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Fostering the antiviral activity of green tea extract for sanitizing purposes through controlled storage conditions

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ABSTRACT

Food-contact surfaces is considered an important vehicle for the indirect transmission of foodborne viral diseases with enteric viruses, especially human norovirus (HuNoV) and hepatitis A virus (HAV). The aim of the present study was to evaluate the antiviral activity of green tea extract (GTE) at room temperature as a function of pH and storage time and to relate it with changes in composition as a consequence of degradation and epimerization reactions in the storage conditions. The obtained results revealed that freshly prepared GTE was very effective in inactivating murine norovirus (MNV) and HAV at neutral and alkaline pH but was ineffective at pH 5.5. Additionally, storage of the solutions for 24 h at various pH conditions significantly increased their antiviral activity. The reduction in MNV and HAV infectivity was related to the formation of catechin derivatives during storage, as demonstrated by HPLC/MS analysis.

In addition, the GTE prepared under the optimal conditions (24 h storage and pH 7.2) was applied at a concentration of 5 mg/mL for only 15 min on stainless steel and glass surfaces for sanitizing purposes, showing a reduction of more than 1.5 log of MNV and HAV infectivity. These findings indicate that GTE can be used as a natural disinfectant for decontamination of food contact surfaces, thus preventing the indirect transfer of enteric viruses to food or persons.

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1. Introduction

In the last decade an increased incidence of foodborne outbreaks have been attributed to human enteric viruses, most notably human norovirus (HuNoV) and hepatitis A virus (HAV). The World Health Organization (WHO) has recently estimated that HuNoV causes approximately 120 million illnesses and 35,000 deaths, while HAV causes 14 million cases and 28,000 deaths attributed to foodborne illness each year (WHO, 2013).

HuNoV and HAV are transmitted through the fecal-oral route, either by direct contact with a virus-infected person, or through ingestion of contaminated water or food (reviewed by Sánchez, 2015; de Graaf, van Beek, & Koopmans, 2016). Additionally, their low infectious dose, ranging from 10 to 100 (Teunis et al., 2008) together with their prolonged stability in the environment, make HuNoV and HAV extremely infectious and highly transmissible through environmental surfaces (fomites).

All these factors justify the highest health risk associated to HuNoVs outbreaks in closed communities (e.g. nursing homes, hospitals, cruise ships, etc.) as exposure sites where common areas and facilities contribute to a rapid virus transmission (European Food Safety, European Centre for Disease, & Control, 2016; Hall, Wikswo, Pringle, Gould, & Parashar, 2014; Hedlund, Rubilar-Abreu, & Svensson, 2000).

In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives or sanitizers, foods and food-contact surfaces treated with natural compounds have become very popular since they are consider safe, cheap, and pose no risk to both the environment and population (Li, Baert, & Uyttendaele, 2013). Many different natural compounds have been used as antimicrobials for food applications, either directly added into the product formulation, incorporated into the packaging material or used as a natural sanitizers (Irkin & Esmer, 2015). Although bactericidal efficacy of most of these natural compounds has been well established, current knowledge of the antiviral

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efficacy for food applications is limited and requires further investigation (reviewed by Li, Baert et al., 2013; Li, Lo et al, 2013).

So far several natural compounds have already been characterized for their antiviral activity (reviewed by Li, Baert et al., 2013; Li, Lo et al, 2013), however limited information is available for their use in food applications. For example, grape seed extract (GSE) was effective in reducing HuNoV surrogates, i.e. feline calicivirus (FCV) and murine norovirus (MNV), as well as HAV infectivity on lettuce, apple juice and milk (Joshi, Su, & D'Souza, 2015; Su & D'Souza, 2013). Likewise, carvacrol was effective in reducing MNV and FCV infectivity in lettuce and lettuce wash water (Sanchez, Aznar, & Sanchez, 2015). Recently, green tea extract (GTE) was proved very effective as a natural sanitizer to control viral contamination since 1.5 log reduction and complete inactivation were recorded for MNV and HAV on stainless steel and glass surfaces treated with 10 mg/mL GTE for 30 min (Randazzo, Falco, Aznar, & Sanchez, 2017). Similarly, GTE demonstrated good effectiveness to decrease contamination with human adenoviruses on lettuces, strawberries and green onions (Marti, Ferrary-Américo, & Barardi, 2017).

GTE is a complex mix of various components; it is a source of polyphenols, especially flavonoids, being catechins the mayor substances which make up approximately 30% of the dry weight of the tea leaf (Yilmaz, 2006). Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are the major catechins in GTE (Abdel-Rahman et al., 2011). Several studies confirmed that these catechins have antioxidative, thermogenic, anticarcinogenic, and anti-inflammatory effects depending on its concentration (Chacko, Thambi, Kuttan, & Nishigaki, 2010). Moreover, recent findings suggest that the antiviral activity of EGCG against HuNoV surrogates and HAV is due to the activity of catechin derivatives rather than EGCG itself (Falcó et al., 2017).

