Food and Environmental Virology (2019) 11:90–95
https://doi.org/10.1007/s12560-019-09367-3

ORIGINAL PAPER

Antiviral Activity of Essential Oils Against Hepatitis A Virus in Soft Fruits

Roberta Battistini[1] · Irene Rossini[1] · Carlo Ercolini[1] · Maria Goria[1] · Maria Rita Callipo[1] · Cristiana Maurella[1] · Enrico Pavoni[2] · Laura Serracca[1]

Received: 26 October 2018 / Accepted: 18 January 2019 / Published online: 25 January 2019

Abstract

Berries have repeatedly been associated with outbreaks of hepatitis A virus (HAV) infection. The fruits are usually minimally processed in the food industry due to their delicate nature. While washing treatments partially remove enteric viruses, the commonly used chemical additives produce toxic by-products. A valid alternative to preserve the food safety of these products could be the use of essential oils (EOs). EOs exert antimicrobial activity and do not interfere with the nutritional characteristics of food products. We investigated the efficacy of four essential oils, lemon (*Citrus limon*), sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), and rosemary cineole (*Rosmarinus officinalis* chemotype 1.8 cineole) in reducing viral loads of HAV in soft fruits. Mixed fruit berries were inoculated with $10^{6.74}$ $TCID_{50}$/ml of HAV, and treated with four different EOs (0.5% lemon, 0.1% sweet orange and grapefruit, and 0.05% rosemary) for 1 h at room temperature. Virus infectivity was then assessed by titration assays for its ability to grow on cell cultures. A statistically significant reduction in HAV titer on the fruit surface was observed after treating the berries with EOs of lemon (2.84 log $TCID_{50}$/ml), grapefruit (2.89 log $TCID_{50}$/ml), and rosemary cineole (2.94 log $TCID_{50}$/ml). Rosemary cineole was the most effective EO in reducing viral titer on berries, followed by grapefruit EO. These results improve our knowledge about the antiviral activity of these EOs and highlight their potential use in fresh produce sanitation.

Keywords Inactivation · Berries · Hepatitis A virus · Essential oils

Introduction

Repeated hepatitis A virus (HAV) outbreaks due to the consumption of frozen berries have occurred in Europe and elsewhere in the world (Chatziprodromidou et al. 2018). Between 2012 and 2014, two multistate outbreaks were associated with the consumption of frozen mixed berries. The first outbreak occurred in four Nordic countries (European Food Safety Authority 2013; Lassen et al. 2013), the second involved Italy, where 1803 cases were reported (Scavia et al. 2017) and 12 European Union (EU) countries (Fitzgerald et al. 2014; Tavoschi et al. 2015). Berry fruits can be contaminated during cultivation or processing by contact with infected workers or by fecal contaminated water (Maunula et al. 2013). HAV, a member of the Picornaviridae family, is the causative agent of infectious hepatitis. Genetically, HAV is classified into six genotypes (I to VI) based on sequences of the VP1-2A region. Genotypes I, II, and III infect humans (Sánchez et al. 2007). Transmission occurs via the fecal–oral route, mainly through contaminated water and food (Carter 2005). The virus can resist on foods at length without losing its infectious dose (Sánchez et al. 2007). Because fruit and vegetables are usually eaten raw, viral contamination of fresh products poses a major risk to consumer health. Treatments commonly used for produce sanitation (e.g., washing with disinfectants, refrigeration, freezing, acidic pH, freeze-drying, UV irradiation, etc.) are ineffective in removing or completely inactivating the viruses (Fino and Kniel 2008; Fraisse et al. 2001; Li et al. 2013; Sánchez 2015). While heat is the most effective means for their elimination, heat treatment, i.e., cooking, is not always appropriate for the commercial characteristics of these products. This is why it is interesting to devise and evaluate innovative treatments to

