日本食品微生物学会雑誌　Jpn. J. Food Microbiol., **35**(2), 88–95, 2018

＝Original＝

Transfer and Heat Resistance of Murine Norovirus at a Simulated Bread Manufacturing Process

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(Received: February 15, 2018)
(Accepted: May 2, 2018)

In the present study, behavior of norovirus during bread manufacturing process was analyzed by using murine norovirus strain 1 (MNV-1). Firstly, the transfer of MNV-1 during bread manufacturing process was investigated. MNV-1 was inoculated to various surfaces and then transferred to other surfaces by contacting repeatedly; slicer to bread dough, bread dough to latex glove, and latex glove to sliced loaf. As a result, MNV-1 was transferrable between various surfaces and bread products. Additionally, the protecting effect of sucrose and milk fat, ingredients of pastries, on the survivability of MNV-1 during heating was investigated. MNV-1 was inoculated into pancake mix with different concentration of sucrose or milk fat and then heated at 60℃ for 5 min. The results indicated that sucrose and milk fat contained in high concentrations in the dough/mix ingredients of pastries have a protective effect on the survivability of MNV-1. The data obtained in this study suggested that breads that had not been recognized as a risk of bacterial food poisoning could be a risk of norovirus-associated gastroenteritis if the failure of employee operation occurred in the bread manufacturing process.

Key words: murine norovirus, bread, contamination, viability

Introduction

Norovirus is a non-enveloped virus, which belongs to the family *Caliciviridae* and causes acute gastroenteritis.[11] In Japan, it was reported that norovirus had caused 30% of food borne outbreaks and 54.3% of food poisoning patients in 2016[13]. As norovirus is highly contagious, only 18 viral copies are enough to cause the onset of symptoms[16]. Therefore, it can easily cause large outbreaks in places where people tend to gather, such as hospitals, schools, and restaurants.[2, 4, 11]

The main infection route of norovirus is oral transmission, which occurs by ingestion of contaminated food or water.[9, 11, 12] Additionally, the number of norovirus gastroenteritis outbreaks, which are caused from cross-contamination of norovirus into food during food manufacturing, has been increasing worldwide.[11, 19]

In January 2014, there was a large gastroenteritis outbreak caused by bread product contaminated with norovirus in Hamamatsu city, Shizuoka, Japan. In that gastroenteritis outbreak, over 1,200 people had been infected with norovirus including elementary school children. Hamamatsu city reported that norovirus genogroup II (GII) was detected from bread products and workers in the bread factory. As a result of phylogenetic analysis, obtained capsid N/S region sequences from detected GII samples showed a high similarity with that from norovirus GII.4 Sydney variant (≥98%). Therefore bread, contaminated with norovirus from workers at the bread factory, was determined as an implicated food.[7]

There are two reports regarding norovirus associated with bread products; an outbreak of norovirus caused by a baker in Netherlands[6] and a recovery method of norovirus from bread products to detect by using real-time PCR.[1] However the behavior of norovirus contaminating bread products or its ingredients has not been revealed.

We have previously investigated the survival and heat resistance of norovirus within bread product by using murine norovirus strain 1

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(MNV-1) as a surrogate for human norovirus. The obtained data showed that MNV-1 survived during storage period (5 days) on the surface of bread, MNV-1 was transferred from gloves of workers to bread, and MNV-1 could survive after insufficient baking. These findings suggested that bread products could be a risk of norovirus-associated gastroenteritis, while bread products had not been recognized as a risk of bacterial poisoning due to its low water activity.[15)]

In order to analyze transfer and survivability of MNV-1 under mimic conditions of a bread manufacturing process, especially transfer of MNV-1 between bread and various surfaces, including latex gloves, and slicer was simulated. We also investigated the protecting effect of ingredient sugar and milk fat on the survivability of MNV-1 during heating and bread baking.

Materials and Methods

1. Virus and cells

MNV-1 was propagated with RAW264.7 cells that were cultured at 37℃ in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, penicillin (100 U/m*l*), and streptomycin (100 μg/m*l*). After the RAW264.7 cells had reached confluent, they were inoculated with MNV-1 at a multiplicity of infection (MOI) of 0.1 and then incubated for 3 days at 37℃ in 5% CO_2. After confirmation of the cytopathic effect, the cells were subjected to freezing and thawing for 4 times, and then centrifuged at 8,000×*g* for 20 min. The supernatant was used as the MNV-1 solution and stored at −80℃ until use.

