Waters™

應用手冊

Authentication of Cocoa Butter by Direct Analysis using RADIAN ASAP with LiveID

Li Yan Chan, Yuwei Chang, Hong Peng, Guangtao Zhang

Waters Corporation, Mars Global Food Safety Center

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

RADIAN ASAP combined with LiveID is a practical solution for authenticating cocoa butter using direct analysis with minimal or no sample preparation. Given the volatility of cocoa butter prices, there is an increase in fraudulent practices such as partial replacement using cocoa butter equivalents (CBEs) for unlawful financial gains. This technology is able to rapidly monitor the presence of CBE at levels relevant to the EU legislation, which allows up to 5% in product weight. The triglycerides (*i.e.* TG, triacylglycerol, TAG, or triacylglyceride) fingerprint of cocoa butter contributes significantly to the successful detection of adulteration. The real-time recognition in LiveID enables results to be ready in minutes for an informed decision to be made quickly.

Benefits

- Easy to use – direct analysis with minimal or no sample preparation
- Quick results – LiveID real-time recognition enables informed decision made in minutes from analysis
- Compact – small footprint allows maximum flexibility in deploying the technology

Introduction

Cocoa butter is the most important and the most expensive component of chocolate products. The unique triacylglycerol (TAG) composition of cocoa butter provides a pleasant mouthfeel, hence explains the very attractive characteristics of chocolate products. Given the volatility of cocoa butter prices, there is an increasing trend of using cocoa butter equivalents (CBEs), as a full or partial replacement in confectionery products in order to reduce production costs. Genuine cocoa butter consists mainly of three TAGs, namely 1,3-dipalmitoyl-2-oleyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS), and 1,3-distearoyl-2-oleoyl-glycerol (SOS). The presence of CBE alters the composition, hence impacting the crystallization and melting characteristics in the mouth.[1] The rules on labelling of chocolate products vary across the globe. In EU, chocolates are allowed to contain up to 5% of CBE.[2] If CBE are added, consumers have to be informed by appropriate labelling. In contrast, current FDA regulations stipulate that products that are labelled as chocolate shall not contain any fat other than cocoa butter or fat from certain dairy ingredients. As such, it is extremely important for food manufacturers to differentiate good quality and authentic cocoa butter from its equivalents. The chemical composition and physical properties of the CBE resembles those of cocoa butter very closely, making them extremely difficult to quantify and in some cases even difficult to detect. Current targeted methods based on gas chromatography are time-consuming and rely on intensive sample preparation.

RADIAN ASAP is a single quadrupole mass detector dedicated to direct analysis with an Atmospheric Solids Analysis Probe (ASAP). This technology is accessible to users of all levels of expertise, because of its simplicity of operation as well as the minimal requirement in sample preparation. Chemometric modelling is performed in real time with LiveID Software and enables a quick turnaround time that is crucial for identifying fraud in raw materials.

Herein, we demonstrate the use of RADIAN ASAP with LiveID to swiftly identify adulterated cocoa butter. LiveID enabled us to transform complex raw MS data into simple-to-interpret chemometric models.

Results and Discussion

The addition of 10% (*w*/*w*) CBE into cocoa butter was calculated to be ~3% CBE in finished products (EU legislation allows <5%), and this level was used as the threshold in this application. The mass spectrometric data of six pure cocoa butter, four pure CBEs, and six adulterated cocoa butter (with 10% CBEs) were obtained in replicates by gently swabbing the glass capillary probe on each sample before introducing into the RADIAN ASAP. The chemometric model was built using the principal component analysis (PCA) and linear discriminant analysis (LDA) with the TAG region (*m/z* 750 to 1050) in focus. The three different classes were well clustered as shown in the 3D PCA-LDA scores plot using components 1, 2, and 3 (Figure 1).