✉ Laura Serracca
laura.serracca@izsto.it

[1] Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

[2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

sanitize berries without altering their organoleptic properties. A valid alternative to sanitize food products could be the use of essential oils (EOs) as "natural" additives. Essential oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, branches, bark, grass, wood, fruit, and roots) mainly by steam distillation or other methods such as pressing, fermentation, enfleurage, or extraction by heat or solvents. By virtue of their antimicrobial, antifungal, and antiviral activity, EOs could be advantageously used by the food industry (Nolkemper et al. 2006; Reichling et al. 2009; Tullio et al. 2012; Chouhan et al. 2017). Furthermore, the effectiveness of the chemical components in combating pathogenic microorganisms has been amply demonstrated (Fisher and Phillips 2008; Ozogul et al. 2015; Piątkowska and Rusiecka-Ziółkowska 2016). Yet, few oils have been tested for these properties in food sector applications, and data on the efficacy of EOs against foodborne viruses are scarce (Kovac et al. 2012; Elizaquível et al. 2013; Gilling et al. 2014; Sánchez and Aznar 2015; Kim et al. 2017) particularly for HAV (Sánchez and Aznar 2015). Here, we investigated the efficacy of four EOs to reduce viral loads of HAV in soft fruits and assessed their potential use to reduce or eliminate viral contamination in fresh fruit production. To do this, EOs of lemon (*Citrus limon*), sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), and rosemary cineole (*Rosmarinus officinalis* chemotype 1.8 cineole) were evaluated. All are declared GRAS (generally recognized as safe) by the U.S. Food and Drug Administration (FDA). The first three EOs are derived from plants belonging to the genus *Citrus*; their fragrance and flavor are well suited to berries and are pleasing to consumers. Essential oils of the genus *Citrus* contain 85–99% of volatile components, including monoterpene (limonene), sesquiterpenes, and hydrocarbons; their oxygenated products include aldehydes (citral), ketones, acids, alcohols (linalool), and esters (Fisher and Phillips 2008). *Rosmarinus officinalis* is an aromatic plant of the *Lamiaceae* family commonly used in European cuisine besides its use in medicinal products thanks to its strong antiseptic properties; rosemary oil is used as a natural food preservative (Satyal et al. 2017; Sirocchi et al. 2017). While the antibacterial and antioxidant activities of *Rosmarinus officinalis* EO have been variously described (Nieto 2017), its antiviral properties have been little investigated (Gavanji et al. 2015) and no study to date has investigated its effect on HAV.

Materials and Methods

Virus and Cell Lines

The ATCC/HM-175 strain of HAV was propagated and assayed in Frp3 cells (kindly provided by Enrico Pavoni IZSLER, Italy). The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich-Life Science, St. Louis, MO, USA) supplemented with 3% fetal bovine serum (FBS; EuroClone, Milan, Italy) and 1% antibiotic/antimycotic (Sigma Aldrich) incubated at 37 °C and 5% CO_2. The HAV stock titer used in the experiments was $1 \times 10^{6.74}$ $TCID_{50}$/ml. Viral titrations were performed using the Reed and Muench method (Reed and Muench 1938; Hoskins 1975) to determine the 50% tissue culture infectious dose ($TCID_{50}$). Briefly, serial 10-fold dilutions of the virus sample were assayed in 24-well tissue culture plates (EuroClone) containing monolayers of Frp3 cells in 900 µl of DMEM supplemented with 3% FBS, incubated at 37 °C and 5% CO_2. Three wells were inoculated with 100 µL of each dilution and each well was checked every day for 15 days for viral cytopathogenic effects (CPE).

Essential Oils

Citrus limon (lemon), *Citrus sinensis* (sweet orange), *Citrus paradisi* (grapefruit), and *Rosmarinus officinalis* chemotype 1.8 cineole (rosemary cineole) EOs supplied by Flora srl (Pisa, Italy) were used in this study. Gas chromatography–mass spectrometry (GC–MS) analysis performed by the producer identified the main chemical compounds of the EOs: limonene (71.18%), β-pinene (8.76%), and γ-terpinene (8.24%) in the lemon EO; limonene 95.74% and 93.45% in the sweet orange and the grapefruit EO, respectively; and 1.8 cineole (51.79%), α-pinene (16.54%), camphor (8.38%), and camphene (4.27%) in the rosemary cineole EO.

