. The studies showed that chitosan could reduce cholesterol levels in animal serum without dietary intervention. In addition, the studies have been tested in humans, yielding the same results [6]. Chitosan's ability to lower cholesterol levels is highly beneficial, particularly for individuals with elevated cholesterol. While cholesterol is a fat vital for various metabolic functions, excessively high levels can pose serious health risks. It can increase the risk of coronary heart disease, for example, if blood cholesterol levels are very high. This has become a serious problem nowadays, especially among physicians, to find the best way to lower the blood cholesterol level and thus reduce the risk of getting coronary heart disease [7].

Numerous studies have highlighted the potential of chitosan, a naturally derived cationic polysaccharide, to lower cholesterol levels. Its positively charged molecular structure enables it to bind with negatively charged lipids, such as cholesterol and bile acids, in the digestive tract. This interaction may inhibit the absorption of dietary cholesterol and promote its excretion, thereby lowering overall cholesterol levels. It is said that chitosan prevents fat from being absorbed into the bloodstream, where cholesterol and other lipids bind to it. Moreover, it has been reported that chitosan can reduce the risk of cardiovascular diseases [8]. Also, chitosan is often used as a dietary supplement to prevent fat absorption and as a weight-control agent [9-10]. Also, chitosan can strongly bind oppositely charged molecules, such as fatty acids and bile acids, via its positively charged tertiary amino group. People who take chitosan as a daily supplement are said to experience lower blood cholesterol levels. Previous studies have shown that chitosan can entrap fat droplets in the stomach as they are consumed.

The effect of radiation on chitosan's cholesterol-lowering properties can be significant, as gamma radiation can alter its chemical structure and physical properties. When exposed to radiation, chitosan undergoes various modifications, such as changes in molecular weight, degree of deacetylation, and the introduction of new functional groups. These modifications can affect its solubility, hydrophobicity, and cholesterol-binding capacity. Also, gamma radiation can modify chitosan to enhance its cholesterol-lowering properties, but the exact effect depends on the specific

radiation conditions (e.g., dose and exposure time). Studying this effect is important for determining the optimal conditions for maximising chitosan's functional properties in cholesterol management.

While this speciality of chitosan's properties in lowering blood cholesterol levels has been demonstrated in many studies, its molecular mechanism of action remains incompletely understood. There is insufficient scientific knowledge about the mechanism by which the modified chitosan works [7,11]. Furthermore, the interaction between chitosan and lipids, such as cooking oil, often leads to heterogeneous mixing, hindering uniform dissolution. This incompatibility makes it challenging to form a stable emulsion, as chitosan's hydrophilic nature contrasts with the hydrophobic nature of fats, leading to phase separation and reduced emulsion stability. To address these problems, this research aims to determine chitosan's ability to lower cholesterol levels. The study hypothesises that chitosan exhibits unique bio-based properties that lower blood cholesterol. The different parameters, including different chitosan concentrations, pH values, and types of cooking oil used, made it distinct from previous studies [12]. This gap in understanding limits the ability to optimise chitosan's use in therapeutic applications and hampers the development of more efficient cholesterol management formulations. Further research is needed to explore these mechanisms in detail and better harness chitosan's potential to improve cardiovascular health. Overall, this study aims to address this by exploring how chitosan modifications can enhance its compatibility with fats, facilitating better emulsification and more efficient cholesterol binding. By focusing on the parameters that influence chitosan's interaction with lipids, this research seeks to bridge the gap between its cholesterol-lowering properties and its ability to form stable emulsions, ultimately improving its practical applications in both dietary and pharmaceutical formulations.

Additionally, the research seeks to assess the interaction between chitosan and cooking oil under different conditions, using various analytical techniques to understand how these factors affect chitosan's performance in lowering cholesterol. Optimisation is crucial in this study because varying chitosan concentrations and pH levels can significantly affect cholesterol binding efficiency and the formation of stable emulsions. The concentration of chitosan affects its ability to interact with cholesterol: too low a concentration may not bind enough cholesterol. At the same time, too high a concentration may cause aggregation or reduce the accessibility of binding sites. Similarly, pH levels influence chitosan's solubility, charge, and overall structure, which, in turn, can affect its interactions with lipids and cholesterol [13].

Specifically, the study addresses the limited understanding of the molecular mechanism of cholesterol binding by modified chitosan, as well as the lack of systematic evaluation of key influencing parameters (concentration, pH, and oil type) under controlled conditions. Additionally, poor emulsification due to the hydrophilic–hydrophobic incompatibility between chitosan and lipids is identified as a critical limitation in prior studies. This work aims to bridge these gaps by integrating physicochemical characterisation (UV-Vis, FTIR, FESEM) with functional analysis (oil entrapment), thereby providing a more comprehensive understanding of chitosan–lipid interactions.

2. Methodology

2.1 Materials

Chitosan powder, three different types of vegetable cooking oils (palm oil, corn oil and sunflower oil), 0.1 M Hydrochloric acid (HCl), 0.1 M Sodium hydroxide (NaOH), ethyl ether and distilled water. All materials were purchased from Fluka, except for the oils, which were sourced from local shops.

2.2 Stock Solution Preparation

Stock solutions of chitosan at different molar concentrations (0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M) were prepared. The required amount of chitosan powder, based on the target molarity, was weighed and dissolved in a beaker containing a specific volume of distilled water. The resulting solution was then transferred to a 1-L volumetric flask.

2.3 Preparation of Sample

For the concentration parameter, samples were prepared by adding 10 ml of palm oil to a 100 ml beaker containing a 0.1 M chitosan stock solution. The mixture of palm oil and chitosan was stirred at a constant speed and maintained at 37ºC using a hot plate and magnetic stirrer. Stirring continued for approximately 30 minutes until an emulsion formed. This process was repeated at different chitosan stock solution molarities, yielding five distinct samples for this parameter.

The samples were prepared at 12 different pH values: 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0. First, 4g of chitosan powder was added to 50 ml of distilled water in a beaker, and the mixture was stirred with a stirring rod. The pH of the solution was measured with a pH meter, and the initial pH of the chitosan solution was estimated at 8.42. To adjust the pH, either 0.1 M HCl or 0.1 M NaOH was added until the desired pH value was reached. HCl was used to achieve an acidic pH, while NaOH was used to create an alkaline medium. The solution was then stirred for 30 minutes at 37ºC at a constant speed using a hot plate and a magnetic stirrer. For the different cooking oil parameters, three types of vegetable oil: palm oil, sunflower oil, and corn oil were used. In each case, 10 ml of the respective oil was added to a beaker containing 100 ml of 0.5 M chitosan stock solution. The mixture was stirred for 30 minutes at 37 °C on a hot plate with constant stirring. The same procedure was followed for the samples with corn oil and sunflower oil.

