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Evaluation of skimmed milk flocculation method for virus recovery from tomatoes

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ABSTRACT

This study aimed to evaluate the elution-concentration methodology based on skimmed milk flocculation from three varieties of tomatoes (*Solanum lycopersicum L.* [globe], *Solanum lycopersicum* var. *cerasiforme* [cherry] and hybrid cocktail [grape tomato]) for further monitoring of field samples. Spiking experiments were performed to determine the success rate and efficiency recovery of human norovirus (NoV) genogroup II, norovirus murine-1 (MNV-1) used as sample process control virus and human adenovirus (HAdV). Mean values of 18.8%, 2.8% and 44.0% were observed for NoV GII, MNV-1 and HAdV, respectively with differences according to the types of tomatoes, with lower efficiency for cherry tomatoes. Analysis of 90 samples, obtained at commercial establishments in the metropolitan region of Rio de Janeiro State, revealed 4.5% positivity for HAdV. Bacterial analysis was also performed with no detection of *Salmonella* spp., *L. monocytogenes* and fecal coliforms. Data demonstrated that the skimmed milk flocculation method is suitable for recovering HAdV from tomatoes and highlights the need for considering investigation in order to improve food safety.

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Introduction

Enteric viruses are described as important contaminants of fresh foods as vegetables and fruits, considering the inadequate system of water irrigation or inappropriate food handling as possible routes of contamination.[1] Among those, noroviruses (NoV) are the main agent causing acute gastroenteritis (AG) outbreaks associated with consumption of fresh products worldwide.[2–7] NoVs are RNA viruses and its genome is composed of RNA single-strand positive-sense, belonging to genus *Norovirus, Caliciviridae* family and classified into seven different genogroups (G) and more than 35 genotypes.[8,9] NoV GI, GII, and GIV can infect human, NoV GII.4 is the most prevalent genotype related to foodborne infection.[10]

Additionally, other viruses such as human adenoviruses (HAdV) have been also investigated in water and food samples.[11,12] Even though they are rarely associated with foodborne illnesses some of them are associated with cases of gastroenteritis.[11,13–16] Currently they have been investigated as indicators of human fecal contamination mainly due to their resistance to adverse environmental conditions, absence of seasonality and its high concentration detected in wastewater samples.[12,16,17] HAdVs are DNA viruses belonging to the *Adenoviridae* family and genus *Mastadenovirus* with 67 types reported.[18]

The increasing number of viruses foodborne outbreaks have resulted in a growing number of studies that evaluate elution and concentration methods from different food matrices, as well as the use of sample processes control viruses (SPCVs) as murine norovirus-1 (MNV-1), bacteriophage PP7 and others.[19–26] In 2013, the International Organization for Standardization (ISO), together with the European Committee for Standardization (CEN), standardized methodologies for recovering NoV and hepatitis A virus from matrix foods[27] that were validated recently.[28]

This study aims to expand previous studies that adapted successfully skimmed milk flocculation method to recover virus from strawberries.[29] Here, we assess NoV, MNV-1 and HAdV success rate and efficiency recoveries from three varieties of tomatoes as well as assess their microbiological quality by investigating NoV GI and GII, HAdV, *Salmonella* spp., *Listeria monocytogenes* and fecal coliforms from samples obtained at market places at the Great Metropolitan Region of Rio de Janeiro State.

Materials and methods

Viruses and food samples

A NoV GII.4 stool sample (GenBank accession number JX975591) was obtained from the Regional Reference Gastroenteritis Laboratory collection, at Oswaldo Cruz Institute, Rio de Janeiro-RJ, Brazil. Murine norovirus-1 (MNV-1) was kindly provided by Dr. Herbert W. Virgin (Washington University School of Medicine) and propagated in RAW 264.7 cells (a macrophage-like Abelson leukemia virus-transformed cell line derived from BALB/c mice; ATCC® TIB-71™), according to de Abreu Corrêa and Miagostovich.[24] HAdVs type 2 was propagated in HEK 293 cells (human embryonic kidney cells; ATCC® CRL1573™) obtained from the Regional Reference Gastroenteritis Laboratory collection, at Oswaldo Cruz Institute, Rio de Janeiro, RJ, Brazil.[30]

Three species of tomatoes as *Solanum lycopersicum* L. (globe), *Solanum lycopersicum* var. *cerasiforme* (cherry) and hybrid cocktail (grape) were obtained from distinct markets in Rio de Janeiro.