Cytotoxicity Assay

Preliminary trials were performed to identify the working solution and EO concentrations that did not produce cytotoxicity on cell cultures (data not shown). Because EOs are hydrophobic, a medium had to be found in which they could be dispersed before applying them to the berries. Sunflower oil, peanut oil, olive oil, and wheat ethylic alcohol were evaluated as carriers for the EOs. DMSO, Tween 80, and ethanol for food use were tested as surfactants. The EOs were first evaluated at concentrations of 0.1%, 0.5%, and 1% (v/v), then decreased as needed according to the results. Only solutions of carrier oils and different surfactants were first tested on Frp3 cells and then the selected working solution was tested in combination with the EOs at different concentrations. Cytotoxicity effects were determined by visual inspection under optical microscopy for 5 days.

Application of EOs in Soft Fruits

Five samples (one for each EO and one control sample) of 12.5 g of frozen commercially mixed berries (each consisting of 3 raspberries, 4 blackberries, 4 blueberries, 4 currants) were used to evaluate the efficacy of the four EOs in reducing viral loads in soft fruits. After thawing, the samples were seeded by distributing 10 µl of HAV (at $10^{6.74}$ $TCID_{50}$/ml) over the fruit surface. Inoculated samples were air dried in a laminar cabinet. The samples were then soaked for 1 h with 20 ml of working solution (66% Tris/glycine/beef extract (TGBE) buffer pH 9.5, 33% peanut oil, 0.1% Tween 80) with the EO at the following concentrations: 0.5% lemon oil, 0.1% orange and grapefruit oils, and 0.05% rosemary cineole oil. After treatment, the viruses were eluted and concentrated from berries according to methods described in the standard method for virus detection in food (section soft fruits) ISO/TS-15216-2:2013. A final clarification step with chloroform-butanol was not performed, however, because it causes cytotoxic effects on cell cultures. All samples of about 500 µl were added with 1% antibiotic/antimycotic (Sigma–Aldrich), stored at − 80 °C and subsequently assayed using the $TDCID_{50}$ method (as described previously) to determine the infectious virus titer. Control samples consisted of soft fruits contaminated with HAV and soaked with working solution without the addition of any EO. The experiments were performed in single and repeated three times at room temperature.

Statistical Analysis

Data are presented as mean ± SD. The Wilcoxon–Mann–Whitney two-sample rank-sum test was used to evaluate the differences between the mean number of virus determined after treatment with each EO and control. Statistical significance was set at 0.05. Data analysis was performed with STATA Software, version 14.2.

Results

Determination of Cytotoxicity on Cell Monolayers

Peanut oil was selected as the carrier oil because it is considered suitable and safe for this purpose, and Tween 80 as surfactant. Orange and grapefruit EOs were found to be cytotoxic for Frp3 cells at concentrations that exceeded 0.1%, while lemon and rosemary cineole EOs resulted cytotoxic at concentrations that exceeded 0.5% and 0.05%, respectively. These values were the maximum concentrations of lemon, orange, grapefruit, and rosemary cineole EOs tested to evaluate their effect on berries contaminated with HAV. The final experiments were performed with the working solution that did not cause a cytotoxic effect on cells as described above.

Effect of EOs on the Infectivity of HAV on Berries

The effect of treatment with EO on berries spiked with HAV is shown in Table 1. Treatment of contaminated berries with all four EOs tested at room temperature reduced the viral titer of HAV as compared with the control. In particular, the HAV titer was reduced by 2.84 log $TCID_{50}$/ml after treatment with lemon EO at 0.5%, by 2.14 and 2.89 log $TCID_{50}$/ml after treatment with orange and grapefruit EO at 0.1%, respectively, and by 2.94 log after treatment with rosemary cineole at 0.05%. Statistical analysis showed a significant reduction ($P < 0.05$) in the samples treated with lemon, grapefruit, and rosemary cineole EOs. Rosemary cineole was the most effective EO in reducing the viral titer on berries followed by grapefruit EO and lemon EO.