2. Measurement of MNV-1 infectivity by plaque assay

The infectivity of MNV-1 was measured by plaque assay, using a method described previously.[8)] RAW264.7 cells were seeded into 6-well plates (Falcon, Becton, Dickinson and Company, Franklin Lakes, NJ) at 6 log cells/m*l*. The cells were then incubated for 18 hr at 37℃ in 5% CO_2. After the cultivation of RAW264.7 cells, 500 μ*l* each of the prepared samples was inoculated into the plates, and the plates were shaken at room temperature for 1 hr. Subsequently, the virus samples were layered with 2 m*l* of 1.5% SeaPlaque Agarose (Lonza Japan, Tokyo, Japan) in DMEM. These plates were incubated at 37℃ for 48 hr in 5 % CO_2.

After the incubation of sample plate, 2 m*l* of 0.03% neutral red solution (Sigma-Aldrich Japan, Tokyo, Japan) was added to each well of the culture plates, and the plates were incubated for 1 hr at 37℃ in 5% CO_2. The plaques formed were then counted.

3. Transfer of MNV-1 during bread manufacturing process

An experimental outline of the transfer of MNV-1 designed to simulate a bread manufacturing process is shown in Fig. 1. MNV-1 was inoculated to slicer, bread dough, and latex glove. Then the inoculated MNV-1 was transferred repeatedly from slicer to bread dough (Section 3.1), bread dough to latex glove (Section 3.2), and latex glove to sliced loaf (Section 3.3), respectively.

3.1 Transfer of MNV-1 from slicer to bread dough

A stainless-steel slicer at the blade length of 18 cm was immersed in MNV-1 solution at the inoculation level of 4.2 log PFU/slicer, dried at room temperature for 30 min. Bread dough, whose composition is shown in Table 1, was prepared by using a bread machine, formed into round portions of 60 g each, and cut four times into a square shape using the slicer contaminated with MNV-1. After the first transfer, the same slicer was used to cut second and third portion. Transferred MNV-1 from slicer to each bread dough was recovered by following method.

Contaminated bread dough was homogenized with 60 m*l* of phosphate buffer saline (PBS) at 230 rpm for 120 sec by using stomacher machine (Stomacher 400 circulator, Seward Ltd., London, UK). The resulting homogenate was then centrifuged at 8,000×*g* for 10 min, and the supernatant was passed through a 0.20 μm filter and applied to plaque assay. The inoculation level of MNV-1 to the slicer was measured by washing the contaminated slicer with 10 m*l* of DMEM and then applied to plaque assay.

3.2 Transfer of MNV-1 from bread dough to latex glove

MNV-1-contaminated bread dough at the inoculation level of 3.7 log PFU/g, whose composition is shown in Table 2, was prepared using a bread machine. A 50 g ball of dough was formed and placed for 5 sec on a latex glove cut into a 3 cm square. After the first transfer, the same