For field analysis 90 tomatoes samples (45 globe and 45 grape tomatoes) were randomly obtained from March to September, 2014 (three–five samples per week). All samples were inoculated with MNV-1, used as SPCV.

Spiking experiments for assessing efficiency of virus recovering using skimmed flocculation method.

Artificial contamination was carried out in duplicate in three independent experiments totaling six assays for each virus. The quantitative PCR (qPCR) TaqMan™ system was used to quantify the absolute number of genome copies (gc)/reaction[31] used for those experiments.

Twenty-five grams of tomato samples were spiked by direct application of 250 μL of NoV GII.4 (1×10^6 gc/reaction), 100 μL of MNV-1 (5×10^5 gc/reaction) and 200 μL of HAdV (1×10^6 gc/reaction) onto food surfaces for 2 h at room temperature. The values of gc/reaction for NoV GII.4 and MNV-1 spikes were obtained according to the formula shown in Eq. (1), where n is the average number of amplified copies, based on the standard curve; D is the dilution of extracted nucleic acid; V (μL) represent the volumes of cDNA produced (V_E); of the eluted nucleic acid (V_C); the suspension of virus particles inoculated in the sample (V_G); of cDNA was added to the TaqMan (V_F) reaction; of the template used for cDNA synthesis (V_D); and nucleic acid extracted from the viral particle (obtained by cell culture or stool suspension) (V_H). For HAdV, the same calculate, excluding V_E and V_D. One negative control (seeded with 350 μL of phosphate saline buffer [PBS] 1×) was included and processed at the same time together with the other samples.

$$N = (nDV_E \times V_c \times V_G/V_F \times V_D \times V_H) \quad (1)$$

Skimmed milk flocculation method was performed as described by Melgaço et al.[29] including the use of cetyltrimethylammonium bromide (CTAB) (Fig. 1).

RNA/DNA extraction and viral detection

Viral RNA/DNA was extracted from 140 μL of concentrated samples, using QIAamp viral RNA mini kit® (Qiagen, Valencia, CA, USA), according to manufacturer's instructions. Synthesis of complementary DNA (cDNA) was performed for NoV and MNV-1 detection using random primers, pd(N)6 (Amersham Biosciences, UK) for RNA virus detection.

QPCR using TaqMan™ assays were carried out using a set of specific primers and probes described previously.[19,32,33] Reactions were performed using TaqMan Universal PCR Master Mix® (Applied Biosystems, California, USA) according to the manufacturer in ABI 7500® (Applied Biosystems).

For all genomic quantification, a standard curve was performed with eight points of serial plasmid dilutions (10^7–10° gc/reaction). All the standard curves yield a slope of −3.59 and a R^2 (reaction efficiency) of 0.90. An ABI PRISM

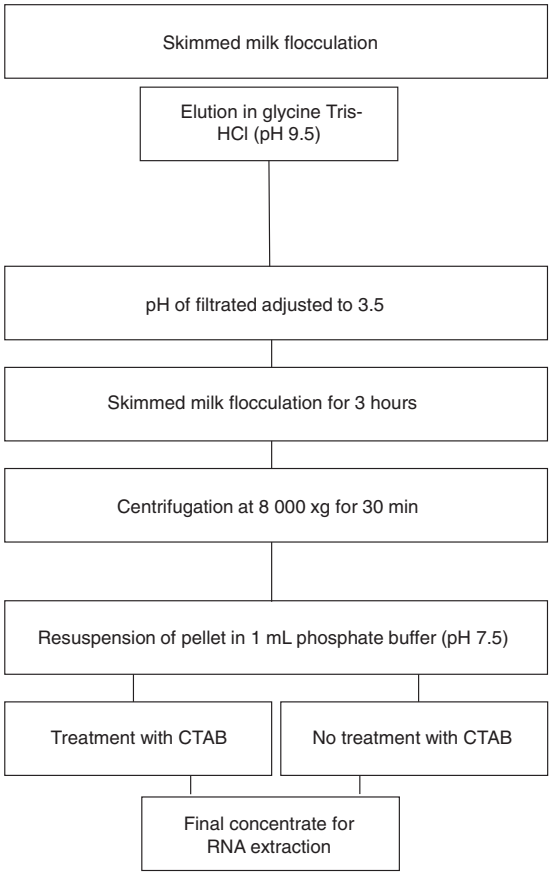

Fig. 1 – Flow-chart of the viral elution-concentration method.