2.4 Quantification of Entrapped Oil

The entrapped and absorbed oil was extracted using a solvent treatment method and quantified gravimetrically [14]. Initially, a filter was used to separate the flocculent formed through filtration. The flocculent was then washed with ethyl ether to remove the absorbed oil, followed by stirring with 10.0 ml of ethyl ether three times to extract the entrapped oil. After solvent removal, the amount of entrapped oil was determined gravimetrically.

2.5 Sample Characterization

Samples were characterised using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and field-emission scanning electron microscopy (FESEM).

3. Results and Discussion

3.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is a proper analytical technique for identifying the functional groups in molecules. Since certain functional groups in organic molecules absorb light at characteristic wavelengths in the Ultraviolet-visible region, this research applies the technique to identify the presence of these groups in each sample. This will be a supportive analysis of structural information obtained from other spectroscopic methods, such as FTIR.

Also, from the UV-Vis spectroscopy test, an absorbance-versus-wavelength plot could be obtained, showing differences in absorbance values between samples with different parameters. The light absorbance values depend on the number of molecules in the samples. The greater the number of molecules that absorb light of a given wavelength, the greater the absorbance values obtained. In this research study, the absorbance-wavelength data for each sample parameter, such as different concentrations and types of cooking oil, are being observed and discussed. Different light-absorbing values for all these samples indicate that the number of molecules in each sample differs. Also, other factors, such as adding HCl and NaOH, varying amounts of chitosan powder, and using various cooking oils, might affect the results.

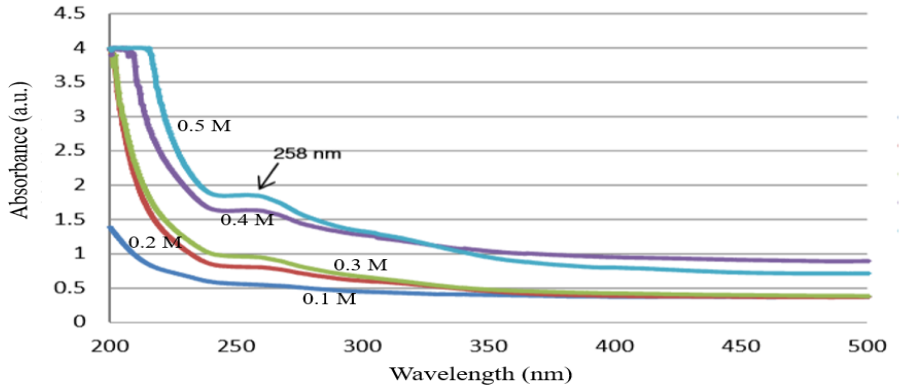

Fig. 1. The graph of absorbance against wavelength for different concentration parameters

Figure 1 shows the graph of absorbance against the wavelength of different concentrations of chitosan powder, which are 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M. This graph pattern indicates that as the concentration increases, the light absorbance also increases. This might be due to the number of molecules in each sample, which varies with molarity. There is a broad peak over the wavelength range 250-300 nm, with a maximum absorbance at 258 nm. The maximum light absorption values for each concentration occur within the same wavelength range. However, the absorbance values depended on the concentration of chitosan powder.

The absorbance values for 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M obtained from the UV-Vis spectroscopy test are 0.550, 0.806, 0.955, 1.633 and 1.848, respectively. This clearly shows that the trend line of absorbance versus wavelength increases with increasing concentration. The graph shows that 0.5 M is placed at the highest line, followed by 0.4 M, 0.3 M, 0.2 M and 0.1 M. The higher absorbance at 0.5 M is due to more molecules, resulting in greater light absorption. Since chitosan contains two groups, N-acetylglucosamine (GlcNAc) and glucosamine (GlcN), it cannot be completely deacetylated. The UV-Vis spectra obtained over the wavelength range 250-300 nm reveal the presence of pure chitosan. This was proved in 1996 by [15].

Meanwhile, no peak is observed in the wavelength range from 300 nm to 500 nm. This shows that the molecules absorb only a small amount of light at that wavelength. Thus, no functional group is detected at that wavelength. The findings show that the molecules in the samples absorb a beam of ultraviolet light spanning 200-400 nm.

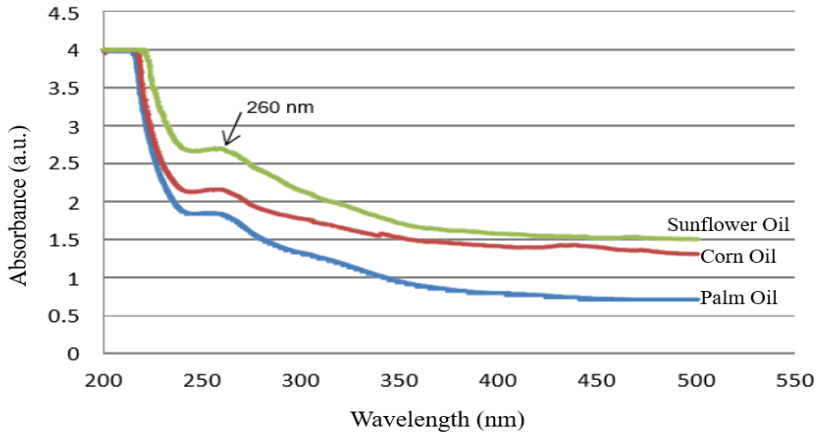

Fig. 2. The graph of absorbance against wavelength of different types of cooking oil parameters

The UV-Vis absorbance versus wavelength spectra for the different cooking oil parameters are shown in Figure 2. Sunflower oil shows the highest absorbance of those three vegetable cooking oils, followed by corn and palm oil. These results might be due to their contents affecting light absorption at specified wavelengths. From this line graph, it can be seen that all the cooking oils analysed show strong absorption in the ultraviolet range, from 200 nm to 400 nm. Additionally, a peak was observed for each cooking oil sample between 250 nm and 300 nm. This absorption peak at this wavelength indicates the presence of a functional group.

