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Short communication

Evaluation of survival of murine norovirus-1 during sauerkraut fermentation and storage under standard and low-sodium conditions

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ABSTRACT

Sodium reduction strategies have raised a few concerns in regards to possible outbreaks in unpasteurised raw fermented vegetables. Among potential outbreak agents, foodborne viruses are recognized as an important cause of food-borne illnesses. As most of them are acid-resistant, evaluation of the efficacy of lactic fermentation in inactivating enteric viruses must be considered to ensure the safety of these foods. In particular with the sodium reduction trend which could impair adequate fermentation in vegetables, we have challenged sauerkraut fermentation at a final concentration of 4 log $TCID_{50}$/mL with the murine norovirus (MNV-1). Three sodium chloride concentrations (1.0%, 1.5%, 2.0%) were evaluated in spontaneous and starter fermentation of sauerkraut and were followed during fermentation and over a storage phase of 90 days. Detection of MNV-1 genetic material was carried out by real-time RT-PCR and the infectivity on cell culture. Real-time RT-PCR results showed that viral RNA was still detected after 90 day in sauerkraut under all the different conditions. Furthermore, MNV-1 viral particles were able to infect RAW cells after 90 days of storage with a non-significant viral charge reduction. Sodium reduction has a significant impact on the fermentation processing of sauerkraut but no influence on the destruction of norovirus particles or on their survival.

1. Introduction

Fermentation of vegetables based on lactic bacteria is one of the oldest forms of food preservation in the world. Lactic fermentation improves the nutritional value of the product and can reduce the microbial risk by preventing the growth of bacterial pathogens keeping the safety of the product. Since few years, consumers demand foods with low-sodium content because links between high sodium intake and adverse effects on human health are clearly demonstrated. Sodium chloride (NaCl) is present in every kind of vegetable fermentations. In sauerkraut, the cabbage is dry-salted at a level of 2–2.5% by weight allowing it to self-brine through its own moisture. Sodium chloride is a key component of the processing by initiating the fermentation as free water and nutrients are pumped out from vegetal cells by osmosis and moreover, it selectively competes foodborne pathogens and spoilage microorganisms while allowing growth of Lactic Acid Bacteria (Hatoum et al., 2012). Those bacteria have a sequential order during the fermentation process to give an appropriate ratio of lactic/acetic acids ensuring not only sensory qualities but also ensuring the safety and the stability during storage. Unbalanced acid ratio may allow the survival of pathogens as well as acid-tolerant yeasts which can raise the pH during the storage (Savard et al., 2002). Sodium reduction in fermented vegetables may negatively impact the fermentation processing, the quality of the product and moreover the recognized safety of this kind of food (Bautista-Gallego et al., 2013).

Fresh produce is increasingly recognized as a source of foodborne outbreaks in the US and throughout the world (Berger et al., 2010; Lynch et al., 2009). Fresh fruits and vegetables can potentially be contaminated directly in the field through contact by foodborne pathogens with irrigation water, organic fertilizer, by human handling, during post-harvest transport (ice) and by washing. Enteric viruses, including Norovirus (NoV), hepatitis A virus (HAV), rotavirus, astrovirus and enteric adenoviruses are recognized as leading cause of foodborne illnesses and outbreaks (Hall et al., 2013; Koopmans, 2008; Newell et al., 2010). Fresh produce consumed raw or minimally processed, provide an ideal route of transmission for those viruses because they are introduced by the fecal-oral route. Unlike most microbiological agents, viruses are strict intracellular parasites which cannot replicate on food, therefore contamination levels do not increase during processing

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or storage. However, very few viral infectious particles, only 10 to 1000 particles of HAV and NoV, are necessary to induce disease (Sair et al., 2002; Teunis et al., 2008). Also, these viruses are known for their stability in various environments and on surfaces and, their resistance to freezing and acid conditions.

Enteric viruses present on fresh produce or in water used for sauerkraut fabrication can potentially survive to lactic fermentation particularly if the sodium hurdle is impaired. There is currently very little data on the presence and survival of enteric viruses during processing and storage of sauerkraut. Only two studies reported the inactivation of murine norovirus and feline calicivirus in Dongchimi and oyster fermentation (Lee et al., 2012; Seo et al., 2014). Since no simple system exists for the propagation of human noroviruses, murine norovirus (MNV) can be used as a surrogate because of its similarity in shape, size, genomic organization, significant persistence and high stability under acid conditions (Bae and Schwab, 2008; Baert et al., 2009; Cannon et al., 2006; Wobus et al., 2006). The aim of this study was to determine the survival of MNV during cabbage fermentation into sauerkraut with starter culture and spontaneous fermentation under standard and sodium reduction conditions. The survival of MNV was also monitored during a period of storage.