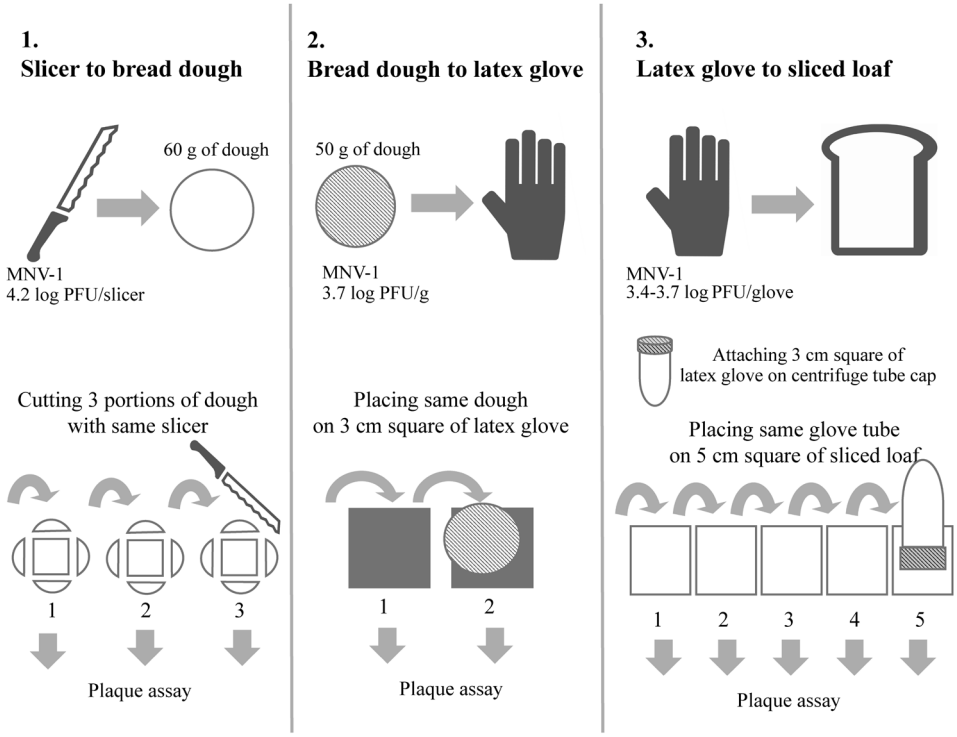

Fig. 1.　Experimental outline of MNV-1 transfer

Table 1.　Ingredients for making bread dough

Ingredient	
Water	20 mL
White sugar	4 g
Salt	0.5 g
Butter	1.7 g
Flour	33.4 g
Dried yeast	0.4 g
Total	60 g

Table 2.　Ingredients for making bread dough contaminated with MNV-1

Ingredient	
MNV-1 solution	16.7 mL
White sugar	3.3 g
Salt	0.5 g
Butter	1.4 g
Flour	27.8 g
Dried yeast	0.3 g
Total	50 g

dough contaminated with MNV-1 was placed on another latex glove.

The latex glove was then washed with 15 m*l* of PBS to recover MNV-1, which was then applied to plaque assay. To measure the inoculation level of MNV-1 in bread dough, MNV-1-contaminated dough was homogenized with 50 m*l* of PBS at 230 rpm for 120 sec. The homogenate was then centrifuged at 8,000×*g* for 10 min, passed through a 0.20 μm filter and applied to plaque assay.

3.3 Transfer of MNV-1 from latex glove to sliced loaf of bread

A latex glove was cut into 3 cm square and attached to a centrifuge cap. The centrifuge tube, attached latex glove on the cap, was standardized to the weight of 20 g and used for transfer of MNV-1 from latex glove to sliced loaf like stamping. Then the surface of the latex glove on centrifuge tube cap was contaminated with MNV-1 by immersing in MNV-1 solution at the inoculation level of 3.4–3.7 log PFU/glove. Two types of contaminated gloves were used in experiments: one was dried at room temperature for 30 min after contamination and another was not dried up. Then the surface of latex glove contaminated with MNV-1 on centrifuge tube was placed on 5 cm square of sliced loaf, purchased at local store in Tokyo, for 5 sec to transfer MNV-1 from latex glove to sliced loaf. After the first transfer, the same latex piece contaminated with MNV-1 was placed on another sliced loaf.

The sliced loaf was homogenized with 64 m*l* of PBS at 230 rpm for 30 sec. After the centrifugation of the homogenate at 8,000×*g* for 10 min, the supernatant passed through a 0.20 μm filter and applied to plaque assay. The inoculation level of MNV-1 on the disk of latex glove was measured by washing the glove with 30 m*l* of PBS and ap-

plied to plaque assay.

4. Viability of MNV-1 in bread dough during baking process

Bread dough contaminated with MNV-1 of the composition shown in Table 2 was prepared using a bread machine. The dough was formed into 50 g balls, left at room temperature for 10 min to simulate bench time, then the temperature was elevated to 40℃ and held for 30 min. The risen dough was then placed in an oven pre-heated at 180℃ and baked for 9 min at an oven temperature of 150℃ until fully baked (until the dough weight decreased by 10%). Bread dough was collected at 0 min (before baking), 4, 6, 7, 8, and 9 min from the start of baking, and the samples were prepared by the same procedure as described above in Section 3.1. The MNV-1 infectivity of these samples was then measured using plaque assay. During baking, the temperature in the center of the bread dough was also measured.

5. Heat resistance of MNV-1 in pancake mix at different content of sucrose and milk fat

A pancake mix, consists of 0.1 g of white flour and 10 m*l* of water, was added 0, 1, and 2 g of sucrose in order to prepare pancake mix with different content of sucrose at 0, 10, and 20%, respectively. Similarly, different content of milk fat solution (0, 3.5, and 35%) was prepared by mixing 0, 1, 10 m*l* of commercially available fresh cream (35% milk fat) and 10, 9, 0 m*l* of water to prepare 0, 3.5, and 35% of milk fat. Then a pancake mix with different content of milk fat was prepared by adding 0.1 g of white flour into milk fat solution. Each pancake mix was inoculated with MNV-1 at 5 log PFU/m*l*, and then 20 m*l* of pancake mix contaminated with MNV-1 was poured into centrifuge tubes. The centrifuge tubes were placed in a water bath at 60℃ and heated for 5 min.