Referring to the UV-Vis absorption of the common functional group, a carboxyl group (C=O) is present in that wavelength range. Although the peaks of each cooking oil fall within the same wavelength range, their absorbance values differ because of their molecular compositions. The raw UV-Vis spectroscopy data show that sunflower oil has an absorbance of about 2.699, followed by corn and palm oil, with absorbances of 2.159 and 1.839, respectively. The differences in absorbance values are due to varying amounts of fat, including saturated, monounsaturated, and unsaturated fats. It is claimed that saturated fat can increase bad cholesterol, also known as low-density lipoprotein (LDL) [16]. This saturated fat has more palm oil than corn and sunflower oil. Because of this high amount of saturated fat, the amount of light the molecules could absorb decreases. It can be said that the chemical composition of cooking oil affects the absorbance values.

3.2 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is used to obtain an infrared spectrum of the absorption, emission, or scattering of a solid, liquid, or gas sample. It is also used to measure how well a sample absorbs light at a specific wavelength and to identify the functional group present in a molecule. FTIR has become one of the most important techniques available to scientists working on chitosan.

FTIR spectroscopy commonly records the energy of the electromagnetic radiation transmitted through a sample as a function of the wavenumber or frequency. Furthermore, this technique allows the qualitative identification of certain bond types present in the sample, as indicated by the energy of each peak in the absorption spectrum, which corresponds to the molecular vibration frequency. In this research, FTIR is used to obtain and analyse the spectra of the selected samples, covering all

three parameters: chitosan powder concentration, pH, and type of cooking oil. In addition, the effect of gamma radiation on the samples was discussed using FTIR analysis.

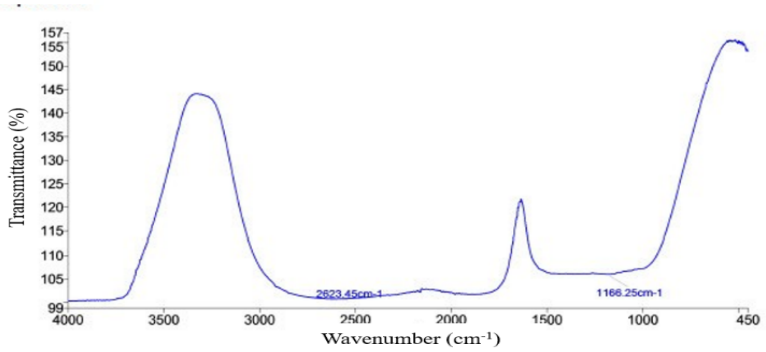

Fig. 3. Analysis of the FTIR spectra of pure chitosan

Pure chitosan, not a chitosan sample, without adding other solvents or substances. The analyses were divided into regions (1000 – 1500 and 2000 – 3000) cm^{-1}. The (1000–1500) cm^{-1} region indicates that carbonyl compounds (C=O) are present in pure chitosan. The peak at 1166.25 cm-1 shows only the (C-O-C) vibration. Meanwhile, the region of (2000 – 3000) cm^{-1} shows significant peaks for analysis. This is where the primary and secondary amines (N-H stretch), carboxyl group (C=O) and the strongly hydrogen-bonded O-H stretch are situated. The band at 2623.45 cm^{-1} indicates the presence of a carboxyl group, as this peak spans the entire region. This region, containing several peaks, is quite similar to the previous study of FTIR spectra of pure chitosan, which showed a peak at 1650 cm^{-1}, attributed to the acetylated amino group. This was due to the C=O stretching vibrations of the amide, and the band at 1590 cm^{-1} was also assigned to NH_2 bending vibrations [17].

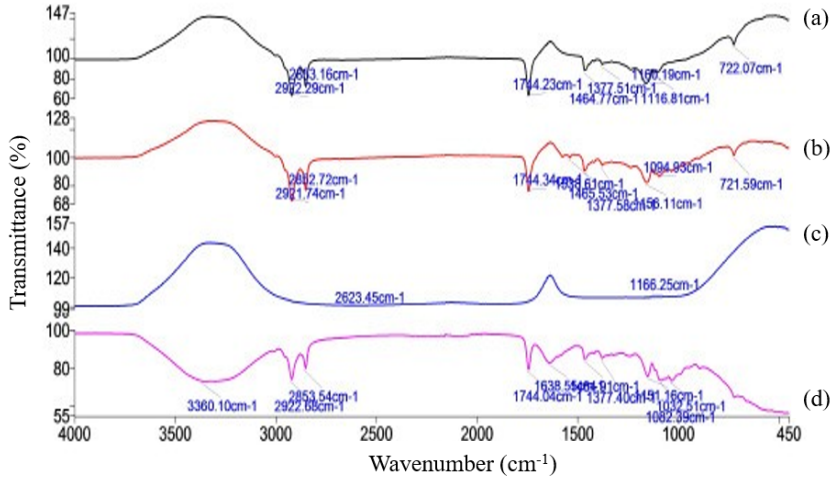

Fig. 4. FTIR spectra of (a) 0.1M of chitosan and palm oil; (b) 0.5 M of chitosan and palm oil; (c) pure chitosan, and (d) 0.5 M of irradiated chitosan with palm oil

The spectra of 2 different concentrations of chitosan and palm oil (0.1 M and 0.5 M) and the spectra of irradiated 0.5 M are compared in the same graph, as shown in Figure 4. From the figure, it can be seen that the peak differences between the four samples, including the pure chitosan, are significant. The differences indicate that the samples contain distinct functional groups at specific

wavenumbers due to the addition of cooking oil to pure chitosan and to gamma radiation. The line graphs for 0.1 M and 0.5 M chitosan and palm oil are quite similar, with only specific peak values that shift slightly in opposite directions distinguishing them. Moreover, there are significant peaks in both samples, indicating the presence of important functional groups, including the carboxyl group (C=O), amino group (N-H), hydroxyl group (-OH), and esters (C=O). Meanwhile, the irradiated 0.5 M sample shows significant changes in the hydroxyl and amino group regions (3200 – 3500 cm^{-1}). Besides that, the sample absorbance rapidly decreases after undergoing gamma radiation (γ-ray). This shows that γ-rays applied to the samples cause systematic changes in the FTIR spectra of chitosan with palm oil.

