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Research paper

Characterisation of Catfish (*Clarias batrachus)* Waste Oil: γ-Cyclodextrin Inclusion Complex

Zaibunnisa Abdul Haiyee[1*], Nor Izzatul Adyani Yahya[1], Norizzah Abd Rashid[1], Norakma Mohd Nor[1], Dzulkifly Mat Hashim[2]

[1] *Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia*
[2] *Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding author E-mail: nisha@salam.uitm.edu.my*

Abstract

Catfish is a cheap source of essential omega-3 fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In this study, catfish waste oil was extracted and clean-up using pressurised liquid extraction (PLE) from viscera of catfish (*Clarias batrachus*). However, the characteristics of catfish waste oils are sticky, strong fishy odour, easily oxidised with short shelf-life. Catfish waste oil was converted into powder by formation of inclusion complex with γ–cyclodextrin. Inclusion complex was prepared by using co-precipitation and kneading methods and compared with physical mixture. The inclusion complex formed were characterised by using Field Emission Scanning Electron Microscopy (FESEM), Differential Scanning Calorimeter (DSC) and Fourier Transform Infrared Spectroscopy (FTIR. Results from FESEM images revealed that formation of inclusion complex using co-precipitation and kneading methods has smaller in crystal sizes and appeared as different morphology compared to physical mixture. DSC proved that co-precipitation method was able to formed new solid phase due to shifting of melting point to the higher temperature (130 ºC). FTIR supported the result by reduction of intensity of C-H band in co- precipitation which results a formation new solid phase. Therefore, co-precipitation method was able to successfully produce inclusion complex of catfish oil: γ-cyclodextrin.

Keywords: *catfish oil; γ-cyclodextrin; co-precipitation; kneading; physical mixture.*

1. Introduction

The walking catfish, *Clarias batrachus* represents a cheap priced food fish and is one of the most preferred fresh water fish consumed by Malaysians [1-2]. In commercial catfish operations, the viscera of Clarias species are considered as waste. Normally, the catfish flesh is utilised for con-sumption, while the viscera are discarded and cleaned from catfish, thus creating waste disposal problems [3]. Most of the oil in catfish is found in the viscera, which contain approximately 33 percent fats or other lipids which could be converted into edible oil [4]. The oil extracted from fish is rich in polyunsaturated fatty acids (PUFAs) especially those of the omega-3 family, mainly eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). The importance of PUFAs in human health and nutrition is well recognised in preventing cardiovascular diseases, Alzheimer's disease and psoriasis [1-5].

One of the major drawbacks of oils containing a high amount of omega-3 PUFA such as fish oils is their susceptibility to oxidation which involves the formation of toxic products such as peroxides or volatile compounds which contribute to undesirable off flavours [5]. Encapsulation is a technology of packing solid, liquid or gaseous materials in miniature sealed capsules for release at controlled rates using desired released triggers [6]. Some researchers have reported that the encapsulation of fish oil significantly retards lipid oxidation. It can also mask the objectionable odours caused by volatile oxidation products and enhance the odours of fish oil–enriched products [5]. Cyclodextrin has been extensively used in areas such as pharmaceutical, cosmetic and food industries because it is inexpensive and nontoxic. Using the molecular inclusion method, cyclodextrin can solubilise and stabilise active compounds on the molecular scale. Cyclodextrin can form solid phases by self-assembly. This process depends on the solubility, temperature, pH, molecular weight and chemical nature of guest materials [6].

By means of enzymatic conversion, cyclodextrins are produced which containing of six α-cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds which act as flavour masker which also can prevent against oxidation, light-induced decompositions and heat-induced changes. Moreover, cyclodextrins improve shelf life of food products and mask or reduce undesired taste [4-6]. However, encapsulation of catfish oil with γ-cyclodextrin has not been carried out before.