Samples were collected at 0 min (before heating), 1, 2, 3, 4, and 5 min from the start of heating. Centrifuge tube was rapidly cooled and homogenized with 180 m*l* of PBS at 230 rpm for 30 sec. The homogenate was then centrifuged at 8,000×*g* for 10 min, and the supernatant was filter sterilized by passing through a 0.20 μm filter. In order to measure the infectivity of MNV-1, the resulting sample was applied to plaque assay.

6. Statistical analysis

All the experiments were performed in triplicate (n=3). The results shown are the mean value±standard error. In the results, significant differences were analyzed by Tukey's test using Microsoft Excel.

Results

1. Transfer of MNV-1 during bread manufacturing
1.1 Transfer of MNV-1 from slicer to bread dough

The infectivity of MNV-1 transferred from slicer to bread dough by each transfer operation is shown in Fig. 2. The inoculation level of MNV-1 on the slicer was 4.2 log PFU/slicer. The infectivity of MNV-1 transferred from slicer to a latex glove resulted 2.0 log PFU/g at the first transfer and 0.4 log PFU/g at the second and third transfer (Fig. 2).

1.2 Transfer of MNV-1 from bread dough to latex glove

The infectivity of MNV-1 transferred from bread dough to latex glove by each transfer operation is shown in Fig. 3. The inoculation level of MNV-1 in bread dough and it was 3.7 log PFU/g. The infectivity of MNV-1 transferred from bread dough to latex glove resulted 2.4 log PFU/glove at the first transfer and 2.0 log PFU/glove at the second transfer (Fig. 3).

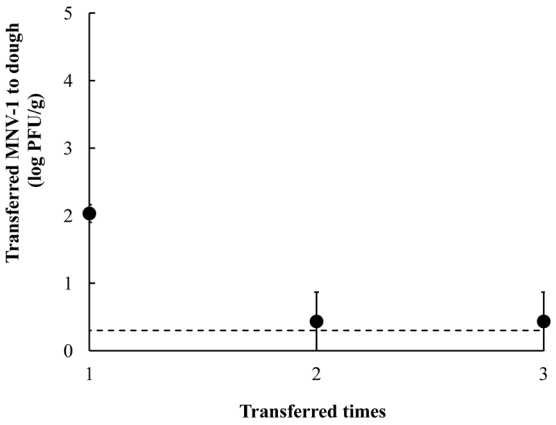

Fig. 2. Transferred MNV-1 from slicer to bread dough. MNV-1 was inoculated onto a slicer and dried for 30 min, then transferred to bread dough by cutting. The inoculation level of MNV-1 on the slicer was 4.2 log PFU/slicer. The dashed line means limit of detection in this experiment. Values are expressed as mean±SEM (n=3).

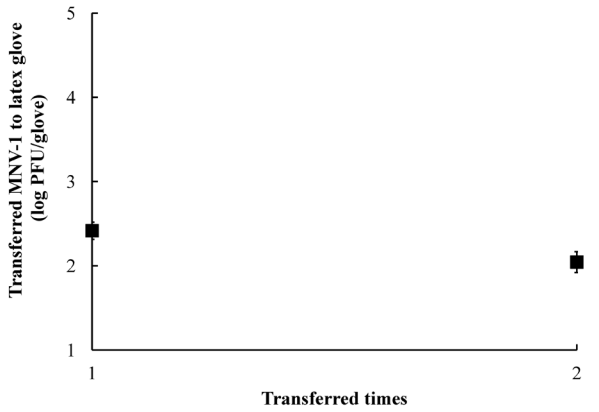

Fig. 3. Transferred MNV-1 from bread dough to latex glove.
MNV-1 was inoculated into bread dough, then transferred to latex glove. The inoculation level of MNV-1 in bread dough was 3.7 log PFU/g. Values are expressed as mean±SEM ($n=3$).

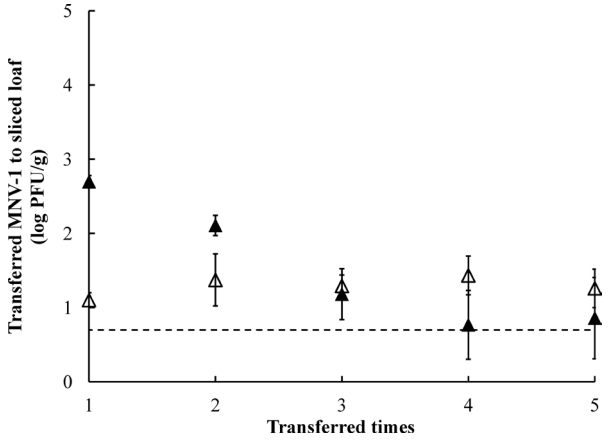

Fig. 4. Transferred MNV-1 from latex glove to sliced loaf.
MNV-1 was inoculated onto latex glove and dried for 30 min (white) or without drying (black), then transferred to a sliced loaf. The inoculation level of MNV-1 on latex glove was 3.4–3.7 log PFU/glove. The dashed line means limit of detection. Values are expressed as mean±SEM ($n=3$).

