Application of Flow Field-Flow Fractionation Coupled With Multiple Detections for Separation and Characterization of Egg Yolk Plasma

Haiyang Dou[1], Emma Magnusson[2], Jaeyeong Choi[1], Sujeong Han[1], Yeongsuk Yoo[1], Lars Nilsson[2], Seungho Lee[1,*]

[1]Department of Food Technology, Engineering and Nutrition, P.O. Box 124, Lund, Sweden

[2]Department of Chemistry, 461-6 Jeonmin-dong Yuseong-gu, Daejeon, Korea

E-mail address: slee@hannam.kr

Abstract: *In this study, the capability of asymmetrical flow field-flow fractionation (AF4) coupled online with a UV/VIS, quasi-elastic light scattering (QELS), multiangle light scattering (MALS) and fluorescence (FS) detectors (AF4-UV/VIS-QELS-MALS-FS) was evaluated for separation and simultaneous characterization of egg yolk plasma. AF4 provided gentle separation of major components of the egg yolk plasma such as free proteins, low density lipoproteins (LDL) and their aggregates, according to their hydrodynamic sizes. Free proteins were completely separated from LDL by a programmed AF4 analysis, where the external field (cross-flow) was gradually reduced during a run. For each component, QELS and MALS yielded hydrodynamic radius (R_h) and radius of gyration (R_g), respectively, allowing study on conformation (e.g., shape) of each component based on R_g/R_h. The R_g/R_h data were confirmed by Cryo-transmission electron microscopy (Cryo-TEM) images of collected AF4 fractions. The R_g/R_h results indicated that LDL has a homogeneous spherical shape, while larger aggregates have swollen micro-gel type structure. Elution of LDL was confirmed by staining them with a fluorescent dye, Nile red. Collected AF4 fractions of free proteins were further characterized using a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Results suggested that the absence of Ca^{2+} leads to aggregation, while the presence of Ca^{2+} leads to bindings of proteins. The effect of heating and enzymatic treatment on egg yolk plasma was also investigated. It was found that LDL undergoes a 'clusters-fusion-gelatin' process under heating treatment (65 ^{o}C), while enzymatic treatment with phospholipase A_2 (PLA_2) significantly enhances the heat stability of LDL.*

Keyword: *Asymmetrical flow field flow fractionation (AF4), Egg yolk plasma, Low density lipoprotein (LDL)*