CORVINUS UNIVERSITY of BUDAPEST

Faculty of Food Science

Microbiological and chemical analysis of organic and integrated grown stone and pome fruits

PhD Thesis

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The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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1. INTRODUCTION

In the last decade, the modern and healthy nutrition has come into view more and more. Serving these kinds of consumer needs requires permanent changes from agriculture and also from food industry. Increasing demand appears for foods that are produced in the spirit of reform diet, and for many other trend products (paleo-diet, vegetarian, low-carb diet, etc.). Gradually, the healthy nutrition has come to the fore worldwide, but especially in Europe. Foods of plant origin form the basis of these healthy diet, and also these fruits form the basis of my study - fruits with outstanding economic and nutritional features. In Hungary apple is the fruit produced in the largest quantity, followed by sour cherry. Pear and sweet cherry are also produced in large amount. The sour cherry is primarily the fruit of Eastern Europe and Hungary has a decisive role in the region. Because of the production value and the large export ratio of cherry, its production is considered as key sector in the agricultural industry. In the coming years, the increase in fruit production can be expected worldwide.

The organic farming is another important trend in the sustainable farming systems, and it becomes more and more widespread. The organic farming systems grow tasty and authentic foods, just as do the conventional or integrated production method, but in the mean time, respects the natural cycles, too. In the organic farming human impact on the environment is reduced, – concerning either plant or livestock farming -, while the most natural functioning of agricultural systems are ensured.

Tracing, cultivating and consumption of fruit varieties with outstanding nutritional and biological activity is becoming increasingly important. For apple and cherry breeding, institutions with high importance are organized, like the Fruit Growing Research and Consulting Office in Újfehértó. High ratio of the apple and cherry samples were received from this institution. The activity of this research center is prominent in the field of conservation and breeding the North Hungarian sour cherry varieties. During my work, four kinds of fruits (sour cherry, cherry, apple, pear) were examined from the point of nutritional value characteristics and parameters of good value. In case of apple and sour cherry comparative examinations were carried out between organic and integrated farming in a three years (2008-2010) period.

Food safety has become increasingly important in the recent years. The European Union imposes strict requirements for food production for all the Member States and also for foods that imported from countries outside the EU. My aim was to examine the fruit surface microbial contamination, regarding to the occurrence of indicator and pathogenic microbes, as well as to examine the determining fruit quality parameters depending on the cultivars, the production site, the production method and the vintage.

2. AIMS OF THE PROJECT

During my work within the framework of the three-year (2008-2010) *Jedlik Ányos* project called *"Biological development of organic and integrated fruit production technologies"* I carried out a comprehensive examination of different fruits. The primary aim was the examination of goods and nutritional value, and secondly the comparison of the two types of cultivation methods from the point of food safety. Accordingly, in the study I report on fruits (apple, pear, cherry, sweet cherry), which are the most common in the Hungarian fruit cultivation practice. The aims of the research work were as follows:

- Determination of physical characteristics which affect the commercial value of the fruits (different apple and stone fruit species/cultivars) and their comparative mathematical analysis. Examination of the effect of the production system (integrated and organic) in case of sour cherry and apple.
- Examination and tracing the nutritional value of the fruits from three vintages and of several cultivars.
- The analysis of microbial surface contamination depending on the fruit cultivar, cultivation method, production site and vintage in case of apple and sour cherry.
- Examination and tracing the polyphenol content and antioxidant capacity in each vintage in case of sour cherry and sweet cherry. The comparison of the above mentioned two parameters in the two different cultivation (organic and integrated) systems. Examination of the effect of cold storage on the change of polyphenol content in case of cherry.
- Measuring the anthocyanin content in case of sour cherry and sweet cherry. Comparison of the anthocyanin profiles.

3. MATERIALS AND METHODS

3.1 Fruits, growing areas, cultivation methods and test methods

- Examinations were carried out with 15 cultivars of sour cherries (in case of 4 cultivars organic and integrated pairs), 3 cultivars of integrated sweet cherries, 33 cultivars of apples (in case of 6 cultivars organic and integrated pairs) and 5 cultivars of integrated pears.

