Microbiological Evaluation of Fruits Certified as Specially Grown Agricultural Products by an Accredited Certification Agency

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Abstract

The partial organic fruits (Japanese apricot, Japanese plum, persimmon, satsuma mandarin, and lemon) grown under restricted inorganic practices and certified as Specially Grown Agricultural products by a Wakayama Prefecture-accredited certification agency were evaluated for microbial quality and safety in 2004 to 2006. Microbial population in most whole fruit or peel and flesh of fruit was below the detection level (2.4 log CFU/g for bacteria and 3.0 log CFU/g for fungi). Among all samples, the peel of satsuma mandarin showed the highest counts of 3.9 log CFU/g for total bacteria in 2004 and 5.0 log CFU/g for fungi in 2005. The microbial counts in the flesh of all fruits were below the detection level and the pH values of flesh of all fruits were as low as 3.2 except for persimmon. Molds comprised approximately 80% of the total isolates in all fruits. The most frequently isolated mold genera were *Alternaria*, *Diaporthe*, *Pestalotia*, *Phialophora*, and *Phanerochaete*, which were phytopathogenic organisms. No human pathogens such as *Salmonella* and *Escherichia coli* O157:H7 were detected from any of the samples. These results suggest that the partial organic fruits do not have high microbial risk due to the farming methods used.

1. Introduction

Food safety of fresh fruits and vegetables is of major concern for consumers. One of potential hazards of fresh produce is excess pesticide-residues. The consumer demand for organic produce is driven by the assurance that no synthetic pesticides and fertilizers are used in its production. According to Ministry of Agriculture, Forestry and Fisheries of Japan, only producers certified by registered certifying bodies accredited by the Ministry are able to label as organic agricultural products through the Japanese Agricultural Standard (JAS) Law [(1)]. The requirements to produce the agricultural organic products include use of composts and no use of prohibited agricultural chemicals and fertilizers during current production periods and previous two years before sowing or planting. However, the Ministry recognizes partial organic products certified as Specially Grown Agricultural (SGA) products by accredited certification agencies, in which the level of pesticides and chemical fertilizers applied are below 50% of those of conventional products[(2)]. The producers certified by accredited SGA products certification agencies should produce the products under some organic practices on behalf of inorganic practices to reduce the synthetic pesticides and chemical fertilizers. Therefore, technical criteria of certification of the SGA products are not so strict for producers as compared to that of the JAS for organic agricultural produce.

Another important potential hazard of fresh produce is foodborne illness. Especially in the USA, the number of foodborne outbreaks associated with fresh produce and produce dishes increased from 20 outbreaks with 1563 cases in 1990 to 85 outbreaks with 3181 cases in 2004 [(3)]. It has been suggested that organically grown products have a greater risk to pathogenic contamination than the conventional counterparts [(4)], because natural fertilizers such as animal manure are used and no chemical treatments are employed to reduce the microbiological loading of the raw product in organic farms. Thus, several researchers have focused on microbial risk assessment of organic products [(5-9)]. However, no reported surveys were undertaken to determine the microbiological quality and safety of partial organic products such as the SGA products.

In this study, the SGA fruits certified at Wakayama Prefecture in 2004 to 2006 were analyzed for the enumeration and identification of microflora and the presence of *Salmonella* and *Escherichia coli* O157:H7, which have been the

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two most common etiological agents responsible for produce-related outbreaks [9,10]. Although this study was not designed to compare partial-organically and conventionally produced fruits, the information from this study could be helpful in formulating microbiological guidelines of fruits and vegetables in Japan.

2. Materials and Methods

2.1 Collection of fruits

Fruit samples were directly collected from partial organic farms, which were certified by a Wakayama Prefecture-accredited SGA products certification agency, in 2004 to 2006. These fruits were partial-organically produced by a cultivation method, in which the frequencies of synthesized pesticides and the quantity of chemical fertilizers as nitrogen content used in the production processes did not exceed 50% of those in standards for each fruit set by Wakayama Prefecture. The list of fruits collected included Japanese apricot from 2 farms, Japanese plum from 1 farm, persimmon from 1 farm, and satsuma mandarin from 2 farms in 2004, Japanese apricot from 5 farms, persimmon from 1 farm, lemon from 1 farm, and satsuma mandarin from 1 farm in 2005, and Japanese apricot from 7 farms in 2006. Twelve fruit of Japanese apricot and 9 fruit of Japanese plum, persimmon, satsuma mandarin, and lemon from each farm were put into sterile zip-lock bags without washing and transferred from the farm to the laboratory at Kinki University within 10 h after sample collection. Microbial analyses of the samples were replicated three times.