1.3 Transfer of MNV-1 from latex glove to sliced loaf of bread

The infectivity of MNV-1 transferred from latex glove to sliced loaf surface by each transfer operation is shown in Fig. 4. The inoculation level of MNV-1 on the latex glove surface was 3.4–3.7 log PFU/glove. The infectivity of MNV-1 from latex glove, dried for 30 min after the inoculation of MNV-1, to sliced loaf resulted 1.1 log PFU/g at the first transfer and 1.4 log PFU/g at the second transfer, then a similar infectivity at the third, fourth, and fifth transfers. In the case of non-dried latex glove after the inoculation of MNV-1, the infectivity of MNV-1 from latex glove to sliced loaf resulted 2.7 log PFU/g at the first transfer and 2.3 log PFU/g at the second transfer. MNV-1 was transferred from latex glove to bread during all 5 consecutive transfer operations, regardless of whether the MNV-1-inoculated latex glove was air-dried or not (Fig. 4).

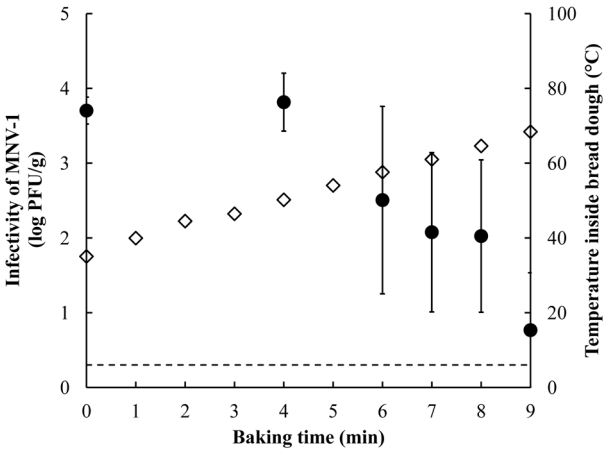

Fig. 5. Viability of MNV-1 in bread dough during baking.
MNV-1 was inoculated into 50 g of bread dough and then baked in oven for 9 min. Black circle and white diamond indicates the infectivity of MNV-1, and the temperature inside bread dough, respectively. The dashed line means detection limit of the infectivity of MNV-1. Values of MNV-1 infectivity are expressed as mean±SEM ($n=3$).

2. Viability of MNV-1 in bread dough during baking

Figure 5 shows the viability of MNV-1 in bread dough during baking. The infectivity of MNV-1 in bread dough was 3.7 log PFU/g at 0 min of baking, 3.8 log PFU/g at 4 min, 2.5 log PFU/g at 6 min, 2.0 log PFU/g at 6 and 7 min, and 0.8 log PFU/g at 9 min (Fig. 5). During baking, the temperature in the center of the dough gradually increased with heating time. The temperature in the center of the bread dough was 35.1℃ immediately before baking and 68.4℃ after 9 min of baking (Fig. 5).

3. Heat resistance of MNV-1 in pancake mix at different content of sucrose and milk fat

Figures 6 and 7 shows the viability of MNV-1 in pancake mix during heating at 60℃ for 5 min with different content of sucrose, and milk fat, respectively. The infectivity of MNV-1 was decreased with heating time. After 5 min of heating, the infectivity of MNV-1 in 0% sucrose pancake mix was reduced by 2.6 log PFU/m*l* while the MNV-1 infectivity in 20% sucrose pancake mix

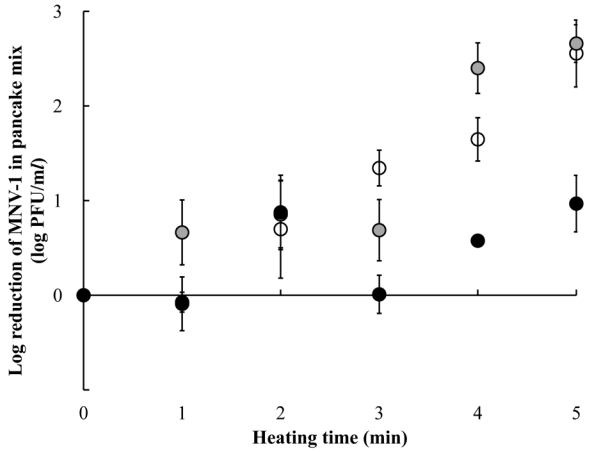

Fig. 6. Viability of MNV-1 in pancake mix after heating under the different concentration of sucrose. MNV-1 was inoculated into pancake mix (5 log PFU/m*l*) which contains 0% (white), 10% (gray), or 20% (black) of sucrose and then heated at 60℃ for 5 min. Values are expressed as mean±SEM ($n=3$).