7500™ real-time PCR system (Applied Biosystems, Foster City, CA, USA) was used. All samples were tested in duplicate using both undiluted and 1:10 diluted RNA, totalizing four qPCR reactions per sample. Samples were considered positive when at least one replica was detected at the cycle threshold (Ct) 40 or lower.

Bacterial analysis

Salmonella spp. analysis was performed using a semi-automated VIDAS® system (BioMérieux, France) kit using VIDAS® *Salmonella* (SLM) according to manufacturer's instructions. For *L. monocytogenes*, the culture method by selective enrichment technique was carried out according to standard methodology (Food and Drug Administration's Bacteriological Analytical Manual online (BAM-FDA).[34] Fecal coliform was investigated using a Petrifilm™ Coliform Count Plate (3M, USA) according to the manufacturer's instructions.

Data analysis

Recovery of NoV GII, MNV-1 and HAdV from tomatoes samples was qualitatively and quantitatively analyzed according.[35] Qualitative analysis of viral recovery was performed to determine recovery success rate, calculated as the number of qPCR reactions with successful NoV GII.4, MNV-1 or HAdV recovery per number of qPCR reactions performed. Quantitative recovery analyses from samples yielded recovery efficiency (%), calculated per individual sample as mean number of recovered viral genomic copies per inoculated number of NoV GII.4, MNV-1 or HAdV, genomic copies.

Statistical analysis of NoV GII.4 and MNV-1 recovery rates was performed using the nonparametric Mann–Whitney (MW-test), and Wilcoxon (t-test) tests followed by a Kolmogorov–Smirnov (KS-test) test. All statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA). Significance levels were set at 0.05.

Results

Efficiency of virus recovering

Table 1 shows success rate and recovery efficiency obtained from spiking experiments. No viruses were detected in PBS negative controls. For NoV GII.4, the recovery success rate was of 100% in all specimens, except for cherry and recovery efficiency that ranged from 5.2% to 33.4% with better results for globe and grape tomatoes (Table 1). CTAB treatment did not show significant increase in recovery success rate for all specimens. However, when comparing globe with cherry tomatoes CTAB revealed an increase in recovery efficiency for the first one ($p = 0.0043$).

For MNV-1 recovery success rate ranged from 45.8% to 87.5%, with lowest values results for cherry tomatoes. Recovery efficiency ranged from 0.6 to 4.2, also with lower results for cherry tomatoes.

For HAdV recovery success rate reach 100% for globe and grape tomatoes with efficiency or recovery of 60.7 and 27.4%, respectively.

Field study

HAdV was detected in four samples, three globe and one grape (4.5%) of the 90 samples tested, with concentrations ranging from 10^5 to 10^6 gc/g in 25 g of tomatoes. All samples were negative for NoV GI and NoV GII. MNV-1 used as SPCV was detected in all samples evaluated. No samples showed contamination by *Salmonella* spp. or *L. monocytogenes* (absence in 25 g). Fecal coliform levels were <10 CFU/g in all samples tested.

Discussion

The use of organic flocculation method for virus recovery from tomatoes showed variable results among viruses and species studied, both for success rate and efficiency recoveries. In general, the method showed higher efficiency recoveries for NoV GII.4 and HAdV from tomato globe, with percentage of 33.4% and 60.7%, respectively. Considering the varieties analyzed the low recovery percentages obtained for cherry tomatoes was remarkable. Due to unsatisfactory results obtained for virus recovery from this variety, cherry tomatoes were not included in the field study. Previously, low virus recovery efficiency of cherry tomatoes was reported by Pan et al.[36] suggesting problems of adsorption of virus on the food surface.

Table 1 – Recovery success rate (%) and recovery efficiency (%) of skimmed flocculation analyzed in 24 qPCR reactions for norovirus genogroup II (NoV GII), murine norovirus 1 (MNV-1) and human adenovirus type 2 (HAdV-2).

