INCLUSION COMPLEXES OF CAROTENOIDS EXTRACTED FROM PEANUT BUTTER FRUITS PULP WITH β-CYCLODEXTRIN TO ENHANCE THE AQUEOUS SOLUBILITY OF CAROTENOIDS

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Carotenoids (car) are naturally occurring multifunctional plant pigments. Car consists of a long-conjugated polyene chain having 40 carbon with eight isoprene units. Peanut butter fruits (Bunchosia glandulifera) are a good source of car, vitamins, polyphenols and natural sugars. β-carotene, lutein, and zeaxantheins are among the major types of car present in Peanut butter fruits pulp. They have excellent antioxidant properties, provitamin A sources, and prevents chronic diseases such as cancer. Despite these functions, carotenoids have major limitations as a nutraceutical supplement, such as less water solubility and susceptibility to processing and storage due to oxygen sensitivity and photosensitivity. To overcome these limitations encapsulation technology is used in the food and pharmaceutical industry. The goal of this study was the formation of inclusion complexes with selected carotenoids extraction from Peanut butter fruits extraction and β-cyclodextrin using nano-encapsulation techniques which are co-precipitation and extended co-precipitation. Inclusion complexes enhance the bioavailability and solubility of β-carotene. β-cyclodextrin acts as a host molecule and the guest molecule is β-carotene. Solvent extraction was used for an efficient β-carotene extraction process from Peanut butter fruits. Characterisation of β-carotene was done by using UV-Visible spectroscopy, FT-IR, and high-performance liquid chromatography. Inclusion complexes were prepared from car and β-cyclodextrin in a 1:1 stoichiometric ratio. The complexes were characterized by using UV-Vis spectroscopy and FT-IR. UV-Vis spectral analysis, it is clear that intensity of maxima in the inclusion complex prepared by extended co-precipitation is more than compared to maxima of inclusion complex prepared by co-precipitation, suggesting a better inclusion by extended co-precipitation method. In the FT-IR spectra important changes in the polymeric hydroxyl stretching region (3000-3600 cm^{-1}) appeared more in both types of inclusion complexes. The obtained data showed that the extended co-precipitation method resulted in better yield compared to co-precipitation.

Keywords – carotenoids (car), β-cyclodextrin, encapsulation, co-precipitation, Peanut butter fruits

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1. INTRODUCTION

Carotenoids (car) are naturally occurring plant pigments that function as light harvesting. These essential pigments are synthesized by plants and some microorganisms but are not synthesized by humans and animals, they are also present in blood and tissues. Car consist long conjugated polyene structure which contain eight isoprene units. They have important nutritional value as the precursor of vitamin A which is formed by cleavage at the central double bond of β-carotene. Consumption of car in sufficient amounts involves preventing many chronic diseases such as cancer, vision impairment, diabetes, cardiovascular diseases, and risk of cataract formation. Car potent antioxidant that scavenges free radicals and reactive oxygen from the system. The major limitations of car are aqueous insolubility and stability. To overcome these limitations encapsulation technology is used in the pharmaceutical, cosmetics, and food industries. Car as an active core molecule can be reversibly entrapped into the cavity of cyclodextrin in an aqueous environment Inclusion complexes are a nano-encapsulation technique which is increased the stability, solubility, and bioavailability of guest molecules. β-cyclodextrin was the host molecule for these inclusion complexes which allow the encapsulation of hydrophobic molecules. β-cyclodextrin is a cyclic oligosaccharide containing seven D-glucose molecules linked together by α 1-4 glycosidic linkages. The uses of these encapsulation techniques with natural extracts have been of great commercial interest in the food and pharmaceutical industries due to the great thermal, chemical, and color stability and less toxicity rather than the controversial uses of artificial pigments. Therefore, the need to use safe natural extract pigment from Peanut butter fruits was used in these inclusion complexes. Peanut butter fruit is a good source of bioactive compounds such as car. Among these car mainly contains β-carotene, lutein, and zeaxanthin as car pigments.