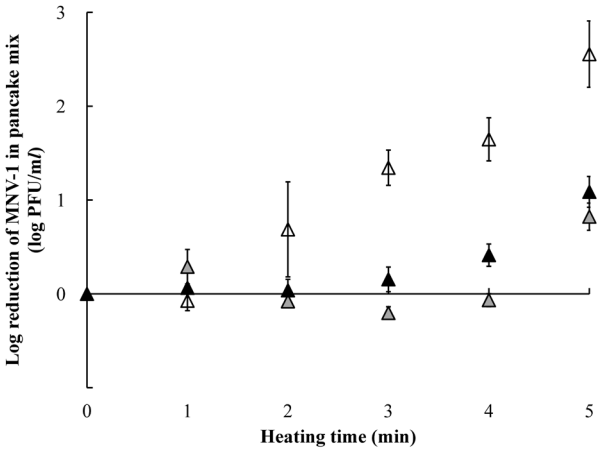

Fig. 7. Viability of MNV-1 in pancake mix after heating under different concentration of milk fat. MNV-1 was inoculated into pancake mix (5 log PFU/m*l*) which contains 0% (white), 3.5% (gray), or 35% (black) of milk fat and then heated at 60℃ for 5 min. Values are expressed as mean±SEM ($n=3$).

was reduced by 1.0 log PFU/m*l* (Fig. 6), showing that MNV-1 survived significantly better in 20% sucrose mix than 0% sucrose mix ($p<0.05$).

In 0% milk fat pancake mix, the infectivity of MNV-1 was reduced by 2.6 log PFU/m*l* after 5 min of heating (Fig. 7). Meanwhile, the infectivity of MNV-1 in 35% milk fat pancake mix was reduced by 1.1 log PFU/m*l* after 5 min, and the MNV-1 infectivity in 3.5% milk fat pancake mix was reduced by 0.8 log PFU/m*l* (Fig. 7). Figure 7 shows that MNV-1 survival was better in pancake mix that contained milk fat ($p<0.05$).

Discussion

Bread is recognized as a low risk food of bacterial poisoning,[14] and the risk of viral food poisoning with bread has received less attention. However, since 2000 there have been outbreaks, both in Japan and overseas, of norovirus gastroenteritis caused by norovirus-contaminated bread, and these outbreaks have been large in scale and involving transmission to over 200 people.[6, 13] As norovirus behavior in bread is currently unknown, the present study showed some aspects of behavior of norovirus within bread manufacturing process. MNV-1 was used as a surrogate for human norovirus as MNV-1 is culturable and is genetically similar to human norovirus[20].

The results showed that infectious amount of MNV-1 was transferred during bread manufacturing process, from slicer to bread dough, bread dough to glove, and glove to sliced loaf (Figs. 2–4). Among the data of MNV-1 transfer, MNV-1 was transferred most frequently from latex glove to sliced loaf (Fig. 4). MNV-1 was transferred from latex glove to sliced loaf for 5 times repeatedly, regardless of whether the latex glove was air-dried after the inoculation of MNV-1.

In the outbreak of gastroenteritis caused by norovirus contaminated bread products in Hamamatsu City, Japan in 2014, it was estimated that norovirus contaminated from gloved hand to bread surface during inspection of bread products, and that process was concluded as one of the causes of secondary contamination.[17] Norovirus has an extremely low infection dose,[16] and based on the results shown in Fig. 4, it is conceivable that a worker could transfer norovirus to multiple portions of bread and cause contamination on each contact.

In the present study, bread dough inoculated with MNV-1 was baked in an oven at 150℃ to simulate the baking of "white bread" that is normally baked without creating a hard crust. The infectivity of MNV-1 in 50 g of bread dough was decreased with baking time, but MNV-1 was also remained viable at the end of baking for 9 min (Fig. 5).

