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Rapid and Sensitive DNA based Method for Detection of Gluten Allergen in Food

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Abstract—*Food allergens are generally naturally occurring substances that triggers abnormal immune response. Gluten is one of the common food allergen present in wheat, rye, barley and their derivatives. However, it could be present in other food items undeclared or as unintentional additives. Celiac disease is an autoimmune response towards gluten and so to avoid allergic reaction it is important to choose the food product wisely. The present work was carried out to identify gluten allergen in food by Real Time PCR method. The genomic DNA was extracted from the wheat, barley, rye and rice flour using the commercially available kit DSS-DNEUS-011 (DSS Imagetech Pvt. Ltd, New Delhi). Briefly, the 40mg of sample was taken in the 1.5 ml tube and 500µl lysis buffer was added along with 15µl of Proteinase-K and incubated at 65°C for 30 min. After incubation, the tubes were centrifuged at 10000 rpm for 10 minutes. The supernatant was collected in fresh tube and 300µl of DNA binding buffer was added to the collected tube. This mixture was passed through the mini spin column by centrifugation. The column was washed twice and finally the DNA was eluted. The gluten was detected using qPCR with highly specific primer pairs. The qPCR reaction set was done as follows: Initial Denaturation at 95 °C for 1 Min, Denaturation at 95 °C for 15 s and Annealing at 60 °C for 30s for 40 cycles which was followed by melt curve analysis. Results showed the gluten presence in wheat, barley, rye but it was not detected in rice flour. The sensitivity and specificity experiment done on different spiked food samples indicated that the primer pairs are very specific to gluten and able to detect gluten in raw as well as processed food. In conclusion, the gluten allergen can be detected with high sensitivity and specificity by Real-Time PCR in different food products which is rapid, accurate and cost effective method.*

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