Epiphytic microbiota of apple in integrated and organic growing

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Summary: The surface microbial contamination is of great interest in case of fruits, since they are they potential sources of spoilage or food-borne diseases. The aim of this work was to compare the epiphytic microbiota of food safety concern of different apple cultivars as a function of cultivation methods (organic versus integrated), production year and place. Investigating 47 samples it was found that the average microbial contamination of the apple surfaces was within a certain range,, practically independent of cultivar, growing area and year. The frequency distribution of the surface bacterial, mould and yeast counts did not show significant differences between growing technologies or varieties. The principal component analysis the samples ranked the apple samples into seven groups on the basis of their microbial contamination level, and the discriminant analysis proved the goodness of grouping. The grouping was independent from cultivars and growing methods. No food-borne pathogen *Salmonella* spp. and *Listeria* spp. were found on the surfaces of apples.

Introduction

Fresh fruits and vegetables are essential components of the human diet, and there is a considerable evidence of the health and nutritional benefits associated with their consumption. Significant rise in the consumption trends of fresh produce are noticed recently partly due to the changing lifestyles, partly as a result of worldwide campaigns on healthy nutrition. As a result, an increase in the popularity of fresh, and also the consumption of the minimally-processed ready-to-eat convenience foods increased (*Abadias*, 2008; *FAO & WHO*, 2008). Exotic, import fresh fruits and vegetables appear on the market, and also the choice of minimally-processed, convenience foods (fresh-cut vegetable and fruit salads with or without packaging) broaden. The elongated food chain, as a result of globality in itself result in microbiological problems, partly as spoilage of the product (qualitative and quantitative losses), partly food safety aspects arise. The growers, producers, handlers and distributors are facing different and altering conditions like weather, pests, market value, trends, and customer requests. Changes in growing and logistic practices may influence microbiological food safety.

More attention is paid to the food-borne outbreaks in the last decades related to the consumption of contaminated plant foods. Fresh produce can be a vehicle of bacterial, parasitic and viral pathogens causing human illnesses. Fresh apple is generally not considered as source of food-borne illnesses, and the presence of mycotoxin patulin is of the main concern in apple juices. However, in some cases food-borne outbreaks due to *E. coli* O157:H7, *Salmonella* spp. were recorded in association with the consumption of unpasteurized apple cider (*Lund & Snowdon*, 2000, *Zhao*, 2005).

The sources of potential contamination of fruits during production are the irrigation water (surface waters), soil, compost and manure, sewage/sludge (*Beuchat*, 1996, *Beuchat & Ryu*, 1997). Survival of bacteria in the soil is influenced by a number of factors (temperature, moisture content, sunlight, pH, available nutrients, etc.) being the temperature the most important parameter – the bacterial survival increases with decreasing temperature. It was also found, that the majority of pathogens in manures applied to land declines below detectable level after 3 months, and the limiting factor of survival is possibly the temperature (*Tyrrel & Quinton*, 2003). Nevertheless, survival of *Listeria* sp. over 6 months (*Nicholson* et al., 2005), *E. coli* O 157 over a year (*Kudva* et al., 1998), and that of *Salmonella* sp. over 1-2 years (*Nicholson* et al., 2005) was noticed in soils amended with manure. Increased number of bacteria and moulds was found in soils treated with treated municipal sewage sludge in different type of soils, owing partly due to the carry-over of microbes, but the effect of increased nutrients on the growth of microbes should also be considered (*Beczner* et al., 2004; *Biró* et al., 2008).

The main vectors of contamination are the water (rain, irrigation water spreading soil or slurry particles with the attached microbes onto the surface of plants, fruits), animals (insects, birds, rodents), and humans while harvesting, handling and processing (*Beuchat*, 1996, *Tyrrel & Quinton*, 2003, *Zhao,* 2005). Association of *Salmonella* contamination with bacterial soft rot of fresh fruits was reported (*Wells and*

Butterfield, 1997), as well as the growth of *Escherichia coli* O157:H7 in the wounds of apple (*Janisiewicz* et al., 1999). Fruit flies are easily contaminated externally and internally with *E. coli*, indicating the possibility of insect transmission of the bacterium (*Janisiewicz* et al., 1999). Presence of protozoa *Giardia lamblia*, *Cryptosporidium parvum* (*Monge & Chinchilla*, 1996), *Cyclospora cayetanensis* and trematode *Fasciola hepatica* (*Abdussalam* et al., 1995) and others is also of concern; their occurrence on raw fruits is likely due to contact with animal and human faeces, untreated sewage and sludge from primary or secondary municipal water treatment facility. Nematode *Caenorhabditis elegans* can act as a vector to *E. coli* O157:H7, *S. enterica* and *Listeria monocytogenes* and might be a potential vector by shedding the pathogens for a considerable length of time (*Anderson* et al., 2006).