In this work, the antiviral activity of GTE over time was evaluated against MNV and HAV at 25 °C, using solutions freshly prepared at different pHs and after different times of storage. Moreover, HPLC analysis of the GTE solutions was performed to disclose if GTE derivatives would explain the differences in antiviral activity displayed. Finally, the optimum GTE solution was evaluated as a natural disinfectant for food-contact surfaces.

2. Materials and methods

2.1. Virus propagation and cell lines

MNV-1 was propagated and assayed in RAW 264.7 cells, both were kindly provided by Prof. H. W. Virgin (Washington University School of Medicine, USA). HAV (strain HM-175/18f) was purchased from ATCC (VR-1402), which was propagated and assayed in confluent FRhK-4 cells (kindly provided by Prof. A. Bosch, University of Barcelona, Spain). Semi-purified MNV and HAV viruses were harvested at 2 days and 12 days after infection, respectively, by three freeze-thaw cycles of infected cells followed by centrifugation at 660 × g for 30 min to remove cell debris. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution and 20 μL of inoculum per well using the Spearman-Karber method (Pinto, Diez, & Bosch, 1994).

2.2. Antiviral activity of GTE solution depending on aging

GTE (Naturex SA, France), listed as GRAS (21CFR or AAFCO), with an EGCG content of approximately 40–50% was used in this study. It was dissolved in PBS (pH 7.2) to obtain a concentration of 1 mg/mL. Antiviral effect of GTE solutions was evaluated at time 0 (freshly prepared), after 24 h and seven days of storage. Each solution was mixed with an equal volume of HAV and MNV suspensions (ca. 5 log $TCID_{50}$/mL) getting a final concentration of GTE of 0.5 mg/mL, followed by incubation at 25 °C in a shaker for 2 h. Positive control was virus suspensions in PBS under the same experimental conditions. Each treatment was performed in triplicate. Confluent RAW 264.7 and FRhK-4 monolayers in 96-well plates were used to evaluate the effect of GTE as described above. Antiviral activity of GTE was estimated by comparing the number of infectious viruses on suspensions without GTE and on the GTE-treated virus suspensions. The decay of HAV and MNV titers was calculated as $\log_{10}(N_x/N_0)$, where N_0 is the infectious virus titer for untreated samples and N_x is the infectious virus titer for GTE-treated samples.

2.3. Effect of GTE depending on aging and pH

In order to elucidate the effect of pH and aging on the antiviral activity of GTE, MNV and HAV suspensions were ten-fold diluted in PBS at different pHs (5.5, 7.2 and 8.5) and immediately incubated with a freshly prepared GTE solution at 0.5 and 5 mg/mL prepared in PBS at pH 5.5, 7.2 and 8.5. Moreover, GTE solutions at different pHs were stored for 24 h and further incubated with virus suspensions (1:1). Samples were incubated for 2 h at 25 °C in a shaker. Positive controls were virus suspensions in PBS under the same experimental conditions. Infectious viruses and effectiveness of the treatments were calculated as described above.

2.4. Time dependent effects of GTE

The effect of time over GTE solution was tested using a concentration of 10 mg/mL solution prepared by dissolving the supplied powder in PBS at pH 7.2 and stored for 24 h. This solution was then mixed with an equal volume of each virus to reach viral titers about 5 log $TCID_{50}$/mL and a final concentration of GTE of 5 mg/mL. Then samples were incubated in a water-bath shaker at 150 rpm at 25 °C for 15, 60, 120, 240 and 480 min. Positive controls were virus suspensions added with PBS at pH 7.2 under the same experimental conditions.

2.5. HPLC analysis

GTE solutions (5 mg/mL) in PBS at different pHs (i.e. 5.5, 7.2 and 8.5) were subjected to HPLC-MS analysis. Samples were analyzed after different storage periods: 0, 1 and 7 days. An Agilent 1290 HPLC system equipped with an Acquity UPLC BEH C18 column (Waters, 50 mm × 2.1 mm, 1.7 μm of particle size) was used, following the method described in Gómez-Mascaraque, Soler, and Lopez-Rubio (2016). The injection volume was 10 μL. Eluent A was water and eluent B methanol, both slightly acidified with 0.1% formic acid. The flow rate was 0.4 mL/min and the elution gradient started with 10% of eluent B during 2 min, followed by 100% eluent B for 13 min, and 10% eluent B for the last 7 min. A TripleTOF™ 5600 system with a DuoSpray™ source operating in the negative mode was used for detection (AB SCIEX). The parameter settings used were: ion spray voltage −4500 V, temperature 450 °C, curtain gas 30 psi, ion source gas 50 psi. Data were evaluated using the XIC manager in the PeakView™ software (version 2.2). The compounds detected were tentatively identified with the aid of the Phenol Explorer and Chemspider databases (http://phenol-explorer.eu, http://www.chemspider.com).

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