Development and validation of a droplet digital PCR targeting intron-based sequence for detection and quantification of buffalo meat substitution in beef

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Introduction: Economically motivated food fraudulency (EMA) like substitution or adulteration of meat food products are of high-risk category in vulnerability assessment of supply chain. Several adulteration/substitutions of cheaper quality food products into high priced commodities occur in a ubiquitous manner. Such fraudulent practices should be assessed for its vulnerability by detection and genetic traceability. This can ensure public health protection as well as authenticity, in addition to the transparency, quality and safety in response to consumers' demand for each food commodity. Further, in instances of EMA, quantification of adulteration in addition to authenticity is of paramount importance, which requires sensitive and precise techniques. Such methods could even quantify the trace amount of DNA present in the food samples. Hence, a highly sensitive technique (Droplet Digital PCR™) was used in this study for precise detection and absolute quantification of buffalo meat substitution in beef.

Materials and methods: In this current study, a droplet digital PCR (ddPCR) assay targeting an intron-sequence of Melanocortin - 1 receptor gene of buffalo was developed to detect and quantify buffalo derived materials in different foods. The assay was standardized using the QX 200 Automated digital droplet PCR (Bio-Rad, USA) and all the experiments were performed in an accredited environment (ISO/IEC 17025: 2017) with quality control materials (QCMs). The ddPCR assay system was optimized for several parameters, including primer annealing temperature, primer-probe concentration ratio and PCR cycle numbers. The exclusive specificity of the primers was confirmed both by In-silico analysis and with cross-reactivity check to other closely related met species.

Results: The LoDabs and LoDrel were found out to be 0.6 copies/µL and 2.5 copies/µL for the assay with a sensitivity of 0.1% for buffalo in relative DNA mixtures. The LoQ of the assay performed with meat mixtures of buffalo and beef was found out to be 4.23 copies/µL. The assay was evaluated for robustness which provided a very robust detection. Regression models for quantitative relation were constructed statistically to establish a correlation between target copy number to DNA weight and finally meat weight proportions. The fitness evaluation of regression curve in quantifying meat samples provided a deviation of -12 to 10% for buffalo and -11 to 12% for the matrix species used. Further, the assay was evaluated for applicability to several food matrices of animal origin (real-world samples) which detected trace-quantities of adulteration in products like milk, ghee and Haleem.

Conclusion: The assay was validated as sensitive and robust following international standards (ISO 20395: 2019; ISO 20813:2019) for biomarker analysis and quantitation. The validated assay entrenched in this study can be valuably used by regulatory bodies in detection of buffalo derived materials in various food matrices.