Research Article
Home Page Journal: ejournal.umm.ac.id/index.php/fths/about

DOI. https://doi.org/10.22219/FTHS
Received: April 2018
Accepted: May 2018
Available online: July 2018

Assessment organic production of kyuri farms focuses on microbiological contamination with good agricultural practices (GAP) standard criteria.

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Abstract The assessment was based on interviews result and experiment laboratory assay with the three organic kyuri farmers in Saga City focus prevention activities of microbiological contamination. Each farm the 21 samples (soil, kyuri seedlings, natural fertilizer, and water resource) were taken during six times visited or one cultivation production cycle. The object was a focus on hygiene attribute (coliforms) and safety (Salmonella spp and E. coli O157: H7), the evaluation activities of farmers' food safety practice used GAP standard as an indicator. The laboratory results in both of three kyuri farmers, coliforms ranging from 3.0 to 4.7 log 10 cfu/g, E.coli O157: H7 was detected only at the farm 3 and Salmonella spp was not detected. The evaluating activities through self-assessment tool describe all three farms have two major hazards focus, one is chemical control such as synthetic substances uses, pesticide residue and chemical contaminations, two is a microbial hazard due to contact with raw material, and natural characteristics. The overall study, the farmers in the middle level of application food safety practice.

Keywords: GAP, Microbiology, Farmers

Introduction

In recently, consumptions of global organic fresh produce have increased with major reason personal preference, healthy, and sustainable food production (FAO, 2012). The organic products should be free from synthetic and chemical substances during planting to post-harvest process (Aquino & Assis, 2007). As a natural characteristic of organic produce is more susceptible to spreading microbiology contaminations, some literature has been increasing concern in microbiological quality of organic produce (Delaquis, Bach, & Dinu, 2007; Oliveira, et al., 2010). In Japan, Japanese cucumber "Kyuri" have used in a diverse processing way on local food and have a huge percentage of organic fresh produce consumption (MAFF, 2016). However, Kyuri more prone to contamination of microbiological due to the soil environment and peel provides potential conditions for fungal growth (Alam, et al., 2015).

The Pathogenic contaminations and fungal growth of fresh produce have frequently through from soil during preparation field, natural fertilizers are not mature when it using, water supply is contaminated with other water resources. (Codex, 2005; Oliveira et al., 2010; Umesha et al., 2017). Another aspect, such as sophisticated facilities and equipment, adequate sanitary conditions and packaging are of utmost

importance attribute to assure the products are not contaminated along the production system.

The data related to microbiological contamination issue in Japan is not specific to organic kyuri consumptions. Due to that, the purpose of our study was to investigate the microbiological preventing activities and food safety management aspect along the production. The assessment and indicator is base on good agricultural practices attribute (GAP). Three organic kyuri farms in Hiroshima Prefecture were selected and visited six times for observations and take a sampling for microbiological contamination assay (Salmonella spp, E. coli O157:H7 and coliforms) during the kyuri production systems. At last visit, comprehensive assessment using a standardized self-assessment questionnaire develop by Lunning, et al., 2011, Kirezieva et al., 2013, and Uyttendaele et al., 2010 is applied to asses level of food safety activities.

Material and Methods

Microbiological sampling test

Critical sampling locations (CSLs) was used to identifying bottlenecks in microbial contaminations preventions. In line with that, 10 CSL (table 1) has modified base on potential risk factors (i.e., soil, water, natural fertilizer, and product handling) could contribute to microorganism growth (Yeni et al., 2016). Which sampling plan was a focus to obtain coliforms as hygiene attribute, and both E. coli O157: H7 and Salmonella spp as food safety attribute. Visit and sampling was conducted from May 2017 until October 2017 with a total of 6 times visited. First visit, one week before cultivation preparation, second visit, on the planting day, third visit, three weeks after planting a day, fourth visit, two weeks before harvest, fifth visit, at harvest day and last visit before shipping. This step was repeated at the respective farms (Table 1 and 2).

