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DEVELOPMENT OF PRIMER SETS FOR DETECTION OF CORN AND BARLEY IN ROASTED COFFEE BY REAL TIME PCR.

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In the last years, the need for certification of origin and authenticity of foods increased the demand for sensitive and selective methodologies to guarantee the consumers' choice, which is not always attended due to different existing types of adulteration. In Brazil, two of the most common adulterants found in roasted coffee are corn and barley. The present study focused on the development and validation of real-time Polymerase Chain Reaction (PCR) systems for the detection of corn and barley. The DNAs from barley, corn and coffee *in natura* (standards) were extracted using a modified version of the DNeasy mini plant Kit protocol. Dilution series of DNA template were tested in 10 replicates for each primer to obtain standard curves. Coffee was roasted in a fluidized bed roaster, corn and barley were roasted in microwave oven. Roasted food samples were submitted to extraction with DNeasy kit/ buffer CTAB. DNA concentrations were determined in all *in natura* and roasted samples by spectrophotometer (Shimadzu UV-1800 Japan) at 260nm. Due to the inhibition of the reaction caused by low molecular weight compounds, such as phenols, carbohydrates, peptides and aromatic compounds, their presence was measured in the isolated DNA, using spectrophotometer at 230 nm, and the absorbance ratio 260:230 was calculated to evaluate the isolate quality[1]. The sequences used as templates to design the primers were obtained in the GenBank. Primer pairs were designed using the program GeneFisher2 and named ZEINA2 for corn and CEVADA3 for barley. The reactions were conducted by qPCR (SDS ABI Prism 7000 - Applied Biosystems USA) using the SYBR Green system (UK). Primers Specificity was determined by running new reactions with genomic DNA from rice, corn, wheat, coffee, soybeans and barley. The dissociation curve for CEVADA3 and ZEINA2 demonstrated that these primer pairs were specific for barley and corn detection. The ratio 260:230 in the *in natura* samples was = 1.6, which is considered of good quality (1). The Limits of Detection (LOD) for corn and barley DNA *in natura* were 8.1pg and 0.33pg, respectively, while the Limits of quantification (LOQ) were 5pg and 0,1pg, respectively. Reaction efficiency[2] was 113.4% and 95%, for corn and barley. Despite the low quality of the DNA isolate from the roasted foods (ratio 260:230 = 0.7)[1], due to the Maillard compounds formed during roasting, which present similar structure to DNA nitrogen bases, it was still possible to detect them in roasted coffee even as residues of materials ground in coffee grinders.

(1) Murray et al, 1980. *Nucleic acid Research*, **8**:4321-4325.

(2) Lube et al, 2010. *Food Chemistry*, **118**, 979-986.