- The samples were grown and transported from different places of Hungary from organic and integrated farming system:

- Integrated sweet cherry samples were from Nagykutas (Alma 2000 Ltd.)
- Integrated pear samples were from Szepetnek and Bánfapuszta (Gyümölcskert Plc.)
- Integrated apple and cherry samples were from Újfehértó (Fruit Growing Research and Consulting Office)
- Organic apple samples were from Debrecen-Pallag (University of Debrecen, Department of Economic and Regional Research Institute, Horticultural Experimental Station and Department of Plant, Pallag)
- Organic sweet cherry samples were from Kabalás (private plantation)

- Microbial examinations: determination of microbial surface contamination during three vintage (2008-2010)

- Analytical studies: dry-extract and acid content (2008-2010), determination of polyphenol content, antioxidant capacity and anthocyanin profile in case of sour cherry and sweet cherry (2008-2010).

3.2 Applied test methods

- Microbial examinations:

- Performed according to MSZ ISO standards (total aerob microbial count, total yeast and mould count, indicator and pathogenic microbes)

-Analytical examinations:

- Dry-extract content determination performed according to MSZ EN standard.
- Acid content determination performed according to MSZ ISO standard.
- Total polyphenol content determination performed according to MSZ 9474-80 standard with the method of spectrophotometry
- Antioxidant capacity determination performed according Brand-Williams method by spectrophotometry.
- Determination of anthocyanin profile performed with HPLC method.

- The experimental results were evaluated with mathematical and statistical methods.

4. RESULTS AND RECOMMENDATIONS

In the course of research I examined four types of fruits – apples, pears, sour cherries, sweet cherries (henceforth cherries) – and carried out a comprehensive investigation focusing on the factors affecting the production of goods value: the nutritional components and the biologically active compounds in three vintages (2008-2009-2010). In the overall study my aim was - with the determination of the above parameters - the comparison of products primarily for cherries and apples originating from organic and integrated cultivation forms. Recently the organic cultivation is spreading more and more. During the research 33 cultivars of apples, 15 cultivars of sour cherries, 5 cultivars of pears and 3 cultivars of sweet cherries were investigated. Part of the apples were the commercially available resistant cultivars (‘*Rewena’, ‘Remo’, ‘Resi’, ‘Releika’*), another category is made up of new cultivars (‘*A 8/31’* (Soltadino), ‘*A11/28’* (Davidino), ‘*M5/98’* (Matika), ‘*AS 10/31’*) and the third one involves commercial cultivars (‘*Gala’, ‘Jonathan’, ‘Idared’, ‘Pinova’, ‘Pink Lady’*, etc.). Most of the sour cherry cultivars originate from the Újfehértó Research Center of Northern Hungary established by land cultivars selection (‘*‘Kántorjánosi 3’’, ‘Debrecen bőtermő’* etc), some cultivars are ‘*Bosnian’* sour cherry cultivars and ‘*Morello’* sour cherry. The pears are commercially known cultivars (‘*Williams’, ‘Abate fetel’, ‘Bosc kobak’, ‘Packham's Triumph’, ‘Conference’*). The cherry samples are all commercially available cultivars (‘*Regina’, ‘Catherine’, ‘Red Firm’*).

During the examination of the fruit parameters affecting goods value (size, weight) in terms of organic and integrated production modes, differences were found in apples. The average fruit weight of organic apples is less by 10% and the average fruit size is 5% smaller than in case of integrated fruits. Each of the four species was classified and illustrated with a hierarchical cluster analysis and graphs. In case of apples six, as for the other fruits two groups can be separated according to the physical parameters. Concerning sour cherries, despite the large number of cultivars, only two groups could be distinguished, one for smaller size and weight cultivars (‘*Morello’, ‘Oblacsinszka’, ‘Bosnian’*) and one for larger ones (‘*Debreceni bőtermő’, ‘Érdi bőtermő’, ‘Kántorjánosi 3’, ‘Éva’*). The cultivar has a dominant role in forming the physical parameters in which the production method and weather play a minor role, only.