Sampling method

Samplings are divided into two aspects, first, aspect the natural material (soil, natural fertilizer, water resource and kyuri seedling) and second, aspect preparation in post-harvest activities (washing treatment, cutting and grading step and transport container box). At each activities step, samples were collected during visiting the farms. In specifically, aspect the natural material, for material soil and natural fertilizer were collected 100 g on the top part of each ground field and composting locations. The samples were stored in sterile plastic bags for advance microbiological analyses in a laboratory. At water resource step, 10 L was pumped to sterile gallon from water tank. The kyuri seedlings were pooled using a seedling tray. Furthermore, post-harvest activities samples were collected using sterilized swabs rubbed two-way streak directions delimited in square 2 cm^2 x 2 cm^2 on a final product at washing treatment, cutting and grading step and transport container box activities. To keep moistened for the microbial growth the test tubes are containing 0.1% peptone water placed in thermal boxes and store in refrigeration (7^0C).

Microbiological analysis methods

Table 2 has presented a method and indicators are used to assess the degree of hygiene and safety attribute in each farm. First, at hygiene attribute is used amount coliforms to analyse the quality level of the sanitation program applied. Second, at food safety attribute is used total plate count colonies describe in ISO 16140:2010 (ISO, 2010) to analyse the level of the contamination at each activity from E. coli O157: H7 and Salmonella spp. The validation characteristic of colonies using a gram stain test method on selective media agar 38^0C and growth at the presence of 4.0% NaCl.

Step first to analyses hygiene attribute, 1 g samples took from natural materials has collected placed in a test tube with 9 ml water. The samples were homogenized in a digital vortex mixer (cole-parmer) for 60 s with 2.000 rpm. Afterwards, the solution used for decimal dilutions until 10^4 and took 1 ml using pour plate method into a petri dish containing nutrient agar Merck (Darmstadt, Germany) and incubated for 48 h at 37^0C, this is prepared in triplicate. However, to analyse water samples using the Most Probable Number (MPN) method which 50 ml water samples filled into the multiple-tube technique (Standard Methods for Examination of Water and Wastewater, 1998).

Questionnaire self-assessment the degree of safety base on good agricultural practices (GAP) standard

The self-assessment tools used a four-set questionnaire to get insight from the interview during the visited farm. These questionnaire designed focus on prevention or control activities of contamination or growth microbiological with a total of 28 indicators required by GAP standard. For each indicator has three levels of assessment low-risk, medium or high-risk level and data will analysis with the statistical method (Jacxsens et al., 2013; Kirezieva et al., 2015). The interviewees are individual have responsible for each activity in the indicator.

The first set of the questionnaire examining management operation of farms through context factors described from performance cultivation process characteristics and orientations of the organization. The second set of the questionnaire cross-examines monitoring activities done by farms have to meet with GAP standard through two aspects performance of monitoring activities and monitoring activities operation. The third set of the questionnaire verification the indicators during the assurance activities. The fourth set of the questionnaire analyzing the system output through the report feedback or complaints.

Result and Discussion

Table 3 presents a result from 63 samples (21 per farm) while table 4 demonstrates results irrigation water assay. Overall both results of three kyuri farmers, coliforms ranging from 3.0 to 4.7 log 10 cfu/g, E.coli O157: H7 was detected only at the farm 3

and Salmonella spp was not detected. Water samples collected in the tank was not detected coliforms in all farms. Moreover, in all farms, examined counts have decreased when approaching to post-harvest. For example, coliform started (T0) 3.5 to 4.7 (T1), 3.5 to 4.2 (T2) 3.4 to 4.1 (T3) 3.4 to 3.9 (T4) 3.3 to 3.7 and (T5) 2.0 to 3.3.

The highlight bold activites observed during the sampling time is hygiene (transport boxes and hands of workers) and safety (soil, natural fertilizer, and kyuri seedlings). These activities are the critical control point from the whole process, all of the kyuri farms presented low microbiological contamination. Moreover, washing used a secure water tank supplies was a tendency of decreasing the microbial potential contamination. The final kyuri samples from all farms meet with a Japanese regulation that maximum coliforms 10 x 2 cfu/g, E.coli O157: H7 and Salmonella spp should negative. However, the presence of E.coli O157: H7 was detected only in natural fertilizer at the farm 3, due to failing decomposition of pathogens period. In line with that, study of Johannessen (2005) findings E.coli O157: H7 is a serious problem which may lead contamination in the irrigation system or spreadings to other kyuri fruits since it is served or eaten in raw.

