Role of *Zingiber Officinale* Essential Oil- Chitosan Based Biopolymer Film For Bioactive Packaging

Sawsan A. Al-Hilifi (sawsan.hameed@uobsarah.edu.iq)
University of Basrah College of Agriculture https://orcid.org/0000-0002-4857-8157
Rawdah M. Al-Ali
University of Basrah College of Agriculture

Research Article

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Abstract

The recent interest in bio-packing at field of food become trending in the development of antimicrobial coatings. The focus of this study was to assess the potential application of *zingiber officinale* essential oil (GEO) in chitosan films (CHf). The data indicated that there were significant differences($p < 0.05$) in the chemical composition of the samples.Forty-seven active compounds of the essential oil were identified from the rhizomes of ginger, which were identified byGC-MS. Fourier transforms infrared spectra (FT-IR) confirmed that an interaction between the hydroxyl groups of the phenolic compounds of the essential oil and the amide groups of polymer matrix. As shown the appearance of peaks at wavenumbers 1639cm^{-1} and 1558cm^{-1} Furthermore, X-ray diffraction results suggested a lower crystallintiyin CHf due to GEO effect. Differential Scanning Calorimetric (DSC) analysis revealed that CHf possessed high thermal stability, especially when different concentrations of GEO added. The bioactive CHf showed distinct activity against both positive and negative gram bacteria. They are *Staphylococcus aurous, Bacillus subtillis, Streptococcus Sp. Escherichia coli, Salmonella Sp. Pseudomonas erugiosa.* This results provides a comprehensive insight on the importance of films incorporated with EOs of interest in food packaging.

1. Introduction

Active packaging(APg) is important in the food industry, as natural antimicrobial materials are combined to prevent or control outbreaks of infectious foodborne-associated, shelf-life lengthening of food products and improve the quality and safety standards of the packaged foods. The demand for bio-degradable and eco-friendly packaging is expected in the food packaging market around the world [1–3]. Biopolymers, such as proteins, carbohydrates and fats cover the requirements for obtaining bio-degradable films. These polymers can used individually or as mixture or mixed with other compounds. The films can cover the entire surface of the food and thus limit the effect of factors causing damage and improves the quality of food [4, 5]. Bio-composite polysaccharide based material is receiving great attention because it is biodegradable and extends the shelf life of food [6]. APg means the change of the passive packaging system to the role of active defense, that conceder as catalyst for interaction between the environment, packaging and the product. APg ordinarily involves the intentional combination of discharging or absorption of certain compounds from or into the food packaging [7, 8]. These active systems frequently contain active substances that can be released into the packaging's interior [9–11]. Moreover, biopolymer based films have proven to be an exceptional matrix for developing functional packaging materials through many additives, such as antioxidants, antimicrobial agents, flavors and colorings, etc., as they restrict the in inhibiting or spread of pathogenic microbes / food spoilage and contamination [12]. Therefore, active films can play an important role in packaging and thus preservation of fresh food ([13]. The essential oils (EOs) containing antimicrobial is a promising bio-active packaging technology, From a chemical point of view, EOs are secondary metabolites that are mixed with most of them terpenes, especially mono terpenes, containing their oxygenated derivatives, and other compounds such as hydrocarbon, ether, alcohol, aliphatic acid esters and phenolic, which are responsible for the antibacterial activities of EOs. [14, 15]. Antimicrobial and antioxidant properties of essential oils are common, and they can be employed as a substitute for synthetic preservatives in the food industry [16]. Furthermore, EOs can

be incorporated into biopolymer materials such as chitosan (CH) to enhance the antimicrobial effect,, i.e., Foodborne pathogens slow down, EOs have Generally Recognized as Safe (GRAS) status. [17, 18]. The essential oil of Ginger (*Zingiber officinale*) is well recognized for its antimicrobial ant oxidative and action [19]. These activities are usually attributed to some active compounds such as Zingiberene, Camphene, α-Curcumene, and α-Phellandrene and The EOs contained mixture of chemical compounds including monoterpine hydrocarbons, oxygenated monoterpine, [20, 21]. CH is a homogenous polymer of N-acetyl-D-Glucose units linked to each by β-(1→4) bounds, we can get it form the exoskeleton of crustaceans, CH is a natural, sustainable and biocompatible material It has exciting and unique antimicrobial properties and is used in pharmaceutical, food packaging, and environmental conservation. [22–24]. Activities of CH is due to the reactive amino and hydroxyl functional groups, CH is frequently blended other polymers(Xu etal.,2005). The resistance of bacteria and fungi to the in varied antimicrobial agents is main challenge within the treatment of the infections demand to the requirement for finding new sources of materials with anti-microbial properties, as the combination of essential oils and chitosan films coating may enhance the antibacterial properties [26]. The aim of this study is to highlight the effectiveness of antimicrobial chitosan films(CHf) as well to study some of their chemical properties. Hence, in this work we focus on develop active bio-packaging films, and assessment the influence of GEOs addition on thermal stability, morpho-structural(FTIR, XRD, and DSC) characteristics of the films produced. Moreover, the efficiency of GEOs-incorporated films in inhibiting the growth of some indicator food pathogenic bacteria against both gram-positive and gram-negative were determined.

