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FT-IR ANALYSIS ON THE INCLUSION COMPLEX OF B-CYCLODEXTRIN WITH QUERCETIN, NARINGENIN, 3-HYDROXYFLAVONE AND CHRYSIN VIA DIFFERENT PREPARATION METHODS

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ABSTRACT

Flavonoids are used widely in the pharmaceutical industry due to their anti-oxidative and anti-inflammatory properties. However, most of the flavonoids are poorly water-soluble. These experiments were conducted to prepare the inclusion complex of β-Cyclodextrin with selected flavonoids such as Quercetin, Naringenin, 3-Hydroxyflavone and Chrysin. The methods used in complexing the host and guest molecules are modified physical mixture, kneading and freeze drying method by using the molar ratio of 1:1. The complexations of the flavonoids tested were analyzed by the Fourier Transform Infrared (FT-IR)spectroscopy. Analysis results suggested that the best method to complex β-Cyclodextrin with Quercetin and Chrysin, were via modified kneading method. Complexation of Naringenin and 3-Hydroxyflavone was best done via modified freeze drying and physical mixture method respectively.

Keywords: inclusion complex; β-cyclodextrin; Quercetin; Naringenin; 3-Hydroxyflavone; Chrysin; Fourier Transform Infrared spectroscopy.

1. INTRODUCTION

The flavonoids are phenolic substances resulted from the isolation of a multiple of vascular plants and more than 10,000 structural variants that have been reported (Uliana, Garbellini & Yamanaka, 2014). These flavonoids can be found in fruits, vegetables and the leaves of herbal plant (Ko, Cheigh & Chung, 2014). Many studies have shown that the biologically active flavonoids molecules possess antiviral, anti-inflammatory, hepatoprotective, antioxidant, antithrombotic, vasodilating and anticarcinogenic activities (Barontini, Bernini, Crisante & Fabrizi, 2010).

Research concluded that most abundant flavonoids present in fruits and vegetables is Quercetin, (3-5-7-3',4'-pentahydroxyflavone). Quercetin can preserve food quality by preventing the oxidative deterioration of lipids due to its antioxidant activity (Liu et al., 2013). Naringenin (5,7,4'-trihydroxyflavanone) can be found in grapefruits and citrus fruits. It induced cytotoxicity and apoptosis in various cancer cell lines and no toxic effect on normal

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cells is shown at a similar dosage. Naringenin too exhibited insulin-like effect which is used to decrease apoliprotein B(ApoB) secretion in hepatocytes (Yang et al., 2013). 3-Hydroxyflavone is a polyphenolic benzo-γ-pyrone compounds, mostly found in the plants and nowadays are the major intake component of diets amongst the human (Davilla, Sancho, Almandoz & Blanco, 2012). Chrysin (5,7-dihydroxyflavone) can be found in many plants extracts, honey and propolis (Kim, Rho, Shin, Park & Kim, 2011). Research stated that apoptosis in a panel of cancer cell lines, including HeLa cervical cancer cells, U937, HL-60 and L1210 leukemia cells are induced by the Chrysin (Rashid, Ali, Nafees, Hasan & Sultana, 2014). Despite of the benefits of the flavonoids mentioned earlier, they are poorly-water soluble.