Another major indicator that can be linked to the microbiological potential contamination is a water resource. The results are ranging from 6.3 to 20 MPN/ml at all times, it means the potential is negative. All the farms obtained the main resource for water is pumping from a well and filtered before depositing to a huge tank. Afterwards, the tank is channelled to pipe network for a sprinkle and faucet. With this system, the irrigation be sufficient to eliminate a contamination potential to avoid using standstill water or pond (Olaimat, et al., 2012).

The evaluating activities through self-assessment tool describe all three farms have two major hazards focus, one is chemical control such as synthetic substances uses, pesticide residue and chemical contaminations, two is a microbial hazard due to contact with raw material, and natural characteristics. In table 5 part I demonstrated that three farms proved operated in high performance of microbial risk. Due to cultivation in the same region and environment, all of the farmers attending organic production practices that were provided by cooperative and food safety authorities and each activity is performed to GAP criteria guidelines. Others, during the interviews the workers have high commitment concerned with quality improvements.

Table 1. Timeline of selected critical sampling locations (CSL). T0: field preparations; T1: Start of the planting; T2: After planting two weeks; T3: After planting four weeks T4: Two weeks before harvest; T5: Harvesting T6: Postharvest handling.

T0		T1		T2		T3		T4		T5	
CSL 1	Manure	CSL 5	Manure Soil	CSL 5	Manure Soil	CSL 5	Manure Soil	CSL 5	Manure Soil	CSL 5	Manure Soil
SL 2	manure Soil	CSL 6	Kyuri	CSL 6	Kyuri	CSL 6	Kyuri	CSL 6	Kyuri	CSL 6	Kyuri
CSL 3	Seedlings in soil	CSL 10	Irrigation Water Tank	CSL 10	Irrigation Water Tank	CSL 10	Irrigation Water Tank	CSL 10	Irrigation Water Supply	CSL 7	Kyuri after washing
CSL 4	Seedlings	CSL 10	Irrigation Water supply	CSL 10	Irrigation Water supply	CSL 10	Irrigation Water supply	CSL 10	Irrigation Water supply	CSL 8	Swab Farmer Hands
CSL 10	Irrigation Water supply									CSL 9	Swab of transport Box of kyuri

Table 2. Identification step in microbiological methodologies sampling of critical sampling locations (CSL)

CSL	Samples	Amount of sampling	Period	Parameters	Method	Peformance of the result
1	Manure	4 Samples	T0	Coliforms	ISO 16140:2010, AOAC (2002)	2.000 NPM/g
				Salmonella		A/25g
				E.Coli O157:H7	ISO 16140:2010	A/25g
2	Manured Soil	9 Pooled Samples	T0	Coliforms	ISO 16140:2010, AOAC (2002)	2.000 NPM/g
				Salmonella		A/25g
				E.Coli O157:H7	ISO 16140:2010	A/25g
3	Seedlings in Soil	3 Pooled Samples	T0	Coliforms	ISO 4832:2006, AOAC (2002)	10^2
				Salmonella		A/25g
				E.Coli O157:H7	ISO 16140:2010	A/25g
4	Seedling	1 Sample	T0	Coliforms	ISO 16140:2010, AOAC (2002)	10^2
5	Manured Soil	9 Pooled Samples	T1	Coliforms	ISO 16140:2010, AOAC (2002)	10^2
			T2	E.Coli O157:H7		A/25g
			T3	Salmonella	ISO 16140:2010	A/25g
			T4			
			T5			
6	Kyuri	9 Pooled Samples	T5	Coliforms	ISO 16140:2010, AOAC (2002)	10^2
				Salmonella		A/25g

				E.Coli O157:H7	ISO 16140:2010	A/25g
7	Kyuri after washing	9 Pooled Samples	T5	Coliforms	ISO 16140:2010, AOAC	10^2
				Salmonella	(2002)	A/25g
				E.Coli O157:H7	ISO 16140:2010	A/25g
8	Swab of farmers hands	3 x 25 cm^2	T5	Coliforms	ISO 16140:2010, AOAC (2002)	≤0.6 log cfu/25 cm^2 (below detection)
9	Swab of Transport container of Kyuri	10 x 50 cm^2	T5	Coliforms	ISO 16140:2010, AOAC (2002)	≤0.6 log cfu/25 cm^2 (below detection)
10	Irrigation water supply	200 ml	T0	Coliforms	20th APHA (1998)	2 x 10^2 cfu/200ml
			T1	Salmonella	ISO 16654:2001	A/25 ml
			T2	E.Coli O157:H7		A/25 ml
			T3			
			T4			

Table 3. Results of microbial samples assay at three organic kyuri farms.