Human pathogenic bacteria might also be part of the bacterial association of plant phyllosphere, as a result of carry over by insects, wind, irrigation water, and survive to certain length of time (*Beuchat*, 1996). Enteropathogens can adapt to the phyllosphere environment, but may also be outcompeted by the epiphytic bacteria (*Cooley* et al., 2006).

The effect of growing technology applied might also have influence on the microbial contamination of fruits. It was noticed, that straw coverage during strawberry growing resulted in an increased microbial contamination (i.e. *Enterobacteriaceae*, moulds and yeasts, coliforms) of the berries under rainy weather conditions, but not in dry weather (*Beczner & Bata-Vidács*, 2009).

Mould contamination might result in mycotoxin production. On apple, mainly during storage, *Penicillium expansum* produces mycotoxin patulin, which arises concern in apple juices and apple based baby foods (*Taczman-Brückner* et al., 2005). The main source of mould contamination of apples is the growing area; and the skin and tissue damages during harvesting, handling and transporting lead to the extensive proliferation and mycotoxin production of the mould during storage at low temperature (cca 10–15 °C).

The ratio of plants from organic farming is increasing in the market following to environmental and customer demand. The organic and integrated systems had higher soil quality and potentially lower negative environmental impacts than conventional systems (*Cassandro*, 2008). The effect of organic production on the quality and safety of the plant foods is scrutinized, and there is an ongoing debate between pros and contras. Organic products have much lower amounts of pesticide residues compared to integrated or conventional ones (*Baker* et al., 2002), however, often criticised for having lower crop, and higher incidence of plant diseases, and greater spoilage during storage. *Weibel* and co-workers (1999) found, that organically grown apple fruits were firmer, had higher technical quality index, higher phosphorous, fibre and phenolic compounds content, and higher scores in the taste panel test. Postharvest quality was also investigated, and it was found, that the parameters determining storability are strongly influenced by the growing conditions (i.e. nutrient supply, watering and plant

protection, etc.). Organically grown crops had slower growth rates and were physiologically more mature at harvest, and had longer storability, and due to their lower respiration rates and enzyme activity had lower storage losses (*Lampkin*, 1990). *Róth* and co-workers (2004) investigated four apple cultivars from organic and integrated productions in Hungary, concerning their -galactosidase and polygalacturonase enzyme activity, firmness and mass loss during storage. The statistical analysis of the data showed, that the effect of cultivar was the main factor in influencing the storability. Investigating the effect of growing area and production system (organic and integrated) on the storability (in air and CA conditions) and quality parameters of apple cv. Jonagold in Belgium, *Róth* and co-workers (2007) found, that storage conditions affected the quality of apples to a much greater degree, than the production system. *Nagy* and co-workers (2009a, b) and *Holb* and co-workers (2009) and *Holb and Nagy* (2009) concluded the same in their investigations.

The aim of this ongoing study was to compare the epiphytic microbiota of food safety concerns of apple as a function of cultivars, growing systems (organic and integrated), growing area and production year in Hungary.

Materials and methods

Sampling

At multiple time points throughout two growing seasons (2008–2009), fruits of *Malus domestica* cultivars were collected from two growing places (Újfehértó, Pallag) from integral and organic orchards *(Table 1)*. Samples were collected by the staff in Újfehértó and Pallag, and transferred to CFRI, Budapest, and were stored in refrigerator (at 4–7 °C) until investigated (generally up to 2–5 days). Altogether 47 samples were investigated.

Microbiological investigation

The average surface of 5–10 fruits per cultivars were calculated by measuring the apple diameters, and the microbiological results were calculated as log colony forming unit per cm^2 (cfu/cm^2, or $\log N\ cm^{-2}$). Microbial determinations were carried out using standard food microbiological methods. Five fruits of each cultivar were washed in 90 ml physiological saline peptone water (pepton 1 g, NaCl 9 g in 100 ml water), and the . mesophylic aerobic total count (MSZ 3640/3-86), moulds and yeasts (MSZ ISO 7954: 1999), *Pseudomonas* spp. (MSZ 3640/7-80), coliforms (MSZ 3640/18-79) and *Escherichia coli* (MSZ 3640/19-79) were quantitatively determined on general or selective media. Another five apples were used for the determination of presence/absence of *Listeria monocytogenes* (MSZ EN ISO 112900:1998) and *Salmonella* spp. (MSZ EN ISO 6579:2002) .