Several methods can be used to produce inclusion complex; co-precipitation, and kneading [7]. Physical mixture product will usually act as a control [8]. Characterisation of the encapsulated products was performed to confirm formation of inclusion complex. The inclusion complex formed was differentiated using FTIR, FESEM and DSC. The stable inclusion complex can be applied as food antioxidant, natural colouring agent and also as nutrient. This encapsulated inclusion complex also will be protected from flavour, aroma and color degradation during storage. The resultant water-soluble fish oil in powdered form has many applications such as food, pharmaceutical and medical fields. In addition, results from this study will be useful for future research that involves other fish oils that contain high PUFAs especially DHA and EPA.

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2. Materials and methods

2.1. Materials

The chemicals used in this study were either analytical or GC grade. Ethanol (96% purity) and n- Hexane (99% purity) used were of European Pharmacopeia (Ph Eur.) grade (MERCK, Germany). Deionised water used was purified by Milli-Q purification system (Millipore) (Massachusetts, USA). EPA Standard (Aldrich, USA) and DHA (Fluka) were obtained from MERCK, Germany. γ-cyclodextrin (BCD) (purity: 99.5%) was purchased from Wacker-Chemie GmbH (Munchen, Germany) and KBr powder (BDH, UK) for FTIR analysis.

2.2. Sample preparation

Fresh catfish (*Clarias batrachus*) weighing around 1.2 to 1.5 kg were obtained from a farm fish located in Kajang, Selangor. The maturity of catfish harvested was in between four to five months. The raw materials were transported to the laboratory under ice. Upon arrival, the fishes were filleted and the viscera was manually removed by using a sharp knife. After filleting, the fish viscera were cleaned by tap water for three times and drained. The fish viscera were then ground with a commercial blender for 10 minutes and frozen at -20 ºC with a maximum storage of less than 2 months before use. Prior to PLE extraction methods, samples were dried using freeze dryer at -50 ºC for 5 days. The dried samples were frozen at -20 ºC until used.

2.3. PLE extraction

The extraction of catfish waste oil was used the combination of purification and extraction using rapid method which is Pressurised Liquid Extraction (PLE). Extraction of catfish waste oil was done by using Accelerated Solvent Extractor ASE 200 (Dionex Ltd. Camberly, Surrey, UK). The mixture of samples and Diatomaceous earth (DE) were placed into the extraction cell. The position of sample, dispersing agent and adsorbents (bentonite clay) in the extraction cell were shown in Fig. 1 before being placed into the carousel of ASE 200 and carried out the extraction process.

The cells were prepared with cellulose filter at the bottom end and addition with different amount of absorbents of 0.8g. About 3 g of catfish dried viscera was thoroughly mixed with diatomaceous earth (DE) in the 22 ml extraction cell. Addition of diatomaceous earth was done to improve dispersion of sample in sample cells thus prevent lumpiness. The sample cells were closed to finger tightness before being placed into the carousel of the ASE 200 system. Sample was extracted using non –polar solvent, n- Hexane of 99% purity (MERCK, Ph. Eur), with temperature of 96°C, pressure of 2196 psi and static time of 17 minutes [9-10].

The extracted analytes were purged from the sample cell using pressurised nitrogen (861- 1034 kPa). Once extraction was completed, nitrogen gas was used to compress and carry all solvent from the extraction cell move to the collecting vial for further process. The extracted oil was evaporated to dryness by using a rotary evaporator BUCHI Evaporator R- 210 (Flawil, Switzerland) to calculate the yield of extracted catfish waste oil prior to being subjected to GC/MS analysis. As for PLE extraction, 20 mL of extract obtained was transferred to 20 mL *n*-hexane portion using liquid-liquid extraction method. This method was done in triplicate and collected before evaporated to 1 mL and subjected to GC/MS analysis.