Visit	CSL	Samples	Amount of sampling	Farm 1			Farm 2			Farm 3		
				Coliforms	E.Coli O157:H7	Salmonella	Coliforms	E.Coli O157:H7	Salmonella	Coliforms	E.Coli O157:H7	Salmonella
T0	1	Manure	3	3.5 (±0.11)	-(0/2)	-(0/3)	5.5 (±0.92)	-(0/2)	+(2/3)	4.5 (±0.33)	+(1/2)	-(0/3)
T0	2	Manured Soil	9	3.4 (±0.70)	-(0/3)	-(0/3)	4.0 (±0.13)	-(0/2)	+(2/3)	4.7 (±0.55)	-(0/3)	-(0/3)
T0	3	Seedling in soil	3	4.5	-(0/1)	-(0/1)	5.7	-(0/1)	-(0/1)	4.0	-(0/1)	-(0/1)
T0	4	Seedling	1	3.5	-(0/1)	-(0/1)	4.3	-(0/2)	-(0/2)	4.2	-(0/1)	-(0/1)
T1	5	Manured Soil	9	3.5 (±0.67)	-(0/1)	-(0/1)	3.9 (±0.33)	-(0/1)	-(0/1)	4.2 (±0.92)	-(0/3)	-(0/3)
T2	5	Manured Soil	9	3.4 (±0.07)	-(0/3)	-(0/3)	4.1 (±0.44)	-(0/3)	-(0/3)	4.2 (±0.30)	-(0/3)	-(0/3)
T3	5	Manured Soil	9	3.4 (±0.72)	-(0/3)	-(0/3)	3.6 (±0.73)	-(0/3)	-(0/3)	3.9 (±1.53)	-(0/3)	-(0/3)
T4	5	Manured Soil	9	3.3 (±1.34)	-(0/3)	-(0/3)	3.7 (±0.92)	-(0/3)	-(0/3)	3.5 (±0.66)	-(0/3)	-(0/3)
T5	5	Manured Soil	9	3.3 (±0.78)	-(0/3)	-(0/3)	3.6 (±1.03)	-(0/3)	-(0/3)	3.1 (±0.93)	-(0/3)	-(0/3)
T5	6	Kyuri	9	3.3(±1.83)	-(0/3)	-(0/3)	3.5 (±0.41)	-(0/3)	-(0/3)	3.0 (±1.23)	-(0/3)	-(0/3)
T5	7	Kyuri after washing	9	3.2 (±0.17)	-(0/3)	-(0/3)	3.3 (±1.63)	-(0/3)	-(0/3)	3.0 (±0.37)	-(0/3)	-(0/3)
T5	8	Worker hands	9	3.1 (±0.62)	-(0/3)	-(0/3)	2.9 (±0.93)	-(0/3)	-(0/3)	2.2 (±1.21)	-(0/3)	-(0/3)
T5	9	boxes	9	3.0 (±0.89)	-(0/3)	-(0/3)	2.0 (±0.06)	-(0/3)	-(0/3)	2.9 (±0.63)	-(0/3)	-(0/3)

Table 4. Results Irrigation water assay at three organic kyuri farms.

Visit	CSL	Samples	Amount of sampling	Farm 1			Farm 2			Farm 3		
				Coliforms NPM/200ml	E.Coli O157:H7 NPM/25ml	Salmonella NPM/25ml	Coliforms NPM/200ml	E.Coli O157:H7 NPM/25ml	Salmonella NPM/25ml	Coliforms NPM/200ml	E.Coli O157:H7 NPM/25ml	Salmone NPM/25
T0	1	Irrigations water tank	1	14	absence	absence	18	absence	absence	20	absence	absence
T1	2	Irrigations water tank	1	13	absence	absence	18	absence	absence	20	absence	absence
T2	3	Irrigations water tank	1	13	absence	absence	11	absence	absence	20	absence	absence
T3	4	Irrigations water tank	1	10	absence	absence	7.1	absence	absence	17	absence	absence
T4	5	Irrigations water tank	1	8.7	absence	absence	6.3	absence	absence	16	absence	absence