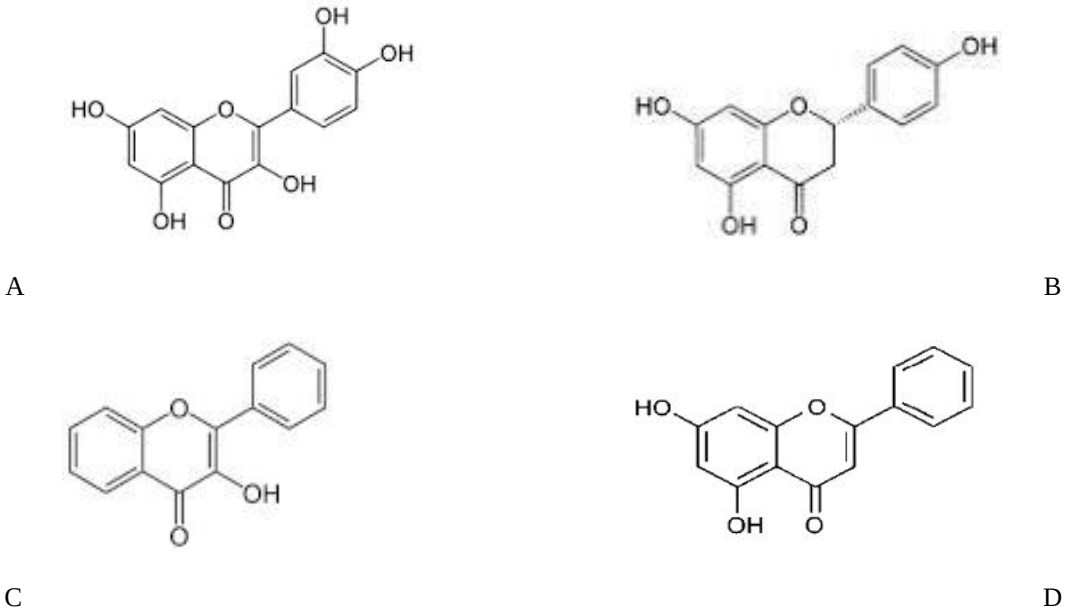

Figure 1: The structures of Quercetin (A), Naringenin (B), 3-Hydroxyflavone (C) and Chrysin (D).

β-Cyclodextrin (β-CD) is a series of cyclic oligosaccharides formed through α(1-4) ether linkages of glucopyranose units and have seven glucoside unities (Valente & Sherman, 2013). β-CD has the shape of a truncated cone with interior cavity hydrophobic that allows the formation of inclusion complexes with hydrophobic guest substances and the outer surfaces is hydrophilic. β-CD has low biotoxicity and high biocompatibility and is a suitable material used for drug inclusion. It has successfully increased the stability, solubility and bioavailibity of poorly soluble compounds in oral drug delivery (Pinho, Grootveld, Soares & Henriques, 2014).

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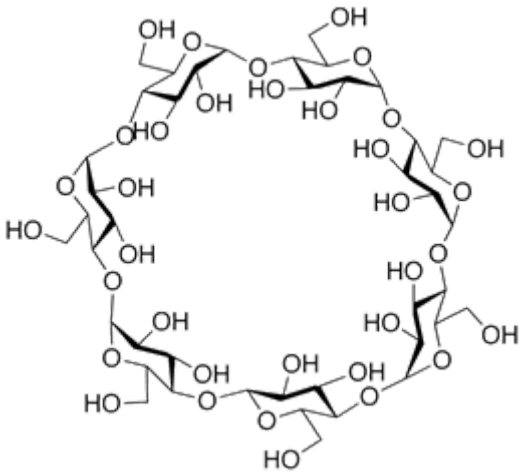

Figure 2: The structures of β-Cyclodextrin (β-CD).

In this study, the inclusion complex of Quercetin, Naringenin, 3-Hydroxyflavone and Chrysin with free β-Cylodextrin were achieved. Three methods were used to complex the host-guest molecules; the Physical Mixture, Kneading and Freeze Drying methods. The complex compounds were characterized by FT-IR spectroscopy.

2. MATERIALS AND METHODS

2.1 Research Materials

Quercetin, MW: 302.24, Naringenin, MW: 272.25, 3-Hydroxyflavone, MW: 238.24, Chrysin, MW: 254.24 and β-Cyclodextrin, MW: 1134 (Sigma Aldrich).

2.2 Preparation of Samples

Three methods were used to complex the host-guest molecules; the Physical Mixture, Kneading, and Freeze Drying. All methods applied 1:1 of host:guest molar ratio.

Physical Mixture: 22.68 mg of β-CD was weighed and the mass needed for Quercetin, Naringenin, 3-Hydroxyflavone and Chrysin were 6.00, 5.50, 5.00 and 5.00 mg, respectively. The β-CD was crushed in a mortar turning it into a powdery form. The respective flavanoid was then introduced to the powdered β-cyclodextrin (in four separate experiments). The mixture was rotated in one direction to ensure that the mixed powder was crushed and well mixed together. The pulverization process took place approximately one hour. The sample was then stored and labelled in a borosilicate glass at room temperature for further analysis.

Kneading: Using the same method as the Physical Mixture above, the next procedure follows. Ten drops of distilled water was added dropwise and mixed in the mortar until it became paste like. Lastly, the sample was stored and labelled in a borosilicate glass and the sample was freeze dried.