Statistics

Frequency distribution of the contamination level was calculated. Principal component analysis (PCA) was used to group the samples according to their total aerobic bacterial, mould and yeast contaminations. After forming the groups, discriminant analysis was used to control the goodness of PCA.

Results

No *Pseudomonas* spp., *Listeria* spp. and *Salmonella* spp. were found on any of the samples investigated. The presence of coliforms was sporadic, and in each case when present, it was around the detection limit (10 cfu/cm^2), and no *E. coli* was found on any of the samples. The dominant mesophylic aerobic bacterial count, the presence of moulds and yeasts were characteristic for the microbiota of apple surface, regardless to growing system, locality and production year.

The mesophylic aerobic total microbal contamination of the apple surfaces was between 10-10^9 cell/cm^2, the majority being between 10^3–10^5 cfu/cm^2, with a Gauss-like distribution. The distribution of cell counts was even in case of organic growing, and varied within a wider range for integral growing (*Table 1, Fig 1*). The frequency distribution of mould counts on the apple surfaces was also Gauss-like, being the majority of samples contaminated by 10^3–10^5 cfu/cm^2, with no difference between the organically and integrally grown samples (*Table 1, Fig 2*). The occurrence of yeast was similar in case of both growing systems, varying between 10^1 and 10^4 cfu/cm^2 (*Table 1, Fig 3*).

Principal component analysis was used to group the apple samples according to their main microbial contamination (aerobic total bacterial, mould and yeast counts). The program ranked the apple samples into 7 groups (except for two samples), according to their microbial load (*Fig 4*).

Table 1. Microbial contamination of apple cultivars

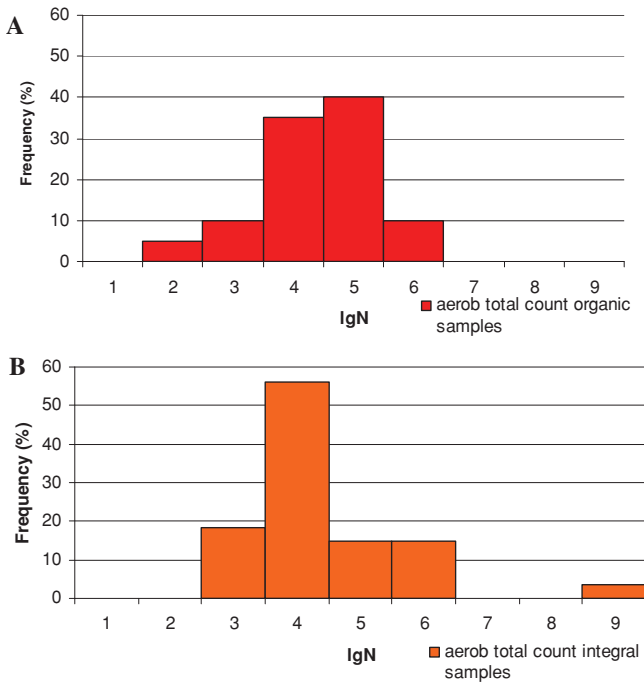| Cultivar	Sample number	Production system	Year	Place of growing	Total aerobic count (cfu×cm^{-2}) lgN	Mould count (cfu×cm^{-2}) lgN	Yeast count (cfu×cm^{-2}) lgN
Gála Must	1	Integrated	2008	Pallag	2.64	3.08	2.3
Gála Must	2	Organic	2008	Pallag	3.97	2.94	0
Prima	3	Integrated	2008	Újfehértó	5.67	3.25	0
Prima	4	Organic	2008	Újfehértó	4.76	2.61	0
Gála	5	Organic	2008	Újfehértó	4.47	3.43	2.8
Remo	6	Integrated	2008	Újfehértó	3	3	2.43
Remo	7	Organic	2008	Újfehértó	1.39	3.71	0
Topáz	8	Integrated	2008	Újfehértó	5.38	4.79	2.53
Topáz	9	Organic	2008	Újfehértó	5.2	3.89	2.44
Idared	10	Integrated	2008	Pallag	5.32	3.64	3.64
Idared	11	Organic	2008	Pallag	5.44	2.44	3.88
Remo	12	Integrated	2008	Pallag	4.25	3.23	3.2
Remo	13	Organic	2008	Pallag	4.38	4	2.71
Prima	14	Organic	2009	Újfehértó	4.69	4.23	0
Prima	15	Integrated	2009	Újfehértó	4.77	2.8	0
Releika	16	Organic	2009	Újfehértó	4.11	2.81	0
Releika	17	Integrated	2009	Újfehértó	3.38	2.44	0
Resi	18	Organic	2009	Újfehértó	4.88	3.11	3.44
Resi	19	Integrated	2009	Újfehértó	3.94	2.08	0
Remo	20	Organic	2009	Újfehértó	4.76	2.84	1.84
Remo	21	Integrated	2009	Újfehértó	3.86	2.08	0
Rubinola	22	Organic	2009	Újfehértó	4.41	2.69	1.3
Rubinola	23	Integrated	2009	Újfehértó	4	2.72	2
Rajka	24	Organic	2009	Újfehértó	3.65	3.3	2.61
Rajka	25	Integrated	2009	Újfehértó	3.44	3.44	2.44
Rewena	26	Organic	2009	Újfehértó	3.6	4.23	3.53
Rewena	27	Integrated	2009	Újfehértó	3	3.44	2.36
Topáz	28	Organic	2009	Újfehértó	3.69	5.44	1.96
Topáz	29	Integrated	2009	Újfehértó	3.43	3.11	2.17
Florina	30	Integrated	2009	Újfehértó	2.46	2.79	1.99
Prima	31	Integrated	2009	Újfehértó	2.3	2	1.2
Rebella	32	Integrated	2009	Újfehértó	2.58	2	1.87
Prima	33	Organic	2009	Újfehértó	2.8	1.9	2
Remo	34	Organic	2009	Újfehértó	2.15	2	2.23
Gála	35	Integrated	2009	Újfehértó	2.65	1.84	1.68
Remo	36	Integrated	2009	Újfehértó	3.2	2.7	2
Rajka	37	Integrated	2009	Újfehértó	3.23	2.62	2
Idared	38	Organic	2009	Újfehértó	3.11	2.75	2.11
Florina	39	Integrated	2009	Újfehértó	3.99	2.8	2.91
Idared	40	Integrated	2009	Újfehértó	3.41	2.95	2.56
Rewena	41	Integrated	2009	Újfehértó	3.28	3.15	2.48
Topáz	42	Integrated	2009	Újfehértó	3.08	4.23	2.57
Remo	43	Integrated	2009	Újfehértó	9.3	2.85	2.49
Remo	44	Organic	2009	Újfehértó	3.45	2.74	2.34
Topáz	45	Organic	2009	Újfehértó	3.46	2.99	2.21
Red Idared	46	Integrated	2009	Újfehértó	3.46	2.98	1.95
Idared	47	Integrated	2009	Újfehértó	3.25	2.93	2.83