Significant peaks appear at 0.5 M of chitosan in the palm oil sample. Three regions are analysed, all of which (700 – 750, 1000 – 2000, and 2500 – 3000 cm^{-1}) exhibit peaks, indicating the presence of certain functional groups. In the area of 700 – 750 cm^{-1}, a peak of 721.59 cm^{-1} is observed. This region shows an aromatic C–H bond type in the samples, involving monosubstituted benzene. This type of bond might be due to the palm oil molecules.

Other than that, about six significant peaks have been observed between the 1000 – 2000 cm^{-1} range. A very sharp peak of 1744.34 cm^{-1} appeared, and this absorption peak shows the existence of the carboxyl group, which is due to C=O stretching vibrations. There were also peaks of 1094.93cm^{-1}, 1156.11 cm^{-1}, 1377.58 cm^{-1}, 1465.53 cm^{-1} and 1538.61 cm^{-1}, which assigned the presence of functional group esters (C – O stretch) and also the amino group with the stretching of N – H vibrations. The peak absorbance spectra of this sample were also observed at the peaks of 2852.72 cm^{-1} and 2921.74 cm^{-1}. These two peaks are in the 2500 – 3000 cm^{-1} region, where any peak that appears here indicates the vigorous intensity of the hydrogen-bonded O-H stretch, which is in a group of carboxyl. Gamma radiation on the sample caused changes in its FTIR spectrum. The main changes were observed in the hydroxyl and primary and secondary amino group regions (2800–3500 cm^{-1}). Here, three peaks have been observed, indicating the presence of (N-H) and (-OH) stretching. This agrees with a previous study that reported the presence of these functional groups in the spectra of UV-irradiated chitosan [18].

Significant peaks between the (1400- 2000) cm^{-1} region could be observed. The peak at 1744.04 cm^{-1} in the spectra indicates the presence of a carboxyl group in the sample, which corresponds to the C=O stretching vibration. Moreover, on the spectrum bands from the amide group, stretching band C=O at 1638.55 cm^{-1} and the bending of CH_3 (1377.40 cm^{-1}) and NH bending vibrations from the amino group were recorded. Also, the sample's FTIR spectra show some other significant absorption peaks at the (1000 – 1200) cm^{-1} region. There are peaks of asymmetric vibrations C-O-C from the glycosidic bonds (1151.16 cm^{-1}) and the skeletal stretching C=O at (1082.39 and 1032.51) $cm^{-1,}$ which belong to the characteristic bands of polysaccharides [19].

Figure 5 shows the differences in the absorption peaks of samples with different pH values (pH 2, pH 6, and pH 8) and the effects of gamma radiation on the FTIR spectra of the pH 8 sample at a specific wave number. The same pattern of line graphs was observed for samples with different pH, and the peaks were also observed in the same region. The only thing that distinguishes them is that the peaks are slightly shifted, which might be due to differences in sample composition caused by different pH values. Meanwhile, there is no significant difference in the FTIR spectra between the samples before and after gamma-ray irradiation. Both samples show the same functional groups, but what makes them different is the percentage of transmission. It shows that an irradiated sample has a lower transmission percentage than a non-irradiated one.

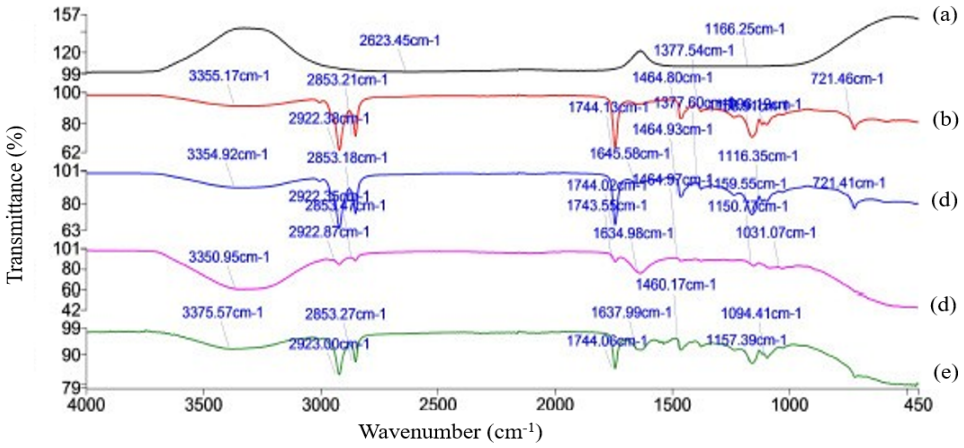

Fig. 5. FTIR spectra of (a) pure chitosan; (b) pH 8 of irradiated chitosan and palm oil; (c) pH 8 of chitosan and palm oil; (d) pH 6 of chitosan and palm oil, and (e) pH 2 of chitosan and palm oil

The FTIR spectrum of pH 8 chitosan with palm oil can be divided into three regions (700–750, 1000–2000, and 2500–3500 cm^{-1}). In the first region, the absorbance peak of 721.41 cm^{-1} is observed. This significant peak indicates the presence of an aromatic C–H bond. Additionally, some peaks appear in the second region, with the sharpest peak at 1744.02 cm^{-1}, assigned to a carboxyl group stretching vibration. Another band at 1645.58 cm^{-1} corresponds to an acetylated amino group due to the C=O stretch of the primary amide; meanwhile, the peak at 1159.55 cm^{-1} is assigned to C-O-C vibrations in the sample. The third region shows peaks at 2853.18 and 2922.35 cm^{-1}. These two significant and sharp peaks indicate the C-H asymmetrical stretching of the chitosan. The broad peak at 3354.92 cm^{-1} corresponds to the NH_2 symmetric stretching mode of amines. As in the FTIR spectra of the non-irradiated samples, pH 8 chitosan with palm oil shows three regions (700 – 750, 1000 – 2000, and 2500 – 3500 cm^{-1}) that contain significant peaks at specific wavenumbers. The only difference between the irradiated and non-irradiated samples is that most of the peaks of the irradiated sample are lower than those of the non-irradiated sample. This might be due to bond rupture in the sample caused by high gamma radiation.

In the region of 700 – 750 cm^{-1}, a peak of 721.46 cm^{-1} appeared. This region indicates an aromatic C – H bond type involving monosubstituted benzene in the sample. Meanwhile, in the area of 1000 – 2000 cm^{-1}, about five peaks were observed. A very sharp peak at 1744.13 cm^{-1} appeared, indicating the presence of a carboxyl group due to C=O stretching vibrations. There was also the peak of 1096.19 cm^{-1}, 1158.91 cm^{-1}, 1377.54 cm^{-1} and 1465.80 cm^{-1}, which assigned the presence of functional group esters (C – O stretch) and also the amino group (N – H stretch).