Discussion

Our knowledge about the efficacy of EOs as an alternative treatment in reducing the risk of HAV contamination of fresh produce is still limited or unknown. Here, we evaluated the antiviral effect of lemon EO at 0.5%, orange and grapefruit EOs at 0.1%, and rosemary cineole EO at 0.05% against HAV on soft fruits that are known to be frequently associated with foodborne outbreaks (Chatziprodromidou et al. 2018). The results showed that lemon, grapefruit, and rosemary cineole EOs significantly reduced the HAV titers after 1 h of incubation at room temperature (as compared with the untreated control). The greatest reduction in cell infectivity was observed for rosemary cineole EO (about 3 log $TCDI_{50}$/ml), followed by grapefruit and lemon EOs, while orange EO, although reducing HAV infectivity by > 2 log $TCDI_{50}$/ml, was not statistically significant. This is the first study to

Table 1 Effects of lemon (L), orange (O), grapefruit (G), and rosemary cineole (R) essential oils against HAV on soft fruits after 1 h of incubation at room temperature

HAV (Log_{10} $TCID_{50}$/ml)		
Treatment	Recovered titer	Reduction
Control	5.83 ± 0.58	
0.5% L	2.99 ± 0.44	2.84*
0.1% O	3.69 ± 1.64	2.14
0.1% G	2.94 ± 0.57	2.89*
0.05% R	2.89 ± 0.61	2.94*

Each treatment was done in triplicate. *reduction was statistically significant ($P < 0.05$) in comparison with the control