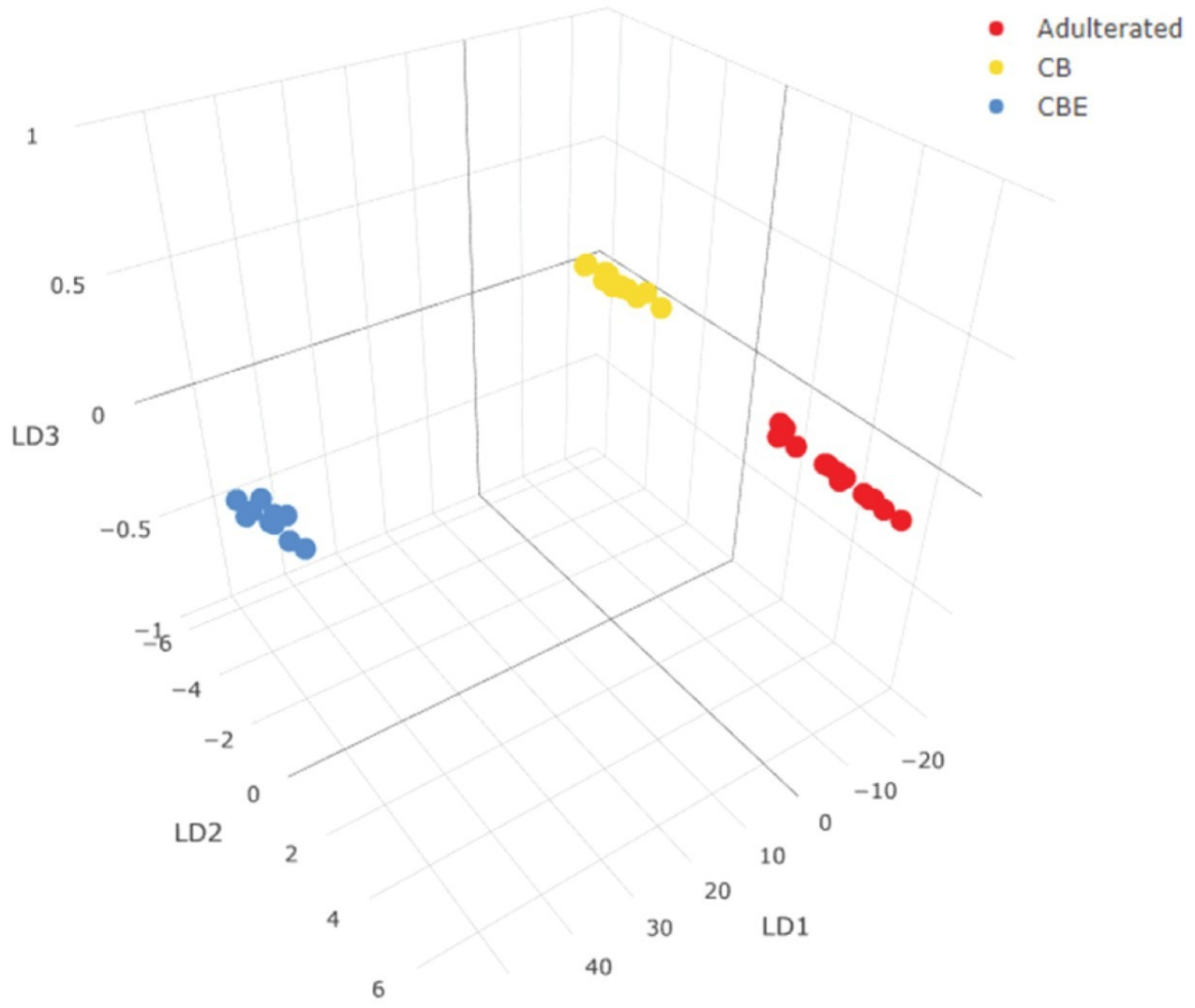

Figure 1. 3D PCA-LDA scores plot of cocoa butter (yellow), CBE (blue), and adulterated cocoa butter (red).

The pure CBE was distinctly differentiated from the cocoa butter using LD1, whereas pure and adulterated cocoa butter, were separated in LD2, as shown in the 2D PCA-LDA scores plot (Figure 2B). The corresponding loadings plot (Figure 2A) shows that the significant ions at 834 (POP), 862 (POS), and 891 (SOS) are the contributory features in LD2. The results were in accordance with the known importance of TAG in cocoa butter fingerprint.[3]