2. Materials And Methods

2.1. Materials

The shrimp shells used in the study were procured ,sourced from the local market in Basrah city, Iraq. Shrimp shells was washed with distilled water and dried at 50°C for 12 hours, after that ground and stored at 4°C in polyethylene bags until the required test performed. Acetic acid, sodium hydroxide, hydrochloric acid, and glycerol were obtained from Sigma(Germany) respectively. *Staphylococcus aureus* PTCC 1337, *Streptococcus Sp., Bacillus subtillis, Salmonella SP., Pseudomonas erugiosa*, and *Escherichia coli* 015:H7 were obtained from the Department of Food Science, University of Basrah, Iraq. All strains were kept in nutrient broth(Sigma, Germany) at − 18°C until further use.

2.2. Extraction of GEO

The essential oil extraction process method befell within the laboratories of the department of Food Science/ college of Agriculture/University of Basrah. The ginger rhizomes (*Zingiber officinale*) were cleaned and then washed and peeled, and the EOs was extracted by steam distillation for 3h using a Clevenger-type apparatus. EOs was dried over anhydrous sodium sulfate. Keep the GEO in tightly closed containers at 4◦C prior to used .

2.3. GC-MS analysis of GEO

GEO were analyzed via GC/MS connected to QP-2010 system and GC-2010 (Shimadzu Co., Kyoto, Japan). A capillary column DB-5 (30 m× 0.25 mm id., film thickness 0.25 µm) was used to separate GEO. The components of EOs were identified by comparing their mass spectra to Wiley7n (Wiley, New York, NY, Technology, Gaithersburg, MD, USA). The peak area were used to calculate the relative percentages for the main components..

2.4. Chitosan preparation

The chitin was prepared to the method mentioned Trung et al. [27] with some modifications. The CH prepared by three treatments in which demineralization and deproteinization the first step: the shrimp shell(7.5g)were treated with 1N HCl in the ratio of 1:4 (W,V) at room temperature after 30 minutes, the shell washed with distilled water four times to remove the mineral and calcium chloride salts. The shrimp shell were then treated with 1N NaOH 1:10 (W:V) at 65°C at 1 h to remove the protein, Then the solution was filtered through Whatman No.1 to get rid of the insoluble parts, Then wash the filtrate with distilled water four times, then it was dried in oven at 50°C to get chitin. The preparation of CH is deacetylation of chitin [28] removing the acetyl groups from chitin was achieved by using 70% NaOH at 8 5°C for 12 hours. The CH were washed with distilled water more than once to get rid of the base, poured into petri dishes and was dried at 50°C, the CH was crushed into a fine powder and stored in light-permeable containers at 5 ± 2°C before the next analysis.

2.5. Determination of chemical composition

Protein, moisture and ash contents of shrimp sells and chitosan were determination according to [29].

2.6. Antimicrobial film preparation

The biodegradable CH- based films in this study were prepared by 1g of CH powder was dissolved in 100 ml (w/v) in a 1% acetic acid and 1% glycerol and then stirred at 50°C for 30 minutes with a magnetic stirrer and was applied till the system entered in the homogenization, afterward, cool the solution to room temperature, and then 0, 0.1,0. 2 and 0.3% (v/w) of GEO were added to solution and homogenized for 30 min using a magnetic stirrer. The polymer solution put in a glass petri dishes to dry at 50°C overnight. The chitosan film without EOs was utilized as a control. The final thickness of 45 ± 2 mm was determined in all samples. The dried films were peeled off and packed at(~ 25°C) with 55 ± 2% RH for 48 h for further characterization [30].

2.7. Film characterization

2.7.1.Fourier-transform infrared spectroscopy (FT-IR)

The effective groups were diagnosed according to the Bonilla et al.(2014)[31], as it included mixing films with potassium bromide KBr in a 100:1 ratio, than high pressure 2500kg/cm^2 to obtain a small tablet of 1mm in diameter and 1–2 mm thick putting the model pressed into FT-IR spectra (Japanese company Jasco). FTIR spectra were recorded in the region 4000 to 400 cm^{-1}.

2.7.2. Differential scanning calorimeter (DSC) analysis

Thermodynamic properties of films was performed using DSC-200F3(Shimadzu, Japan) at a flow rate of 120mL/min in a nitrogen atmosphere. Approximately 10mg of films were placed in aluminum pans, and scanned at range of 30–400◦C with a heating rate of 20°C/min. The curves of calorimetric analysis were recorded., the melting enthalpy (ΔH_m), melting temperature (T_m), and thermal decomposition (T_d) of the films were calculated from the resulting thermo grams.

2.7.3. X-Ray diffraction analysis

X-ray diffraction (XRD) patterns was analyzed with a X per T-pro pw 3050 in ambient condition using Cu-Kα radiation and a nickel monochromator filtering wave at 40kv and 20mA. The bio-films were scanned from 2θ = 5–50° with a steep angle of 0.04C/min.[32].