2.2 Microbial counts and isolations

Each sample collected in 2004 to 2006 was assessed for counts of total bacteria, coliform groups, and fungi as previously described [11]. A 10-g sample of whole fruit or peel and flesh of fruit was macerated separately in 90 ml of saline solution (0.85% NaCl water) in a sterile stomacher bag using an Elmex stomacher (Pro-media SH-001, Eiken Kizai, Tokyo, Japan) for 4 min at room temperature. The serial dilutions from each sample were made in sterile saline solution and then poured in duplicate standard method agar (SMA; Nissui Pharmaceutical, Tokyo, Japan) plates for enumeration and isolation of total bacteria, in duplicate desoxycholate agar (DA; Nissui Pharmaceutical) plates for enumeration of coliforms, and in triplicate potato dextrose agar (PDA; Nissui Pharmaceutical) with 100ppm chloramphenicol plates for enumeration and isolation of fungi. Incubation conditions were 48-72 h at 37°C for SMA, 24 h at 37°C for DA, and 5-7 days at 26°C for PDA. Bacterial, mold, and yeast isolates from each sample collected in 2004 were selected from different appearing colonies by a stereoscopic observation for microbial identification. Since yeasts within fungi were rarely found in fruit samples, only yeasts in Japanese apricot and satsuma mandarin were identified to genus and species.

2.3 Microbial identification and pathogen analysis.

The MicroSeq Microbial Identification and the MicroSeq D2 LSU rDNA Fungal Identification (Applied Biosystems, CA, USA) were used for identification of bacteria and molds, respectively, as previously described [11]. The sequencing data were analyzed using Analysis Software (MicroSeq Analysis Software v. 1.40 and MicroSeq 16S rDNA Sequence Databases v.1.01) and the nucleotide database at GenBank using BLAST to determine bacterial and fungal identities, respectively. A cutoff of the lowest distance score from the sequence in the database was chosen for species identity. The Biolog system, consisting of MicroStation and Microplate Reader (Biolog Inc, CA, USA), was used for yeast identification as previously described [12]. The YT MicroPlate (Biolog Inc) inoculated with yeasts was read by the Microplate Reader (Biolog Inc) at a single wavelength of 590 nm to compare with the yeast database (MicroLog System Release 4.2). Reports that indicated a probability were chosen only for species identity.

The foodborne pathogenic bacteria, *Salmonella* and *E. coli* O157:H7, were identified by loop-mediated isothermal amplification (LAMP) method as described by Notomi et al. [13]. The LAMP reactions were performed using Loopamp *Salmonella* Screening Kit (LMP601, Eiken Chemical, Tokyo, Japan) and Loopamp Verotoxin-producing *E. coli* Screening Kit (LMP621, Eiken Chemical) followed to the manufacturer's instruction. Briefly, the LAMP reaction mixture contained *Bst* DNA Polymerase and Reaction Mix. Sal or Reaction Mix. VT was mixed with the DNA sample extracted using the Extraction Solution for Foods for screening *Salmonella* or *E. coli* O157:H7, respectively. The LAMP reaction was carried out by incubation at 65°C for 60 min using Loopamp Realtime Turbidimeter (LA-200, TERAMECS, Tokyo, Japan). The gene amplification was monitored by measuring turbidity of white precipitates of magnesium pyrophosphate formation [14]. The LAMP assay for detection of *Salmonella* has been shown to be more sensitive than PCR and real-time PCR assays [15].

3. Results and Discussion

Microbial population in most whole fruit or peel and flesh of fruit was below the detection level (2.4 log CFU/g for total bacteria and coliform bacteria and 3.0 log CFU/g for fungi) (Table 1). Exception to this included the peel of Japanese apricot, persimmon, satsuma mandarin, and lemon, which the counts of total bacteria ranged from 2.9 to 3.6 logs CFU/g and those of fungi from 3.3 to 5.0 logs CFU/g. The peel of satsuma mandarin showed the highest counts of 3.9 log CFU/g for total bacteria in 2004 and 5.0 log CFU/g for fungi in 2005 among all samples. The microbial counts in the flesh were below the detection level with all fruits, although the counts of Japanese apricot in 2005 and 2006 were analyzed in whole fruit, where 5 of the 12 samples collected contained detectable level of fungi ranging from 3.0 to 4.6 logs CFU/g. The pH values of peel varied among fruits and ranged from 2.9 of Japanese plum to 6.3 of Japanese apricot, while the values of flesh were as low as 3.2, except for persimmon with a pH value of about 5.5.

We previously reported that total bacterial counts of 3.0 to 6.5 logs CFU/g were found in some vegetables such as cucumber, lettuce, spinach, and carrot [16], and counts of bacteria and fungi of tree fruits such as satsuma mandarin and persimmon were close to or below the detection level [17, 18]. The results of microbial counts and pH values in the present and previous studies corroborate the other report demonstrating that bacterial count is higher in vegetables than in tree fruits due to external barriers such as peel and the interior low pH in flesh, which prevent microorganisms from entering and growing, respectively [19].

