Modification of food additive titanium dioxide (TiO_2 E171) with honey and thyme

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Abstract

Titanium dioxide (TiO_2) is a distinguished semiconductor that is used as a coloring agent in many products including paints, plastics, papers, cosmetic creams, and food products. In the food industry TiO_2 is Labelled as E171 according to the European Union legislation and it is used in many products such as sugar confectionery, chewing gums, candies, some cheeses and sauces, low-fat products such as skimmed milk, ice creams, and pastries with a percentage up to 3.88 mg of TiO_2 per g of product. According to the literature, around 36% of TiO_2 E171 particles are nanoparticles (NPs), which can cross the intestinal barrier reach the blood circulation then accumulate in the liver, spleen, kidneys, and lung tissues resulting in serious damage to these organs. The aim of this study is to use honey and thyme as natural coating materials to enhance the biocompatibility of three types of TiO_2 E171 (anatase, rutile, and food grade). The physiochemical characteristics and bioactivity of TiO_2 E171 before and after modification with honey and thyme were measured. The obtained results demonstrate that the modification of TiO2 E171 with honey and thyme didn't alter its physicochemical properties and the modified TiO_2 E171 showed higher antioxidant activity and lower toxicity.

Highlights

- Honey and thyme were used as natural coating materials to enhance the biocompatibility of three types of TiO_2 E171 (anatase, rutile and food grade).
- The modification of TiO_2 E171 didn't alter the structure of three types of TiO_2 E171 on the contrary it improves the crystallinity and the plasmonic resonance.
- An enhancement in the cell viability for TiO_2 E171 modified with both honey and thyme. However, samples modified with honey showed lower cytotoxicity than those modified with thyme.
- Antioxidant activity results reflect an increase in the radical scavenging activity in TiO_2 E171 after modification with honey and thyme with a higher activity of thyme modified $TiO_{2'}$ E171.
- Food-grade TiO_2 E171 modified with thyme posed the highest radical scavenging activity among the three types of TiO_2 E171.

Introduction

Food Additives are any substance or a mixture of substances other than a basic food stuff that added in food to enhance properties such as texture, taste, and color with potential applications including protection from microbial contamination and oxidation [1]. The European Union legislation defines it as: "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose results in it or its by-products becoming directly or indirectly a component of such foods"[2]. There are two categories of food additives according to their source; natural and synthetic food additives. The natural as the name imply is found naturally as extracts from plants, animals and

minerals. While the synthetic food additives are either synthetic identical copies of substances found naturally or produced synthetically and not found naturally[3]. The exposure to food additives may be have short term effects such as headaches, change in energy level, and alterations in mental concentration, behavior, or immune response or may be long term effects such as the increase risk of cancer, cardiovascular diseases and other degenerative conditions[4].

Titanium dioxide TiO_2 is one of the most common food additives and widely used as white coloring agent with many applications ,it exists in crystalline and amorphous forms, crystalline in either the rutile or anatase or brookite form or mixture of them [5]. It used in foods with number E171 to improve the color of food products such as candies, chewing gums, white sauces, and icing[6]. It is also added to fat products such as skimmed milk, pastries, and ice creams [7]. It has unique physicochemical properties such as brightness, high refractive index, resistance to discoloration .US Food and Drug Administration allows weight of TiO_2 E171 does not exceed 1% of overall food weight. average adult's ingestion of TiO_2 0.2–1 mg/kg daily, while an average child's ingestion 1–3 mg/kg[8]. TiO_2 E171 has a wide range of particle size with around 36% of the particles being in the nanoscale. These nanoparticles can alter the biodistribution of TiO_2 E171 and hence give rise to a toxicological potential[9]. Many studies investigate the circulation of TiO_2 E171 in the body[10][11][10][12][13]. Its reported that TiO_2 E171 found in the liver, kidneys, spleen, and lung tissues and showed that TiO_2 particles could be transferred to other tissues after reuptake by digestive tract and it promotes colonic tumorigenesis and induces change in gut microbiota composition. Moreover, TiO_2 NPs can generate reactive oxygen species (ROS), which can induce a carcinogenic risk[14]. The International Agency for Research on Cancer (IARC) excluded the link between occupational exposures to TiO_2 particles and risk for lung cancer in humans but categorized TiO_2 as group 2B carcinogenic compound experimentally[15].

Recently, green nanotechnology was utilized to produce nanoparticles with enhanced biocompatibility and bioactivity. The biocompatibility of green modified NPs offers very interesting applications in the biomedical filed [16]. Moreover the coating of biological molecules on the surface of NPs makes them biocompatible in comparison with the NPs prepared by chemical methods [17][18].

Honey is a sweet viscous fluid produced from bees, is one of the healthiest food sources since immemorial time. It consists of 80–85% carbohydrate (mainly glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash, and minor quantities of amino acids, enzymes, and vitamins as well as other substances such as phenolic antioxidants However, the precise chemical composition and physical properties of natural honey differ according to the plant species on which the bees foraged, differences in climatic conditions, and vegetation [16].