evaluate the effectiveness of these four EOs against HAV infectivity. To date, only oregano and zataria EOs have been evaluated on HAV suspensions in a tissue culture medium. The results showed only a slight reduction in virus infectivity (> 1 log) (Sánchez and Aznar 2015). Various natural additives tested for reducing HAV infectivity have resulted without much success: carvacrol (Sánchez et al. 2015), thymol (Sánchez and Aznar 2015), Korean red ginseng extracts, and ginsenosides (Lee et al. 2013). Other natural compounds have given good results: grape seed extract (GSE) (Su and D'Souza 2011), blueberry juice and blueberry proanthocyanidins (Joshi et al. 2016), cinnamaldehyde (Fabra et al. 2016), and green tea extract (GTE) (Randazzo et al. 2017). Grape seed extract reduced HAV infectivity at 37 °C and room temperature by a maximum of > 3 log, depending on the initial virus and grape seed extract concentration. Blueberry juice reduced HAV titers by about 2 log PFU/ml after 24 h, and blueberry proanthocyanidins reduced HAV to undetectable levels after 30 min at concentrations of 2 and 5 mg/ml. Cinnamaldehyde at 1% was effective in reducing HAV titers by 1 $\log_{10}$ TCID/ml after 2 h at 37 °C, and by 3.4 $\log_{10}$TCID/ml after overnight incubation, while cinnamaldehyde at 0.5% reduced HAV titers by 2.7 $\log_{10}$ TCID/ml after overnight incubation. GTE at 5 mg/ml incubated at 37 °C for 2 h reduced by 1.08 log HAV infectivity and after overnight exposure to GTE completely inactivated HAV at 37 °C and 25 °C. Furthermore, few studies have investigated the application of natural compounds on fresh products for foodborne viral reduction. Two relatively recent studies have been performed on HAV (Su and D'Souza 2011; Randazzo et al. 2017). Su and D'Souza (2011) assessed the efficacy of grape seed extract washes on HAV on food product (lettuce and jalapeno peppers). They found that GSE washes had a limited effect on HAV infectivity; indeed, after 1 min of contact, 0.25 to 1 mg/ml GSE reduced high and low HAV titers from 0.7 to 1.1 and 1 to 1.3 log10 PFU, respectively, on both food products. Randazzo et al. (2017) evaluated the efficacy of GTE as a natural disinfectant on fresh-cut vegetable surfaces and showed that 10 mg/ml GTE reduced HAV titers in lettuce and spinach by more than 1.5 log after treatment for 30 min. Decontamination of produce with sanitizers is a critical step to reduce the levels of microorganisms and to ensure food safety (Burnett and Beuchat 2001). The process the food industry currently uses for different types of fresh produce includes a step of washing with tap water supplemented with chlorine or other chemical agents at concentrations of 50–250 ppm for 1–10 min, followed by a rinsing step (Gil et al. 2009). Freshly harvested berries will also be washed before they are further processed into frozen berries, puree, or other products. It has been shown that these decontamination procedures reduce the virus titer by approximately 1 to 2 log on fresh produce and that the efficacy of sanitizers varies between viral strains and depends on produce type (Butot et al. 2008; Baert et al. 2009). HAV is known to be more resistant than other enteric viruses to all washing treatments. HAV inactivation on berries washed with chlorinated water (200 ppm free chlorine) is reported to vary between a log reduction of 1.8 in strawberries to 2.4 in blueberries, while chlorinated water had a limited effect on HAV titers when used to decontaminate raspberries (reduction of 0.6 log) probably because of their complex surface topography (Butot et al. 2008). For instance, raspberries have hair-like projections and crevices that may shield the viruses against environmental conditions. Washing raspberries with water containing 5 or 10 ppm chlorine dioxide for 10 min inactivates HAV by less than 1 log (Butot et al. 2008). In another study, HAV on strawberries treated with 200 ppm free chlorine for 3 or 5 min reduced the titer by 1.2 log and 2.6 log, respectively (Casteel et al. 2008). The HAV concentrations detected in food samples varied greatly from less than 100 genomic copies to more than 100,000 per gram of food analyzed. These concentrations were by far greater than the infective dose for HAV, estimated to be around 10 and 100 viral particles (Yezli and Otter 2011). Regarding fresh produce only data on parsley, green onions, and coriander are reported. The HAV concentrations in these products are reported to range between 2.8×10^2 and 2.4×10^3 genomic copies per gram (Felix-Valenzuela et al. 2012). To our knowledge, no published data exist for soft fruits. On the basis of previous studies, it is clear that the use of chlorinated water is not effective in reducing the HAV load in fresh produce under the possible infective dose. Furthermore, concerns have been recently raised regarding the safety of chlorine. It is known that chlorine quickly loses its effectiveness when soil, dirt, and organic materials are present and that it produces carcinogenic disinfection by-products, including trihalomethanes (Richardson 1998). Due to the environmental and health risks posed by chlorine, there is a need to find ways to reduce or eliminate its use from the disinfection process. Our results show that the use of lemon, grapefruit, orange, and rosemary cineole EOs for sanitizing berries contaminated with HAV gave better results in inactivating the virus (by about 2–3 log) as compared with those reported for the current methods of disinfection with chlorine. In addition, EOs are non-toxic, preserve the nutritional characteristics of foods, have less environmental impact, and are preferred by consumers as sanitization agents over synthetic chemicals. Treatment with EOs could be a potential non-chemical alternative to chlorine washing to inactivate or reduce HAV contamination on berries when the contamination level is below 2 to 3 log. These procedures alone would be insufficient to inactivate/decontaminate foods contaminated with higher virus concentrations, however. Hence, EOs should be considered for use in food sanitation in combination with other treatments. It must also be taken into account that EOs have an intense taste and smell, which can

modify the taste and aroma of food products (Laranjo et al. 2017). The industrial application of EOs to soft fruits should be followed by a rinse phase before their commercialization. Further studies are needed to assess the organoleptic impact of such treatments on soft fruits through sensory screening and to determine the minimum necessary amount of EO that can still maintain antimicrobial activity without changing the organoleptic characteristics of food products. It will also be necessary to evaluate the minimum time EOs take to reduce the maximum HAV titers in order to ensure food product safety without affecting the production timing in current industrial practices. Finally, studies will need to investigate how EOs can influence the organoleptic properties of the product in terms of storage and distribution, since soft fruits have a high metabolic rate and a very short shelf life (Vicente and Sozzi 2007). The use of EOs in preserving fresh fruits has attracted increasing interest in recent years owing to their relatively safe status (Lanciotti et al. 2004; Vergis et al. 2015). It is widely documented that these compounds play an important role in food preservation and contribute to the safety and shelf-life extension of food products (Tzortzakis 2007; Serrano et al. 2008; Ulukanli and Oz 2015; Martinez et al. 2018; Lee et al. 2019). Overall, our results improve current knowledge about the antiviral activity of these EOs and highlight their potential use to reduce viral contamination in berries.