Freeze Drying: 37.00 mg of β-CD was weighed and the mass needed for Quercetin, Naringenin, 3-Hydroxyflavone and Chrysin were 10.00, 8.90, 7.80 and 8.30 mg, respectively. β-CD was diluted in the 10 mL of distilled water while the flavonoid (in separate experiments) was dissolved in 10 mL of NaOH solution. The β-CD-distilled water solution

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was allowed to stirr followed by the addition of flavonoids-NaOH solution dropwise to it. The mixture solution was left to stirr for 24 hours. The mixture solution was filtered by using the micro filter syringe and the solution was stored and freeze dried.

2.3 Spectral Investigations and Characterizations

An IR spectrophotometer (Thermo Scientific Nicolet 6700 FT-IR spectrometer) was used for the IR analysis. All spectras were recorded within a range of 4000–500 cm^{-1}. OMNIC software was used to show all the spectras.

3. RESULTS AND DISCUSSION

The FT-IR spectra of free β-CD, free flavonoids, complexed Physical Mixture, Kneading and Freeze Drying method are shown below. The complexations between β-CD and flavonoids were confirmed by the FT-IR spectroscopic analysis. The changes in the characteristic bands pure substances confirm the existence of the complex as a new compound with different spectroscopic bands.

3.1 β-Cyclodextrin and Quercetin

The IR spectrum of β-CD is identified by absorption peaks at 3250 cm^{-1} (OH_{str}), 2921 cm^{-1} ($CH_{aliphatic}$), 1610 cm^{-1} ($C{=}O_{str}$), 1400 cm^{-1} ($C{=}C_{aromatic}$), 1152, 1077, 1020 cm^{-1} ($C\text{-}O_{str}$). IR spectrum of Quercetin 3210 cm^{-1} (OH_{str}), 2700 cm^{-1} ($CH_{aliphatic}$), 1750 cm^{-1} ($C{=}O_{str}$), 1590 cm^{-1} ($C{=}C_{aromatic}$), 1550, 1500, 1470, 1090 cm^{-1} ($C\text{-}O_{str}$).