The microbial contamination of the surface of the fruits is an important test parameter concerning food safety. During the microbiological examination indicator organisms, (*coliforms, E. coli, Pseudomonas aeruginosa, enterobacters*) pathogenic microbes, (*Listeria* spp. *Cronobacter sakazakii, Salmonella* spp.), total aerobic plate count, yeast and mould number were determined. The presence of indicator microbes - mainly that of *coliforms* - were sporadically detected. *Pseudomonas aeruginosa* surface contamination was detected in the year of 2009 in apples (2 cultivars) originating from organic growing system and one type of sour cherry (1 cultivar) originating from the integrated growing system. There were not any pathogenic microbes

detectable in the samples. The microbial surface contamination was evaluated in case of each fruit (sour cherry, cherry, apple, pear) on the basis of the detected total aerobic microbial counts and the total combined yeasts/moulds counts. I examined the impact of individual factors - cultivar, vintage, production methods - on the microbiological contamination in case of apples and sour cherries. Vintage proved to be dominant regarding the microbial contamination. It can be observed that in the rainy and rather extreme year of 2010 low microbial count was characteristic. Examining the modes of production a significant ($p< 0,05$) difference was found. In case of organic farming the high total aerobic microbial count and total combined yeasts/moulds count occurred more frequently on apples. In case of sour cherries originating from organic farming, approximately half orders of magnitude higher microbial contamination was observed (except for 2009 '*Debreceni bőtermő'* and '*Érdi bőtermő'*), however, the difference was not significant ($p<0,05$).

The aim during the examination of the fruit for acid content and dry-extract content was to define the effect of cultivar, vintage, cultivation method and the ratio of these factors. Among the investigated fruits the sour cherries (17.25 ° Brix) have the highest dry-extract content followed by cherries (average 15.7 ° Brix), pears (14.8 ° Brix) and finally apples (13.7 ° Brix). According to Szabó and co-*workers (2010), in those years, where there was a higher temperature fluctuation between day and night, the dry-extract content of the sour cherry increased, however, this relationship no longer existed in those years when there were excessive high temperature differences. In the year of 2010 during the time of cherry blossom the weather was extremely inclement, which caused smaller crop than the average yield. In 2010 the weather was rather extreme in the later developmental stages of the fruit compared to the previous two years. Despite the divergences in the years in case of sour cherries and cherries the difference is not significant ($p< 0,05$). In case of apples there were no significant ($p<0,05$) differences between the organic and the integrated production methods, but difference between the vintages was observed. The positive benefit of the weather of the year 2009 is significant in apples, too. The detected dry-extract content in the year of 2009 was at least 5% higher in case of each cultivar and cultivation method, than in the extreme year of 2010. The difference between the apple dry-extract contents in each year is significant ($p<0,05$).

Comparing the annual results of each fruit cultivars, it may be concluded that the weather and the cultivar characteristics are together dominant factors in the dry-extract content values. The production methods did not affect the dry-extract content. Investigating the acid content the difference is greater between the cultivars than the differences originating from the production mode. The acid content of sour cherries is the highest between the examined fruits, with an average of 18 g/kg. In case of apples the difference in acid-content is significant ($p< 0,05$)

between vintages. The highest acid content values - probably due to the lot of rainfalls – were measured in 2010.

The biologically active substances of fruits greatly contribute to their health care impact. The polyphenol content and antioxidant capacity were determined from sour cherries and sweet cherries in each vintage and in each cultivation method. Confirming the data published so far, highly significant ($p<0,05$) correlation was proven between the polyphenol content and the antioxidant capacity in the examined fruits. Based on the measured data the decisive role of vintage, cultivar and cultivation method in nutritional value was investigated. In case of the two stone fruits significant ($p<0,05$) differences were determined between the vintages in terms of polyphenol content and antioxidant capacity. However, certain component ratios are typical of the varieties, and with some fluctuations - attributed to the effect of vintage - practically considered permanent. The sour cherry has extremely high polyphenolic content (average 1100 - 1900 mg/kg) and antioxidant capacity is in accordance. Applying a hierarchical cluster analysis six groups can be distinguished in case of sour cherries.

Anthocyanins are an important group of plant dyes. Their most important property is the capacity to act as antioxidants protecting the body against reactive oxygen species due to their ability to scavenge free radicals. Comparing fruits from organic and integrated production processes, the results showed that the content of total anthocyanins was slightly higher in case of organic fruits from Nyíregyháza, than in fruits from the integrated cultivation in the year of 2009, except for the cultivar '*Debreceni bőtermő'*, where no difference was detected between the two cultivation methods in that year. Taking into account the measured composition data, certain component ratios are typical of the cultivars with smaller and larger fluctuations, which are practically considered permanent. In relation to phenolic acids non-significant differences were found between the organic and integrated production methods. The anthocyanin profile of sour cherries and sweet cherries were compared and examined using cluster analysis. The anthocyanin composition of '*Csengődi'* and '*VN-4*' closely resembles the anthocyanin composition of cherries, primarily, that of Regina cherries. It is conceivable that the anthocyanin composition data together with molecular genetic examinations can be applicable for tracing the relationships of cultivars.