Acknowledgements This study was supported by Grants from the Italian Ministry of Health (IZS PLV 14/13).

References

Baert, L., Debevere, J., & Uyttendaele, M. (2009). The efficacy of preservation methods to inactivate foodborne viruses. *International Journal of Food Microbiology, 131*(2–3), 83–94.

Burnett, S. L., & Beuchat, L. R. (2001). Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *Journal of Industrial Microbiology & Biotechnology, 27*(2), 104–110.

Butot, S., Putallaz, T., & Sanchez, G. (2008). Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology, 126*(1–2), 30–35.

Carter, M. J. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology, 98*(6), 1354–1380.

Casteel, M. J., Schmidt, C. E., & Sobsey, M. D. (2008). Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. *International Journal of Food Microbiology, 125*(3), 267–273.

Chatziprodromidou, I. P., Bellou, M., Vantarakis, G., & Vantarakis, A. (2018). Viral outbreaks linked to fresh produce consumption: a systematic review. *Journal of Applied Microbiology, 124*(4), 932–942.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines (Basel), 4*(3), 58.

EFSA. (2013). Hepatitis A virus infection in four Nordic countries, 15 April 2013. Joint ECDC/EFSA rapid outbreak assessment.

Elizaquível, P., Azizkhani, M., Aznar, R., & Sánchez, G. (2013). The effect of essential oils on norovirus surrogates. *Food Control, 32*(1), 275–278.

Fabra, M. J., Castro-Mayorga, J. L., Randazzo, W., Lagarón, J. M., López-Rubio, A., Aznar, R., & Sánchez, G. (2016). Efficacy of cinnamaldehyde against enteric viruses and its activity after incorporation into biodegradable multilayer systems of interest in food packaging. *Food and Environmental Virology, 8*(2), 125–132.

Felix-Valenzuela, L., Resendiz-Sandoval, M., Burgara-Estrella, A., Hernández, J., & Mata-Haro, V. (2012). Quantitative detection of hepatitis A, rotavirus and genogroup I norovirus by RT-qPCR in fresh produce from packinghouse facilities. *Jounal of Food Safety, 32*(4), 467–473.

Fino, V. R., & Kniel, K. E. (2008). UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions and lettuce. *Jounal of Food Protection, 71*(5), 908–913.

Fisher, K., & Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in Food Science & Technology, 19*(3), 156–164.

Fitzgerald, M., Thornton, L., O'Gorman, J., O'Connor, L., Garvey, P., Boland, M., Part, A. M., et al. Hepatitis, A., Outbreak Control Team (2014). Outbreak of hepatitis A infection associated with the consumption of frozen berries, Ireland, 2013-linked to an international outbreak. *Euro Surveillance, 30*(43), pii: 20942. 19).

Fraisse, A., Temman, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., Vialette, M., et al. (2001). Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus and hepatitis A virus on lettuce. *International Journal of Food Microbiology, 151*(1), 98–104.

Gavanji, S., Sayedipour, S. S., Larki, B., & Bakhtari, A. (2015). Antiviral activity of some plant oils against herpes simplex virus type 1 in Vero cell culture. *Journal of Acute Medicine, 5*(3), 62–68.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology, 134*(1–2), 37–45.

Gillesberg Lassen, S., Soborg, B., Midgley, S. E., Steens, A., Vold, L., Stene-Johansen, K., Rimhanen-Finne, R., et al. (2013). Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013. *Euro Surveillance, 18*(17), 20467.