2.8. Antimicrobial activity of bio- films

In vitro antimicrobial activity of GEO incorporated chitosan-based films was assessed by agar diffusion method to previous study of [33, 34]. Six various spoilage bacteria and pathogenic consisting of *Escherichia coli* 015:H7, *Staphylococcus aurous*, *Salmonella* Sp., *Bacillus* subtilis, *Pseudomomus aeruginosa*, and *Streptococcus* Sp., were used as tested. All bacterial strains were cultured on nutrient broth slant for 24 h at 4°C. The bio-active films sterilized with UV light were cut into circular shapes of 6mm diameter, and the nutrient agar was poured into 15ml petri dishes for each plate and left to solidify. Then 0.1ml of inoculums which contains approximately 10^4 -10^6 CFU were spread and the films cut were placed in the center, than the Petri dishes were incubated for 24 hours at 37 ° C. The efficiency of the antibacterial agents was determined by the formation of an inhibitory zone around the disc samples. Films without GEO were used as a control and were treated according to the same methodology.

2.9. Statistical analysis

The results were analyzed statistically using a complete randomized design (CRD) with one factor, and the significant differences between the averages were compared using the LSD test at the 0.05 level and using the (SPSS version 13.0, 2012)

3. Results And Discussion

3.1. Chemical Component of GEO

GC-MS analysis of GEO showed different components (Table 1). Oxygenated monoterpenes, monoterpene hydrocarbons, diterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpene hydrocarbons, and fatty acid esters were all present in the EOs. The most essential components were Eucalyptol (19.36%), (−) − Camphene (15.07%), β-Bisabolene (11.52%), Zingiberene (9.58%), and Cineol (9.18%) Fig. 1.Several compounds found in this GEO, such as(−) − Camphenein, alpha-curcumen, *α*-Zingiberene have known anti-bacterial properties. Moreover, the anti-bacterial properties may also be attributed to α.-Pinene, β-Selinenol, β-Sesquiphellandrene, and linalool. The major chemical constituents of GEO found in the current study were similar to those found in previous studies, we can notice that minor variations in concentrations due to changes in internal and external factors related to the growth

environment, harvest season, and extraction process used. Wang et al. (2020) found that the β-Phellandrene, α-Curcumene, and *α*-Zingiberene in the GEO were 2.56, 12.04, and 35.65 %, respectively. α-Zingiberene has also been identified by[35] to be one of the most abundant components of this oil.

Table 1. Chemical composition of ginger essential oil constituents using GC/GC-MS analysis

Components	RT/min	(%)
α-Pinene	5.510	4.69
Camphene	5.925	15.07
α-Phellandrene	6.494	0.23
β- Pinene	6.590	0.77
β-Myrcene	7.018	2.03
Eucalyptol	8.191	19.36
Norborneol	12.181	4.47
α-Terpineol	12.840	1.97
β-Bisabolene	15.038	11.52
Cineol	14.149	9.18
Lemomol	14.533	0.63
Borneol	15.232	0.33
α-Curcumene	20.296	1.25
α-Zingiberene	20.708	9.58
α-Farnesene	20.930	1.81
Terpinolene	20.971	1.21
γ-Elemene	21.203	0.71
β-Sequiphellandrene	21.357	3.31
Zingiberone	21.933	0.45
Nerolidol	22.265	0.29
Spathulenol	22.570	0.59
Globulol	22.749	0.48
Elemol	23.854	0.46
β -Selinenol	24.494	1.42
Geraniol	25.250	0.57
Geranialdehyde	26.042	0.29
6-Gingerol	26.400	0.15

3.2. Chemical composition of chitosan

The chemical compounds of fresh shrimp shell and extracted of chitosan are shown in Table 2. The chemical content of shrimp shell of moisture, protein and ash is 44.25%, 33.57% and 31.40% respectively. While values of, protein and ash decreased in the prepared chitosan as its values from 3.72% and 1.48 % compared to raw shells. The shell sample contained a high ash and protein, so the ash percentage, directly related with the calcium carbonate in the exoskeleton [36]. These results indicate the efficiency of the demineralization, which is an essential step in prepared chitosan, as this treatment led to the removal of the largest possible amount of calcium carbonate and calcium phosphate, whose concentration in shell about 30–50% [37]. These result were similar to what [38] found, as composition of moisture, protein and ash of shrimp shell and chitosan particle size that were different as follow 45.65%, 32.45%,32.77% respectively.