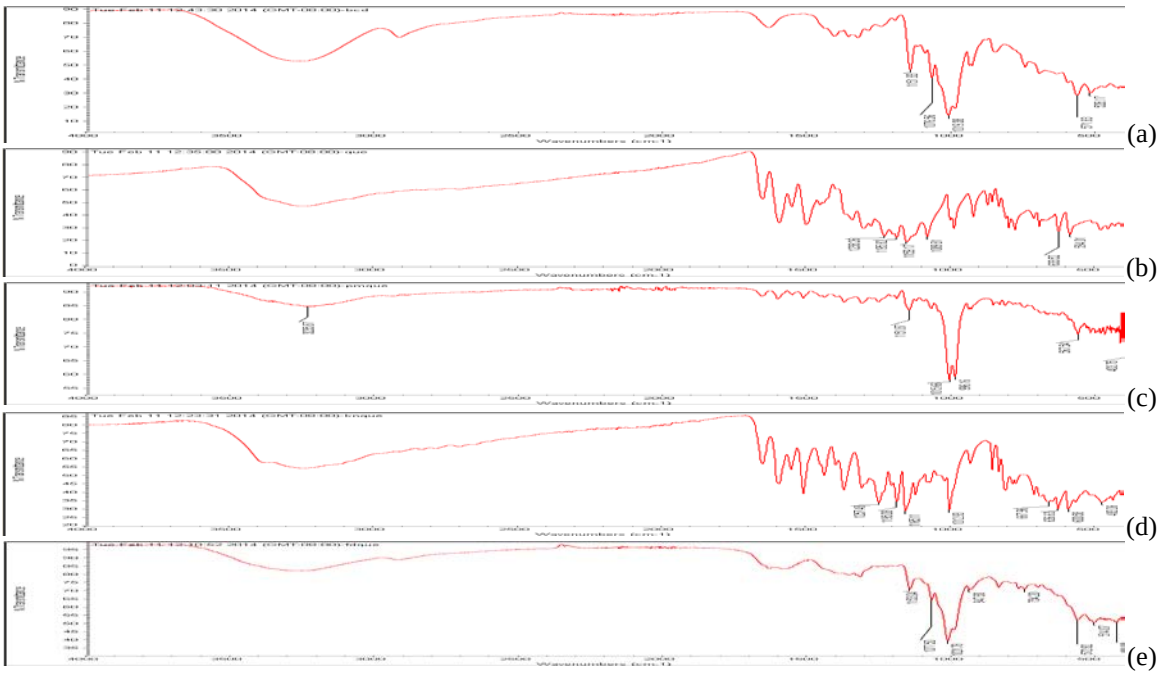

Figure 3: FT-IR spectra of free β-CD(a), free Quercetin(b), complexed Physical Mixture(c), Kneading(d), and Freeze Drying(e).

In the Physical Mixture Method at 3236 cm^{-1} (OH_{str}), the peak was broader compared to the peak of free Quercetin. The peak at 1550, 1470, and 1159 cm^{-1} disappeared greatly in the Physical Mixture Method. For Kneading method, the peak at 3590 cm^{-1} (OH_{str}) was almost

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similar to the free Quercetin but broader compared to the free Quercetin peak. The peak at the 1750, 1590, 1550, 1500, 1470, 1300, 1238 and 1196 cm^{-1} have shifted to 1650, 1600, 1500, 1350, 1300, 1258, 1196, and 1165 cm^{-1} respectively. In the Freeze Drying method, the peak at 3290 cm^{-1} is less sharp than free Quercetin and is broader than the Physical Mixture method peak. The peak at 1750, 1590, 1550, 1500, 1470, 1300, 1238 and 1196 cm^{-1} have shifted to 1690 ($C=O_{str}$), 1610 ($C=C_{aromatic}$), 1590, 1310, 1153, 1078, 1022 ($C-O_{str}$) and 948 cm^{-1}.

The suitable method for the inclusion complex between Quercetin and β-CD is suggested to be the Kneading method. This is due to the great difference in peaks shifting compared to the Physical Mixture and Freeze Drying method. Kneading method has the sharpest peak amongst the methods applied.

3.2 β-Cyclodextrin and Naringenin

IR spectrum of Naringenin is identified by absorption peaks at 3370 cm^{-1} (OH_{str}), 2900 cm^{-1} ($CH_{aliphatic}$), 1640 cm^{-1} ($C=O_{str}$), 1460 cm^{-1} ($C=C_{aromatic}$), 1390, 1300, 1210, 1154, 1009 cm^{-1} ($C-O_{str}$).