Thymus Vulgaris is known as a perennial plant characterized by small grey-green leaves with purple flowers and one of the preferable cooking herbs. It serves as anti-asthma, anti-cough, stomachic stimulant and intestinal catarrh. thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial ,antifungal ,antioxidative ,and antiviral. The chemical composition of its leaves

contains proteins, sugars, polyphenols, terpiines and vitamins and bioactive phytochemicals such as thymol, Carvacrol, Pinene Alfathiogon, Cineol and Terpenoids [19].

This work aimed to study the effect of modification of TiO_2 E171 with honey and thyme on its physiochemical characterizations and bioactivity.

Materials And Methods

Materials

Three types of TiO_2 E171; anatase, rutile, and Food grade used throughout the experiments were bought from Nerol Company (Cairo-Egypt) . Thyme was supplied by local market (Qena-Egypt), and honey from department of agriculture (Luxor ,Egypt).

Colon carcinoma cells (Caco2 cell lines) was supplied from Vacsera ,Giza, Egypt.

3-(4,5-dimethylthiazol-z-yl)-2,5-diphenyl-tetrazotiumbromide (MTT) assay (SERVA Electrophoresis GmbH ,Heidelberg, Germany), PBS (Serox , Germany), DPPH (Himedia, India) , methanol (Diochem, Egypt), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from in vitro, Life Science Production (LSP.LSG-1016A, UK), and 1 % penicillin- streptomycin (Lonza, ,Lot No:8MB114 ,Germany)

Methods

Modification with honey

The modification of TiO_2 E171 with honey was done according to M.K. El-Bisi[20] protocol with a slight modification. Briefly, 2 g of TiO_2 E171 was dissolved in 20 mL of deionized water under stirring and 20 g of honey was dissolved in 85 ml of deionized water under stirring to obtain a homogeneous aqueous solution of honey.

After preparing the two solutions, 20 ml of honey aqueous solution were added in a dropwise manner to 20 ml of TiO_2 E171 solution under vigorous stirring for 1 h and left to precipitate for 24 h. The resulting precipitate was then collected by centrifugation and dried at 65 °C for 90 minutes.

Modification with thyme

The modification of TiO_2 E171 with honey was thyme according to Ghena Mohammad [21] protocol with a slight modification. Briefly, 20 g of well-washed thyme leaves were, added in beaker containing 85 ml of distilled water under stirring at 60 °C for 1h. The solution was then cooled and filtered three times with Whitman No.1 paper (pore size 25 µm). TiO_2 E171 solution was prepared by dissolving of 0.7 g of TiO_2 E171 in 100 ml of deionized water under stirring for 1 h.

After preparing the two solutions, 20 ml of thyme aqueous solution were added in a dropwise manner to 80 ml of TiO_2 E171 solution under vigorous stirring for 1 h then left to precipitate for 24 h. The resulting precipitate was then collected by centrifugation and dried at 65 °C for 90 minutes.

Characterization

(XRD) measurement were performed on Bruker D8 Advance using Cu-Kα radiation with radiation 40 mA, 40 kV from Position [2 θ°] 4.01 to 89.99by step 0.02 with Scan Step Time [s] 0.6000 ,the crystallite size was calculated using the Scherer equation. Mean average size and shape of primary particles were determined by Transmission Electron Microscope(TEM) JEM- 2100 (JEOL , Japan), operating at 200 kV and Images were captured at 30,000 X magnification. The optical absorption spectra were obtained using UV spectrometer SPECORD 200 (Analytik Jena, Germany). The functional groups were measured using FTIR-4100 type A in the 349.053 -7800.65 cm^{-1} range, with a resolution of 4 cm^{-1} at room temperature.

Cytotoxicity

Caco2 cell lines were used to assess the cytotoxicity of all types of TiO_2 E171. Cells were cultured in DMEM (4.5 g/l, Glucose with L-Glutamine) supplemented with 10 % heat inactivated FBS and 1 % penicillin- streptomycin. Cells were grown in 75 cm^2 flasks and subculture at approximately 80 % confluency. The cells were incubated at 37° C, 5% CO_2. Cells in passages 6, 7, 9 were used in this research.

Cytotoxicity was screened via the 3-(4,5-dimethylthiazol-z-yl)-2,5-diphenyl-tetrazotiumbromide (MTT) assay [22]. Cells were seeded in 96 well plate (1x 10^5 cells/well) and incubated at 37°C , 5% CO_2. 24 h later cells were exposed to TiO_2 E171 particles with different concentrations (0.5, 0.25, 0.125, 0.0625 and 0.03125 mM). After 24 h of exposure, cells were washed with PBS and 100 µL of 50 µL of serum-free media and 50 µL of MTT solution were added into each well. The plate was then Incubated at 37°C 5% CO_2 for 3 hours. After incubation, 100 µL Of Demso were added into each well and the plate was put on an orbital shaker for 10 minutes. The absorbance was measured with UV spectroscopy at OD=590 nm for each well. Cell viability % was calculated using the following equation:

$$cell\ viability\% = \frac{\text{mean OD of untreated cells} - mean\ ODof\ treated\ cells}{mean\ OD\ of\ untreated\ cells}$$