2. Material and methods

2.1. Viral inoculum

Murine norovirus (MNV-1) was kindly provided by Dr. Yvan L'Homme from the Canadian Food Inspection Agency and propagated in the RAW 264.7 cell line (ATCC TIB-71) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin/streptomycin, 1% L-glutamine and 10% FBS (Wisent Bioproducts, Saint-Jean Baptiste, Qc, Canada). The plates were incubated at 37 °C with 5% CO_2 for 48 h. Viruses were purified through three freeze–thaw cycles to lyse cells and the supernatant containing the viruses was recovered by centrifugation at 2500 × g for 15 min, filtered on 0.22 μm Millex PES and stored at −80 °C until use. The viral titer was obtained by the determination of the 50% Tissue Culture Infectious Dose ($TCID_{50}$/ml) as described by Payment et al. (Payment and Trudel, 1993).

2.2. Sauerkraut preparation and experimental design

The sauerkraut was prepared with 9.45 kg of organic green cabbage (Agri-choux Inc. Quebec, Canada) and 3.15 kg of brine (varying concentrations), hand mixed and incubated at room temperature for 10 min. After the first maceration, each sample composed of 925 g of drained cabbage and 575 g of brine were placed in 2 L plastic pails. At this step, the 3 different sodium concentrations (low sodium content 1.0%, 1.5% and standard content 2.0% (w/v)) and the 2 fermentation processes (spontaneous and starter fermentation culture (BLAC 1, CBFC Inc. Sainte-Edwidge, Qc, Canada)) were applied. The half of the production for each condition was spiked with MNV-1 for a final concentration of 10^4 $TCID_{50}$/mL. All experiments were replicated three times (3 independent sauerkraut productions) and were followed during fermentation (7 days at 19 °C) and over a curing storage phase (83 days at 4 °C).

2.3. Lactic acid bacteria

Differential and selective microbial counts were done on MRS x-Gal (BD, New Jersey, USA) and MSE (Mayeux et al., 1962) for lactic acid bacteria and YM-tetracyclin-chloramphenicol for yeasts. MRS and MSE plates were incubated at 30 °C for 48 h and YM plates were incubated 72 h at 26 °C. After incubation plates were counted. Microbial counts have been followed during fermentation and curing phases.

2.4. Recovery of viral particles from sauerkraut juice

Twelve milliliters of sauerkraut juice were sterilely collected by a syringe through a septum located at the base of the pails from each sample at 0, 1, 3, 7, 22, 48 and 90 post-fermentation days. A volume of 3 ml of sauerkraut juice sample was used to determine the pH (pH meter SevenEasy, Mettler Toledo, Mississauga, ON, CA) at each sampling time and 9 ml were used for the viral titration and real time RT-PCR detection. Recovery and purification of viral particles from sauerkraut juice were performed by filtration with a 0.45 μm GD/X 25 mm syringe filter (Whatman, Toronto, ON, CA) and by the use of an ultrafiltration device Amicon Ultra-15 (Millipore, Billeria, MA, USA) followed by a centrifugation 5 min, 5000 × g. The obtained concentrate was recovered and diluted in DMEM in a ratio of 1:5 for a final volume in 1 ml. A volume of 140 μl was used for nucleic extraction and 860 μl were kept for vial titration. All samples were stored at − 80 °C until further use.

2.5. Titration of MNV-1

MNV-1 was titrated on RAW 264.7 cells prepared to confluence in 96-well microtiter plates (Sarstedt, Newton, Nc, USA). Each sauerkraut juice concentrate was filtered on 0.22 μm pore size with syringe filter before it was serially diluted in DMEM. A volume of 50 μl of each dilution was inoculated into four replicate wells and incubated at 37 °C with 5% CO_2. Plates were checked daily by microscopy and the virus titer was estimated from cytopathogenic effect (CPE) after a maximum of 8 days. The viral titer was quantified by tissue culture infective dose ($TCID_{50}$/ml). Non-inoculated sauerkraut juices for each condition as negative controls were diluted in DMEM (1:5) and were incubated with RAW 264.7 cells in order to ensure that some components present in the matrix cannot affect the viability of the cells.

2.6. Viral RNA extraction and real-time RT-PCR detection

RNA extraction form virus particles contained in sauerkraut juice concentrate was carried out with QIAamp viral RNA kit (Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations from 140 μl of each sample and was eluted in a final volume of 60 μl. Detection and semi-quantification were performed by real-time RT-PCR with the primers and probes system MNV-1 described by Baert et al., 2008 (Baert et al., 2008). The TaqMan RT-PCR assays were performed in 25 μl of a reaction mixture comprising 2.5 μl of extracted DNA and 22.5 μl of mastermix. Mastermix was prepared with the Brillant III One-step RT-PCR core kit (Agilent, Santa Clara, CA, USA) and RT-PCR amplification was performed with a Stratagene Mx 3005 system (Stratagene, La Jolla, CA, USA) in a 96-well format under the following conditions: 50 °C for 30 min for the reverse transcription step, 95 °C for 10 min for initial denaturation followed by 45 cycles of amplification with denaturation at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. A standard curve was generated using 10-fold serial dilution (10^8 to 10^0 genomic equivalents) of purified DNA plasmid containing cDNA of MNV-1 in a 5 ng ml^{-1} salmon sperm DNA solution.

2.7. Statistical analysis

A one-way analysis of variance, performed by Sigma plot 12.5, was used to calculate the significance of the titer reduction of MNV-