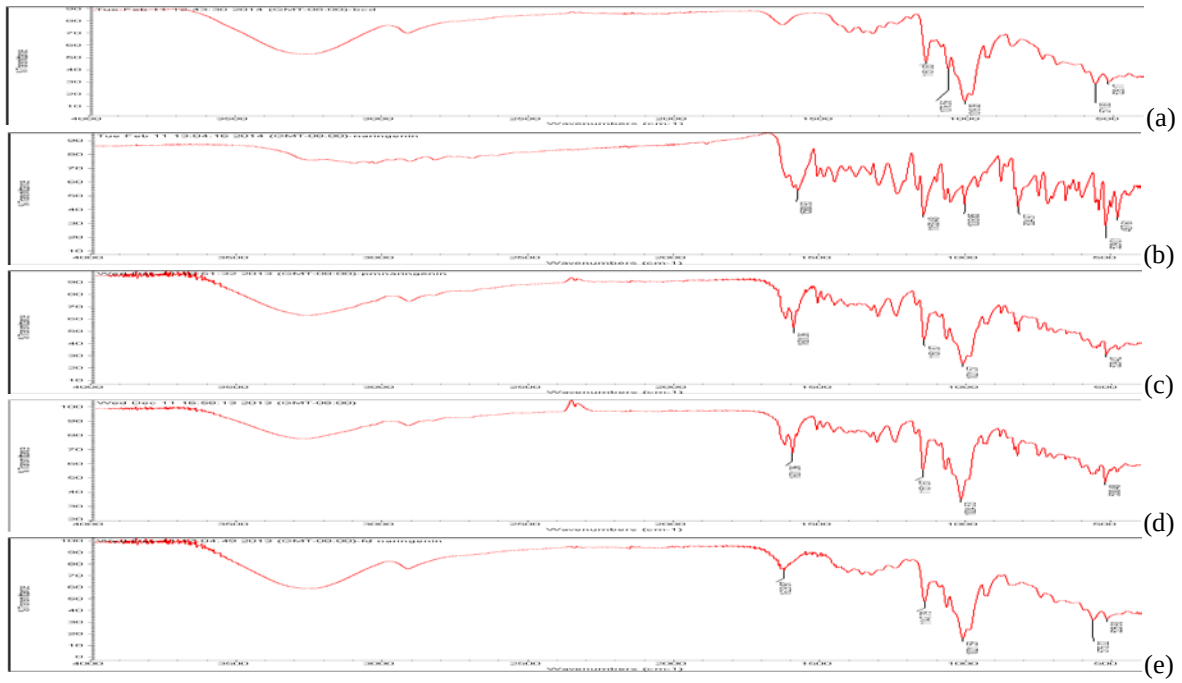

Figure 4: FT-IR spectra of free β-CD(a), free Naringenin(b), complexed Physical Mixture(c), Kneading(d), and Freeze Drying(e).

In the Physical Mixture method, at 3290 cm^{-1} (OH_{str}), the peak was sharper compared to the peak of free Naringenin. The sharp peaks at 1460, 1390 and 1300 cm^{-1} were found to be disappeared. The peak at 1620, 1581, 1460, 1390, 1300, 1210, 1154 and 1009 cm^{-1} have shifted to 1640 ($C=O_{str}$), 1610, 1600 ($C=C_{aromatic}$), 1300, 1210, 1152, 1022 ($C-O_{str}$) and 910 cm^{-1} respectively. For Kneading method, the peak at 3280 cm^{-1} (OH_{str}) was sharper compared to the free Naringenin but broader compared to the Physical Mixture method and the peak of free β-CD. The peak at the 1620, 1581, 1390, 1300, 1210, 1154 and 1009 cm^{-1} have shifted to 1640 ($C=O_{str}$), 1503 ($C=C_{aromatic}$), 1300, 1152, 1090, 1025 ($C-O_{str}$) and 820 cm^{-1} respectively. In the Freeze Drying method, the peak at 3270 cm^{-1} is sharper than free

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Naringenin and almost similar to the free β-CD peak. At the peak 1640 cm^{-1} ($C=C_{aromatic}$) has less sharp peak compared to the free Naringenin peak. The peak at 1620, 1460, 1390, 1300, 1210, 1154 and 1009 cm^{-1} have shifted to 1640 ($C=O_{str}$), 1400 ($C=C_{aromatic}$), 1305, 1148, 1080, 1022 ($C-O_{str}$) and 905 cm^{-1}.

The best method for inclusion complex between Naringenin and β-CD is suggested to be the Freeze Drying method because it shows a sharper and smoother peak compared to the Kneading Method peak and Freeze Drying Method peak.

3.3 β-Cyclodextrin and 3-Hydroxyflavone

IR spectrum of 3-Hydroxyflavone is identified by absorption peaks at 3175 cm^{-1} (OH_{str}), 2990 cm^{-1} ($CH_{aliphatic}$), 1750 cm^{-1} ($C=O_{str}$), 1467 cm^{-1} ($C=C_{aromatic}$), 1270, 1209 and 1119 cm^{-1} ($C-O_{str}$).