Table2. Chemical composition of shrimp shell and chitosan prepared in different particle size

Samples	Parameters (%)		
	Moisture	Ash	Protein
Shrimp shell	44.25±2.21	31.40±3.11	33.57±2.87
Chitosan	6.89±1.21	1.48±1.92	3.72±1.89

3.3. Fourier transform infrared spectroscopy (FT-IR)

FTIR analysis of the films was used to describe the changes generated by the incorporation of GEO into bio-film interactions by separating the IR bands and vibrational shifts related to the incorporation of GEO into film. The spectrum of bio- films (Figure. 3) revealed distinct bands at around 3500 to 3000cm^{-1} (NH bond) and at 1630 – 1400 cm^{-1} (C = O bond) [39, 40], at 3398cm^{-1} (axial stretch of –OH);at 3271cm^{-1} (an asymmetric extension of –NH group). The wide band in the range3400- 3000 cm^{-1} is attributable to O-H and N-H stretching vibration. Band at2939 to 2875 cm^{-1} (C–H bond to –NHCOCH3 of the methyl group); at 1639cm^{-1} (amide I, C = O stretching); at 1558cm^{-1} (amide II,N□□□H bending); at 1423-1382cm^{-1} (CH2 bending); at 1083 to 898 cm^{-1} (skeletal vibration including the stretching of the C–O group) and at 1155 to 1265cm^{-1} (asymmetric stretching of the C–O–C). [41–44].General, the incorporation ginger oil did not in many differences in spectra compared to the control CHf, perhaps this is due small amount incorporated, all peaks prevailed chitosan characteristic and all samples, slight changes in absorption peaks were recorded, which is due to the overlapping of chemical bonds and thus are considered evidence of an interaction between the molecules of different components. The FT-IR spectra of the GEO composition films show a new peak between1745 to 1743 cm^{-1} that corresponds to the vibration of the C = O bond stretch, it is an indication of interaction between the hydroxyl and amine groups of CH with the phenolic compounds in the essential oil [45].

3.4. Thermal property of chitosan films

The thermal analysis method is one of the physical method for evaluating a polymer endurance and stability in temperature range. In this study a DSC was used to estimate the tolerances of chitosan and

chitosan/ ginger oil films as showed in Fig. 3 and Table 3.The result show that the CHf recorded the lowest melting point (T_m) at 88.51°C and endothermic peak at 314.03°C which may be attributed to the moisture loss associated with the hydrophilic groups in chitosan and polymer decomposition, and which accounts for its crystallinity [46]. It is noticed that the highest value of the enthalpy of melting (ΔH) in the CH /0.3%GEO, as it was 526.02J/g and the lowest in the GH is 69.01 J/g. It is noticed from the figures that the greater the value of the change in the enthalpy in the model, the greater its ability to withstand high temperatures, In other words, the change in the model's entropy value increases with the increase in the degree of crystallization, so the more crystallized model is more resistant to higher temperatures. Increased crystallinity results in higher elasticity and in more breakable (lower TS) [47]. It is evident from the results that there is a direct relationship between the oil concentration in the films and crystallization, it may be caused by the movement of CH molecules to polymer segments, thus making them easier to arrange the polymer chain [48].

Table 3
Differential scanning calorimeter characteristics

Characteristics	Films sample			
	Chitosan (A)	Chitosan/ginger01% (B)	Chitosan/ginger0.2% (C)	Chitosan/ginger0.3% (D)
ΔH(J/g)	69.01	104 .16	181.55	526.02
Tm	88.51	275.11	280.21	260.01
Ton	279.55	186.71	180.42	188.24
Tp	314.03	281.81	274.53	245.79

3.5. X-ray Diffraction

X-ray diffraction analysis is used to ascertain the crystal structure of a substance by knowing the internal atomic space. XRD patterns of CHf with different amounts of GEO are shown in Fig. 4. Showed blunt peaks at (2θ(ranging between 20 – 5 this is in agreement with the assumption that CH is a molecularly crystallized polysaccharide that contains some crystals from the embedded in the amorphous region [49]. CH has the advantage of containing three forms: hydrated crystalline, anhydrous crystalline and no crystalline [50].The control CHf appeared in a crystalline state with a major diffraction peak at 5.7° (2θ(,these results were consistent with [51] diffraction peak at 8.44°. According to what a previous study [52], the diffraction peak at 11.54° is attributable to the anhydrous crystalline, at 18.34° the diffraction peak is the aqueous crystal character [53]. Moreover, it was observed that diffraction peak at 22.8° considered as typical fingerprint for CHf [54]. It was observed the diffraction peaks became flatter and less discernible when adding essential oil to the films, indicating a decrease in crystallinity with increasing concentration Usually, the crystallinity index values of films are lower when combined with EOs, it decrease the close packing in the polymer chains, this may be happen due to the stronger interaction between active

compounds and biopolymers(i.e. H-bonds, Van der waals) this leads to improve the crystalline 3D-network [55, 56].