The frequency of bacteria (9 species) and yeasts (4 species) was few and molds (42 species) comprised approximately 80% of the total isolates in all fruits investigated in this study (Table 2), as noted with other fruits such as grapefruit [20] and mango [21] due to low pH and high sugar content. With Japanese apricot, the diversity of mold flora was less in the flesh than in the peel. The mold flora consisted of phytopathogenic organisms such as genera *Colletotrichum*, *Fusarium*, *Mycosphaerella*, *Penicillium*, *Pestalotia*, *Phialophora*, and *Septoria* and soil borne organisms such as genera *Cercophora*, *Cladosporium*, and *Ochroconis*. The genera *Penicillium* and *Phialophora* were found in both the peel and flesh. Only soil bacteria such as *Curtobacterium* species were found in the peel, while only yeasts living in plant-soil environment such as *Candida fructus* and *Issatchenkia terricola* were detected in the peel (data not shown). Many of these microorganisms originate from the soil and may spread by wind or rain [17, 18].

The characteristic on microflora of Japanese apricot was similar to those of Japanese plum, persimmon, and satsuma mandarin. The most frequently isolated mold genera were *Alternaria*, *Diaporthe*, *Pestalotia*, *Phialophora*, and *Phanerochaete*, which were plant pathogenic organisms. The few bacteria and yeasts isolated in the peel were also plant-soil organisms including bacteria species *Terrabacter tumescens* in persimmon and *Pantoea agglomerans* in satsuma mandarin and yeast species *Occultifur externus* and *Pichia guilliermondii* in satsuma mandarin (data not shown). The microflora of these fruits were similar to those of conventionally farmed tree fruits such as satsuma mandarin [17], persimmon [18], and plums [22].

No human pathogens such as *Salmonella* and *E. coli* O157:H7 were detected from any of the samples based on LAMP assay. The absence of pathogens (*Salmonella*, *E. coli* O157:H7, *Campylobacter*, and *Listeria monocytogenes*) was reported in commercially available organic vegetables in the UK [5, 6]. When microbiological quality was compared between fresh products produced by organic and conventional farmers in the USA, no difference in microbiological quality was found between organically and conventionally managed orchards [7, 9]. However, Mukherjee *et al.* [8] reported that organic fruits and vegetables from farms that used manure or compost aged less than 12 months had a prevalence of *E. coli* 19 times greater than that of farms that used older materials, and *Salmonella* was isolated from one organic lettuce and one organic green pepper but not from conventional produce. From our results, the absence of enteric pathogen in all samples was encouraging and the SGA fruits during 3 seasons would be designated microbiologically safe. However, since the fertilizer with manure and compost were sometimes used with the production, it is important that producers be aware of the risks associated with untreated manure as a fertilizer. This study was not a comparative study of partial-organically and conventionally produced fruits, and does not support the suggestion that foods from partial organic practices possess high risk due to the farming methods used.

Table 1 Microbial population and pH of fruits certified as Specially Grown Agricultural products by an accredited certification agency in 2004 to 2006.

Year of analysis	Fruit sample (Farm name)	Part of fruit	pH	Microbial population (Log CFU/g)		
				Total bacteria	Coliforms	Fungi
2004	Japanese apricot (A)	Peel	6.3	2.9	<2.4 [a]	<3.0 [b]
		Flesh	2.6	<2.4 [a]	ND	<3.0
	Japanese apricot (B)	Peel	5.2	<2.4	<2.4	<3.0
		Flesh	2.4	<2.4	ND	<3.0
	Japanese plum	Peel	2.9	<2.4	<2.4	<3.0
		Flesh	2.7	<2.4	<2.4	<3.0
	Persimon	Peel	6.1	<2.4	<2.4	3.3
		Flesh	5.6	<2.4	ND	ND
	Satsuma mandarin (A)	Peel	5.9	<2.4	ND	<3.0
		Flesh	3.2	ND	ND	<3.0
	Satsuma mandarin (B)	Peel	5.2	3.9	<2.4	3.5
		Flesh	3.2	<2.4	ND	<3.0
2005	Japanese apricot (A)	Whole	2.5	<2.4	<2.4	3.6
	Japanese apricot (B)	Whole	2.5	<2.4	<2.4	3.3
	Japanese apricot (C)	Whole	2.4	2.9	ND	3.3
	Japanese apricot (D)	Whole	2.4	<2.4	ND	<3.0
	Japanese apricot (E)	Whole	2.2	<2.4	ND	<3.0
	Persimon	Peel	6.2	<2.4	ND	<3.0
		Flesh	5.5	<2.4	ND	<3.0
	Lemon	Peel	5.9	3.6	<2.4	4.0
		Flesh	2.1	ND	ND	<3.0
	Satsuma mandarin	Peel	5.9	3.6	ND	5.0
		Flesh	3.1	<2.4	ND	<3.0
2006	Japanese apricot (A)	Whole	2.6	<2.4	ND	<3.0
	Japanese apricot (B)	Whole	2.8	<2.4	ND	4.6
	Japanese apricot (C)	Whole	2.6	<2.4	ND	3.0
	Japanese apricot (D)	Whole	2.3	<2.4	ND	<3.0
	Japanese apricot (E)	Whole	3.2	<2.4	ND	<3.0
	Japanese apricot (F)	Whole	2.5	<2.4	ND	<3.0
	Japanese apricot (G)	Whole	2.2	<2.4	ND	<3.0