Antioxidant activity

Antioxidant activity determined by 1, 1 Diphenyl 2- Picryl Hydrazyl (DPPH) assay [23]. DPPH is a stable free radical with red color, when react with antioxidants material it reduced to DPPH-H and turns to yellow. The degree of discoloration indicates the scavenging potential of the antioxidant compounds. To assess the scavenging ability on DPPH, 4mg from DPPH dissolved in 100 ml from methanol and wrapped in foil. The samples were dissolved in methanol with different concentrations (400, 200, 100, 50 and 25 µg/ml) then 3ml of DPPH solution added to 2 ml of each concentration and incubated in dark

condition at room temperature for 30 min. The absorbance was measured with UV spectroscopy at OD= 517 nm then the radical scavenging activity was calculated %using the following formula

$$RSA\% = \frac{\mathrm{Abs_{control}} - \mathrm{Abs_{sample}}}{\mathrm{Abs_{control}}}$$

Results And Discussion

Modified TiO_2 Characterization

X-ray diffraction (XRD)

XRD pattern of the three types of TiO_2 E171 samples were shown in figure 1. All the characteristics peaks for anatase TiO_2 appeared in the XRD pattern of anatase TiO_2 E171 and modified with thyme and honey ; see figure (1-A). All the assigned peaks were in a good agreement with the JCPDS no (73-1764) for anatase TiO_2 [24]. The three samples of anatase TiO_2 E171 showed the anatase phase in the tetragonal crystal structure with lattice parameter a=b=3.776 nm, 3.774 nm , 3.777 nm for anatase TiO_2 E171 and modified with thyme and honey respectively. While the lattice parameter c is 9.47 nm, 9.479 nm, 9.481 nm for anatase TiO_2 E171 and modified with thyme and honey respectively. A small increase in the lattice parameter c was observed in modified anatase TiO_2 E171 samples, which suggest the enlargement of the crystal dimension in this direction and increase of the particle size.

Figure (1-b) represents XRD pattern of rutile TiO_2 E171, all the assigned peaks were in a good agreement with the JCPDS no (87-0920) for rutile TiO_2 [25]. The rutile phase with tetragonal crystal structure were detected in the three samples of rutile TiO_2 E171 and modified with thyme and honey. The calculated lattice parameters were a=b= 4.553 nm, 4.588 nm, 4.584 nm for rutile TiO_2 E171 and modified with thyme and honey respectively and lattice parameter c=2.95 nm, 2.956 nm, 2.956 nm for rutile TiO_2 E171 modified with thyme and honey respectively. A small increase in the lattice parameter a=b was observed in modified rutile TiO_2 E171 samples, which suggest the enlargement of the crystal dimension in this direction and increase of the particle size.

Figure (1-c) represents XRD pattern of food grade TiO_2 E171, all the assigned peaks were in a good agreement with the JCPDS no (87-0920) for rutile TiO_2 [25]. The rutile phase with tetragonal crystal structure were detected in the three samples of food grade TiO_2 E171 and modified with thyme and honey. The calculated lattice parameters were a=b= 4.594 nm, 4.5879 nm, 4.5369 nm for food grade TiO_2 E171 and modified with thyme and honey respectively and lattice parameter c=2.9558 nm, 2.9578 nm, 2.9519 nm for rutile food grade E171 modified with thyme and honey respectively. A small decrease in both lattice parameter a=b and c was observed in modified food grade TiO_2 E171 samples, which suggest the quenching of the crystal dimension of modified food grade TiO_2 E171with thyme and honey and decrease of the particle size.

The effect of modification with thyme and honey on the structure of TiO_2 E171 was observed as increase in the peak intensity and decrease in peak broadening with peak shift to lower 2θ. This result may be due to the increase of the particle size and improvement the crystallinity of TiO_2 E171 after modification with thyme and honey [26] [27].

The average crystalline size of TiO_2 E171 was calculated by Scherer's equation[28] (see table 1).

$$d = \frac{0.89\lambda}{\beta Cos\theta} \quad \ldots\ldots\ldots\ldots (1)$$

where d is the average mean diameter of NPs, λ is the wavelength of X-ray radiation source, 0.89 is a constant crystalline shape factor, θ is the Bragg's diffraction angle, and β is the angular full width at half maximum (FWHM) of XRD peaks recorded at diffraction angle 2θ.

The modification of anatase and rutile TiO_2 E171 with thyme and honey causes an observed increase in the calculated crystal size, while it causes decrease in the crystal size of food grade TiO_2 E171. This change in the particle size after biomodification may attributed to the interaction kinetics of biomolecules with nanoparticles [29] [30].