This investigation aimed to prepare inclusion complexes between from car from Peanut butter fruits extract and β-cyclodextrin using two different procedures namely co-precipitation and extended co-precipitation. The obtained complexes were characterized using FT-IR and UV-Visible spectroscopy.

2. METHODOLOGY

2.1 Materials

Acetone and Petroleum Ether (B.P. 40 – 60 °C), anhydrous sodium sulphate, KOH, N_2 gas, β-cyclodextrin, ethanol were purchased analytical grade chemicals.

2.2 Carotenoids extraction from Peanut butter fruits

Specimens of fruits were collected from home gardens from same natural conditions. Red sticky fruit pulp (20.00 g) following standard extraction procedures using cold acetone and filtration by suction. The extracted car partitioned using petroleum ether. The partitioned solution was saponified with 10% KOH. The saponified solution was concentrated using a rotary evaporator (BUCHI Rotary R-114) at 37 °C. Remaining petroleum ether evaporate by using a Nitrogen stream.

It is noted that the entire car extraction, partition, solvent evaporation procedures were carried out under subdued light and all the glass wares were covered with aluminium foils. In a nitrogen atmosphere reduce the impact of atmospheric oxygen thereby preventing isomerisation and direct exposure to the sunlight is caused to photo-degradation of car. (Rodriguez-Amaya, 2001)

2.3 Preparation of Inclusion complex

2.3.1 Co-precipitation

Equimolar concentration of car extract and β-cyclodextrin mix with 5 ml of solvent ethanol: water. 25:75 (v/v). The two solvents were mixed and stirred at room temperature in 24 h. Then the mixture was kept in 5 °C water bath for 1 h to crystallization. The mixture was centrifuged at 4000 rpm for 20 min. The supernatant was decanted and formed precipitate was collected. The crystals were dried at 37 °C until dry mass was obtained. (Cruz & Flores, 2020)

2.3.2 Extended co-precipitation

Equimolar concentration of car extract and β-cyclodextrin mix with 10 ml of ethanol and water 50: 50 (v/v).The solution of Peanut butter fruits extract was transferred drop wise in to β-cyclodextrin solution under cold condition and continuously stirring for 15 min. After that the solution mixer was ultasonicated using ultrasonic bath in cold condition. The obtained suspension was kept overnight at 4 °C for crystals formation. The suspension was concentrated using vacuum evaporation. The crystals were dried at 37 °C until dry mass was obtained. (Kaur, Bawa, & Singh, 2016)

2.4. HPLC analysis of car extracted from Peanut butter fruits pulp

Peanut butter fruits pulp was characterised using HPLC series 1260 (Agilent waldbronn Germany), equipped with a photodiode array/ multi-wavelength detector. Reversed phase HPLC on a C_{18} column was used for identification of car. The column was packed in a 150×4.6 mm column. Car extracted from Peanut butter fruits pulp (0.003 g) dissolved in 1.5 ml absolute ethanol from which 10 µm. The mobile phase consists of acetonitrile, methanol and ethyl acetate containing 0.05%. A gradient was applied from 95:05:00 to 60:20:20 in 20 min maintain with gradient elution from 60 min.

2.5 Characterization of inclusion complexes

The inclusion complexes were characterized by using UV-Visible spectroscopy and FT-IR spectroscopy.

2.4.1 UV-Vis spectroscopy

The electronic absorption spectra were recorded using a UV-Vis double beam spectrophotometer. The UV-Visible absorption spectrum was recorded from 190 nm to 600 nm. Samples dissolved in absolute ethanol.

2.4.2 FT-IR spectroscopy

The spectra of the sample were obtained in the range of 600-4000 cm^{-1} using KBr pellets.

3. RESULTS AND DISCUSSION

The HPLC analyses of the Peanut butter fruits pulp extract as shown in figure 1. The main pigment of the Peanut butter fruits pulp extract was found to be β-carotene and the present area for peak was 20.14% at the retention time 52.202 min. The other main pigment was found to be lutein, showed permanent peak 19.3529% at 36.193 min.

