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Preparation and Thermal Stability of α-Tocopheryl Acetate and Strawberry Anthocyanins Complexed with Starch and β-Cyclodextrin

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Short Communication

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Abstract: For the sake of enhancing the thermal stability of α-Tocopheryl acetate (α-TA) and Strawberry Anthocyanins (AN), their encapsulation with starch or β-Cyclodextrin was prepared and characterized by UV-Vis and IR spectra in addition to thermal analysis. Thermal stability was investigated by comparing the thermographs of each host and guest with that of the prepared complex. Both hosts provided extra thermal stability to both guests where α-TA is stabilized from 230 to 291ºC by complexation with starch and to 285ºC by encapsulation with β-CD. AN was also stabilized from 40.02ºC to 75.57 and 79.46ºC by complexation with starch and β-CD respectively.

1 Introduction

α-Tocopherol and anthocyanins are potent antioxidants well distributed in the plant kingdom; they have complimentary effects where α-tocopherol is fat soluble whereas anthocyanins are water-soluble. Although α-Tocopherol is found in most oils and fats; oils are richer in α-Tocopherol, particularly in canola, corn, soybean and sunflower oils (Choe and Min 2009). Its role is mainly protecting oils from thermal and oxidative degradation; hence it is regularly used to enhance fatty foods during food processing (Suárez-Jiménez et al 2016). However, α-Tocopherol was observed to undergo degradation upon thermal treatment (Vaidya and Choe 2011). The light was also observed to assist thermal degradation (Hategekimana and Zhong 2015). In addition, enhancing α-Tocopherol solubility is also required in its application in the cosmetic industry (Buschmann and Schollmeyer 2002).

Anthocyanins are natural-colored pigments found in many fruits and vegetables characterized by red to purple colors (de Rosso and Mercadante 2007). They expressed a wide range of biological and pharmaceutical activities (Smeriglio et al 2016) which are attributed to their strong antioxidant activity (Ali and Ali 2017). On the other hand, the stability of anthocyanins was found to decrease by thermal treatment; the degradation rate takes place according to first-order reactions and increases gradually by increasing each temperature and pH (Sui et al 2014).

In addition, both starch and β-cyclodextrin are considered non-toxic and thus suitable for food and pharmaceutical applications. Accordingly, the present work targets enhancing the thermal stability of α-Tocopherol and anthocyanins by preparing their complexes with starch and β-cyclodextrin then examining the stability by thermogravimetry (TG) and differential scanning calorimetry (DSC).

2 Materials and Methods

2.1 Chemicals and instruments

β-Cyclodextrin (β-CD) and starch were purchased from Sigma-Aldrich and El-Nasr Pharmaceutical Chemicals companies respectively. α-Tocopheryl acetate (α-TA) was obtained from Pharco Pharmaceuticals. All other chemicals used were reagent grade. To record UV-Vis spectra, a Thermo Fisher instrument model Evolution 300 was used. FT-ATR-IR model Bruker vertex 80/80V was used to measure the IR spectra. Thermographs were conducted using the TA SDT Q600 instrument under nitrogen gas. The operation condition was initial temperature, 50°C; final temperature, 550°C and heating rate 10°C min^{-1}. Fragments were assigned based on the fragment percentage of the molecular weight (MW) in the TG analysis.

2.2 Extraction of AN from strawberry fruits

Strawberry anthocyanins (AN) were extracted and purified using the method described by Macz-Pop et al (2006) with some modifications. Briefly, 1 Kg of fruits was extracted by methanol or ethanol (80%) containing 0.1% HCl. The extract was defatted by washing with *n*-hexane and purified by Sep-Pak C18 cartridge (Waters Corp., Milford, MA).

2.3 Preparation of starch complexes

Starch complexes were prepared according to the method described by Kim and Huber (2016) with some modifications. Starch (5.0 g) was suspended in 100 mL distilled water; then 1.0 g α-tocopheryl acetate (α-TA) or AN dissolved in 50 mL ethanol was added. The mixture was distributed in tubes with screw caps and covered by aluminum foil then heated in oven at 150°C) for 75 min. Tubes were cooled and refrigerated overnight. The precipitates were filtered, combined and dried in an oven at 50°C for 3 hrs.

2.4 Preparation of β-cyclodextrin complexes

Encapsulation with β-cyclodextrin was performed using the method described by Gomes et al (2014) with modification. To a β-cyclodextrin water solution (20 mL, 15 mM), α-TA (0.1419 g or 1.0 g AN) dissolved in ethanol was added and placed in an ultrasonic for 5 hrs. and then subjected to magnetic stirrer for 4 hrs. The mixture was frozen overnight and then freeze-dried; the product was washed with cold methanol.