Table 1: Particle size of TiO_2 E171 samples as calculated from Scherer's equation

Sample	Particle size (nm)
Anatase TiO_2 E171	53.36 ± 9.52
Anatase TiO_2 E171 modified with thyme	81.48 ± 24.3
Anatase TiO_2 E171 modified with honey	92.43 ± 15.85
Rutile TiO_2 E171	90.5 ± 16.22
Rutile TiO_2 E171 modified with thyme	97.5 ± 12.27
Rutile TiO_2 E171 modified with honey	106.5 ± 12.61
Food grade TiO_2 E171	8102.76 ± 27.6
Food grade TiO_2 E171 modified with thyme	70.97 ± 10.72
Food grade TiO_2 E171 modified with honey	92.08 ± 15.78

High resolution transmission microscope (HRTEM)

The HRTEM images and their corresponding particle size distributions of all TiO_2 E171 samples are shown in figure 2. It's observed in HRTEM images that all TiO_2 E171 samples have a non-uniform spherical shape. While the modification of anatase TiO_2 E171 with thyme and honey causes decrease in

primary particle size (120.23 ± 34.5 nm, 188.9 ± 62.9 nm, 128.79 ± 37.62 nm for anatase TiO_2 E171 and modified with thyme and honey respectively), the modification of rutile and food grade TiO_2 E171 with thyme and honey causes increase in primary particle size (189.147 ± 42 nm, 168.44 ± 59.61 nm, 158.66 ± 34.62 nm for rutile TiO_2 E171 and modified with thyme and honey respectively and 136 ± 44.5 nm, 111.07 ± 38.55 nm, 110.77 ±17.78 nm for food grade TiO_2 E171 and modified with thyme and honey respectively).

UV- visible spectroscopy

The UV–visible absorption spectra of all E171 samples are shown in Fig 3 (a, b and c). The absorption peaks were (282.35, 283.17 and 283.17 nm) for anatase, rutile, and food grade TiO_2 (E171) respectively. The absorption peak of honey extract was observed at 280.53 nm, while the absorption peak of thyme detected at 483.12 nm. After modification with honey the three types of TiO_2 showed a blue shift with the absorption peaks at 222.4, 269.63 and 223.91 nm for anatase, rutile, and food grade TiO_2 (E171) respectively. Similar after modification with thyme all the three types of TiO_2 showed a blue shift with the absorption peaks at 261.76, 268.12 and 268.12 nm for anatase, rutile, and food grade TiO_2 (E171) respectively. This blue shift is the result of changing the particle size after modification, which confirm the modification of the three types of TiO_2 with honey and thyme [31].

The presence of broad peaks in all the spectra of honey and thyme modified TiO_2 samples can attributed to two reasons; the first reason is the presence of bioactive molecules of honey and thyme. It has been concluded that honey has several important peaks in the region 250 – 450 nm due to the absorption of benzoic, salicylic and aryl-aliphatic acids [32]. Also, the reported result for thyme revealed that it has various absorption peaks in the UV-vis region due to the presence of carvacrol, thymol. While, the second reason is the excitation of plasmonic resonances as absorption spectra of larger metal colloidal dispersions can exhibit broad peaks or additional bands with the lower absorbance in the UV-visible range due to the excitation of plasmonic resonances or higher multiple Plasmon excitation [33].

FTIR spectroscopy

Different functional groups can be observed in the FTIR spectrum of thyme and honey due to the presence of bioactive groups such as carvacrol, thymol, carbohydrates, organic acids and sugar, see fig. 4 (a ,b and c). The broad band observed approximately between 3000 to 3500 cm^{-1} corresponding to OH vibration of hydroxyl group. Band observed approximately at 2900 cm^{-1} due to C–H stretching of methyl and isopropyl groups on the phenolic ring. Band at approximately 1730 cm^{-1}, 1600 cm^{-1} due to C=O and C=C functional groups respectively. The vibrational band due to C–C in ring of aromatics group were observed at 1496 cm^{-1}, 1319 cm^{-1} and 1010 cm^{-1}, while C-O were observed at 1171 cm^{-1} [34] [35].

The functional groups belonging to TiO_2 were observed approximately at 458 cm^{-1} corresponds to the Ti - O – Ti vibrational band and around 693 cm^{-1} correspond to TiO_2 Modes [36] [37].

The presence of functional groups belonging to honey and thyme in the FTIR spectrum of the three types of TiO_2 particles confirm the success of modification. New bands where observed in FTIR spectrum of anatase modified with honey due to the presence of honey such as the bands at 3279,1647, and 1074cm^{-1} which corresponding the stretching vibration of the –OH group, vibrations of the –OH groups and the stretching vibrations of C–O in C–OH group and stretching of C–C in the carbohydrate structure respectively. Bands at 3244,1651cm^{-1} corresponding OH vibration of hydroxyl group, the C=C bonds respectively. All these bands are shifted from their original position in honey, this confirming honey and thyme interaction with the surface of anatase TiO_2.

Cytotoxicity

Cell viability against Caco2 cells for all types of TiO_2 E171 was evaluated using MTT assay , fig. 5. At low concentration (0.125, 0.0625 and 0.03125 mM) a small reduction in cell viability approximately from 1 to 6% were observed. While at high concentration (0.25 and 0.5 mM) the reduction of cell viability increased approximately from 10 to 20%. Similar result was obtained by Ji-Soo Hwang et.al.[38]. An enhancement in the cell viability was observed in the three types of TiO_2 E171 after modification with honey and thyme, which is in a good agreement with the reported result for honey [39] and thyme [40]. However, samples modified with honey showed lower cytotoxicity than that modified with thyme.