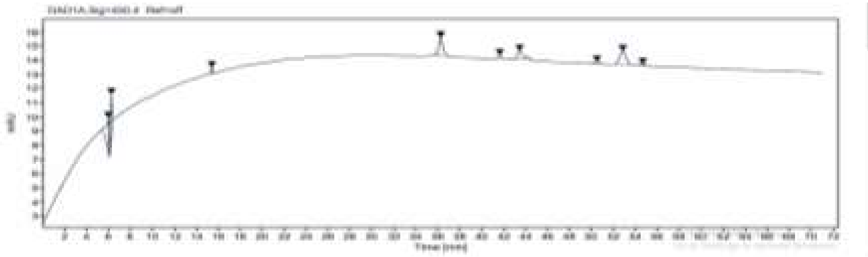


Figure 1: Reversed phase HPLC chromatogram of the car extraction from Peanut butter fruits pulp

UV-Vis spectrum of inclusion complex preparation by using co-precipitation method is shown in Figure 2. The UV-VIS spectrum of β-carotene in ethanol was scanned from 190 to 600 nm, and maximum absorption was obtained at 451 nm. The UV-VIS spectra of Peanut butter fruits extract showed the maximum UV-VIS absorption at the same wavelength. The co-precipitation complex show maximum absorbance to 200-250 nm when compared with absorbance of β-carotene at 450 nm. Complex prepared by using extended co-precipitation was shifted to 200-250 nm when compared with absorbance of β-carotene at 450 nm. According to the maximum absorption of the both spectrum of inclusion complexes, extended co-precipitation method is better than the co-precipitation method.

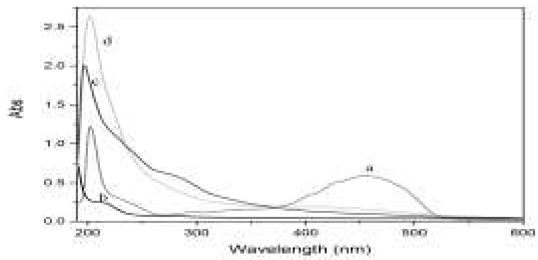
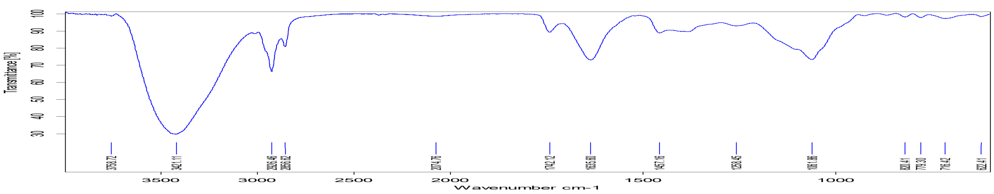
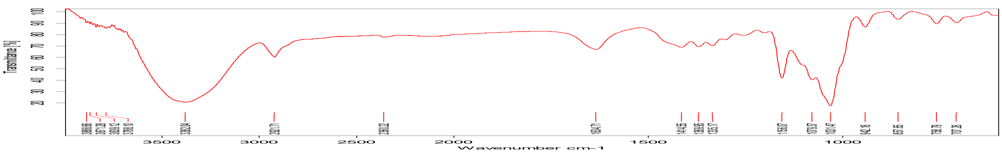


Figure 2: UV-Vis spectra of a) Car extracted from Peanut butter fruits pulp, b) β-cyclodextrin, c) Co-precipitation, d) Extended Co-precipitation