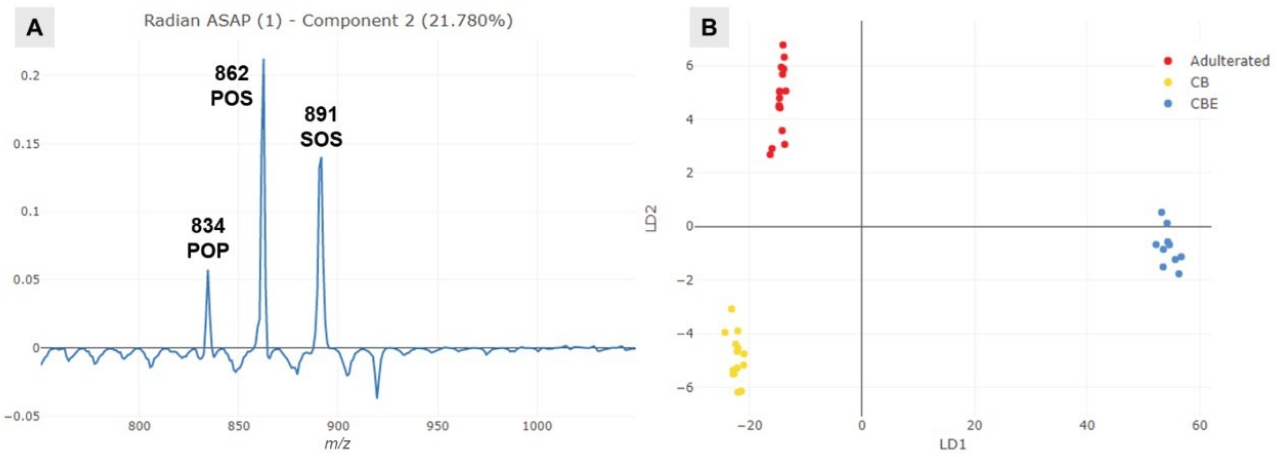

Figure 2. A. Single component loadings plot; and B. 2D PCA-LDA scores plot.

Independent validation of the model was carried out by analyzing triplicates of one pure and one adulterated cocoa butter sample that were not used in the model building. The model correctly classified the blind samples with 100% match confidence in the real-time recognition mode as depicted in Figure 3.

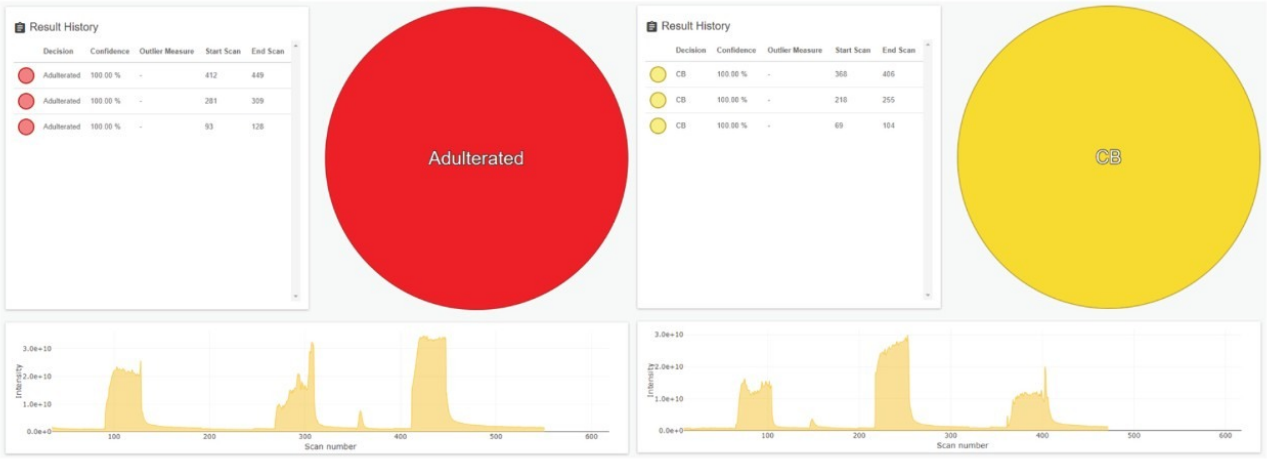

Figure 3. LiveID real-time recognition of pure cocoa butter or adulterated cocoa butter.

As with other types of direct analysis techniques, the RADIAN ASAP does not involve prior sample clean-up or chromatographic separation. A protocol for effective source cleaning is recommended to ensure reproducibility of results and reduction of carryover from runs to runs.

Conclusion

The RADIAN ASAP combined with LiveID was used successfully for cocoa butter quality monitoring. This technology is able to rapidly capture the presence of CBE at levels relevant to legislation. The detection of adulterated cocoa butter with the threshold as low as 10% CBEs was achieved in minutes with simple operation and easy-to-visualize models. The real-time recognition feature enabled informed decision to be made immediately which is crucial in the process of raw material authenticity.

References

1. Perez M, Lopez-Yerena A, Vallverdú-Queralt A. *Crit Rev Food Sci Nutr.* 17 (2020), 1–15.
2. Council of the European Union, Directive 2000/36/EC.
3. Lipp M, Simoneau C, Ulberth F, Anklam E, Crews C, Brereton P, de Greyt W, Schwack W, Wiedmaier C. *J Food Compost Anal* (2001), 399–408.

Acknowledgements

This is part of a collaborative project between Waters International Food and Water Research Centre (IFWRC) and Mars Global Food Safety Center (GFSC).

Featured Products

- MassLynx MS Software <https://www.waters.com/513662>
- RADIAN ASAP Direct Mass Detector <https://www.waters.com/waters/nav.htm?cid=135073413>
- LiveID Software <https://www.waters.com/waters/nav.htm?cid=134939519>

720007100, January 2021