3.6. Antimicrobial activity

The antimicrobial activity of chitosan bio-active films containing GOE at were exhibited in the Table 4. Control compound CHf The film (without essential oil) had the lowest antimicrobial activity compared to other films against the studied microorganisms. Mechanism for anti-microbial activity of CH is based on its positive charges (NH_3^+), which interfere with the electro negative charged on the cell membrane permeability. Leakage of proteinaceous, ionic, and other intracellular materials causes cell death [57–60]. Since CH cannot disperse through agar media and only species that come into contact with the active areas of CH are affected [61–63]. In this study, The growth of the six pathogens was inhibited by all active bio-films in a way depends on concentration EOs. Zone of inhibition increased significantly ($p < 0.05$) as the concentration of GEO in the films increased. CHf with the highest concentration of GEO (0.3 % w/v) effectively affected the growth of tested bacteria, resulting in halos between 9.02 to 18.35 mm. The anti-bacterial activity of EOs is thought to be due to their high phenolic content [64, 65]. In general, EOs have a higher susceptibility to Cram-positive bacteria than to Cram-negative bacteria [66]. GEO was found to have antibacterial activity against S. by [67], while Singh et al. (2008)[68] found strong antimicrobial activity against *E. coli, S. aureus*, and *Yersinia enterocolitica*. This could be the results of synergism generated by the mixture of CH and EOs maybe due to the presence of activity compound and terpenoid that could have antibacterial effect such as Zingerone, shogaol, nerolido and anther Phenolic compounds [68, 69], when analyzing red ginger oil with the GC MS technique by[70] they found that it contains the following compounds Curcumene (15.6%); zingiberene (10.3%); β-Sesquiphellandrene (8.74%); Cineole (7.52%); α-Pinene (3.12%); and borneol (0.46%), these compounds have anti-bacterial properties. The effect of EOs on microorganisms is due to several mechanisms, including their effect on the permeability of the cell membrane, disruption of enzyme systems, which affects the genetic material of the bacteria, as the aromatic and phenolic compounds of the oils affect the cytoplasmic membrane and change its function, and to the inhibition of intracellular metabolic pathways [71–73].This is confirmed by [74], as thy showed that adding essential oils such as GEO improves the anti-bacterial properties of CHf.

Table 4
Antimicrobial activity of edible films incorporated GEO.

Bacterial strains						
Films with oil conc.%	*Escherichia coli* 015:H7	*Staphylococcus aurous*	*Salmonella* Sp.	*Bacillus subtilis*	*Pseudomonas erugiosa*	*Streptococcus* Sp.
inhibition zone(mm)						
Control	8.81 ± 0.00	11.44 ± 0.11	9.77 ± 0.36	12.80 ± 0.006	10.72 ± 0.008	11.38 ± 0.010
0.1%	9.02 ± 0.01	12.62 ± 0.00	11.84 ± 0.017	14.66 ± 0.005	13.27 ± 0.008	15.25 ± 0.00
0.2%	11.38 ± 0.01	14.91 ± 0.005	12.57 ± 0.008	15.26 ± 0.021	13.36 ± 0.015	15.44 ± 0.015
0.3%	13.11 ± 0.02	17.35 ± 0.01	14.95 ± 0.017	17.52 ± 0.008	14.90 ± 0.012	18.35 ± 0.012

Declarations

CRediT authorship contribution statement

Sawsan A. Al-Hilifi: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Review, Writing, and Editing. **Rawdah M. Al-Ali**: , Formal analysis, Conceptualization, Investigation, Software, Data curation.

Declaration of competing interest

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

This article does not contain any studies with human participants performed by any of the authors.

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Conclusion

In this study, the incorporation of GEO into chitosan was successfully prepared bioactive polymers. Characterization of bio composite active films was determined by using Several methods of analysis in order to assess the effect of incorporating GEO in the polymer. Bioactive films based on CH/GEO showing both melting temperature (T_m) and endothermic peak (T_p) had a semi-crystalline structure. The contact angle decreased with EOs addition, so, CHf /GEO coating films have a more hydrophobic surface than pristine CHf. A semi-crystalline polymer crystallographic morphology is normally noticeably changed by EOs effect. The molecular interactions that occurred after the addition of GEO were characterized using FTIR analysis. The films were effective against foodborne pathogens in general. Food packaging is critical for preventing bacterial contamination. Biodegradable food packaging, on the other hand, is more promising in the long term because it is green, recycled, and environmentally friendly. In light of the results, it can be concluded that the addition GEO to antimicrobial films can provide a promising future for biodegradable food packaging applications.