[ab] Below the detection level for each organism
ND, not detectable

Table 2 Bacteria and molds isolated from fruits certified as Specially Grown Agricultural products by an accredited certification agency in 2004.

Fruit sample (Farm name)	Part of fruit	Bacteria Genus	Bacteria Species	Molds Genus	Molds Species
Japanese apricot (B)	Peel	*Curtobacterium*	*albidum*	*Cercophora*	*mirabilis*
			citreum	*Cladosporium*	*sp.*
			flaccumfaciens	*Colletotrichum*	*gloeosp*
				Fungal	*sp.*
				Fusarium	*bactridioides*
				Mycosphaerella	*rabiei*
				Myrothecium	*roridum*
				Ochroconis	*sp.*
				Penicillium	*digitatum*
					minioluteum
				Pestalotia	*sp.*
				Phialophora	*sp.*
				Septoria	*calendulae*
	Flesh	Not detectable		*Hyphoderma*	*setigerum*
				Penicillium	*aculeatum*
				Phialophora	*repens*
Japanese plum	Peel	*Staphylococcus*	*epidermidis*	*Arecophila*	*sp.*
				Lanspora	*coronata*
				Melanotus	*phillipsii*
				Peniophora	*cinerea*
				Phanerochaete	*sordida*
				Xylaria	*acuta*
	Flesh	Not detectable		*Alternaria*	*sp.*
				Tilletiopsis	*albescens*
Persimon	Peel	*Curtobacterium*	*flaccumfaciens*	*Aschersonia*	*sp.*
		Terrabacter	*tumescens*	*Coprinus*	*micaceus*
				Diaporthe	*melonis*
				Dichomitus	*squalens*
				Eutypa	*sp.*
				Phanerochaete	*sordida*
				Phialemonium	*aff*
				Phialophora	*sp.*
				Trichoderma	*inhamatum*
	Flesh	*Pediococcus*	*parvulus*	Not detectable	
Satusma mandarin (B)	Peel	*Pantoea*	*agglomerans*	*Alternaria*	*sp.*
			ananatis	*Diaporthe*	*melonis*
				Fungal	*sp.*
				Glomerella	*cingulata*
				Letendraea	*helminthicola*
				Nalanthamala	*squamicola*
				Pestalotia	*photiniae*
				Plectosphaerella	*cucumerina*
	Flesh	Not detectable		*Fungal*	*sp.*

4. Conclusion

The SGA fruits certified at Wakayama Prefecture in 2004 to 2006 were grown under partial organic practices so that the level of pesticides and chemical fertilizers applied could be below 50% of those of conventional products set by Wakayama Prefecture, and showed no risk for human illness and low microbial contamination. This result reinforces government recommendation for ensuring a high-quality fruits as SGA products.

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和文抄録

公認認定機関から特別栽培農産物として認証された果実の微生物学的評価

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制限された無機栽培により、和歌山県公認の認定機関から特別栽培農産物として認証された部分的有機栽培の果実（ウメ、スモモ、カキ、ウンシュウミカンおよびレモン）について、2004 年から 2006 年にかけて、その微生物的品質と安全性を調査した。多くの全果あるいは果皮および果肉部の微生物数は、検出限界値（細菌では 2.4 log CFU/g および真菌では 3.0 log CFU/g）以下であった。すべてのサンプルの中で、ウンシュウミカンの果皮は、2004 年の一般生菌数が 3.9 log CFU/g、2005 年の真菌数が 5.0 log CFU/g となり、最も高い微生物数を示した。すべての果実の果肉部の微生物数は検出限界値以下で、pH の値はカキを除いて 3.2 以下であった。すべての果実において、分離菌の約 80%はカビが占めた。最もよく分離されたカビ属は、*Alternaria*、*Diaporthe*、*Pestalotia*、*Phialophora* および *Phanerochaete* で、これらはいずれも植物病原菌であった。ヒトの病原菌である *Salmonella* および *Escherichia coli* O157:H7 は、いずれのサンプルからも検出されなかった。これらの結果から、部分的有機栽培果実は、その栽培方法から由来されるような高い微生物リスクは有していないことが示された。

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