Figure 6(a) shows the FTIR spectra of corn oil with chitosan. Instead of palm oil and sunflower oil, corn oil is used across different cooking methods to observe chitosan's interaction with other oils. From this FTIR test, it can be seen that slightly different absorption peaks appear among the three cooking oils. The FTIR analysis clearly shows an ester group with (C- O) stretch at 1032.23 and 1082.37 cm^{-1} peaks, which refers to the composition of cooking oil. The next peak appears at 1151.10 cm^{-1} and is assigned to the antisymmetric stretching of the C-O-C bridge. At the same time, the carboxyl functional group is detected at 1743.97 cm^{-1}. Besides that, in the band between 2850 – 2960cm^{-1}, an alkyl group with the stretching of C-H vibrations is observed. The peaks in this band for this sample are 2854.24 and 2923.47 cm^{-1}. The sample also indicates the presence of an amino group at an absorption peak at 3361.04 cm^{-1}.

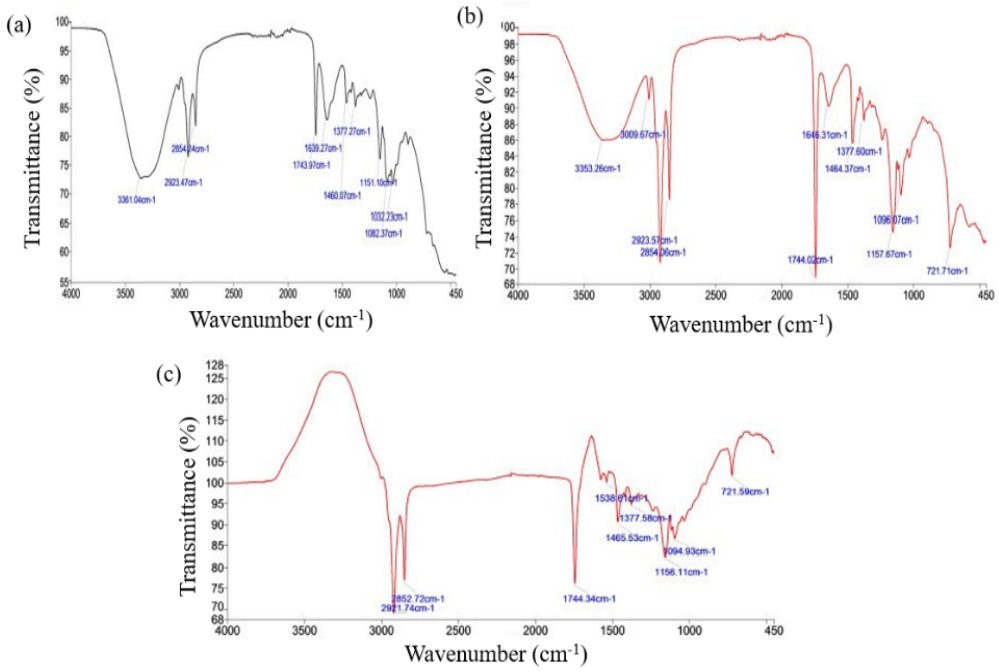

Fig. 6. FTIR spectra of (a) corn oil with chitosan, (b) sunflower oil with chitosan, (c) palm oil with chitosan

From Figure 6(b), it can be seen that two peaks are very sharp, 2923.57 and 1744.02 cm^{-1}, which indicates the carboxyl group with the stretching of hydrogen-bonded O-H and C=O stretch, respectively. Furthermore, there is also a broad peak at 3353.26 cm^{-1}. This peak refers to the NH_2 stretching of the amine functional group. While from the region of 1000 – 1200 cm^{-1}, there are peaks of 1096.07 and 1157.67 cm^{-1} that show the presence of esters with the stretching vibration of C-O. Also, another functional group of amides was recorded at 1646.31 cm^{-1} with stretching band C=O, bending of CH_3 (1377.60 cm^{-1}) and N-H stretching vibrations from the amino group. Figure 6(c) shows several significant peaks at specific wavelengths. Three regions are to be analysed, each with peaks (700–750, 1000–2000, and 2500–3000 cm−1), indicating the presence of certain functional groups in these regions.

In the 700–750 cm−1 region, there is a peak at 721.59 cm^{-1}. This region indicates an aromatic C – H bond type involving monosubstituted benzene in the samples. This type of bond might be due to the palm oil molecules. Besides that, within the range of 1000–2000 cm−1, a very sharp peak at 1744.34 cm−1 appears, indicating the presence of a carboxyl group due to C=O stretching vibrations. There were also peaks of 1094.93cm^{-1}, 1156.11 cm^{-1}, 1377.58 cm^{-1}, 1465.53 cm^{-1} and 1538.61 cm^{-1}, which assigned the presence of functional group esters (C – O stretch) and also the amino group with the stretching of N – H vibrations. This sample's peak absorbance spectra also showed peaks at 2852.73 cm^{-1} and 2921.74 cm^{-1}. These two peaks are in the 2500 – 3000 cm^{-1} region, where any peak that appears here indicates the vigorous intensity of the hydrogen-bonded O-H stretch, which refers to the carboxyl group.