3 Results and Discussion

3.1 Preparation of complexes

The IR spectrum of starch-α-TA complex showed the main characteristic peaks of both precursors where α-TA gives the carbonyl peak of α-TA at 1757cm^{-1} while starch showed the hydroxyl group peak at 3297 cm^{-1}; these peaks in starch- α-TA complex appeared at 1761and 3305 cm^{-1} respectively.

The β-CD-α-TA showed also, in the IR spectrum, both the hydroxyl peak of β-CD at 3323 cm^{-1} and the carbonyl peak of α-TA at 1759 cm^{-1}. UV-Vis spectra of α-TA showed two absorbance bands at 230 and 287 nm while in the complex appeared at 277 and 225 respectively.

The main difference among the spectra of starch, AN and starch-AN complex is the position of the O-H_{str} band (3297, 3288 and 3304cm^{-1} respectively) which is known to be sensitive towards involvement in H-bonding; in addition, the aromatic peak of AN appeared also in the complex at 1636 cm^{-1}.

The IR spectrum of the β-CD-AN complex showed a hydroxyl peak at 3294 cm^{-1} and an aromatic peak at 1627 cm^{-1}. The UV-Vis spectrum of the complex showed two shifted bands (276 and 507 nm) from respective AN bands (270 and 517 nm) indicating complex formation.

3.2 Thermographical analysis

Thermogravimetric analysis (TG and DTG) of the prepared complexes along with their precursors **(Fig 1)** were performed not only to confirm the success of complexation but also to shed light on their thermal stability. Thermographs of starch showed three steps; first, a little weight loss (11.09%) at low temperature (65.85°C) as presented in the TG and DTG thermographs while the DSC indicated that the process is endothermic which could be due to water evaporation. In the second step, the main weight loss (69.39%) appeared at 322.27°C in the DTG with an endothermic peak at 329.72°C as shown by the DSC thermograph. The third step is a steady slow degradation over the range of 400-700°C which represents 6.51% of the sample. The total degradation of organic matter is 86.99% while the remaining inorganic residues represent 13.01%; similar results were observed by Hornung et al (2014).