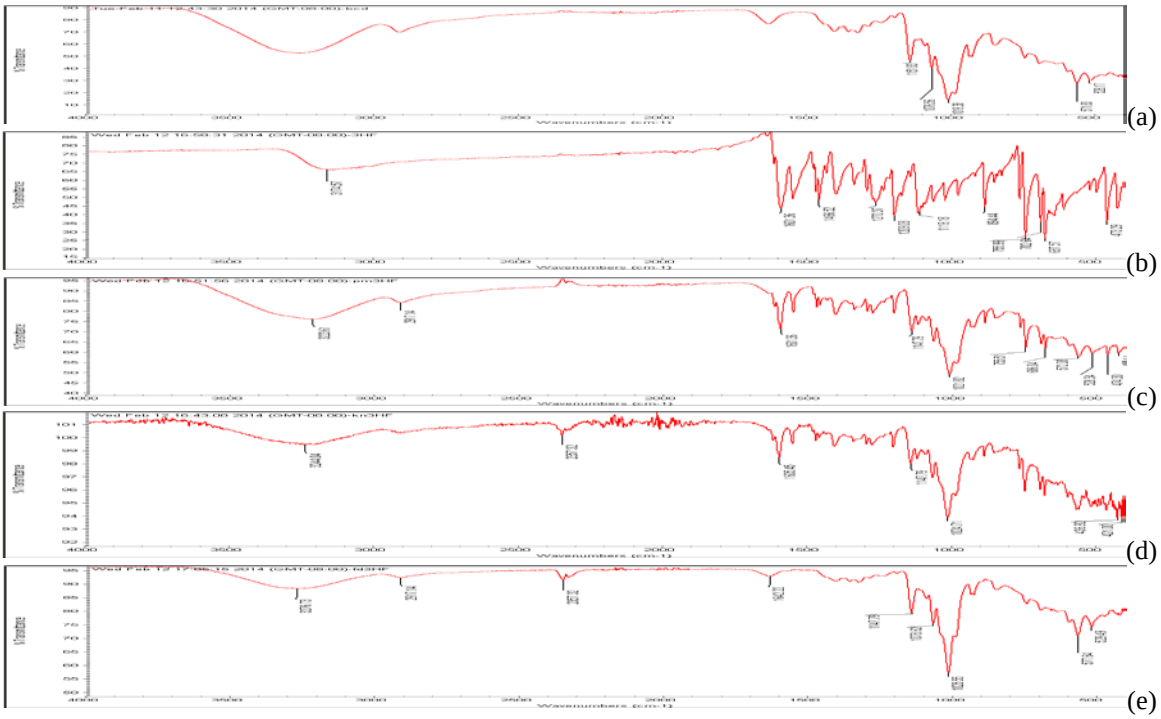

Figure 5: FT-IR spectra of free β-CD(a), free 3-Hydroxyflavone(b), complexed Physical Mixture(c), Kneading(d), and Freeze Drying(e).

In the Physical Mixture method, at 3224 cm^{-1} (OH_{str}), the peak was less sharp compared to the peak of free 3-Hydroxyflavone. The peaks at 1750, 1467, 1270, 1209 and 894 cm^{-1} have shifted to 1640 ($C=O_{str}$), 1410 ($C=C_{aromatic}$), 1148, 1022 ($C-O_{str}$) and 756 cm^{-1} respectively. For Kneading method, the peak at 3244 cm^{-1} (OH_{str}) was broader compared to the free 3-Hydroxyflavone and the Physical Mixture method. The peaks at 1750, 1601, 1467, 1270, 1209 and 1119 cm^{-1} have shifted to 1800 ($C=O_{str}$), 1606, 1405 ($C=C_{aromatic}$), 1400, 1148 and 1025 cm^{-1} ($C-O_{str}$) respectively. In the Freeze Drying method, the peak at 3277 cm^{-1} (OH_{str}) is less sharp than the free 3-Hydroxyflavone. The peaks at 1750, 1601, 1467, 1270, 1209 and 1119 cm^{-1} have shifted to 2357, 1642 ($C=O_{str}$), 1401 ($C=C_{aromatic}$), 1148, 1079 and 1026 cm^{-1} ($C-O_{str}$) respectively.

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The suitable method for the inclusion complex between 3-Hydroxyflavone and β-CD is suggested to be the Physical Mixture method as it inhibits the sharpest peak compared to the Kneading method peak and Freeze Drying method peak.

3.4 β-Cyclodextrin and Chrysin

IR spectrum of Chrysin is identified by absorption peaks at 2627 cm^{-1} (OH_{str}), 2880 cm^{-1} ($CH_{aliphatic}$), 1646 cm^{-1} ($C=O_{str}$), 1447 cm^{-1} ($C=C_{aromatic}$), 1350, 1167 and 1029 cm^{-1} ($C-O_{str}$).