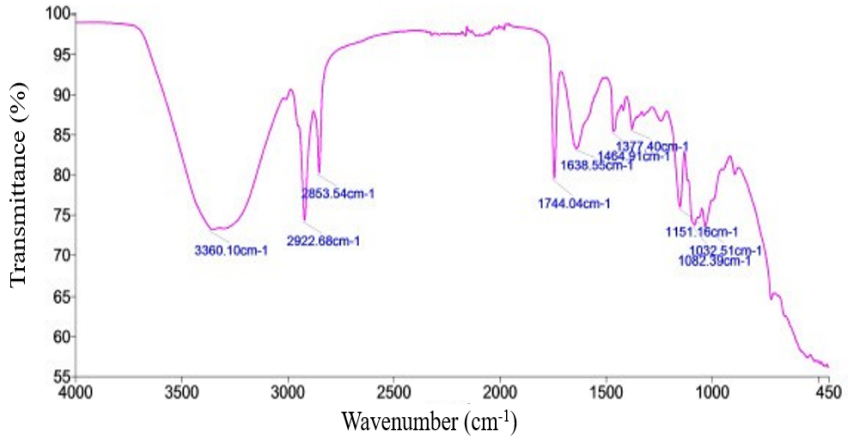

Fig. 7. FTIR spectra of irradiated palm oil with chitosan

Due to the irradiation, there could be significant changes in hydroxyl and both primary and secondary amino group regions (2800 – 3500) cm^{-1} (figure 7). Three peaks have been observed, indicating the presence of (N-H) and (-OH) stretching. This is in good agreement with a previous study that reported the presence of these functional groups in the spectra of chitosan under UV irradiation [18]. Significant peaks could also be observed in the region of (1400- 2000) cm^{-1}. The peak at 1744.04 cm^{-1} in the spectra indicates the presence of a carboxyl group in the sample, which corresponds to the C=O stretching vibration. Moreover, on the spectrum bands from the amide group, stretching band C=O at 1638.55 cm^{-1} and the bending of CH_3 (1377.40 cm^{-1}) and NH bending vibrations from the amino group were obtained [19].

These FTIR spectra results indicate several other significant peaks at the (1000 – 1200) cm^{-1} region. There are peaks of asymmetric vibrations C-O-C from the glycosidic bonds (1151.16 cm^{-1}) and the skeletal stretching C=O at (1082.39 and 1032.51) $cm^{-1,}$ belonging to the polysaccharide bands. The entire graphical data clearly shows that the absorbance of the irradiated samples is lower than that of the non-irradiated ones. The previous study supports this. Gamma irradiation alters several bands in FTIR analysis, significantly alters physicochemical properties, and simultaneously induces degradation and crosslinking [20].

3.3 Field emission scanning electron microscope (FESEM)

A field-emission scanning electron microscope is used to study and analyse particle morphology. The energy-dispersive X-ray spectroscopy (EDS) system enables the elemental composition of particles or samples to be easily determined. This study uses FESEM to examine morphological differences in selected chitosan and oil samples before and after gamma irradiation. Unlike previous studies, which mostly used a scanning electron microscope (SEM) to examine sample morphology, this research used FESEM. A JEOL JSM-7600F determined the morphology of chitosan and chitosan oil. Each sample was coated with gold (Au) to enhance conductivity before scanning.

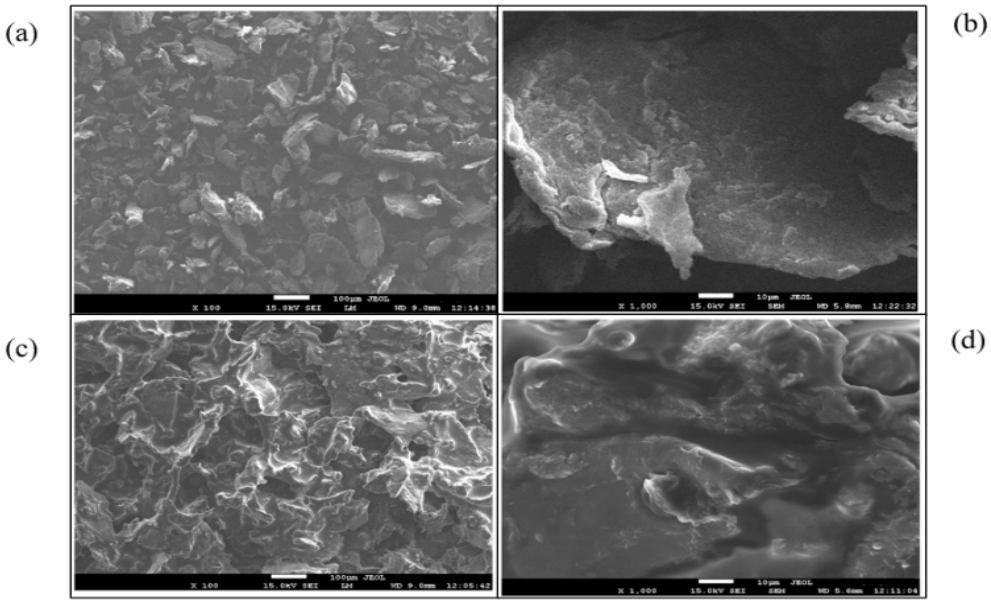

Fig. 8. The FESEM image of (a) chitosan at magnification of x100, (b) chitosan at magnification x1000, (c) chitosan-oil at magnification of x100, (d) chitosan-oil at magnification of x1000

Figures 8(a) and 8(b) show FESEM images of chitosan at magnifications of x100 and x1000, respectively. The photos, especially the one magnified x1000, show that the chitosan is agglomerated and forming flake particles. This result agrees well with the previous study by [20]. The sample's surface morphology is not smooth, as it was prepared by placing chitosan powder on a glass slide and affixing it with adhesive tape. Moreover, the rough surface observed might be due to the unflattened distribution of the chitosan powder during the sample preparation.

The morphology of chitosan oil is shown in Figures 8(c) and 8(d). The morphology shows an unsmooth surface, with a network of non-uniform particles. The chitosan particles had already accumulated with the oil's fat. According to the pioneer's studies, when fat and chitosan are mixed, they form a flocculus shape. Chitosan is said to entrap fat, making it well-known as a fat binder [21]. Also, Figure 8(d) shows the oily surface of the particles, indicating the presence of cooking oil in the chitosan powder.

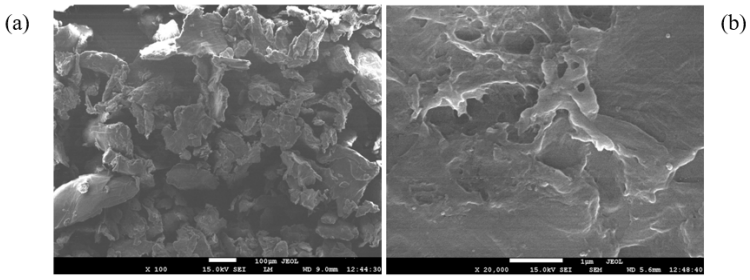

Fig. 9. (a) The FESEM image of irradiated chitosan with a magnification of x100 at 30 mCi of Cesium-137 (b) The FESEM image of irradiated chitosan with a magnification of x20,000 at 30 mCi of Cesium-137

Figure 9(a) shows the effect on the morphology of gamma-irradiated chitosan at a magnification of x100, while Figure 9(b) shows the morphology at a magnification of x20000. The surface morphology differs from that of the non-irradiated sample. The surface forms an irregular

arrangement of molecules. That is because, after the irradiation of this Cesium-137, the sample chains are observed to be ruptured. The chitosan structure appears scattered and blistered [20].