Antioxidant activity

Free radical scavenging activity of TiO_2 E171 after modification of thyme was measured with DPPH assay and plotted in fig. 7. An increase approximately from 10 to 15% was observed in all samples at low concentrations and approximately from 5 to 10% at high concentrations. Between the three types of TiO_2 E171 food grade has the largest radical scavenging activity at all concentrations, while the other two types have a similar radical scavenging activity. The modification of TiO_2 E171 with thyme appears to increase the radical scavenging activity of the three types of TiO_2 E171. The calculated IC_{50} is 19.6 mM, 81.6 mM and 6.8 mM for anatase, rutile and food grade modified TiO_2 E171 respectively. This agreed with the previous studies, which report that thyme extract can be used as a natural antioxidant to prolong stability of oils [41].

In the body, honey can take up free radicals and contribute to better health. The radical scavenging activity after modification with honey increased by approximately 2 to 6% with lower concentration and increased by approximately 1 to 4% with higher concentration. The calculated IC_{50} is 62.6 mM, 93.56 mM, and 60.16 mM for anatase, rutile and food grade modified TiO_2 E171 respectively [42].

In comparison of the effect of modification with honey and thyme on the radical scavenging activity of the three types of TiO_2 E171, thyme was found to have the higher radical scavenging activity. Moreover, food grade TiO_2 E171 modified with thyme have the highest radical scavenging activity among the three

types of TiO_2 E171, fig. 8. These results demonstrate that there no correlation between cytotoxicity and radical scavenging activity [43].

Conclusion

To our best knowledge, this is the first study that deals with the modification of TiO_2 E171 with honey and thyme. Our study was built on the sophisticate that using some bioactive molecules to coat the surface of TiO_2 E171 could reduce its side effect. In this study, three different types of TiO_2 E171 (Anatase, Rutile, and food-grade) were successfully modified by honey and thyme. The primary size of the three particles was 53.36 ± 9.52 nm, 90.5 ± 16.22, and 102.76 ± 27.6 for anatase TiO_2 E171, rutile TiO_2 E171, and food-grade TiO_2 E171, respectively. After modification anatase and rutile TiO_2 E171 showed an increase in the average particle size, while food-grade TiO_2 E171 showed a decrease in the average particle size. The modification of TiO_2 E171 didn't alter the structure of three types of TiO_2 E171 on the contrary it improves the crystallinity and the plasmonic resonance. Moreover, an enhancement in the cell viability for particles modified with both honey and thyme. However, samples modified with honey showed lower cytotoxicity than those modified with thyme. Antioxidant activity results reflect an increase in the radical scavenging activity in the three types of TiO_2 E171 after modification with honey and thyme with a higher activity of thyme modified TiO_2 E171. Also, food-grade TiO_2 E171 modified with thyme posed the highest radical scavenging activity among the three types of TiO_2 E171. These results provide a primary solution to the side effect associated with exposure to TiO_2 E171, but still, need further information about the behavior of the modified TiO_2 with honey and thyme in vivo.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Hager Youssef Ahmed did the experimental part and Alaa Hassan Said analyzed the data and wrote the original manuscript. I. H. Ibrahim and Abdelsattar M. Sallam revised the original manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

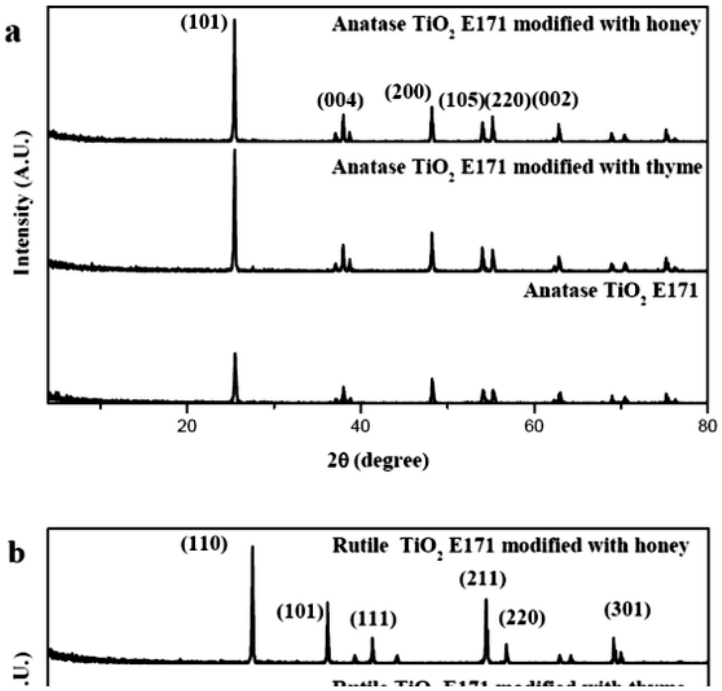

Figure 1

XRD pattern of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

Figure 2

Transmission electron microscopy image and the particle size distribution of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

