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EVALUATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE DETECTION OF VIRUSES IN READY-TO-EAT FOODS

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ABSTRACT

The ability of viruses to persist in the environment and on fresh produce, as well as their low infectious doses, allows even a small amount of contamination to cause foodborne viral outbreaks. Human Adenoviruses (hAdVs) of subgroup F (enteric serotypes 40 and 41) are known to be associated with 5–20% worldwide of acute gastroenteritis cases among infants and young children. The present study aimed to evaluate a simple and cost-effective, one-step, single-tube adenovirus type 40/41 specific loop-mediated isothermal amplification (LAMP) assay for the detection of hAdV40/41 DNA in a series of ready-to-eat food samples (strawberries, sour cherries, lettuce, cherry tomatoes and green onions). No LAMP assay has previously been reported for the detection of these virus types in food samples. Results were obtained within 60 min, under isothermal conditions at 69°C. After amplification, the products were detected either by observing a ladder pattern following gel electrophoresis, or a color change with the addition of SYBR Green I to the reaction tube. The limits of detection of hAdV40/41 in food samples were found to be >30 GCs per reaction, when a nucleic acids extraction step was included in the analysis. A 1 log higher (>100 GCs/reaction) detection limit was found for lettuce, cherry tomatoes and green onions, without a nucleic acids extraction step included before the isothermal amplification. The LAMP assay for the virological analysis of food samples is expected to provide a robust, innovative, powerful, cheap and fast monitoring tool, without the need of sophisticated equipment, which will be available for food safety testing by the food industry and the public food health authorities.

Keywords: Loop-mediated isothermal amplification (LAMP), ready-to-eat food matrices, human adenoviruses 40/41, molecular detection, food safety

INTRODUCTION

Ready-to-eat (RTE) fruits and vegetables are considered important components of a healthy and balanced diet and recognized as an important source of nutrients for humans (**Meng and Gerba, 1996, Nuanualsuwan and Oliver, 2003a**). It is well known that food handlers with poor personal hygiene play an important role in contaminating fresh products (**D'Souza *et al*., 2006**). The pathogens associated with environmental transmission routes, through water and food, include a wide diversity of bacteria, protozoa, as well as viruses, such as Norovirus (NoV), hepatitis A virus (HAV), hepatitis E virus (HEV) and adenoviruses (AdV). As a consequence, the use of indicator viruses is important for investigating food safety and quality (**Kokkinos *et al*., 2012**). There is no strict evidence indicating which stage of the food production process is the most vulnerable for virus contamination. However, in the majority of contamination cases, fresh produce becomes contaminated on the farm during growing or harvesting (**Heaton and Jones, 2008, Kokkinos *et al*., 2012**). Recent foodborne outbreaks in Europe have been caused by Noroviruses present in lettuce (**Feng *et al*., 2011**) or HAV in semidried tomatoes (**Gogate, 2007**). There is a need to provide rapid, sensitive, and easy screening for pathogen detection in food companies.

Although the majority of adenoviruses cause respiratory tract diseases, some of them are associated with gastroenteritis (**Van *et al*., 1992**). It is estimated that more than 90% of the human population is seropositive for different types of adenoviruses (**Fong *et al*., 2010**). HAdVs species F types 40 and 41 are enteric pathogens which are associated with 5–20% of acute gastroenteritis cases among infants and young children (**Haramoto *et al*., 2007, Bányai *et al*., 2009, Haramoto *et al*., 2010, Sdiri-Loulizi *et al*., 2009, Lee *et al*., 2012, Rodríguez-Lázaro *et al*., 2012**).

In 2000, **Notomi *et al***. developed a novel nucleic acid amplification method, designated loop-mediated isothermal amplification (LAMP). This novel assay is advantageous since it has high specificity, sensitivity, low cost and it can be used as an easy diagnostic tool without the need of sophisticated equipment, by the food industry and the Public health authorities (**Notomi *et al*., 2000**).

The aim of the present study was the evaluation of a simple, cost-effective, one-step, single-tube adenovirus types 40/41 specific loop-mediated isothermal amplification (LAMP) assay, which has been recently published by our group (**Ziros *et al*., 2015**), for the detection of hAdV40/41 in a series of fresh ready-to-eat (RTE) food samples. It is the first time that a LAMP assay is reported for the detection of these virus types in two main categories of RTE matrices (vegetables and soft fruits).

MATERIAL AND METHODS

Food samples collection and processing

Five (5) different food samples, were purchased from a local supermarket (Patras, Greece) the day of the experiment, and stored under refrigerated conditions (4°C) until the time of the experiment. Specifically, fresh ready-to-eat products, such as romaine lettuce (*Lactuca sativa L. var. longifolia*), green onions (*Allium spp*), cherry tomatoes (*Solanum lycopersicum var. cerasiforme*), strawberries (*Fragaria x ananassa*), and sour berries (*Prunus cerasus*), were used for the specificity and evaluation tests of the developed LAMP assay.

Sample inoculation-Viral attachment

All food samples were rinsed with sterile water to remove some of the natural flora or impurities before treatment. For the inoculation of the samples of RTE products, a spot-inoculation method was applied to inoculate the hAdVs on their surface. Briefly, 10 μL (5 drops) of AdV40/41, corresponding to concentrations of 10^4 GCs/ 10μl, 10^3 GCs/ 10μl, 10^2 GCs/ 10μl, 10^1 GCs/ 10μl, 10^0 GCs/ 10μl (which were determined by quantitative QPCR) were spotted with a micropipette on 5 different areas of the surface of each food sample weighing 25g. After spiking, the samples were dried in a class II biosafety cabinet (Cytair 155, FluFrance), for 1 hour at 22±2°C, to allow viral attachment.