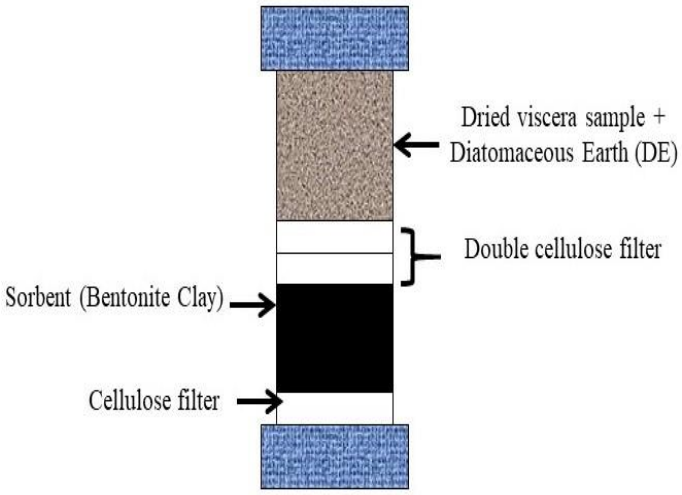

Fig. 1: A Schematic Diagram of ASE 200 Extraction Cell with In-Situ Clean Up

2.4. Inclusion complex

The inclusion complex of catfish oil: GCD (1:8) was prepared using the kneading and co-precipitation methods. The kneading method and physical mixture as control were as reported by [7]. The method for co-precipitation was that of [8].

2.4.1. Kneading method

Extracted catfish waste oil and γ-cyclodextrin with molar ratio 1:8 was placed in a mortar and kneaded for 45 minutes. During the kneading, 40% ethanol: water (27:75 v/v) mixture was combined to the mixture to maintain a proper consistency. The product then was dried at 40ºC for 24 hour and gently sieved through 150 μm mesh (Endecotts Ltd., England) [7].

2.4.2. Physical mixture

Physical mixture of molar ratio 1:8 of extracted catfish waste oil and γ-cyclodextrins was also prepared by dry-pestling in a mortar and kneaded for 5 minutes as a control. The inclusion complex then was dried at 40 ºC for 24 hour and gently sieved through 150 μm mesh (Endecotts Ltd., England) [7].

2.4.3. Co- precipitation method

Extracted catfish waste oil was placed to screw capped vials containing γ-cyclodextrin in ethanol: water (25:75 v/v) mixture (5 ml) with the molar ratio for extracted catfish waste oil: γ-cyclodextrins (1:8). The vials were stirred at 30ºC until equilibrium reached, i.e. for 48 hours, on shaking water bath (Memmert, Germany). Samples were stored at 5ºC for 1 hour. The samples were centrifuged using Clement (Sydney, Australia) at 3000 rpm for 10 min and the supernatant was decanted to provide the complex as microcrystalline precipitate. The product was dried at 40ºC for 48 hours. The dried mass was pulverized and sieved through 150 μm mesh (Endecotts, England [8].

2.5. Characterisation of the inclusion complex

2.5.1. Differential Scanning Calorimetry (DSC)

The inclusion complex of DSC analyses was carried out using Perkin Elmer DSC1 STAR System. 5 mg sample was placed in aluminium pans and heated at 10°C/min in the temperature range of 0 °C to 300 °C. The measurements were carried out under dry nitrogen at a flow rate of 50 mL/min [8].

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of catfish waste oil, GCD, their physical mixture and the inclusion complex were collected between 4000 and 400 cm^{-1} using a Perkin Elmer Model GX FTIR spectrophotometer (Waltham, MA, USA), with 16 scans at a resolution of 1 cm^{-1} [8].

2.5.3. Field Emission Scanning Electron Microscopy (FESEM)

The surface morphology of the dried samples was examined using a scanning electron microscope, Model Supra 40VP (GEMINI). The samples were attached to SEM aluminium stubs via 2-sided adhesive tape. The attached powder was then sputter coated for 50 s at a beam current of 0 mA to obtain a 15 nm layer of gold-palladium alloy and examined using a scanning electron microscope operating at 5 Kv [8].

2.6. Statically analysis

A one-way analysis of variance (ANOVA) and Duncan's Multiple Range test ($p < 0.05$) were used to establish the significance of differences in analysis data. The analyses were performed using SPSS 16.0 for Windows software.