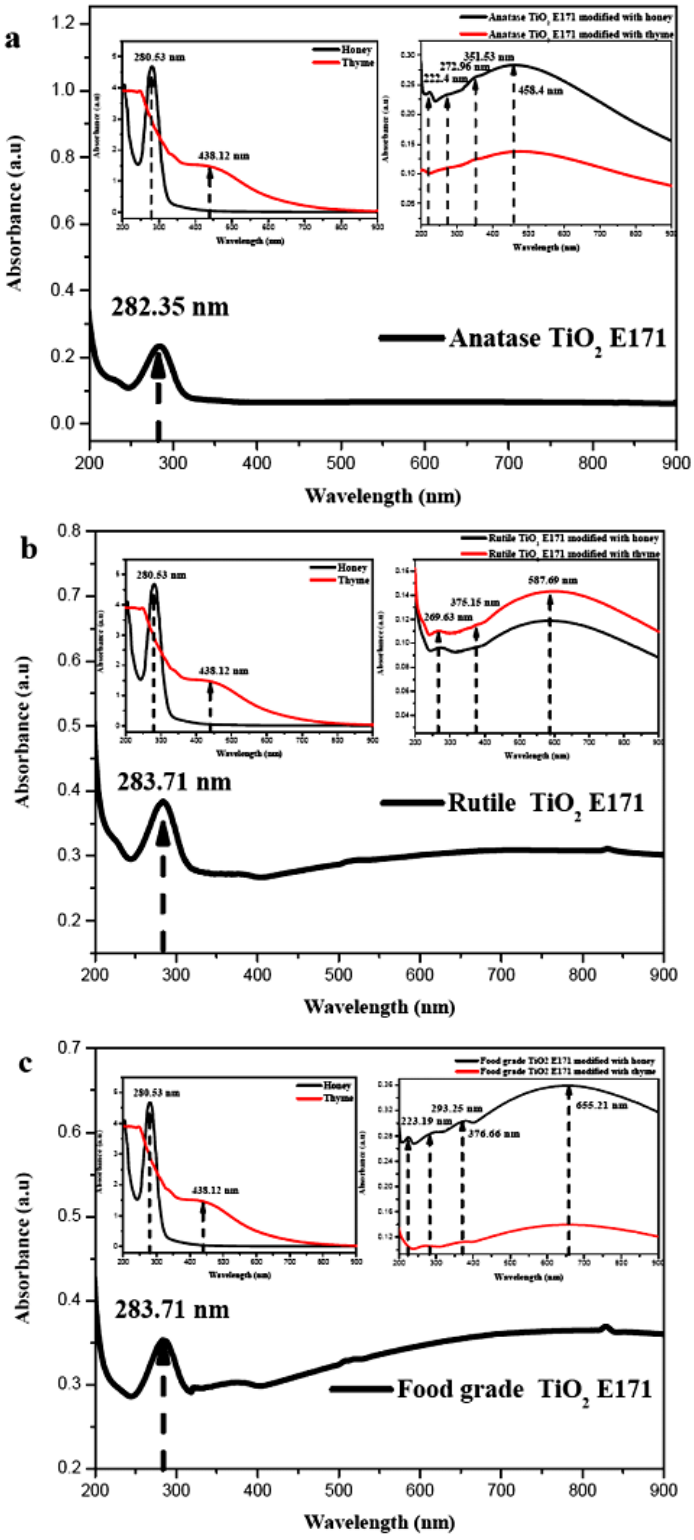

Figure 3

UV–visible absorption spectra of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