Table 5. Self-assessment result degree based on good agricultural practices (GAP) standard attribute

Attribute Descripsions	Assumption linked to indicator (based on Kirezieva, Jacxsens et al., 2013, Kirezieva, Nanyunja et al., 2013)	Farm 1	Farm 2	Farm
	PART I. Context factors			
	Cultivation process characteristics			
Risk of raw materials (microbial)	Raw materials used in kyuri seedlings that can lead to microbial contamination due to natural characteristics of materials are concern during cultivation practices step and follow GAP requirement criteria	3	3	3
Risk of final product (microbial)	Kyuri surface are prone to pathogen or fungal growth, final treatment such microbiological hygiene or pathogen prevalence follow GAP requirement criteria increase food safety performance	3	3	3
Production system	Open field cultivation system contact with the environments and the soils that can minimalize risk to microbial contamination follow GAP requirement criteria which less direct.	3	3	3
Water supply	Water supplies tank should prevent contamination others water resourche likelihoods (i.e., rainwater, uncontrolled surface water, water from ponds) and treatment follow GAP requirement criteria	3	3	3
Mean Cultivation process characteristics		3.0	3.0	3.0
	Organization and chain			
Presence of technological staff	Farms having internal expertise staff with technology technician support in food safety attribute follow GAP requirement criteria.	2	3	3

Variability in workforce composition	Workforce composition variability 20% part-time and 80% permanent workers which rotation is limited with specific safety tasks at cultivation process step attribute follow GAP requirement criteria.	1	1	1
Sufficiency of workers competences	Hiring technical, operators and supervisior having specific skill, training aspect and education in food hygiene and safety attribute follow GAP requirement criteria.	2	2	2
Extent of management commitment	Management very eager commitment improving on food safety control system following adequately improving workers issues solving (i.e., welfare) follow GAP requirement criteria.	2	2	2
Degree of employee involvement	Workers attending machine operation instructions workshop, hygine and safety attribute traning lead to improving motivated and skills in commitment follow GAP requirement criteria.	2	3	1
Sufficiency supporting information systems	Information systems such as book record, or computerize consist of cultivation activity, hygine assesment, safety tasks and assurance activities in real time activity record requiring follow GAP requirement criteria	3	3	3
Severity of stakeholder's requirements	Stakeholders (association organizations, government, retailers, wholesalers) requird differing requirements, selective, sophisticated, higyne and safety easy monitoring by computerized follow GAP requirement criteria.	3	2	2
Extent of power in supplier relationships	Incoming materials from supplier requiring attribute such update, advance and traceable which higyne and safety monitoring under control supplier follow GAP requirement criteria	2	2	2
Food safety information exchange	Sophisticated information sharing system must deal with all recording data of hygiene and ssafety activity follow GAP requirement criteria.	3	3	2
Logistic facilities	Logistic facilities should have adequate and strictly maintaining to preventing microorganisms and contamination on environmental facilities which is follow GAP requirement criteria.	3	3	3
Inspections of food safety authorities	Inspections system by authorities food safety control body should adequate and give properly feedback for improving perofmanc of the hygiene and safety activity follow GAP requirement criteria	3	3	3
Specific external support	External specific support on production system should giving adequate consider such as opinion, observation and assesment related hygiene and safety attribute may improving the food safety activity decisions follow GAP requirement criteria.	2	2	3
Specific legislation	Farms adopting detailed national food legislation on food hygiene and safety attribute adequate following with GAP requirement criteria.	2	3	3
Mean organization and chain		2.2	2.7	2.3