Figures

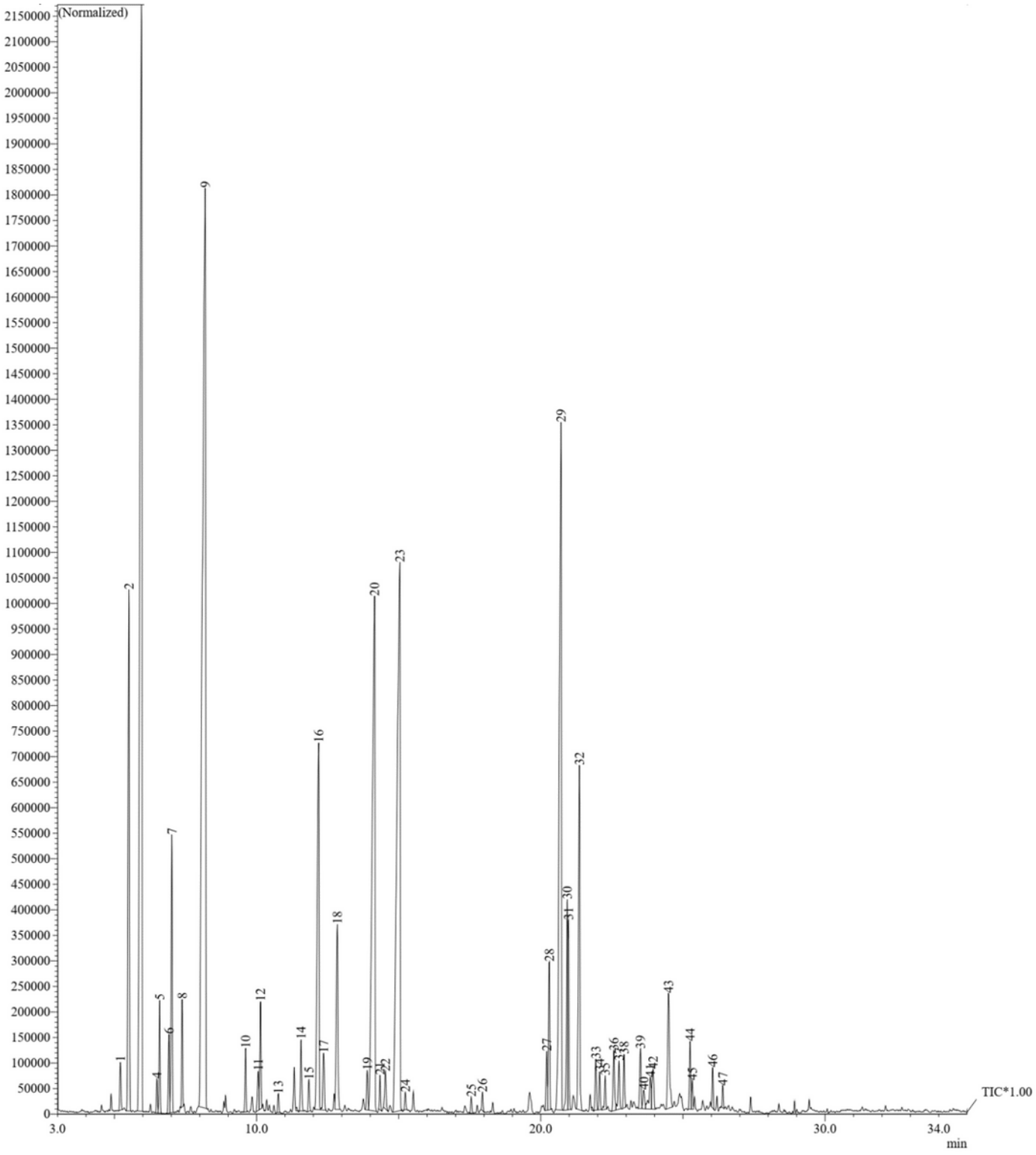

Figure 1

Typical Chromatogram of Total ion current plot of essential oil isolated from the rhizomes of ginger from GC–MS analysis.