Gilling, D. H., Kitajima, M., Torrey, J. R., & Bright, K. R. (2014). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *Journal of Applied Microbiology, 116*(5), 1149–1163.

Hoskins, J. M. (1975). *Diagnosi virologica principi e metodi*. Milano: Casa Editrice Ambrosiana.

Joshi, S. S., Howell, A. B., & D'Souza, D. H. (2016). Reduction of enteric viruses by blueberry juice and blueberry proanthocyanidins. *Food and Environmental Virology, 8*(4), 235–243.

Kim, Y. W., You, H. J., Lee, S., Kim, B., Kim, D. K., Choi, J. B., Kim, J. A., et al. (2017). Inactivation of norovirus by lemongrass essential oil using a norovirus surrogate system. *Jounal of Food Protection, 80*(8), 1293–1302.

Kovač, K., Diez-Valcarce, M., Raspor, P., Hernández, M., & Rodríguez-Lázaro, D. (2012). Natural plant essential oils do not inactivate non-enveloped enteric viruses. *Food and Environmental Virology, 4*(4), 209–212.

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. E., & Gardini, F. (2004). Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in Food Science & Technology, 15*, 201–208.

Laranjo, M., Fernández-Léon, A. M., Potes, M. E., Agulheiro-Santos, A. C., & Elias, M. (2017). Use of essential oils in food

preservation. In Antimicrobial Research: Novel bioknowledge and educational programs (Microbiology Book Series #6) (pp. 177–188). Badajoz: Edited by A. Méndez-Vilas, Formatex Research Center. http://www.microbiology6.org/ebook.php.

Lee, J. Y., Jang, S., Aguilar, L. E., Park, C. H., & Kim, C. S. (2019). Structural packaging technique using biocompatible nanofiber with essential oil to prolong the shelf-life of fruit. *Journal of Nanoscience and Nanotechnology, 19*(4), 2228–2231.

Lee, M. H., Lee, B. H., Lee, S., & Choi, C. (2013). Reduction of hepatitis A virus on FRhK-4 cells treated with Korean red ginseng extract and ginsenosides. *Journal of Food Science, 78*(9), 1412–1415.

Li, D., Baert, L., & Uyttendaele, M. (2013). Inactivation of food-borne viruses using natural biochemical substances. *Food Microbiology, 35*(1), 1–9.

Martínez, K., Ortiz, M., Albis, A., Gilma Gutiérrez Castañeda, C., Valencia, M. E., & Grande Tovar, C. D. (2018). The effect of edible chitosan coatings incorporated with *Thymus capitatus* essential oil on the shelf-life of strawberry (*Fragaria* × *ananassa*) during cold storage. *Biomolecules*. 21,8(4).

Maunula, L., Kaupke, A., Vasickova, P., Söderberg, K., Kozyra, I., Lazic, S., van der Poel, W. H., et al. (2013). Tracing enteric viruses in the European berry fruit supply chain. *International Journal of Food Microbiology, 167*(2), 177–185.

Nieto, G. (2017). Biological activities of three essential oils of the Lamiaceae family. *Medicines (Basel), 4*(3), 63.

Nolkemper, S., Reichling, J., Stintzing, F. C., Carle, R., & Schnitzler, P. (2006). Antiviral effect of aqueous extracts from species of the Laminaceae Family against Herpes simplex Virus type 1 and type 2 in vitro. *Planta Medica, 72*(15), 1378–1382.

Ozogul, Y., Kuley, E., Ucar, Y., & Ozogul, F. (2015). Antimicrobial impacts of essential oils on food borne-pathogens. *Recent Patents on Food, Nutrition & Agriculture, 7*(1), 53–61.

Piątkowska, E., & Rusiecka-Ziółkowska, J. (2016). Influence of essential oils on infectious agents. *Advances in Clinical and Experimental Medicine, 25*(5), 989–995.

Randazzo, W., Falco, I., Aznar, R., & Sànchez, G. (2017). Effect of green tea extract on enteric viruses and its application as natural sanitizer. *Food microbiology, 66*, 150–156.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoint. *American Journal of Hygiene, 27*(3), 493–497.