Figure 1. Frequency distribution of the surface aerobic total count contamination of apple from organic (A) and integrated (B) production
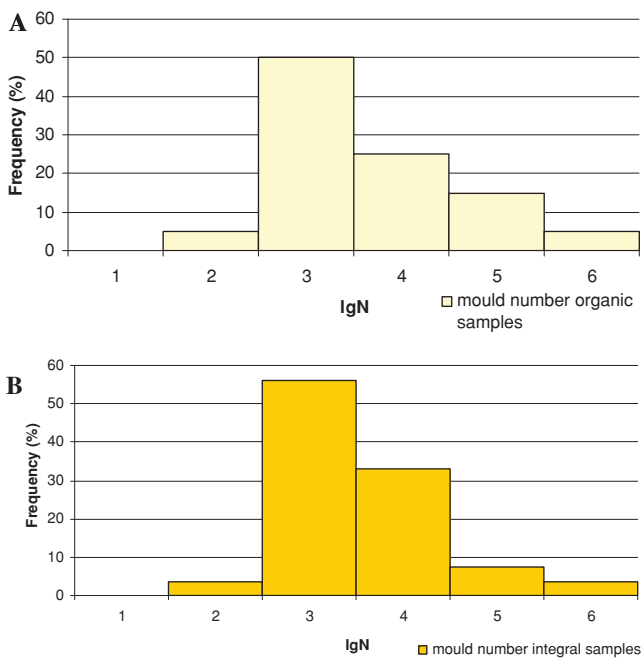

Figure 2. Frequency distribution of the surface mould contamination of apple from organic (A) and integral (B) production