Method	Types of tomatoes	Treatment	NoV GII.4		MNV-1		HAdV-2	
			Positive samples (% recovery success)	Recovery efficiency (%) mean range	Positive samples (% recovery success)	Recovery efficiency (%) mean range	Positive samples (% recovery success)	Recovery efficiency (%) mean range
Skimmed milk flocculation	Globe Tomato	CTAB	24 (100.0)	33.4 7.9–66.3	18 (75.0)	4.1 1.7–9.5	24 (100.0)	60.7 9.8–92.7
		No CTAB	22 (91.6)	18.1 5.8–31.6	21 (87.5)	4.1 1.6–5.2	–	– –
	Cherry tomato	CTAB	20 (83.3)	5.2 0.4–12.9	11 (45.8)	0.6 0.0–1.2	–	– –
		No CTAB	21 (87.5)	9.8 0.5–27.0	11 (45.8)	1.9 0.0–5.7	–	– –
	Grape tomato	CTAB	24 (100.0)	16.9 0.3–52.7	18 (75.0)	2.3 0.05–5.2	24 (100.0)	27.4 2.8–53.2
		No CTAB	24 (100.0)	29.6 2.5–110.9	19 (79.2)	4.2 0.2–11.1	–	– –

(–) Not done.

Concerning MNV-1, although the average of efficiency recoveries obtained were less than 5%, independently of the variety, its use as SPCV in field study was successful, with 100% detection in samples without dilution. MNV-1 experiments were also performed to evaluate success rate and recovery efficiency using methodology described by ISO 15216:2017 with results lower than those obtained by the organic flocculation method (data not shown). MNV-1 has been used as SPCV in other matrices, showing a good recovery percentage ranging from 7.78% to 75.65%[35] and 8.4% to 66.4%.[24]

In this study CTAB treatment showed no improvement for NoV GII.4 and MNV-1 efficiency recovery. Although for NoV GII.4 CTAB treatment achieved a higher recovery rate when compared to data reported previously obtained for strawberry samples.[29] In this study, we considered CTAB treatment once its use was efficient for strawberry samples.[29] CTAB is a reagent described to eliminate possible inhibitors of qPCR reaction, as organic compounds, pigments and sugars present in food samples.[37]

The initial evaluation of the method with NoV GII.4 and MNV-1 focused on experiments performed later with HAdV, carried out only with globe and grape tomatoes and always using CTAB treatment. Another point to consider is detection limit of the method. As values of detection limit was lower for HAdV (10^2–10^3 gc/reaction) and for NoV GII (1.8×10^3 gc/reaction), the high recovery rate and detection of the natural contamination of these viruses in samples evidence the importance of using the organic flocculation method (data not shown).

In relation to monitoring the microbiological quality of tomatoes obtained in the markets of the Greater metropolitan area of Rio de Janeiro, it is important to emphasize that detection of HAdV in samples met Brazilian Standards (a maximum of $10^2\,g^{-1}$ for fecal coliforms and absence of *Salmonella* spp./25 g). Low levels of fecal coliforms found in this study can be attributed to good agriculture practices. In Brazil, cherry and grape tomatoes are cultivated within a closed system and in greenhouses, thus reducing the possibility of contamination.[38,39] The absence of *Salmonella* spp. and *L. monocytogenes* in tomatoes also corroborated quality standard of this production demonstrated in studies carried out in the country.[40] However, it is necessary to observe different possibilities of contamination until this product reaches the consumer, especially food handling.[41,42] HAdV resistance to adverse environmental conditions as well as the absence of seasonality of these viruses reinforced their use as indicators of human fecal contamination in environmental samples,[12,17,43] unlike NoVs, detected in association with outbreaks.[44,45] Virus detection in tomatoes was described previously in Italy when NoV GII contamination was detected in commercially available tomatoes[46] and when a consumption of dried tomatoes contaminated with HAV resulted in fulminant hepatitis.[47]

Concluding, based on our findings, this method has been proved as an alternative for detecting viruses and can be used for improving food safety programs, although further studies need to be performed in order to meet[28] standards.