3. Results and discussion

3.1. Differential Scanning Calorimetry (DSC)

The thermal behavior of catfish waste oil with γ-cyclodextrin (GCD). inclusion complex was studied using Differential Scanning Calorimetry (DSC). The thermograms of pure GCD and catfish waste oil-BCD physical mixture, kneading, co-precipitation and pure waste catfish oil, were represented in Fig. 2.

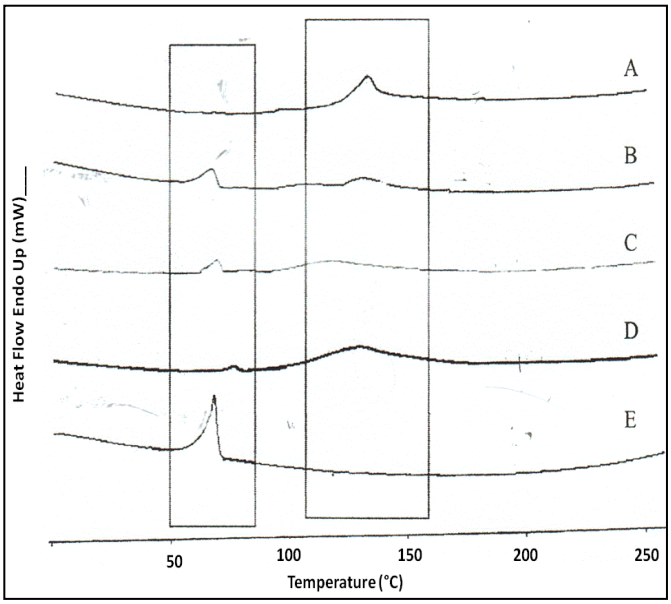

Fig. 2: The DSC thermograms of GCD (A), GCD-catfish waste oil physical mixture (PMM) (B), GCD-catfish waste oil kneading method (KM) (C), GCD-catfish waste oil co-precipitation method (CPM) (D), and catfish waste oil (Oil) (E).

The appearance of melting peak for the catfish waste oil was at 69 °C (Fig. 2E). The thermograms show the endothermic peak reduced intensity in all treatments which indicated the formation of new complexes (Fig. 2B− D). The melting peak catfish waste oil was reduced in intensity for physical mixture and kneading, whereas this melting peak cannot be seen in co-precipitation complexes. These results indicate that there are interactions between catfish oil and GCD in inclusion complex. The DSC thermogram of GCD exhibited an endothermic peak in the 120− 140 °C interval (corresponding to its melting point) (Fig. 2A). As for physical mixture (Fig. 2B), occurrence of two melting peaks correspondence to pure GCD and catfish waste oil indicates no interactions exist between those two compounds [11].

The almost disappearance of GCD melting peak can be seen in thermogram of kneading method (Fig. 2C). This indicates to a partial complexation of catfish waste oil in GCD cavity, with formation of new crystalline structure in which not all of catfish waste oil was complexed with GCD. This result supported the study by [12] where the disappearance of Risperidone endothermic peak occurred for kneaded sample. Formation of a new single broad melting peak of co-precipitation at 130 °C indicates the formation of a new solid phase of catfish waste oil-GCD complex (Fig. 2D). Formation of inclusion complex causes increased in melting temperature, interaction of the guest with the cyclodextrin provides a higher energy barrier to overcome volatilization [13]. These DSC results were coupled with further analytical analysis to verify the existence of inclusion complex formation.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a relevant method to differentiate the inclusion complex [15]. Hence, the complexation between γ-cyclodextrin and catfish oil was investigated by using FTIR in this study

The stretching region of hydroxyl group, -OH was shown at the band range of 3600- 3200 cm^{-1}. As shown in Fig. 3, the band at 3400 cm^{-1} indicates the presence of hydroxyl group in the catfish oil. The presence of water in GCD resulted in the presence of broad peak of O-H which masks the presence of –OH in catfish waste oil.