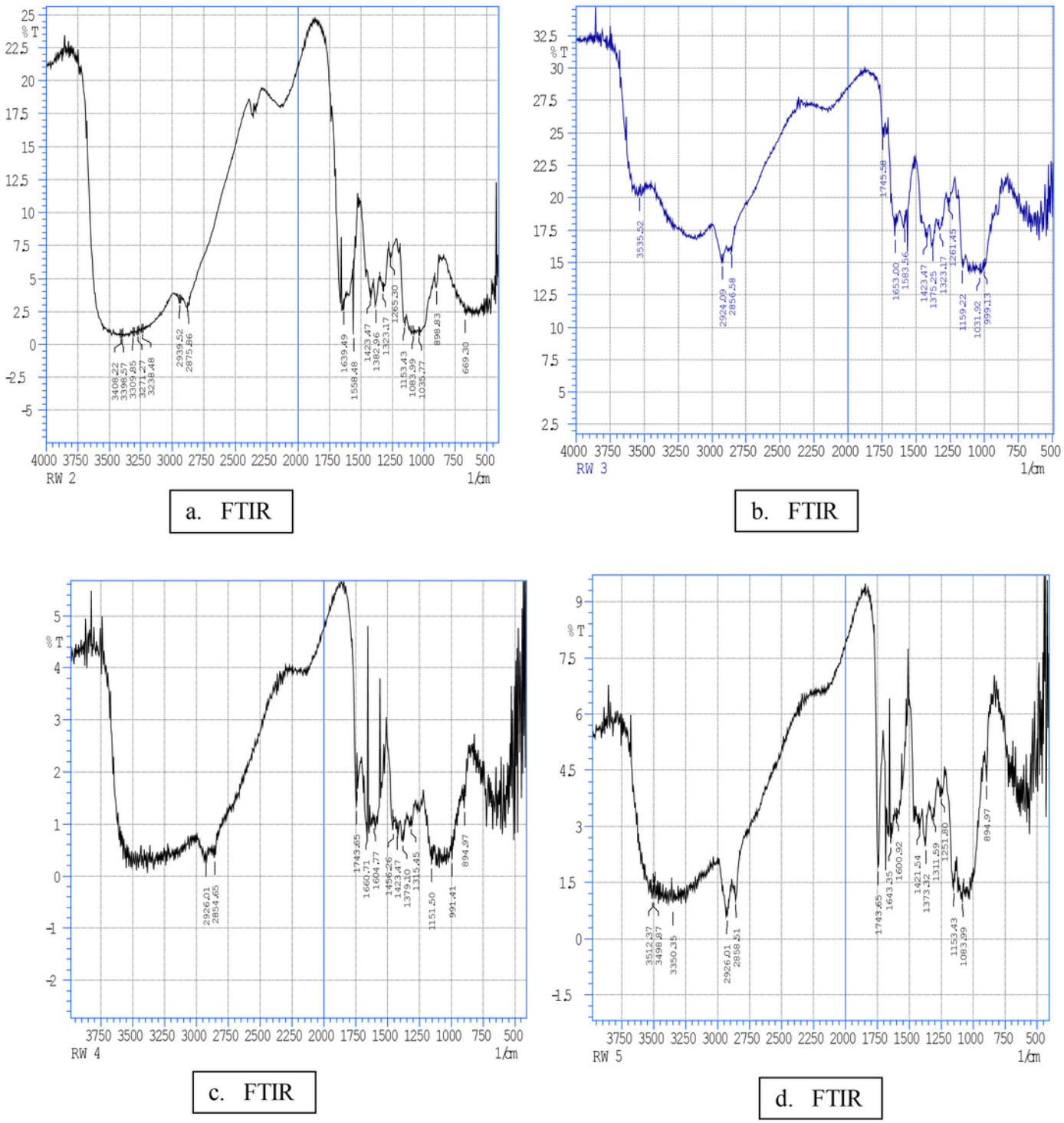

Figure 2

FT-IR spectrum of chitosan film incorporated with ginger oil at different concentration.(a)control chitosan film,(b) ginger oil 0.1%,(c) ginger oil 0.2%,(d) ginger oil 0.3%.

