BIOACTIVE COMPOUNDS IN SMALL FRUITS AND THEIR INFLUENCE ON HUMAN HEALTH

I. Badjakov[1], M. Nikolova[2], R. Gevrenova[3], V. Kondakova[1], E. Todorovska[1], A. Atanassov[1]
AgroBioInstitute, Sofia, Bulgaria[1]
Bulgarian Academy of Sciences, Institute of Botany, Sofia, Bulgaria[2]
Medical University, Faculty of Pharmacy, Department of Pharmacognosy and Pharmaceutical Botany, Sofia, Bulgaria[3]
Correspondence to: Ilian Badjakov
E-mail: ibadjakov@gmail.com

ABSTRACT

The international tendency for growing and production of small fruits shows a permanent increasing. Bulgaria is a traditional important producer of small berries in Europe. A large variety of small fruit products are wide spread and typical for Bulgarian nutriment. Aside with the growing demand in production of small fruit, there is an obvious tendency in food quality, breeding and technology requirements improvement. Breeding purposes comprise improvement of many traits, but selection of disease resistant cultivars, with higher yield and improved consumers properties such as fruit color, shape, smell and transportation ability are among the most important tasks.

Recent progress in molecular analyses and agriculture biotechnologies has enormous impact on selection, technology, testing, preservation and processing of agricultural products. Metabolomics assay as a new dimension in these studies and practice focuses the attention on the biochemical contents of cells and tissues, and has a rapidly growing significance in knowledge of small fruits value for human health.

Berry fruits are very rich sources of bioactive compounds as phenolics and organic acid. Bioactive berry compounds, their characterization and utilization in functional foods and clinical assessment of antimicrobial properties for human health are among the major targets of contemporary research. Phenolic compounds in berries inhibit the growth of range of human pathogens. Especially raspberry, strawberry, cranberry, crowberries showed evidence of antimicrobial effects against bacterial pathogens as Salmonella and Staphylococcus.

The evaluation of small fruit genetic resources for the presence of bioactive compounds and their properties as natural agents is of doubtless significance and will be with great benefit for breeders, food and pharmaceutical industry.

Keywords: metabolomics, bioactive compounds, human health, small fruits

Introduction

Generally, plants are rich source of diverse functional biochemicals and metabolomics technologies already proving them valuable in an applied context (15). These technologies which aimed to giving us full coverage of total genome sequence or the complete transcriptional analysis of an organism are already expanding research horizons just 5-10 years ago. In the recent years, plant metabolomics has been a valuable technology applied in the global knowledge of the molecular organization of multicellular organisms. Many significant advances have been made in metabolomics applications in last years.

Metabolomics is supposed to play a significant role in bridging the phenotype-genotype gap. The rising number of publications in this domain demonstrated that metabolomics is not just a new "omics" but a valuable emerging tool to study phenotype and changes in phenotype caused by environmental influences, disease or changes in genotype (6, 9, 10, 54,77).

More detailed information on the biological and biochemical composition of plant tissues has great potential value in a wide range of scientific and applied fields (14, 56, 58). The quality of plants is a direct function of their metabolite content (existence of definite metabolic profiles, even in single analyses) and indicate their commercial value (20, 26, 48, 51). