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Application of a simple method using minute particles of amorphous calcium phosphate for recovery of norovirus from cabbage, lettuce, and ham

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A B S T R A C T

In this study, the amorphous calcium phosphate (ACP) method developed previously for calicivirus concentration from water was applied for norovirus detection from food. The viral recovery from cabbage, lettuce, or ham (10 g of each) was firstly examined in seeding experiments with feline caliciviruses (FCVs). The viruses were concentrated by viral adsorption to ACP particles (0.3 g) in the eluent solution (40 ml) from foods, collection of the particles by centrifugation, followed by dissolution of the particles with 3.3 M citric acid (3 ml). In ham, FCV recovery was improved by addition of ascorbic acids into the eluent solution before ACP-particle adsorption. Quantitative real-time reverse transcription-PCR (qRT-PCR) revealed that FCV recoveries were 32–33%, 50–55%, and 37–46% from cabbage, lettuce, and ham, respectively, when seeded with 10^3–10^4 viruses, and detection limits were estimated ~10^3 genomic copies in all 3 foods. Subsequently, the ACP-concentration method was evaluated for norovirus (NoV) detection from these 3 foods. The recoveries and detection limit of NoVs determined by qRT-PCR were 12–41% and 10^3 (genomic copies) from cabbage, 30–57% and 10^3 from lettuce, and 20–26% and 10^4 from ham, when seeded with 10^3–10^5 viruses. This simple method may be suitable for NoV detection from these foods.

1. Introduction

Noroviruses (NoVs) are positive-sense RNA viruses which belong to the genus *Norovirus* in the family *Caliciviridae*. NoVs are classified genetically into five genogroups (GI–GV). GI, GII, and GIV infect humans, whereas GIII and GV infect bovine and murine species, respectively. Most NoVs that cause human gastroenteritis belong to GI and GII. It is well known that NoVs are the most common causative agent of viral foodborne gastroenteritis worldwide (Lopman et al., 2003; Widdowson et al., 2005). The outbreaks of viral foodborne gastroenteritis chiefly occur due to the consumption of food products such as (i) bivalve molluscan shellfish (especially oysters) (Le Guyader et al., 2006; Webby et al., 2007), (ii) soft fruits and fresh vegetables growing or cultivated in sewage-contaminated water (Guévremont et al., 2006; Le Guyader et al., 2004a), and (iii) food products contaminated by NoVs during harvesting, packaging, or processing by workers with poor hand sanitation (Baert et al., 2009; Daniels et al., 2000; Friedman et al., 2005; Morioka et al., 2006; Sakon et al., 2005; Sala et al., 2005; Schmid et al., 2007). However, it is difficult to identify NoV-contaminated food products, because of the contamination with low numbers of viruses and the presence of inhibitory substances for the viral detection in foods (Demeke and Adams, 1992; Leggitt and Jaykus, 2000; Pandey et al., 1996; Sair et al., 2002; Schwab et al., 2000). So far, several methods based on polyethleneglycol (PEG) precipitation, ultracentrifugation, ultrafiltration, adsorption–elution using positively charged or negatively charged filter, receptor-binding capture and magnetic sequestration, and immunomagnetic separation techniques have been reported for identifying contamination-route or for monitoring viruses in food products (Table 1). Recently, a simple virus-concentration method using minute particles of amorphous calcium phosphate (ACP) has been developed previously for NoV monitoring from water (Shinohara et al., 2011) and is expected to be applicable for NoV detection in virus-contaminated foods.

ACP is composed of $Ca_3(PO_4)_2 \cdot nH_2O$ ($n = 1$ or 2) with sizes of 40 nm × 100 nm to 300 nm and is a precursor of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The ACP particles are made in globular shape with diameter of 5 to several hundred micrometers by microparticulation of ACP. The particles are multipored and their surface area is 2–10-fold wider than that of hydroxyapatite, indicating the larger capacity of ACP particles for adsorption of fatty acid,

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Table 1
Viral recovery and detection limit of various methods from vegetables, fruits, and ham.

Concentration method	Food sample (weight)	Viral agents	No. of viruses seeded	Recovery (%)	Detection limit	Reference
PEG	Raspberries (50 g)	NoV	10–10^4 RT-PCRU		10^2 RT-PCRU	Baert et al. (2008)
	Strawberries (5 g)	NoV	0.48–4800 RT-PCRU	50	4.8 RT-PCRU	Cheong et al. (2009)
	Lettuce (25 g)	NoV	0.48–4800 RT-PCRU	2.9	48 RT-PCRU	
	Berries, lettuce (30 g)	NoV	6×10^1 to 6×10^3 RT-PCRU		1.2×10^3 RT-PCRU	Dubois et al. (2002)
	Raspberries (100 g)	PV[a]	5.3×10^5 to 4.3×10^7 $TCID_{50}$	15.5	44 $TCID_{50}$	
	Raspberries (100 g)	HAV[b]	4.0×10^5 $TCID_{50}$	20.1	40 $TCID_{50}$	
	Strawberries (20 g)	NoV	4×10^4 RT-PCRU	8		Kim et al. (2008)
	Grape (20 g)	NoV	4×10^4 RT-PCRU	80		
	Raspberries (20 g)	NoV	4×10^4 RT-PCRU	6		
	Lettuce (50 g)	PV	2×10^2 to 2×10^5 PFU	10–53	2×10^2 PFU	Leggitt and Jaykus (2000)
	Lettuce (50 g)	HAV	1×10^3 to 2×10^5 PFU	2–4	1×10^3 PFU	
	Lettuce (50 g)	NoV	10^3–10^5 RT-PCRU		1.5×10^3 RT-PCRU	
	Lettuce (10 g)	NoV	1–100 RT-PCRU/g		10 RT-PCRU/g	Le Guyader et al. (2004b)
	Lettuce (5 g)	CaCV[c]	2.5×10^5 $TCID_{50}$	1		Rutjes et al. (2006)
	Lettuce (10 g)	NoV	2×10^6 RT-PCRU	23	2×10^1 RT-PCRU	Scherer et al. (2010)
	Raspberries (10 g)	NoV	2×10^6 RT-PCRU	7	2×10^2 RT-PCRU	
	Ham (10 g)	NoV	2×10^6 RT-PCRU	24	2×10^1 RT-PCRU	
Trizol extraction	Ham (30 g)	NoV	10–10^4 RT-PCRU		10^2 RT-PCRU	Schwab et al. (2000)
Ultracentrifugation	Lettuce (5 g)	CaCV	2.5×10^5 $TCID_{50}$	10		Rutjes et al. (2006)
	Salami, gammon, roast pork chop (20 g)	FCV	1.3×10^4 $TCIU_{50}$	3.4–12.5		Rzeżutka et al. (2008)
Ultrafiltration	Vegetables (15 g)	NoV	540 RT-PCRU	9.5–43.9	5.4 RT-PCRU	Butot et al. (2007)
	Strawberries (5 g)	NoV	0.48–4800 RT-PCRU	40		Cheong et al. (2009)
	Lettuce (25 g)	NoV	0.48–4800 RT-PCRU	2.9	48 RT-PCRU	
	Lettuce (5 g)	CaCV	2.5×10^5 $TCID_{50}$	1		Rutjes et al. (2006)
	Lettuce (10 g)	NoV	2×10^6 RT-PCRU	9	2×10^2 RT-PCRU	Scherer et al. (2010)
	Raspberries (10 g)	NoV	2×10^6 RT-PCRU	3	2×10^3 RT-PCRU	
	Ham (10 g)	NoV	2×10^6 RT-PCRU	7	2×10^2 RT-PCRU	
Adsorption–elution	Strawberries (5 g)	NoV	0.48–4800 RT-PCRU	4.8		Cheong et al. (2009)
	Lettuce (25 g)	NoV	9.5×10^3 to 1.9×10^7 genomic copies	5.2–72.3	9.5×10^3 genomic copies	Fumian et al. (2009)
	Lettuce (50 g)	MNV[d]	10^5 PFU	94.39	10^1 PFU	Morales-Rayas et al. (2009)
	Berries (50 g)	MNV[d]	10^5 PFU	74.46–83.90	10^1 PFU	
Receptor-binding capture and magnetic sequestration	Cherry tomato, fruits and vegetable salad (5 g)	NoV	20–500 RT-PCRU	8.75	0.056 RT-PCRU	Pan et al. (2012)
Immunomagnetic separation	Lettuce (25 g)	NoV	10^1–10^4 genomic copies		10^3 genomic copies	Morton et al. (2009)
	Green onion (25 g)	NoV	10^1–10^4 genomic copies		10^2 genomic copies	
	Strawberries (25 g)	NoV	10^1–10^4 genomic copies		10^4 genomic copies	
	Ham (25 g)	NoV	10^1–10^4 genomic copies		10^3 genomic copies	
	Strawberries (20 g)	NoV	2.58×10^4, 5.87×10^6 RT-PCRU	14–30		Park et al. (2008)

[a] PV: poliovirus.
[b] HAV: hepatitis A virus.
[c] CaCV: canine calicivirus.
[d] MNV: murine norovirus.