Although the dough surface temperature approaches the oven temperature during baking, the temperature inside the dough remains below 100℃, and there are temperature gradients between dough surface and inside dough signifi-

cantly.[5] Our data also showed a large temperature gradient between oven temperature and inside dough, and center of the dough remained below 70℃ at the end of baking (Fig. 5). Even though we baked 50 g of bread dough, commercially available loaves of bread are heavier at approximately 400 g. There could be a large temperature gradient between oven and inside dough during baking. Thus it should be necessary to prevent secondary contamination of norovirus from various surfaces to bread ingredients.

We also investigated differences in the viability of MNV-1 effected by sucrose or milk fat content in order to simulate a manufacturing process of pastries. In that experiment, heating temperature was set at 60℃ to clarify the relationship between heating time and infectivity of MNV-1. Results showed that MNV-1 could survive under the condition with higher sucrose concentration after heating at 60℃ (Fig. 6) Jarke *et al.* previously reported that the heat resistance of MNV-1 increased in PBS by adding sucrose or milk fat. Specifically, when PBS containing 50% sucrose is inoculated with MNV-1, then heated at 60℃ for 5 min, the infectivity of MNV-1 infectivity was around 4 log PFU/m*l*, higher than that in PBS containing 0% sucrose[10]. Jarke *et al.* presume that the reason for this increase in the heat resistance of MNV-1, in the presence of a high concentration of sucrose, is that sucrose has a protective effect on MNV-1. The details of the mechanism of this effect are unknown, but it may be speculated that a high concentration of sucrose reduced the content of free water molecules in solution, which may affect to the solubility of virus particle in solution.[10]

Our data of the survivability of MNV-1 in pancake mix containing different content of milk fat during heating was consistent to that of sucrose (Fig. 7). Jarke *et al.* also reported the reduction of MNV-1 viability in PBS was greater than in milk during heating[10]. In the case of other virus, Bidawid *et al.* reported the heat resistance of hepatitis A virus (HAV) in liquid that contains milk fat. In that report, skimmed milk and table cream with different milk fat contents were inoculated with HAV and were heated at 71℃ for 10 min. The reduction of HAV infectivity was smaller in table cream with a high milk fat content than in skimmed milk with a low milk fat content.[3]

Sugars and milk fat are essential ingredient of pastries, and pastries commonly contain 25–37 % sucrose, while sliced loaves contain 2–8% sucrose.[18] This indicates that the risk of norovirus could be higher in pastries compared to other breads, since their major ingredients have protective effect on norovirus.

From the obtained data, MNV-1 was highly transferrable, regardless of the low water activity of bread, suggesting the possibility of secondary contamination from food workers. The results also revealed that sucrose and milk fat contained in high concentrations in the dough/mix ingredients of pastries had a protective effect on MNV-1 survivability. If norovirus contaminates bread dough, it can be inactivated by baking, but our data showed that norovirus could be survive under inadequate baking condition.

Although mechanization of bread manufacturing process has been developed along with the increasing of bread consumptions, manual operations are still required. We consider that the obtained data will be useful for workers in bread manufacturing factories to recognize the risk that norovirus could contaminate bread products during bread manufacturing process, as seen in the norovirus gastroenteritis outbreak occurred in Hamamatsu city, Japan. Therefore it is essential that contamination of norovirus be prevented during bread manufacturing process. We hope the data presented here will be utilized by factory authorities or companies manufacturing bread and other similar dry food products.

Acknowledgement

This study was supported by the Tojuro Iijima Foundation for Food Science and Technology.

References

1) Arita, T., Kimura, H., Noda, M., Nishio, O.: Study on recovery of norovirus from bread. J. Jap. Ass. Infect. Dis., **82**, 473–475 (2008).
2) Bernard, H., Faber, M., Wilking, H., Haller, S., Höhle, M. et al.: Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. Euro. Surveill., **19**, 20719 (2014).
3) Bidawid, S., Farber, J. M., Stattar, S. A., Hayward, S.: Heat inactivation of hepatitis A virus in dairy foods. J. Food Prot., **63**, 522–528 (2000).
4) Chen, M. Y., Chen, W. C., Chen, P. C., Hsu, S. W., Lo, Y. C.: An outbreak of norovirus gastroenteritis associated with asymptomatic food handlers in Kinmen, Taiwan. BMC Public Health, **16**, 372 (2016).