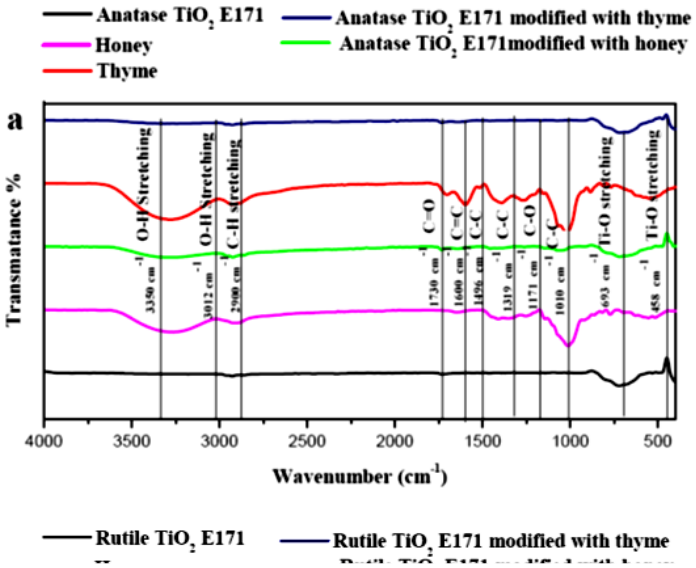

Figure 4

FTIR spectra of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

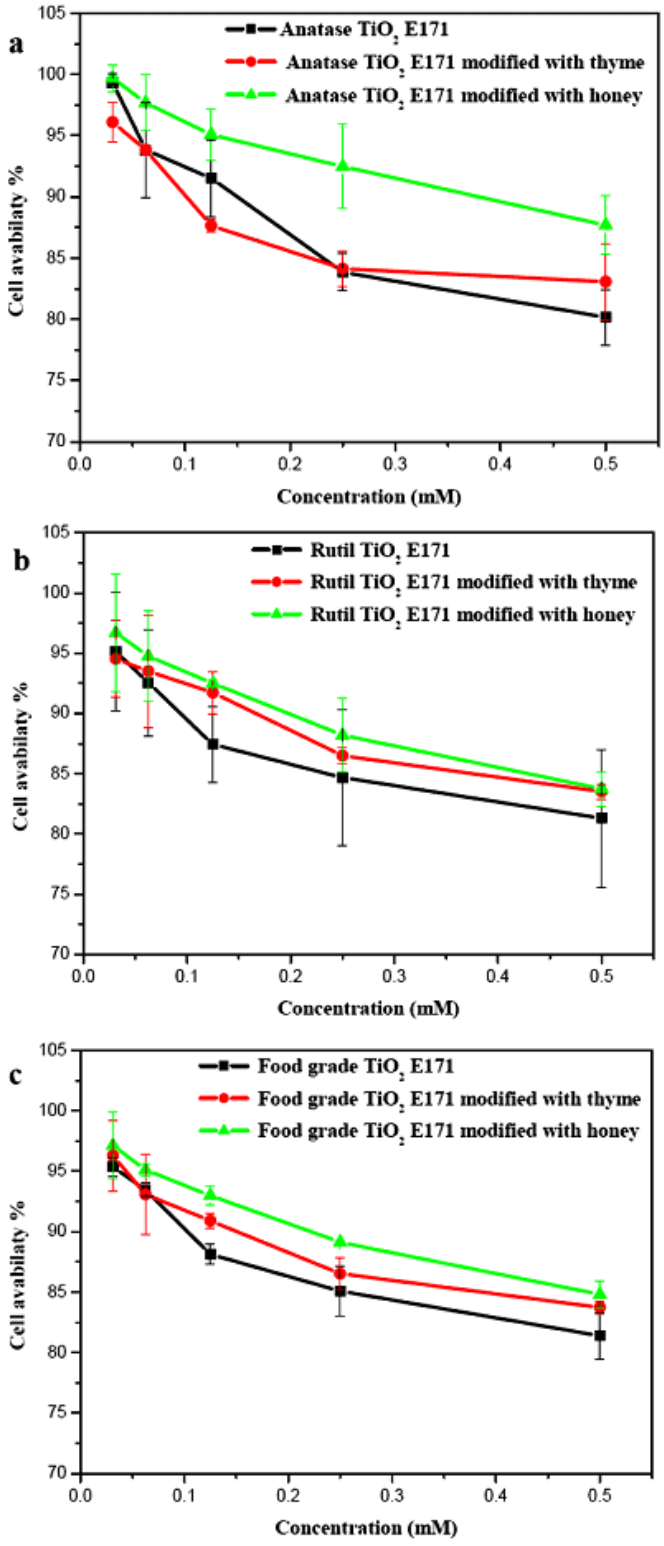

Figure 5

Caco-2 cell viability after treatment with of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