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Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. FAO/WHO [Food and Agricultural Organization of the United Nations/World Health Organization]. *Viruses in food: scientific advice to support risk management activities: meeting report. Microbiological Risk Assessment Series Nr. 13*. Rome: FAO/WHO; 2008, 58 pp. Available from: http://www.who.int/foodsafety/publications/micro/mra13/en [accessed 23.11.17].
2. Ethelberg S, Lisby M, Böttiger B, et al. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. Rapid communications. *Euro Surveill*. 2010;15(6), pii=19484.
3. Loury P, Le Guyader FS, Le Saux JC, Ambert-Balay K, Parrot P, Hubert B. A norovirus oyster-related outbreak in a nursing home in France. January 2012. *Epidemiol Infect*. 2014:1–8.
4. Maunula L, Roivainen M, Keränen M, et al. Detection of human norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks. Rapid communications. *Euro Surveill*. 2009;14(49):19435.
5. Mäde D, Trübner K, Neubert E, Höhne M, Johne R. Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food Environ Virol*. 2013;5:162–168.
6. Moore MD, Goulter RM, Jaykus LA. Human norovirus as a foodborne pathogen: challenges and developments. Review article. *Annu Rev Food Sci Technol*. 2015;6:411–433.
7. Rodriguez-Manzano J, Hundesa A, Calgua B, et al. Adenovirus and norovirus contaminants in commercially distributed shellfish. *Food Environ Virol*. 2013;6(1):31–41.
8. Vinjé J. Advances in laboratory methods for detection and typing of noroviruses. *J Clin Microbiol*. 2015;53(2):373–381.
9. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus classification and proposed strain nomenclature. *Virology*. 2006;346:312–323.
10. Verhoef L, Hewitt J, Barclay L. Norovirus genotype profiles associated with foodborne transmission, 1999–2012. *Emerg Infect Dis*. 2015;21(4):592–599.
11. Maunula L, Rönnqvist M, Aberg R, Lunden J, Nevas M. The presence of norovirus and adenovirus on environmental surfaces in relation to the hygienic level in food service operations associated with a suspected gastroenteritis outbreak. *Food Environ Virol*. 2017;9(3):358–359.
12. Wyn-Jones AP, Carducci A, Cook N, et al. Surveillance of adenoviruses and noroviruses in European recreational waters. *Water Res*. 2011;45(3):1025–1038.
13. Ahmad T, Arshad N, Adnan F, et al. Prevalence of rotavirus, adenovirus, hepatitis A virus and enterovirus in water samples collected from different region of Peshawar, Pakistan. *Ann Agric Environ Med*. 2016;23(4):576–580.
14. Azcona OM, Gómez LV, Sánchez PB, Soto RD, Suárez LMM. Acute gastroenteritis and enteric viruses: impact on the detection of norovirus. *An Pediatr (Barc)*. 2016, pii:S1695-4033(16)30253-3.
15. Portes SAR, Volotão EM, Rocha MS, et al. A non-enteric adenovirus A12 gastroenteritis outbreak in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz*. 2016;111(6):403–406.
16. World Health Organization (WHO). *Guidelines for drinking-water quality*. 4th ed; 2011, 518p. ISBN: 9789241548151.
17. Hewitt J, Greening GE, Leonard M, Lewis GD. Evaluation of human adenovírus and human polyomavírus as indicators of human sewage contamination in the aquatic environment. *Water Res*. 2013;47(17):6750–6761.
18. Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. *Clin Microbiol Rev*. 2014;27:441–462.
19. Baert L, Wobus CE, Coillie EV, Thackray LB, Debevere J, Uyttendaele M. Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time reverse transcription-PCR before and after heat exposure. *Appl Environ Microbiol*. 2008;74(2):543–546.
20. Fumian TM, Leite JP, Marin VA, Miagostovich MP. A rapid procedure for detecting noroviruses from cheese and fresh lettuce. *J Virol Methods*. 2009;5(1):39–43.
21. Mattison K, Brassard J, Gagne MJ, et al. The feline calicivirus as a sample process control for the detection of food and waterborne RNA viruses. *Int J Food Microbiol*. 2009;132: 73–77.
22. Comelli HL, Rimstad E, Larsen S, Myrmel M. Detection of norovirus genotype I.3b and II.4 in bioaccumulated blue mussels using different virus recovery methods. *Int J Food Microbiol*. 2008;127:53–59.
23. Stals A, Baert L, Van Coillie E, Uyttendaele M. Extraction of food-borne viruses from food samples: a review. *Int J Food Microbiol*. 2012;153:1–9.
24. de Abreu Corrêa A, Miagostovich MP. Optimization of an adsorption – elution method with a negatively charged membrane to recover norovirus from lettuce. *Food Environ Virol*. 2013;5(3):144–149.
25. Brandão MLL, Almeida DO, Marin VA, Miagostovich MP. Recovery of Norovirus from lettuce (*Lactuca sativa*) using an adsorption-elution method with a negatively charged membrane: comparison of two elution buffers. *Visa Debate*. 2014;2(3):58–63.
26. Iturriza-Gomara M, O'Brien SJ. Foodborne viral infections. *Curr Opin Infect Dis*. 2016;29(5):495–501.
27. ISO/TS 15216-1. *Microbiology of Food and Animal Feed-Horizontal Method for Determination of Hepatitis a Virus and Norovirus in Food Using Real-time RT-PCR – Part 1: Method for Quantification*. Geneva, Switzerland: International Organization for Standardization; 2013.
28. ISO 15216-1. *Preview. Microbiology of the food chain – horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR – Part 1: Method for quantification*. Geneva, Switzerland: International Organization for Standardization; 2017.
29. Melgaço FG, Victoria M, Corrêa AA, et al. Virus recovering from strawberries: of a skimmed milk organic flocculation method for assessment of microbiological contamination. *Int J Food Microbiol*. 2016;217:14–19.

