116594 - DEVELOPMENT AND EVALUATION OF MOLECULAR METHODOLOGY BASED ON POLYMERASE CHAIN REACTION (PCR) FOR IDENTIFICATION OF *Campylobacter* spp. IN ARTISANAL MINAS CHEESE

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Artisanal Minas Cheese (AMC) is a potential carrier of zoonotic pathogens. *Campylobacter* spp. has been the most frequent pathogen responsible for outbreaks of foodborne diseases worldwide. Molecular methods, especially Polymerase Chain Reaction (PCR), are promising tools for rapid and direct detection of *Campylobacter* in food. The aim of this study was to develop a molecular method based on multiplex PCR for direct identification of *Campylobacter* in AMC. In the first PCR assay, the samples were artificially contaminated with an equal mix of *C. jejuni* (CAMP 492) and *C. coli* (CAMP 1004) to adjust the PCR conditions (>10^8 CFU/g) and to set the detection limit (0.1, 10, 10^3 and 10^5 CFU/g). The DNA of these samples was extracted by phenol/chloroform method. Afterwards, the multiplex PCR assays were performed using *primers* for the genus *Campylobacter* (16SrRNA), *C. jejuni* (*hip*O) and *C. coli* (*ceu*E). Five protocols were tested, running in different concentrations of $MgCl_2$, 10x buffer, DNA ng/µl in the PCR reactions, annealing temperature, agarose gel concentration, and voltage of electrophoresis running. The presence of *Campylobacter* spp. was evaluated in 81 AMC samples from different sources. A multiplex PCR protocol was adjusted for the artificially contaminated samples (4mM of $MgCl_2$, 1.2mM of buffer, 5.0ng/µl of DNA, agarose 1%, 80V and annealing temperature of 62°C).The detection limit was estimated at 10^3 CFU/g. *Campylobacter* was not found in any of the 81 samples using this multiplex PCR protocol. In contrast, 29 (35.8%) of these 81 samples were positive, when only *primers* of the 16S rRNA gene were used in the reactions. The developed method is effective to detect *Campylobacter* in artificially contaminated samples but is not robust enough for detection in samples from different sources, probably due to the complexity of these cheese matrixes. Despite this, the presence of positive samples using the *primer* 16s rRNA is an important public health problem.
Keywords: Artisanal Minas Cheese, *Campylobacter,* PCR multiplex.

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