Reichling, J., Schnitzler, P., Suschke, U., & Saller, R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties: an overview. *Forschende Komplementarmedizin, 16*(2), 79–90.

Richardson, S. (1998). Drinking water disinfection by-products. In: Encyclopedia of environmental analysis and remediation. In R. A. Meyers (Ed.), *Encyclopedia of environmental analysis and remediation* (Vol. 3, pp. 1398–1421). New York: Wiley.

Sánchez, C., Aznar, R., & Sánchez, G. (2015). The effect of carvacrol on enteric viruses. *International Journal of Food Microbiology, 192*, 72–76.

Sánchez, G. (2015). Processing strategies to inactivate hepatitis A virus in food products: a critical review. *Comprehensive Reviews in Food Science and Food Safety, 14*(6), 771–784.

Sánchez, G., & Aznar, R. (2015). Evaluation of natural compounds of plant origin for inactivation of enteric viruses. *Food and Environmental Virology, 7*(2), 183–187.

Sánchez, G., Bosch, A., & Pintó, R. M. (2007). Hepatitis A virus detection in food: current and future prospects. *Letters in Applied Microbiology, 45*(1), 1–5.

Satyal, P., Jones, T. H., Lopez, E. M., McFeeters, R. L., Ali, N. A., Mansi, I., Al-Kaf, A. G., & Setzer, W. N. (2017). Chemotypic characterization and biological activity of *Rosmarinus officinalis. Foods, 6*(3), 20.

Scavia, G., Alfonsi, V., Taffon, S., Escher, M., Bruni, R., Medici, D., Pasquale, S. D., et al. National Italian Task Force On Hepatitis A (2017). A large prolonged outbreak of hepatitis A associated with consumption of frozen berries, Italy, 2013-14. *Journal of medical microbiology, 66*(3), 342–349.

Serrano, M., Martinez-Romero, D., Guillén, F., Valverde, J. M., Zapata, P. J., Castillo, S., & Valero, D. (2008). The addition of essential oils to MAP as a tool to maintain the overall quality of fruits. *Trends in Food Science & Technology, 19*, 464e471.

Sirocchi, V., Devlieghere, F., Peelman, N., Sagratini, G., Maggi, F., Vittori, S., & Ragaert, P. (2017). Effect of *Rosmarinus officinalis* L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. *Food Chemistry, 221*, 1069–1076.

Su, X., & D'Souza, D. H. (2011). Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology, 77*(12), 3982–3987.

Tavoschi, L., Severi, E., Niskanen, T., Boelaert, F., Rizzi, V., Liebana, E., Dias, G., et al. (2015). Food-borne diseases associated with frozen berries consumption: a historical perspective, European Union, 1983 to 2013. *Euro Surveillance, 20*(29), 21193.

Tullio, V., Mandras, N., Allizond, V., Nostro, V., Roana, J., Merlino, C., Banche, et al. (2012). Positive interaction of Thyme (Red) essential oil with human ply-morphonuclear granulocytes in eradicating intracellular *Candida albicans. Planta Medica, 78*(15), 1633–1635.

Tzortzakis, N. G. (2007). Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative Food Science & Emerging Technologies, 8*, 111e116.

Ulukanli, Z., & Oz, A. T. (2015). The effect of oleum myrtle on the fruit quality of strawberries during MAP storage. *Journal of Food Science and Technology, 52*(5), 2860–2868.

Vergis, J., Gokulakrishnan, P., Agarwal, R. K., & Kumar, A. (2015). Essential oils as natural food antimicrobial agents: a review. *Critical Review in Food Science & Nutrition, 55*(10), 1320–1323.

Vicente, R. A., & Sozzi, G. (2007). Ripening and postharvest storage of 'soft fruits'. *Fruit, Vegetable and Cereal Science and Biotechnology, 1*, 95–103.

Yezli, S., & Otter, J. (2011). Minimum infective dose of the major human respiratory and enteric viruses transmitted through food and the environment. *Food and Environmental Virology, 3*(1), 1–30.