Meanwhile, by the EDS system of the FESEM, elements in the chitosan sample were detected in the percentage of weight (weight) and percentage of atomic (atomic %). It was found that the chitosan sample had about 59.58 weight% of carbon and 66.25 nuclear% of carbon, and 40.42 weight% of oxygen and 33.75 atomic% of oxygen. The same elements were detected in the oil sample, but at different percentages. It was about 69.68 weight % with 75.38 atomic % of carbon and 30.32 with 24.62 atomic % of oxygen. Detecting these two elements enhances the carbon and oxygen content of chitosan and oil molecules. For an irradiated chitosan sample, about 52.3% of the carbon was detected, with an atomic% of 59.36%. For the oxygen element, 47.70 weight % and 40.64 atomic % had been observed in the sample.

3.4 Microscopic View Study

Samples of each emulsion of cooking oil and chitosan were observed with an MB1-1600X microscope to examine the image characteristics. Figure 10 shows different microscopic views of the samples at pH 2, 6, and 8. All three samples show that chitosan and palm oil interact differently at various pH levels. At pH 2, a few emulsified oil drops were observed. This acidic condition confirmed that chitosan emulsified palm oil in the gastric environment, as observed in the body when they were eaten together.

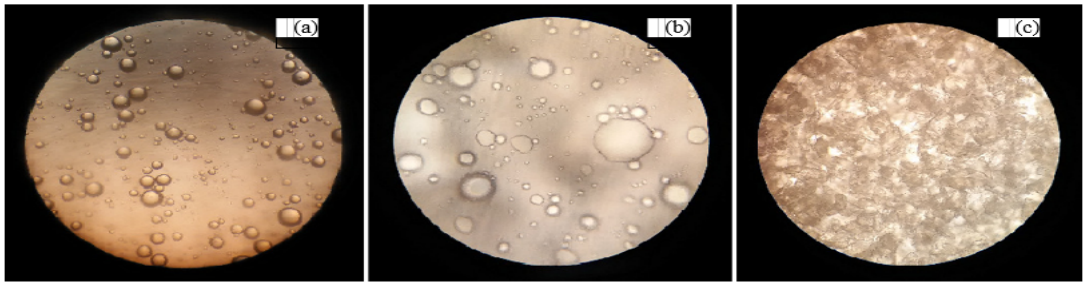

Fig. 10. Microscopic views of the emulsion of palm oil with chitosan at (a) pH 2, (b) pH 6 and (c) pH 8

At pH 6, the view image shows the improvement of chitosan emulsion-forming capacity. This pH condition is equivalent to the duodenal environment in the body. It can be observed that the samples produced more emulsified oil droplets. These findings are supported by a previous study by [22], which showed that the emulsion-forming capacity of chitosan was improved at a pH of about 5.8. Meanwhile, at pH 8, it shows isolated floccules forming, as at this pH range (7.0–8.5), the oil is claimed to be entrapped by the chitosan, resulting in floccules. This observation suggested that chitosan precipitated in the duodenal environment, further strengthening the claim that chitosan is suitable for entrapping the oil.

Figure 11 shows microscopic images of the samples at the highest (0.5 M) and lowest (0.1 M) chitosan concentrations. A sample at 0.1 M shows the formation of an agglutination structure. This indicates that when chitosan and palm oil are mixed, the chitosan will entrap the palm oil, resulting in clumps. The same view can be seen for the 0.5 M chitosan with palm oil sample, but it is more complex than for the 0.1 M sample. The image shows more clump shapes. This is due to the number of chitosan molecules in a 0.5 M solution. As the number of chitosan molecules increases, the formation of agglutination between chitosan and palm oil increases.

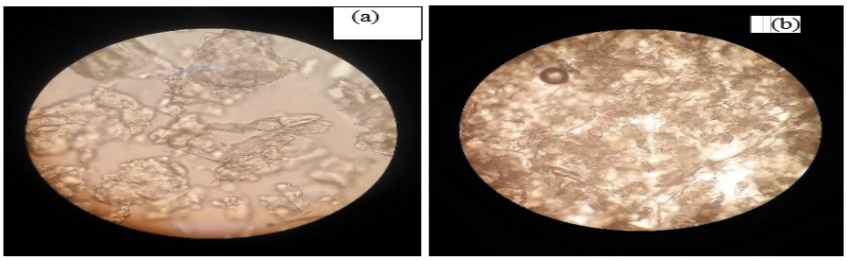

Fig. 11. Microscopic views of the emulsion of palm oil with chitosan of (a) 0.1 M and (b) 0.5M

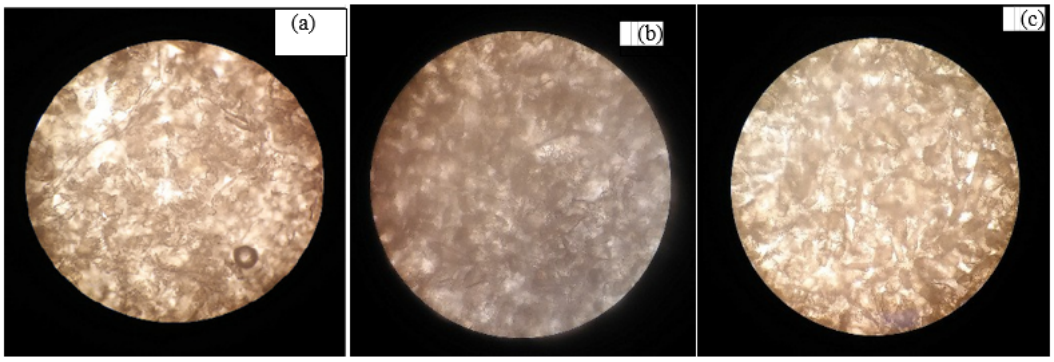

Fig. 12. Microscopic view of chitosan with (a) palm oil, (b) corn oil, and (c) sunflower oil

There are a few differences in the microscopic view of chitosan compared with the three types of cooking oil, as shown in Figure 1. All three images show the agglomeration structure between chitosan and cooking oils. Theoretically, palm oil will produce the highest percentage of entrapped oil by chitosan, followed by corn and sunflower. This is due to its high content of total fats, including saturated, monounsaturated, and unsaturated fats. These fat contents affect the interactions between the chitosan and cooking.