In the **group 1** (six samples) the contamination was low (10^2/cm^2 for aerobic total count, yeasts and moulds). Sample distribution between organic and integral growing is cca 50–50%, and samples were mostly from 2009. **Group 2** (four samples) had somewhat higher contamination, than group 1 (10^3/cm^2 for aerobic total count, yeasts and moulds), and 75% of the samples were from integral growing and all of them from year 2009. For **group 3** (fourteen samples) contamination level of 10^3/cm^2 – 10^4/cm^2 was characteristic for each microbial
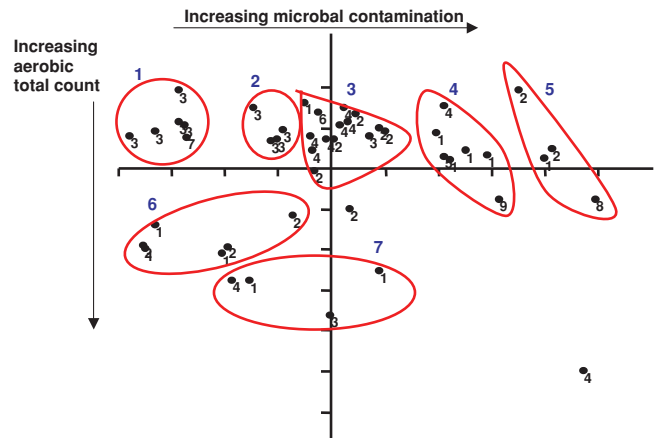

Figure 4. The principal component analysis (PCA) of apple cultivars of organic and integral production, on the basis of their microbial surface contamination (for decoding samples see Table)
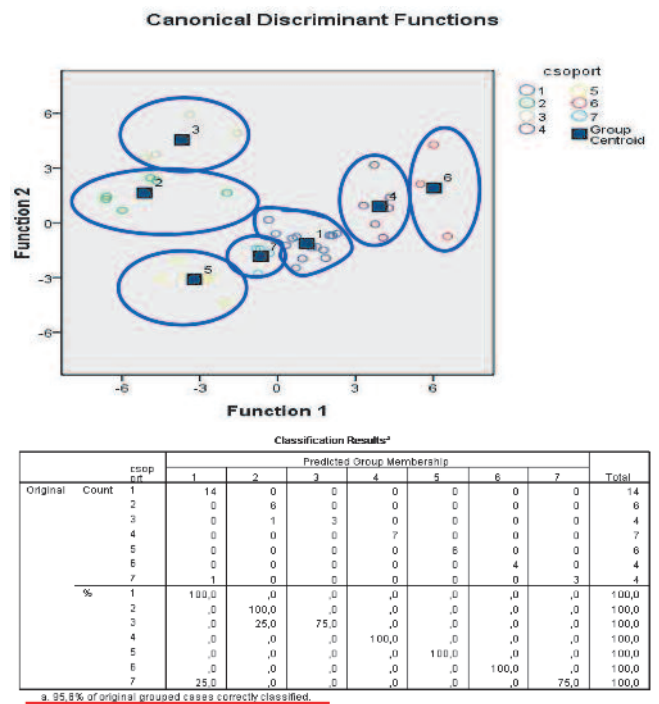

Figure 5. Discriminant analysis of apple samples (the goodness of PCA analysis)

groups, 80% of the samples were from integral growing and 80% from the year 2009. **Group 4** (seven samples) had a contamination level of 10^5/cm^2 for aerobic total count, and 10^3/cm^2 for moulds and yeasts, and 70% of the samples were from organic system. **Group 5** (four samples) had higher contamination level, than group 4 (aerobic total count 10^5/cm^2, moulds and yeasts 10^4/cm^2), 50% organic, year 2009 – 50% integral, year 2008. **Group 6** (six samples) had a dominance of aerobic total count (10^4/cm^2), mould contamination was low (10^2/cm^2) and yeasts were totally absent. All samples were from year 2009, 50%–50% organic and integral growing. **Group 7** (four samples) had high aerobic total count and moulds numbers (10^4–10^5/cm^2), yeasts were totally absent. All samples were cv. *Prima*, 50–50% integral and organic growing.

The PCA did not rank two samples (Remo 2009, Újfehértó, one organic and one integrated sample, harvested at different times) into any of the groups formed, having either extremely high aerobic total bacterial count (sample 43), or because of the combination of cell counts being different from the others (sample 20). Except for these two samples, the discriminant analysis proved the goodness of grouping (95.6% level) (*Fig 5*). These results also show, in harmony with the distribution patterns of the microbial contamination, that there are no statistically detectable differences between the epiphytic microbial contamination of the apple fruits concerning the growing system, year and growing territory. No differences were found also among the cultivars.

Acknowledgements

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