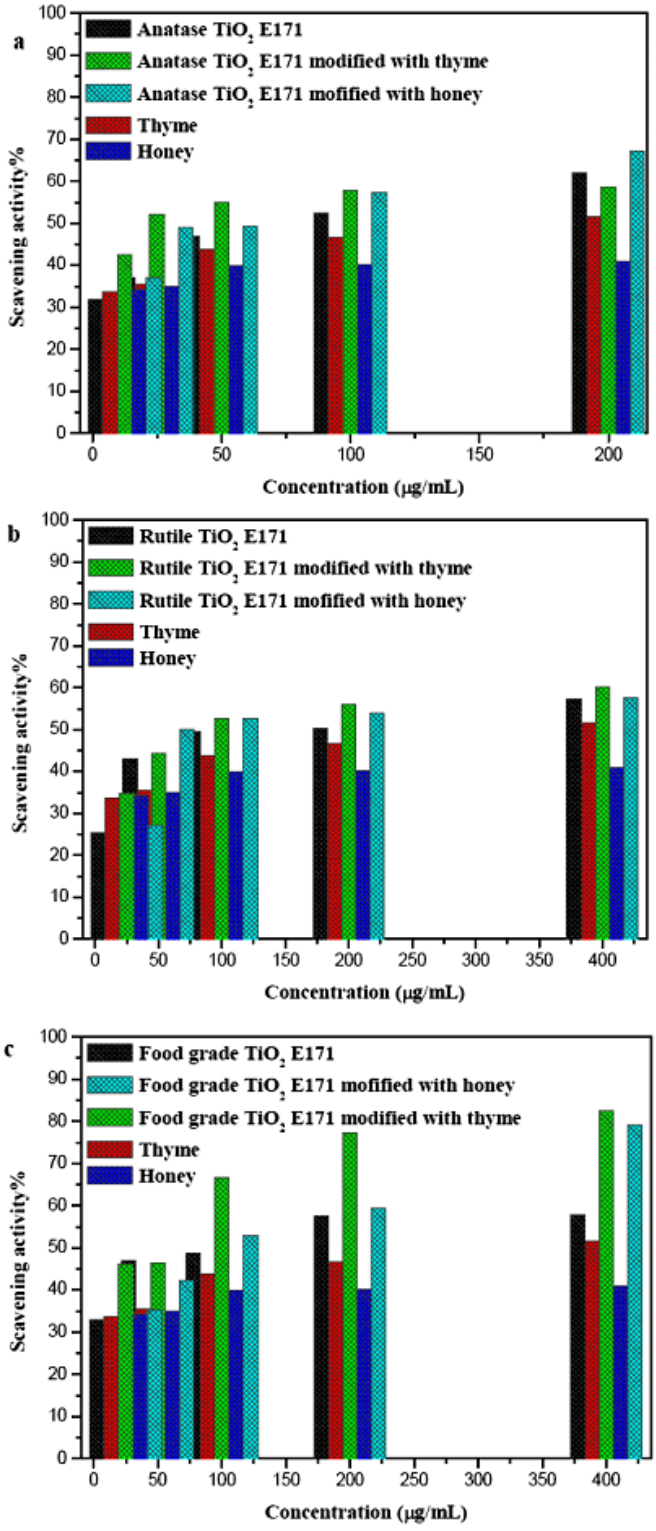

Figure 6

The activity of DPPH with different concentration of (a) Anatase TiO_2 E171 before and after modification with honey and thyme, (b) Rutile TiO_2 E171 before and after modification with honey and thyme and (c) Food grade TiO_2 E171 before and after modification with honey and thyme.

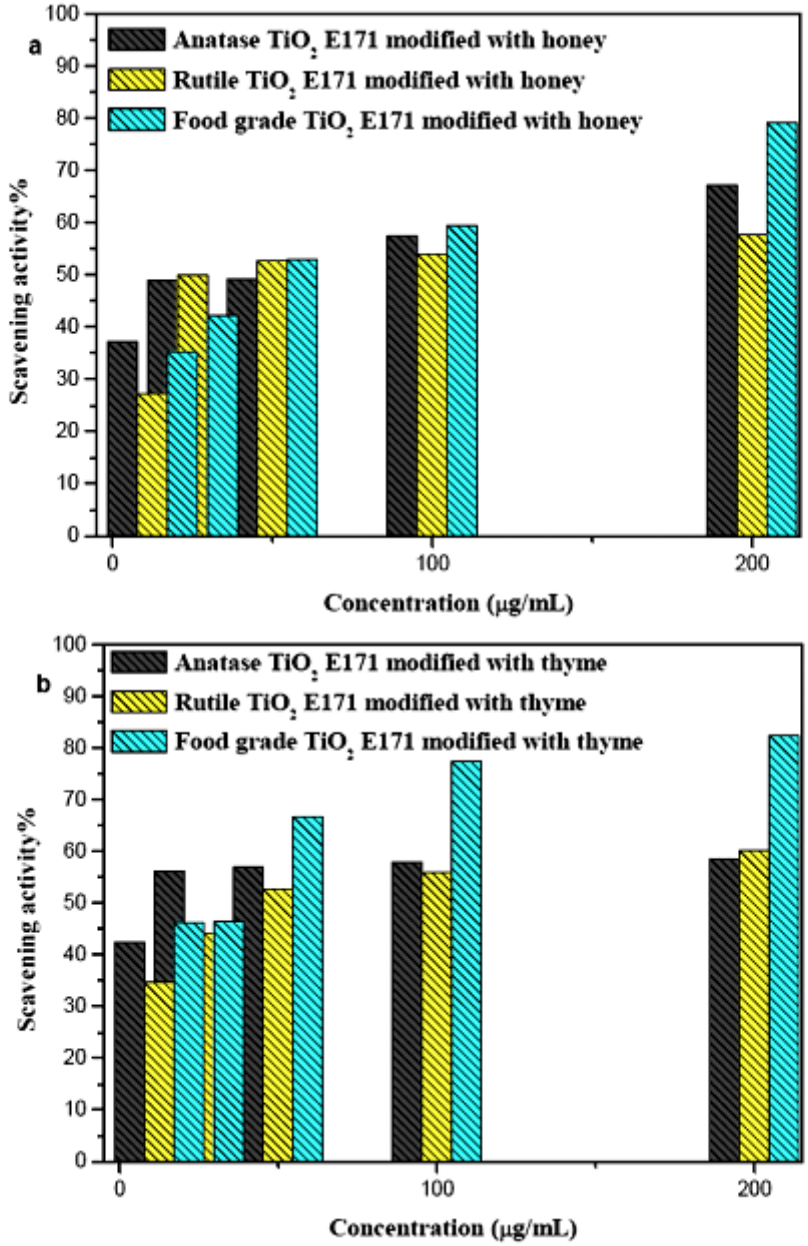

Figure 7

Comparison of the effect of modification the three types of TiO_2 E171 with (a) honey and (b) thyme on the radical scavenging activity.