PART II. Monitoring activities				
Performance of Monitoring activities				
Hygienic design of equipment and facilities	Facilities designs focus on critical hygiene and equipment considering efficient cleaning and preventing cross-contamination following with GAP requirement criteria.	3	3	3
Maintenance and calibration program	The maintenance program should have schedule period and specific manual guide depent on machine follow with GAP requirement criteria.	2	2	3
Storage facilities	Storage facilities designs for keep humidity, air circulation and temperature contidions to preventing cross-contamination or microorganisms' growth follow GAP requirement criteria.	2	2	2
Sanitation program(s)	Sanitation facilities should have adequate equipment for cleaning period following specific aspect instructions to preventing cross-contamination or microorganisms growth follow GAP requirement criteria.	3	3	2
Personal hygiene requirements	Farms should have specific personal hygiene guidance to prevent contamination follow GAP requirement criteria.	2	2	2
Incoming material monitoring	Farm should have incoming material monitoring record and verifications before using the material in every single step follow GAP requirement criteria.	3	2	2
Packaging materials	Packaging materials using compatible material product friendly to preventing product harm or damange follow GAP requirement criteria.	3	3	2
Supplier monitoring	More specific criteria and systematic evaluation process for selecting supplier material follow GAP requirement criteria.	3	3	3
Organic fertilizer program	Adequate facilities and equipment to support organic fertilizer programs follow GAP requirement criteria.	2	2	1
Irrigation method	Irrigation methods should have limited direct contact among water and edible parts to preventing contamination follow GAP requirement criteria.	3	3	3
Analytical methods to assess pathogens	More adequed rapid pathogen identification methods fullil GAP requirement criteria.	2	2	2
Sampling plan for microbial assessment	Periodical sampling data program on real time product and treatment activities information follow GAP requirement criteria.	2	2	2
Mean Performance of Monitoring activities		2.4	2.2	2.3
Monitoring activities operation				
Actual availability of procedures	Accesable of traceability procedures and programing follow GAP requirement criteria.	2	2	2
The actuality of compliance to procedures	The detail activities at all steps record in computerized lead to compliance behavior in monitoring follow GAP requirement criteria.	3	3	3

Actual hygienic performance of equipment and facilities	Equipment and facilities should mainaining hygienic performance follow GAP requirement criteria.	2	2	3
Actual storage/cooling capacity	Storage/cooling facilities should have stable temperature to reduce risk of contaminations follow GAP requirement criteria.	2	2	2
Actual process capability of packaging	Consistent packaging product material base on the suitability product follow GAP requirement criteria.	3	3	3
Actual performance of analytical equipment	Periodicals calibrations equipment program to avoid incompatibility measument during analysis activities follow GAP requirement criteria	3	2	2
Mean monitoring activities operation		2.5	2.4	2.5
	PART III. Assurance activities			
Validation of preventive measures	A laboratory evidance and verification observation base preventive measure act taken follow GAP requirement criteria.	3	3	3
Verification of equipment and methods related performance	Competence verificated from the third part follow GAP requirement criteria.	2	2	2
Documentation system	All information and documen have data base which is support for sharing to other stakholders follow GAP requirement criteria.	3	3	2
Record-keeping system	Computerized record system on real time with documentation at every step follow GAP requirement criteria.	3	3	3
Mean assurance activities		2.8	2.8	2.6
	PART IV. System output			
Evaluation of good agricultural practices	Inspection and evaluation current applications which auditing by competence organization body follow GAP requirement criteria.	2	2	3
Seriousness of remarks	All inditaros in good or excellent marking by competence organization body follow GAP requirement criteria.	3	3	3
Hygiene-related and microbiological food safety	Complaints or feedback regarding hygiene and safety product issues in small number.	3	3	2
Product sampling for microbiological performance	Microbiological performance measuring with adequate methods with accurate result follow GAP requirement criteria.	3	2	2
Judgment criteria for microbiological results	Considering thrid party analyses to assement the microbiological performance follow GAP requirement criteria.	3	3	3
Non-conformities	All non-conformities indicator at low level or good or excellent marking follow GAP requirement criteria.	3	2	2
Mean assurance activities		2.7	2.4	2.5

The self-assessment questionnaire in water control is in high performance with grade point 3. Besides the independent water system, environmental surrounding field cultivations (forest and rural areas) identically with a catcher water area and far away from chemical or household waste. Other, the advantage of this system are considered a critical point to eliminate contaminations potential which is the consumer may not be use hygiene water for washing, preferably eat directly (James, 2006; Kavyashri, et al., 2016; Huang, et al., 2016)

Concussion

These findings demonstrated and confirmed, all of the local farms have medium performance in application of good agriculture practice. Particularly, preventing the Salmonella spp and maintaining the hygiene attribute through food safety activities and following the GAP criteria. However, the assurance activities are still limited information about the improving program and lack of core activities in laboratory checking system. Other, local government and cooperatives collaborative support is considered as a credit to improving the comprehensive aspect (knowledge, funding support) of food safety.

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