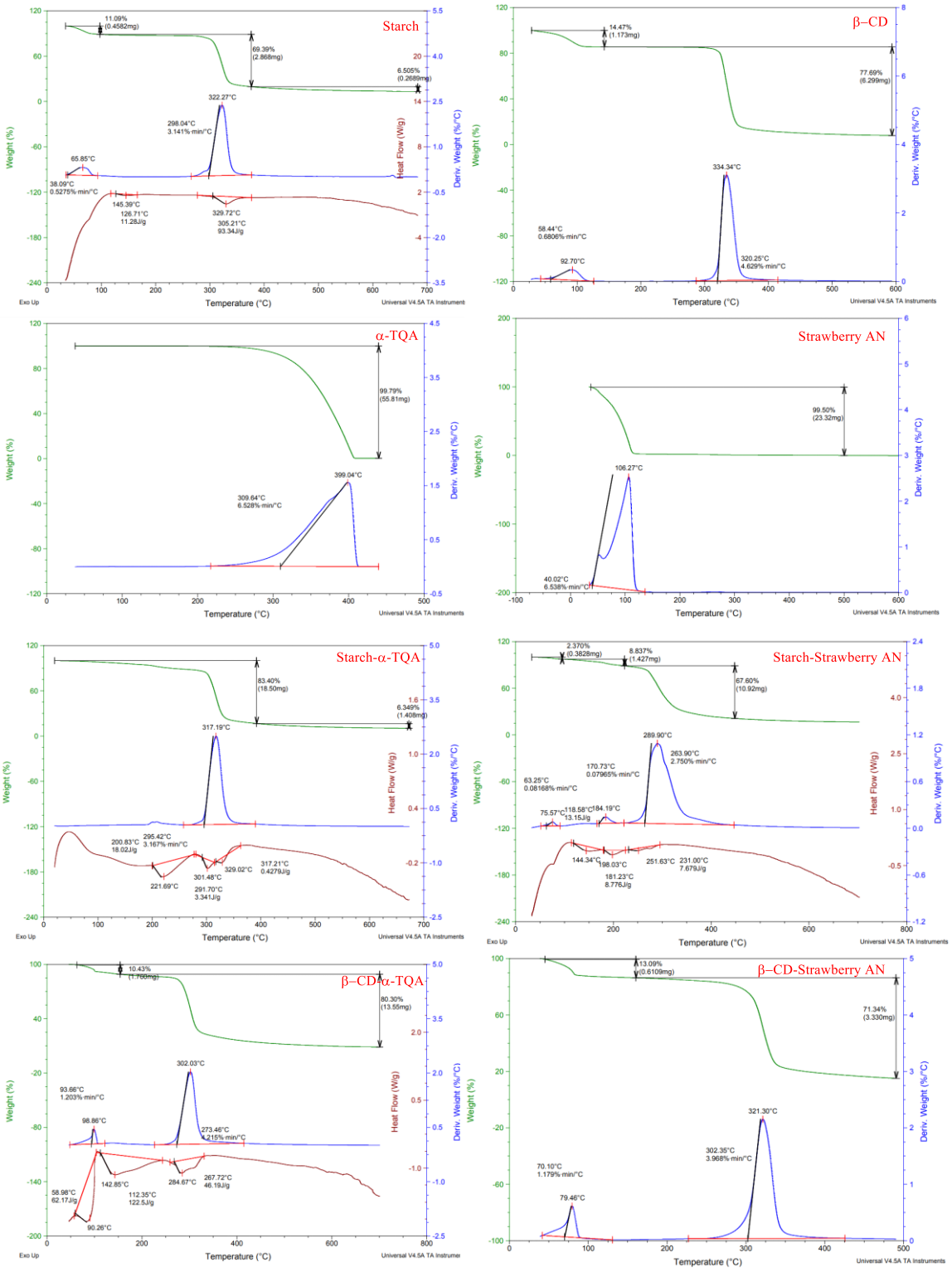

Fig 1. Thermographs of starch, β-CD, pure α-TA, strawberry Anthocyanins and their complexes

Thermographs of pure α-tocopheryl acetate (α-TA, MW 488.71) showed complete degradation (99.79%) presented by tailing peak at 399.04ºC with the onset of starting degradation at 230ºC; similar thermographs were reported previously (de Almeida et al 2010). Thermographs (TG and DTG) of starch-α-TA complex showed weight loss presented by sharp peak at 317.19ºC while this degradation step is manifested in the DSC by two endothermic peaks; the second one is similar to that of starch degradation at 329.02ºC while the first one is for α-TA degradation (301.48ºC) with on-set temperature 291.20ºC indicating the more stability of α-TA in complex compared to the pure form (230ºC). DSC shows also an extra endothermic peak at 221.69ºC which could be for amylose helix rewinding and release of α-TA.

Thermal analysis (TG and DTG) of strawberry Anthocyanins (AN) indicated degradation at low temperature (106.27ºC) with an on-set temperature of 40.02ºC and weight loss of 99.50%. On the other hand, thermographs (TG and DTG) of starch-AN complex presented in **(Fig 1)** showed degradation peaks at 75.57ºC (higher than that of pure AN, 40.02ºC) and 184.19ºC in addition to the main degradation peak at 289.90ºC indicating better stability by complexation. DSC showed also an additional endothermic peak at a lower temperature of 144.34ºC suggesting amylose helix rewinding and release of AN. The total degradation of the complex is 78.81 less than each starch and AN (86.99 and 99.50% respectively) confirming the complex formation.

Thermogravimetric analysis (TG and DTG) of β-CD (MW 1135.0) is similar to that reported previously (Ai et al 2019). The thermograph shows two clear steps of thermodegradation; the first one is at low temperature (92.70ºC) and account for 14.47% weight loss; it could be attributed to losing one of the glucose units (calculated loss of 14.27%). The Second step appeared at a high temperature (334.34ºC) accompanied with 77.69% weight loss while the total observed weight loss is 92.16% i.e. the remaining is 7.84% suggesting three (CHOH) units (calculated 7.93%).

Although pure α-TA started complete degradation (99.79%) at 230ºC with a maximum rate at 399.04ºC, the thermographs of the inclusion complex (β-CD-α-TA) showed different behavior indicating the effective complexation process where two degradation steps appeared at low temperatures (43.33 and 84.55ºC) both account for 12.37% weight loss, comparable with the low-temperature degradation step of β-CD (92.70ºC, 14.74%), and two steps at high temperature (285.23 and 330.07ºC) responsible for the main weight loss (75.41%) where the total weight loss of the complex is 87.78% less than those of both the two precursors (92.16 and 99.79%) indicating also different behavior and some stability of the complex.

While thermal analysis of strawberry AN showed degradation starts at 40.02ºC with weight loss 99.50%, thermographs of the complex β-CD-AN **(Fig 1)** indicated more thermal stability where two degradation steps are observed; the first peak appeared at 79.46ºC with on-set temperature 70.10ºC, lower than that of pure AN at 40.02ºC, and the second degradation step at 321.30ºC with total weight loss only 84.43% less than those of both the two precursors (92.16 and 99.50%) indicating the formation of complex with better thermal stability.

4 Conclusion

It could be concluded from the present study that complexation provided extra thermal stability to both α-TA and strawberry AN where α-TA is stabilized from 230 to 291ºC by complexation with starch and to 285ºC by encapsulation with β-CD. Complexation of strawberry AN showed also an enhancement in thermal stability where AN was stabilized from 40.02ºC to 75.57 and 79.46ºC by complexation with starch and β-CD respectively. The thermal stability of complexes could be due to the protection from heat by encapsulation in addition to the requirement of more energy to break the host-guest interactions e.g. hydrogen bonding and/or polar interaction.

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