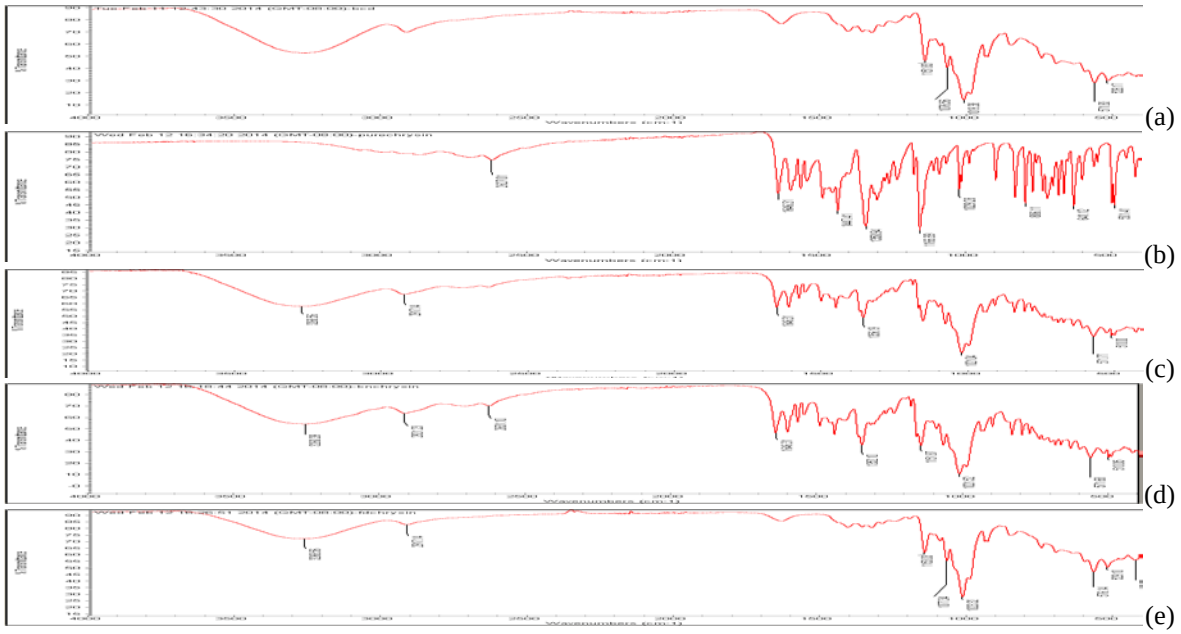

Figure 6: FT-IR spectra of free β-CD(a), free Chrysin(b), complexed Physical Mixture(c), Kneading(d), and Freeze Drying(e).

For the Physical Mixture method, at 3269 cm^{-1} (OH_{str}), the peak has a sharper peak compared to the peak of free Chrysin which is broad. The peaks at 1646, 1447, 1350, 1167 and 1029 cm^{-1} have shifted to 2605, 1646 ($C=O_{str}$), 1620 ($C=C_{aromatic}$), 1356, 1021 ($C-O_{str}$), 572 and 510 cm^{-1} respectively. In the Kneading method, the peak at 3260 cm^{-1} (OH_{str}) was sharper compared to the free Chrysin and slighty sharper than the β-CD. The peaks at 1447, 1350, 1167 and 1029 cm^{-1} have shifted to 1620 ($C=O_{str}$), 1352, 1152, 1022 ($C-O_{str}$), 572 and 510 cm^{-1} respectively. For the Freeze Drying method, the peak at 3269 cm^{-1} (OH_{str}) is sharper than the free Chrysin. The peaks at 1646, 1447, 1167 and 1029 cm^{-1} have shifted to 1620 ($C=O_{str}$), 1400 ($C=C_{aromatic}$), 1153, 1077 ($C-O_{str}$), 910 and 576 cm^{-1} respectively.

The suitable method for the inclusion complex between Chrysin and β-CD is suggested to be the Kneading method as it illustrated a sharper peak compared to the Physical Mixture method peak and Freeze Drying method peak.

4. CONCLUSION

From this study, it is suggested that the inclusion complexes between β-CD and the selected flavonoids (Quercetin, Naringenin, 3-Hydroxyflavone and Chrysin) have taken place. These were proven by the significant changes in data gained from the FT-IR analysis. However, different methods used in the complexation procedure affect the complexation process

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between the host and guests. Thus, the inclusion complex between β-CD and the guest molecules is a promising method to produce flavonoid base-loaded product with improved solubility in water. Kneading method was suggested the best method in the complexation between β-Cyclodextrin and Quercetin as well as Chrysin. However, Freeze Drying and Physical Mixture method were suggested best in the complexation with Naringenin and 3-Hydroxyflavone respectively.

5. ACKNOWLEDGEMENT

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