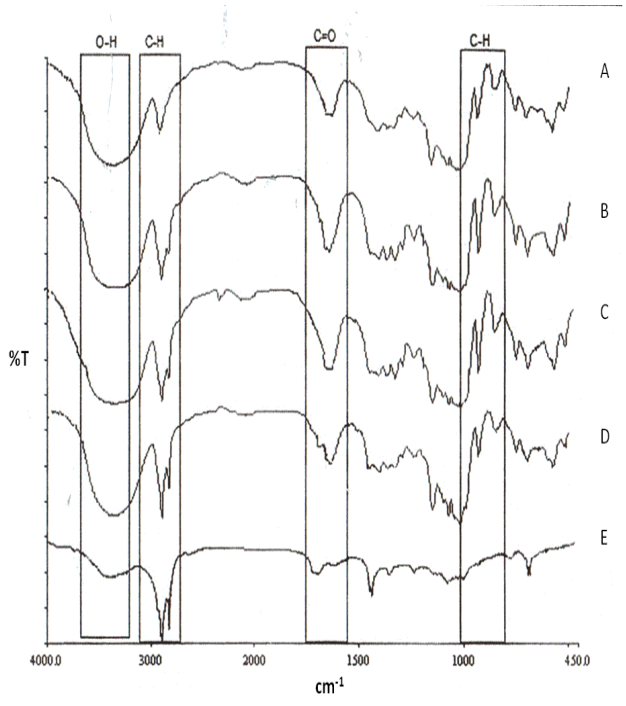

Fig. 3: A FTIR spectrum of GCD (A), GCD- catfish waste oil co-precipitation method (B), GCD-catfish waste oil kneading method (C), GCD- catfish waste oil physical mixture (D) and catfish waste oil (E).

The FTIR spectra of complexes (Fig. 3B- D) appeared quite the same for pure GCD, but less similar to pure catfish waste oil. This phenomenon was reported by [15] where the toroidal cavity structure of GCD act as a shield to the guest catfish waste oil. During encapsulation, the molecules of catfish waste oil rearrange themselves to fit the host, this give a final configuration almost similar to the pure GCD as the host. The prominent FTIR spectra of GCD can be seen in the region of 3800- 3070 cm^{-1}, related to the vibra-

tion of bonded hydroxyl O-H group and 1700- 1500 cm^{-1} assigned for carbonyl C=O stretching. In the carbonyl region, spectrum of physical mixture (Fig. 3D) appear as double peak at 1650 cm^{-1} which indicates that the catfish oil (peak at 1740 cm^{-1}) properties was still in the detected. Thus, complexation process was not completely occurred.

A prominent characterisation of catfish waste oil (Fig. 3E) can be seen in the band range 3100- 2800 cm^{-1} where the stretching of C-H band took place. The physical mixture spectrum shows almost similar to pure catfish waste oil, which indicates that no complexes between GCD and catfish oil waste been developed. The least intensity of the other two complexes co-precipitation (Fig. 3B) and kneading (Fig. 3C) relates to which the C-H band of the catfish waste oil has been used during complexation process. A stronger interaction of inclusion complex might be seen in co-precipitation process due to the almost similar peak to the pure GCD as compared to kneading [17]. The small changes in FTIR spectra of all treatments suggest a weaker interaction of GCD for catfish waste oil.

3.3. Field Emission Scanning Electron Microscopy (FESEM)

FESEM provides qualitative information on study of pure substance and complexes formation by different methods of preparation. In recent study, FESEM observation was done in collaboration with DSC and FTIR to verify the occurrence of GCD-catfish waste oil complexes formation. This helps to assess existence of any products during the process [8]. The FESEM photographs of pure GCD; co-precipitation, kneading and physical mixture products at 2500 times magnification are shown in Fig. 4.