30. Filho EP, da Costa Faria NR, Fialho AM, et al. Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. *J Med Microbiol*. 2007;56:313–319.
31. Yin JL, Shackel NA, Zekry A, et al. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for measurement of cytokine and growth factor mRNA expression with fluorogenic probes or SYBR Green I. *Immunol Cell Biol*. 2001;79(3):213–221.
32. Hernroth BE, Conden-Hansson AC, Rehnstam-Holm AS, Girones R, Allard AK. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel. *Mytilus edulis*: the first Scandinavian report. *Appl Environ Microbiol*. 2002;68:4523–4533.
33. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol*. 2003;41:1548–1557.
34. Hitchins AD, Jinneman K, Chen Y. Detection and enumeration of *Listeria monocytogenes* in foods. In: *Bacteriological analytical manual Online, Chapter 10. [S.l]*. FDA; 2017. Available from: http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm071400.htm [accessed 23.11.17].
35. Stals A, Baert L, Van Coillie E, Uyttendaele M. Evaluation of a norovirus detection methodology for soft red fruits. *Food Microbiol*. 2011;28:52–58.
36. Pan L, Zhang Q, Li X, Tian P. Detection of human norovirus in cherry tomatoes, blueberries and vegetable salad by using a receptor-binding capture and magnetic sequestration (RBCMS) method. *Food Microbiol*. 2012;30:420–426.
37. Demeke T, Jenkins GR. Influence of DNA extraction methods. PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Anal Bioanal Chem*. 2010;396(6):1977–1990.
38. Gusmão MTA, Gusmão SAL, Araújo JAC. Produtividade de tomate tipo cereja cultivado em ambiente protegido e em diferentes substratos. *Hortic Bras*. 2006;24:431–436.
39. Anvisa. *Agência Nacional de Vigilância Sanitária, Ministério da Saúde. Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA), Relatório Complementar, 2012*. Brasil: Brasília-DF; 2014. Available from: http://portal.anvisa.gov.br/wps/portal/anvisa/anvisa/home/agrotoxicotoxicologia [accessed 14.7.15].
40. Ferreira SMR, Freitas RJS, Silva CA, Karkle ENL, Maia CB. Microbiological quality of organic and conventional tomatoes. *Rev Inst Adolfo Lutz*. 2011;70(4):647–650.
41. Codex alimentarius, Standard for Tomatoes. CODEX-STAN 293-2008. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B293-2008%252FCXS_293e.pdf [accessed 26.11.17].
42. Maunula L, Kaupke A, Vasickova P, et al. Tracing enteric viruses in the European berry fruit supply chain. *Int J Food Microbiol*. 2013;167:177–185.
43. Verhaelen K, Bouwknegt M, Lodder-Verschoor F, Rutjes SA, de Roda Husman AM. Persistence of human norovírus GII.4 and GI.4, murine norovirus, and human adenovirus on soft berries as compared with PBS at commonly applied storage conditions. *Int J Food Microbiol*. 2012;160:137–144.
44. Morillo SG, Luchs A, Cilli A, Timenetsky MCST. Rapid detection of norovirus in naturally contaminated food: foodborne gastroenteritis outbreak on a cruise ship in Brazil, 2010. *Food Environ Virol*. 2012;4:124–129.
45. Morillo SG, Luchs A, Cilli A, et al. Norovirus GII.Pe genotype: tracking a foodborne outbreak on a cruise ship through molecular epidemiology, Brazil, 2014. *Food Environ Virol*. 2016:1–7.
46. Serracca L, Rossini I, Battistini R, et al. Potential risk of norovirus infection due to the consumption of “ready to eat” food. *Food Environ Virol*. 2012;4:89–92.
47. Chi H, Haagsma EB, Riezebos-Brilman A, Van den Berg AP, Metselaar HJ, Knegt RJ. Hepatitis A related acute liver failure by consumption of contaminated food, case report. *J Clin Virol*. 2014;61:456–458.