Recommended further examinations

In addition to the present research it might be worth expanding the examinations with more fruit species and cultivars in the future, particularly with those where there are fruit samples from both - organic and integrated - crop productions. Further, storage experiments should be carried out with samples of the two production methods. Tracing the surface microbial contamination of fruits from different production systems during the vegetation season and during storage, as well as to compare this data to the spoilage data during storage would be highly informative.

5. NEW SCINETIFIC RESULTS

1. During the trial (2008-2010) data obtained from physical parameters measurement of Hungarian and foreign varieties of apple, pear, cherry and sweet cherry were analysed. **I proved that apples from organic farming system have a 10 % smaller fruit weight and 5 smaller fruit size than apples originating from integrated farming system.**

2. **On the basis of the measurements, it can be established that nutritional contents (dry-extract and acid content) are dominantly affected by the cultivar, vintage and the growing method are less significant.**

3. The two different growing systems were analysed also from the point of view of microbial surface contamination, in case of apple and cherry cultivars. **It was proved that there was no significant difference between the two farming systems in terms of microbial surface contamination in case of apple and cherry cultivars.**

4. **Occurrence of *Cronobacter sakazakii, Listeria monocytogenes, Salmonella* spp. and other indicator microbes were first investigated in order to compare the microbial surface contamination of organic and integrated farming system in case of apple and cherry varieties. I proved that the pathogenic microbes listed above were not present on any of the samples investigated.**

5. **Anthocianine profile examination was applied first in case of organic and integrated grown sour cherry.**

6. Measuring and analyzing the data of sour cherry's biologically active compounds **showed that the cultivar plays the dominant role in the polyphenol content and antioxidant capacity, and other factors (the cultivation method, location and the vintage) are less dominant**. The significant correlation between the antioxidant capacity and polyphenol content described in the literature was also proven by my results.

PUBLICATIONS CONNECTED WITH THE SUBJECT OF THE DISSERTATION

Publication in review:

Article in review with IF:

Sz. Pintér, M. Petrik, I. Bata-Vidács, J. Beczner: ***Biodegradation of packing materials*** In: Acta Microbiologica et Immunologica Hungarica, 56 (Suppl.), pp. 1-111 (2009), DOI: 10. 1556/ A Micr. 56. 2009. Suppl.1. pp. 82-83. (IF: 0,646)

Pintér, Sz., Bata-Vidács I., Beczner J.: ***Epiphytic Microbiota of sour cherry (*Prunus cerasus*) in integrated and organic growing***. Acta Alimentaria, Vol. 42 (4), pp. 618–630 (2013) DOI: 10.1556/AAlim.42.2013.4.16 (IF: 0,475)

Article in review without IF:

Pintér, Sz., Beczner, J., Szabó, Z., Nyéki, L.: ***Epiphytic microbiota of apple in integral and organic growing***, Acta Horticulturae Vol. 16 , Number 3.,2010 pp.85-89.

Publication in conference issue:

In Hungarian language (abstract):

Bata-Vidács, I., **Pintér Sz.**, Balázsdi Szabó E., Beczner J. : ***Fals pozitív Listeria ALOA agaron***, XVI. Élelmiszer Minőségellenőrzési Tudományos Konferencia konferenciakiadványa, Tihany, 2008. április 25., 281-288.

International conference (proceeding):

Sz.Pintér, J.Beczner: ***Epiphytic microbiota of fresh fruits in Hungary***. In: EFFoST Conference Book (Budapest, 2009. november 11-13.)(DVD).

International conference (abstract):

Cseh J., **Pintér Sz.,** Beczner J., Szeitz-Szabó M.: **Emergenging Microbal Agents in Plant-Associated Human Outbreaks –** ***Preliminary study on the occurence of Cronobacter spp. in some food stuffs of plant origin.*** FoodMicro 2010 – Copenhagen 30August – 3 September, Poster section.