3.5 Quantification of entrapped oil: Chitosan can entrap and bind to cooking oil when mixed

The entrapped oil will then form a flocculus. Each parameter in this research study has a distinct analysis of entrapped oil. The entrapped oil in each sample's chitosan can be determined and analysed using the entrapped oil quantification method. The trapped oil refers to the amount of oil entrapped by the chitosan. When chitosan and oil or fats are taken together, chitosan prevents the absorption of the fat through the intestinal wall. Instead, it will be excreted by the body, as had been proved by [22]. Because of its role as a fat binder, it is also claimed to lower blood cholesterol levels.

Table 1 shows that the highest percentage of trapped oil appears at pH 8, neglecting the irradiated one. This is due to the previous study's findings that claimed the fat-binding capacity of chitosan increases by irradiation [20]. It shows that chitosan's fat-binding function was best at pH 8. This is because it was found that, in the duodenal environment (pH 7.0–8.5), the flocculus of the entrapped oil in the chitosan would form. It is then followed by pH 6, pH 4, and pH 2 in decreasing sequence. At pH 10 and pH 12, the percentages of trapped oil were 11.1% and 6.67%, respectively. Although there is no reference to the interaction between chitosan and fat at these pH values, pH 8 showed the highest percentage. So, it can be concluded that chitosan works best as a fat binder at pH 8.

Table 1
Percentage of trapped oil by different pH values

pH values	Oil (g)	Entrapped oil (g)	Trapped oil (%)
pH 2	4.5	0.3	6.67
pH 4	4.5	0.4	8.89
pH 6	4.5	1.7	37.8
pH 8	4.5	1.8	40.0
pH 10	4.5	0.5	11.1
pH 12	4.5	0.3	6.67
Irradiated pH 8	4.5	1.9	42.2

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Table 2
Percentage of trapped oil by different concentrations of chitosan

Concentration of chitosan	Oil (g)	Entrapped oil (g)	Trapped oil (%)
0.1 M	4.5	0.7	15.6
0.2 M	4.5	1.4	31.1
0.3 M	4.5	1.5	33.3
0.4 M	4.5	1.6	35.6
0.5 M	4.5	1.8	40.0
Irradiated 0.5 M	4.5	2.1	46.7

Table 2 shows the percentage of trapped oil for samples with different concentrations. It was observed that an irradiated 0.5 M sample had the highest rate compared to the non-irradiated samples. This is consistent with the study by [20], which proposed that the fat-binding capability (FBC) of irradiated chitosan at 100 kGy was 1573.35%, compared to 574.03% for the non-irradiated sample.

Besides that, from the table, it could also be seen that 0.5 M has a 40% trapped oil percentage, which shows the highest rate among the non-irradiated samples, followed by 0.4 M (35.6%), 0.3 M (33.3%), 0. 2 M (31.1%) and 0.1 M (15.6%). This result is due to the greater number of chitosan molecules in the sample at 0.5 M concentration. Here, it can be noted that increasing the chitosan molarity in the solution increases the amount of entrapped oil in the chitosan, thereby increasing its oil-trapping efficiency.

Table 3
Percentage of trapped oil by different types of cooking oil

Type of cooking oil	Oil (g)	Entrapped oil (g)	Trapped oil (%)
Palm oil	4.5	1.3	28.9
Corn oil	4.5	0.7	15.6
Sunflower oil	4.5	0.6	13.3
Irradiated palm oil	4.5	1.5	33.3

Table 3 shows the percentage of trapped oil by different types of cooking oil, including irradiated and non-irradiated palm oil, corn oil, and sunflower oil. It was found that palm oil has the highest percentage of trapped oil, at 28.9%, compared with corn oil at 15.6% and sunflower oil at 13.3%. This difference in the percentage of trapped oil might be due to differences in their contents. Since palm oil has the highest fat content, it increases the interaction between chitosan molecules and oil, thereby increasing the amount of oil trapped by chitosan.

4. Conclusion

The results demonstrate that the maximum oil entrapment was achieved at pH 8 (40.0%) and at a chitosan concentration of 0.5 M (40.0%), with a further increase to 46.7% following gamma irradiation, indicating enhanced binding performance under these conditions. In terms of oil type, palm oil exhibited the highest binding capacity (28.9%) compared to corn and sunflower oils, suggesting a stronger interaction between chitosan and oils with higher fat content. Additionally, FTIR analysis confirmed the presence of key functional groups, including C=O, N–H, and O–H, which are believed to facilitate interactions between chitosan and lipid molecules. Additionally, FESEM images reveal agglomeration and flocculation structures, confirming the presence of physical entrapment within the chitosan matrix. The higher binding capacity observed for palm oil is discussed more cautiously as correlated with its higher saturated fat content, supported by differences in UV-Vis absorbance and the existing literature, rather than as a definitive mechanistic conclusion.

Key limitations of the study include the use of oil entrapment as an indirect proxy for cholesterol binding, which may not fully replicate physiological conditions, and the lack of in vitro digestion models or in vivo validation. In addition, the results may be influenced by potential confounding factors such as emulsion stability and phase separation. There is also limited discussion on the practical feasibility of gamma irradiation for real-world applications. Acknowledging these limitations enhances the transparency and overall scientific rigour of the study.

Modified chitosan shows potential for use in functional foods, particularly as a fat-binding additive, as well as in dietary supplements aimed at cholesterol management. However, practical application requires careful consideration of factors such as appropriate dosage, formulation stability, and safety. Furthermore, it is important to note that additional validation, including clinical studies or gastrointestinal simulation models, is necessary before these applications can be implemented in real-world settings. Future research should include investigations using simulated gastrointestinal models or in vivo studies to reflect physiological conditions better. In addition, there is a need to quantify actual cholesterol binding rather than relying solely on oil entrapment measurements. Further work should also focus on optimising gamma irradiation parameters to ensure safe and effective application, as well as developing stable chitosan-based formulations suitable for industrial use. These directions highlight the study's contribution while providing clear pathways for further research.

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Author Contribution
Siti Nur Farahayu Abd Rahman– experimental, conceptualisation, data curation; Siti Amira Othman – supervision, review and editing.

Conflict of Interest
The authors declare that they have no conflict of interest regarding the publication of this work.

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