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DETECTION OF CORN AND BARLEY AS ADULTERANTS IN ROASTED COFFEE USING REAL-TIME PCR.

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Coffee is one of the main commodities in Brazil and, as such, it has been a target for fraudulent admixtures with a diversity of cheaper materials, such as twigs, spent coffee, roasted barley and corn. Many techniques have been developed in order to establish suitable parameters and markers for adulteration of ground roasted coffee and instant coffee. However, these methods usually present low sensitivity and specificity. Although the recombinant DNA technology has shown to be a promising tool to determine the authenticity of processed foods, it has not been used to detect coffee adulteration. The objective of this study was to use a real-time Polymerase Chain Reaction (PCR) based method for detection of two among the main adulterants in coffee: corn and barley. For this purpose, ground coffee blends containing 80% of *C. arabica* and 20% of *C. canephora* were intentionally adulterated with corn and barley, in the proportions of 0.05; 0.5; 5 and 50mg per g of coffee and thoroughly mixed. In addition to these samples, roasted corn, barley and plain coffee were separately assayed for standard curves preparation. DNA extractions were performed using a modified version of DNeasy kit protocol and CTAB buffer. The DNA concentrations were determined by spectrophotometry (Shimadzu UV-1800 Japan) at 260 nm. Primers for corn (ZEINA2) and for barley (CEVADA3) were designed through the on line program GeneFisher2. The reaction was conducted using qPCR (SDS ABI Prism 7000 - Applied Biosystems USA), with SYBR Green I system (Applied Biosystems, UK), in quadruplicate. The method was sensitive and specific to detect down to 0.02 ng of corn DNA and 0.005ng of barley DNA per g of roasted coffee, which converted to mass could be estimated in approximately 307 µg corn/g coffee and 45 µg barley/g coffee. The methodology based on real-time PCR appears to be more efficient to detect small amounts of adulterants, compared to other existing and routinely used methodologies, showing to be a promising tool for the detection of corn and barley in commercial coffees.

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