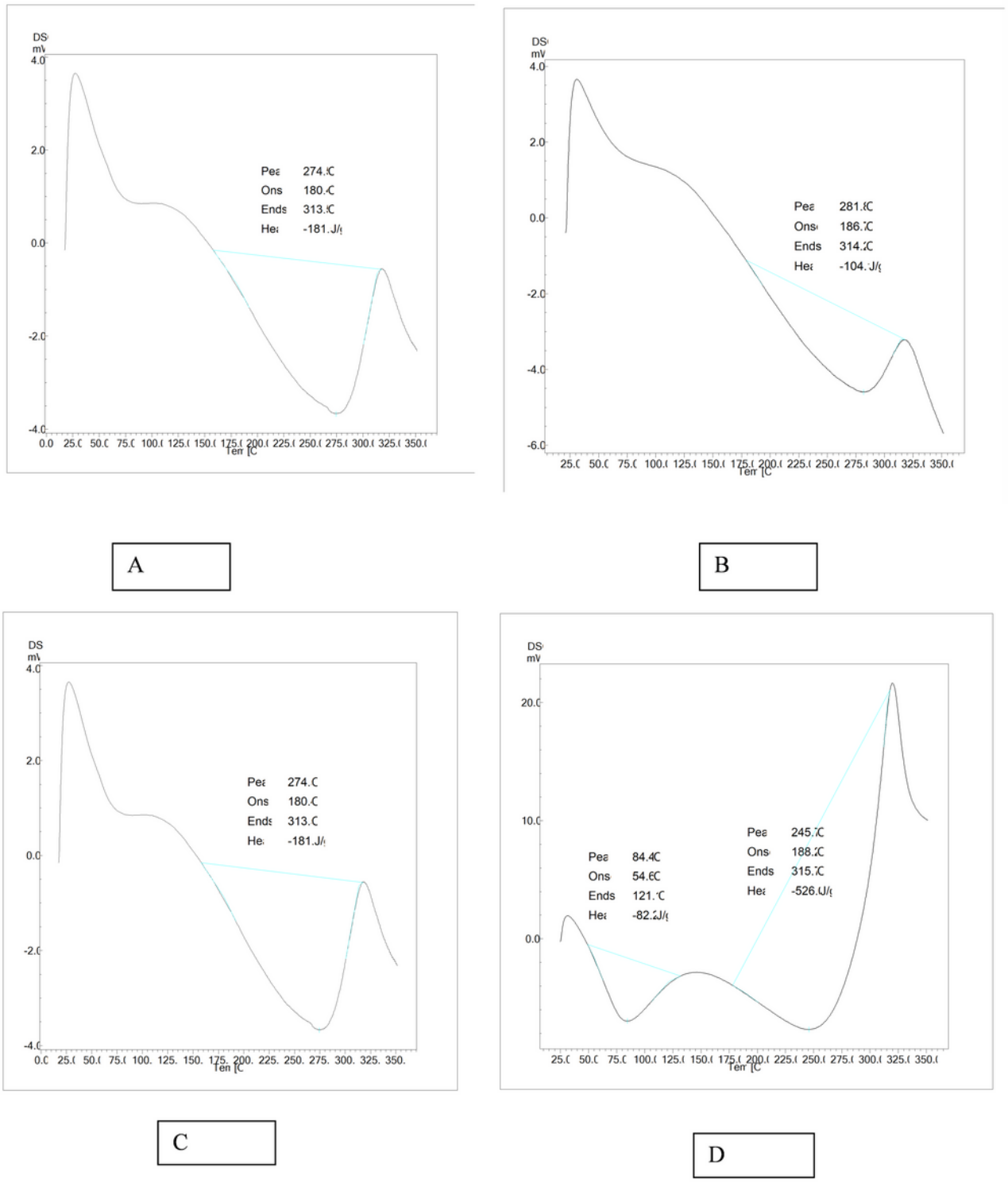

Figure 3

Differential scanning calorimeter of (a)control chitosan film,(b) ginger oil 0.1%,(c) ginger oil 0.2%,(d) ginger oil 0.3%.

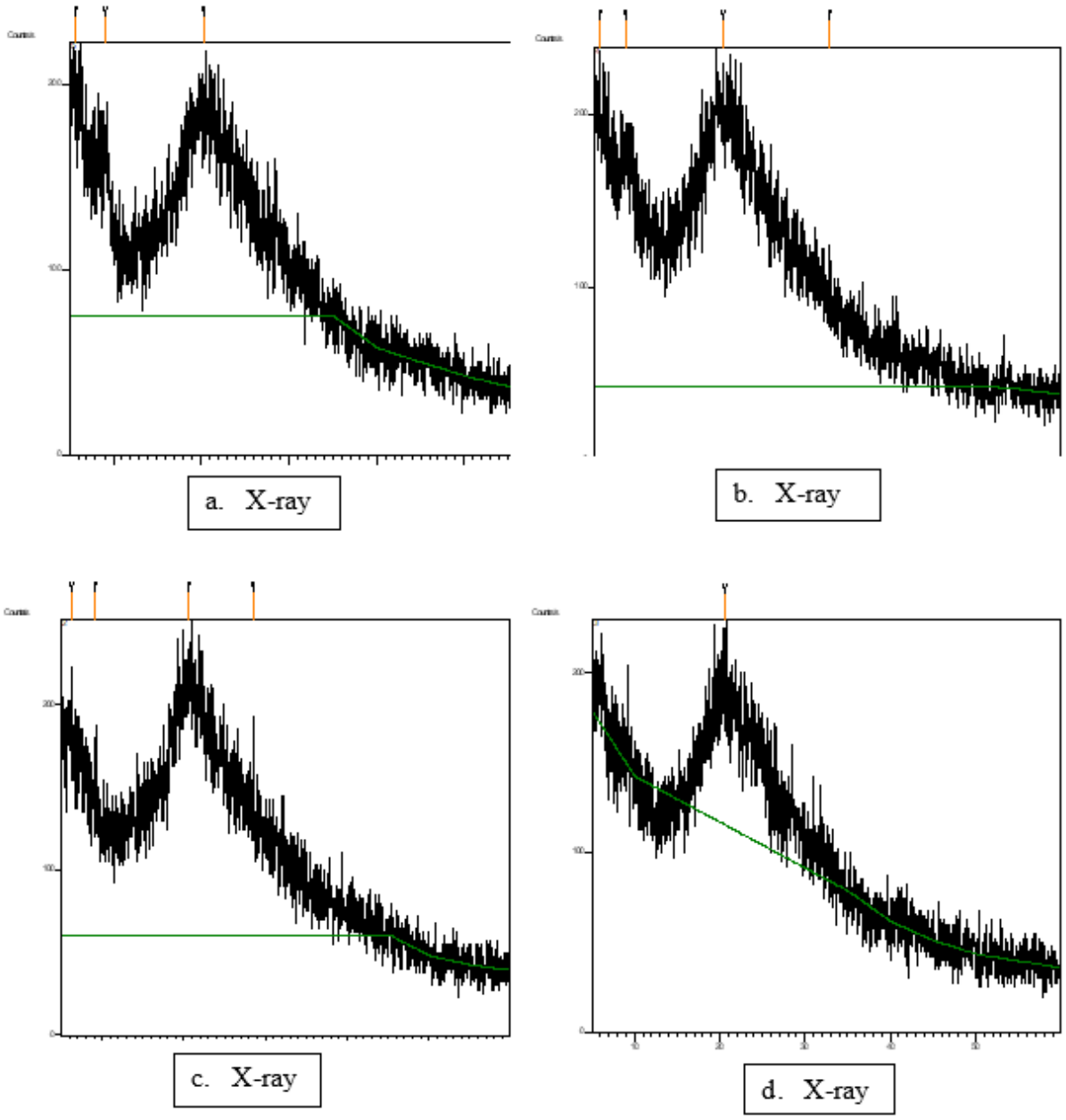

Figure 4

X-ray Diffraction patterns of (a)control chitosan film, (b) ginger oil 0.1%, (c) ginger oil 0.2%, (d) ginger oil 0.3%.