5） Derde, L. J., Gomand, S. V., Courtin, C. M., Delcour, J. A.: Moisture distribution during conventional or electrical resistance oven baking of bread dough and subsequent storage. J. Agric. and Food Chem., **62**, 6445–6453 (2014).
6） de Wit, M. A. S., Widdowson, M. A., Vennema, H., de Bruin, E., Fernandes, T., Koopmans, M.: Large outbreak of norovirus: The baker who should have known better. J. Infect., **55**, 188–193 (2007).
7） Furuta, T., Ohta, K., Terada, Y.: An outbreak of norovirus in Hamamatsu, Shizuoka. IASR, **35**, 164–165 (2014). (in Japanese)
8） Gonzalez-Hernandez, M. B., Bragazzi Cunha, J., Wobus, C. E.: Plaque assay for murine norovirus. J. Vis. Exp., **22**, 1–6 (2012).
9） Iritani, N., Kaida, A., Abe, N., Kubo, H., Sekiguchi, J., Yamamoto, S. P., Goto, K., Tanaka, T., Noda, M.: Detection and genetic characterization of human enteric viruses in oyster-associated gastroenteritis outbreaks between 2001 and 2012 in Osaka city, Japan. J. Med. Virol., **86**, 2019–2025 (2014).
10） Jarke, C., Petereit, A., Fehlhaber, K., Braun, P. G., Truyen, U., Albert, T.: Impact of Sodium Chloride, Sucrose and Milk on Heat Stability of the Murine Norovirus and the MS2 Phage. Food. Environ. Virol., **5**, 135–143 (2013).
11） Koopmans, M., Duizer, E.: Foodborne viruses: An emerging problem. Int. J. Food Microbiol., **90**, 23–41 (2004).
12） Loury, P., Le Guyader, F. S., Le Saux, J. C., Ambert-Balay, K., Parrot, P., Hubert, B.: A norovirus oyster-related outbreak in a nursing home in France, January 2012. Epidemiol. Infect., **143**, 2486–2493 (2015).
13） Ministry of Health, Labour and Welfare: Food poisoning surveillance report (2016). Available in Japanese: http://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/syokuchu/04.html#4-2 (Accessed on Feb. 15, 2018).
14） Scott, E.: Food Safety in the home: Safe handling of foods. Farber, J. M., Todd, E. C. D. (eds.), p.320–321, Marcel Dckker, Inc. New York (2000).
15） Takahashi, M., Takahashi, H., Kuda, T., Kimura, B.: Viability and heat resistance of murine norovirus on bread. Int. J. Food Microbiol., **216**, 127–131 (2016).
16） Teunis, P. F. M., Moe, C. L., Liu, P., Miller, S. E., Lindesmith, L., Baric, R. S., Le Pendu, J., Calderon, R. L.: Norwalk virus: how infectious is it? J. Med. Virol., **80**, 1468–1476 (2008).
17） Tsuchiya, Y., Sahara, A., Jinbo, T., Nakano, T., Kato, K., Ogai, T., Kosugi, K.: A foodborne outbreak of norovirus caused by bread. Jpn. J. Food Microbiol., **32**, 153–158 (2015). (in Japanese)
18） Uchida, M.: Sugar and bread. Agriculture and Livestock Industries Corporation (2010). Available in Japanese: http://sugar.alic.go.jp/japan/view/jv_9911b.htm#2-2 (Accessed on Feb. 15, 2018).
19） Wang, Y., Zhang, J., Shen, Z.: The impact of calicivirus mixed infection in an oyster-associated outbreak during a food festival. J. Clin. Virol., **73**, 55–63 (2015).
20） Wobus, C. E., Thackray, L. B., Virgin, H. W.: Murine norovirus: a model system to study norovirus biology and pathogenesis. J. Virol., **80**, 5104–5112 (2006).

製パン現場を想定した ノロウイルスの移行性と加熱耐性について

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本研究では製パン現場を想定したノロウイルスの挙動を詳細に解析することを目的とした．代替ウイルスであるMurine norovirus strain 1(MNV-1)を用いて，製パン現場におけるMNV-1の移行性や焼成中のパン生地におけるMNV-1の生残性を調査した．はじめに，製パン現場で起こり得るラテックスグローブやスライサー等の表面からパン生地や焼成後のパンへのMNV-1の各種移行性を調査した．その結果，MNV-1はスライサーやラテックスグローブ表面から食パンへ複数回連続して移行した．さらに，生地に含まれる砂糖や乳脂肪分含量がMNV-1の加熱耐性に与える影響を検証した．異なる濃度のスクロースあるいは乳脂肪を含むパンケーキ生地にMNV-1を接種し，60℃で5分間加熱した．生地に著量の砂糖や乳脂肪分が含まれることで，生地中におけるMNV-1の生残率が高まった．本研究で得られたデータは，細菌性食中毒のリスクとして認識されてこなかったパン類がノロウイルス食中毒のリスクになりうることを認識するための有用な情報になると考えられる．