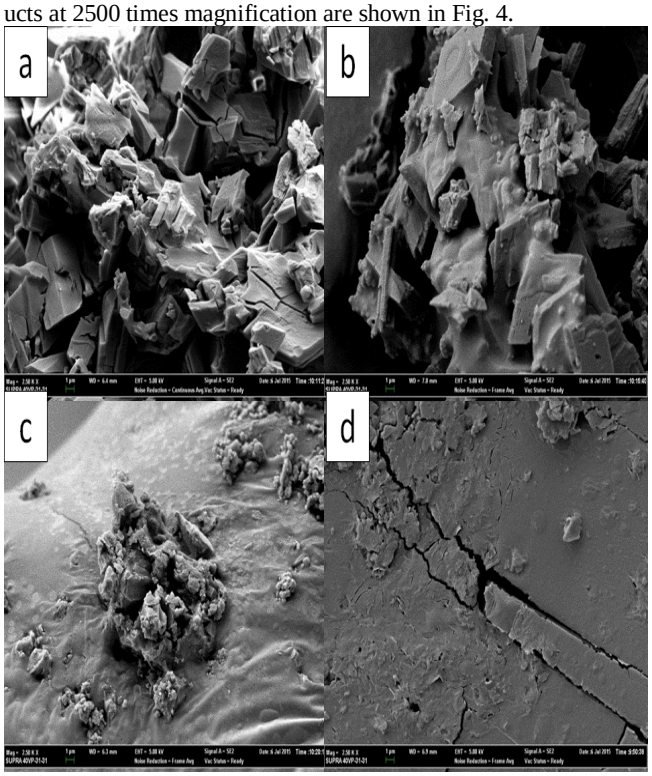

Fig. 4: A FESEM illustration of a) γ-cyclodextrin (GCD), b) co-precipitation, c) kneading and d) physical mixture at magnification x 2500.

As a general observation, the complexes were found slightly distorted (Fig 4). The expected change of the crystal habit and shape due to the inclusion of catfish waste oil with cyclodextrin can be seen. As shown in Fig.4 (a), particle size for pure GCD was appeared bigger than other samples. It appears that the commercial pure GCD was in structured, plated and parallelogram shape under high FESEM magnification (Fig. 4(a)). The shape of kneaded complexes GCD as shown in Fig. 4(c) appear quite different from the pure cyclodextrins (Fig. 4(a)). The kneaded products of GCD-catfish waste oil are more translucent and appears as irregular. This shows that catfish waste oil might not be fully form complexes with GCD that is the bonds formed inside GCD cavity are very weak [15]. Catfish waste oil might only appear on the surface of GCD. From the micrographs (Fig. 4(b)), a uniform structured shape of co-precipitation products can be observed in GCD-catfish waste oil which indicates a formation of new solid compound. This finding is similar to [16] where in their study, the complexation of alpinetin with hydroxypropyl beta cyclodextrin appears as compact and plate–like structure which differs from the pure compounds. Physical mixture of GCD with catfish waste oil (Fig. 4 (d)) forms sticky and clumps to each other. This appears as big oily agglomerates under FESEM observation. Not only that, the oiliness of structure can be seen on the surface of the complexes and therefore, inclusion complex may not form for GCD using this physical mixture method.

Co-precipitation and kneading methods give evidence formation of new solid phase. In the co-precipitation samples, the original morphology of the raw materials disappeared, and it was not possible to differentiate the final products from the starting materials [17]. Thus, it concludes that the data obtained from FESEM indicated that inclusion complex been slightly formed using co precipitation and kneading methods. However, physical mixture was unable to form inclusion complex. This finding supported by [17] which stated that the new solid phase of oleoresin and cyclodextrins formed using the co-precipitation method appeared as a crystal structure which was completely different from the original morphology of its pure compounds.

4. Conclusion

The interaction between C-H and GCD demonstrated in the formation of an inclusion complex. The formation of the inclusion complex was investigated and confirmed using DSC, FTIR and FESEM. The results obtained from this study will be very useful for producing odourless catfish oil powder from cheap source. Kneading and physical mixture were able to form loose interaction inclusion complex. However, co-precipitation was the chosen method.

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