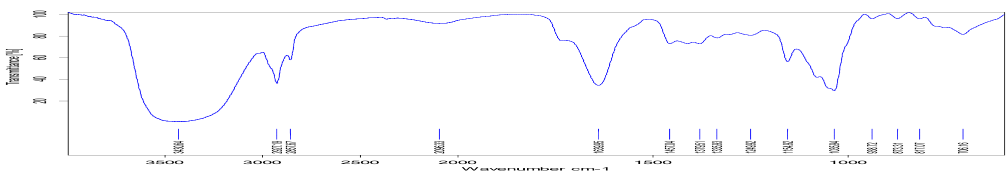
a) Car extracted from Peanut butter fruits pulp



b) β-cyclodextrin



c) Co-precipitation



d) Extended Co-precipitation

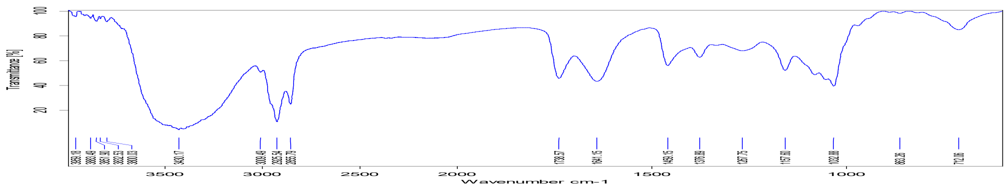


Figure 3: FT-IR spectra of a) Car extracted from Peanut butter fruits pulp, b) β-cyclodextrin, c) Co-precipitation, d) Extended Co-precipitation

Table 1: Absorption peaks (in cm^{-1}) obtained by FT-IR spectroscopy for Car extracted from Peanut butter fruits pulp, β-cyclodextrin, Co-precipitation, Extended Co-precipitation

	Extract from Peanut butter fruits	β-Cyclodextrin	Co-precipitation inclusion complex	Extended co-precipitation
OH stretch	3421	3382	3430	3430
C-H stretch	2926	2921	2927	2925
OH bend	1635	1634	1639	1641
C-H deformation	1457	1414,1369, 1335	1457, 1379, 1335	1459,1376
C-O-C and C-OH stretch	-	1156,1076,1031	1154,1035	1157,1032
C-H out of plane bending	-	857	873	863
Pyaranose ring Vibration	-	758	706	712

FT-IR alone or other vibrational analyses can be used to analyse the interactions between the host and guest materials based on changes in the four different spectra. Figure 3 shows the comparison between the FT-IR spectra obtain for a) car extracted from Peanut butter fruits b) β-cyclodextrin c) co-precipitation complex d) extended co-precipitation complex. Important changes in the polymeric hydroxyl stretching region (3000-3600 cm^{-1}) appeared more in both types of inclusion complexes. The O-H stretch band shifted from 3421 cm^{-1}(car extract from Peanut butter fruits) and 3382 cm^{-1}(β-cyclodextrin) to 3430 cm^{-1}. Both these complexes exhibited changes that seemed to be related to the complex formation between the OH groups of the host molecule and the Peanut butter fruits extraction. C-H aliphatic bands were apparent in the 2921-2927 cm^{-1} region as seen in all spectra. The bands of the inclusion complex prepared by the extended co-precipitation complex exhibit a different profile compared to the co-precipitation complex, suggesting that different behaviour is related to this kind of link when there is inclusion complex formation. The 1742-1738 cm^{-1} region, can be observed to have a vibrational bond in the region of the C=O bond in the carbonyl group of the compounds in the Peanut butter fruits extract. At the fingerprint region, terpene compound including β-carotene at 1457-1459 cm^{-1} which reported for C-H deformation. β-cyclodextrin exhibited sharp peaks at 1031-1035 cm^{-1} and 1151-1154 cm^{-1} are associated for C-C and C-O-C stretching. The peak at 1634 cm^{-1} in β-cyclodextrin is due to O-H bending of water molecules within the cyclodextrin cavity.

4. Conclusions

The characterization analyses revealed the occurrence of car extract from Peanut butter fruits is dock into the cavity of the host molecule which is β-cyclodextrin in both procedures. The complexes were characterized by using UV-Vis spectroscopy and FT-IR spectroscopy. The applications of such inclusion complexes in the food and pharmaceutical industries and biocompatible carotenoid health supplement and development of functional food in Vitro studies are suggested.

5. REFERENCES

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6. ACKNOWLEDGMENTS

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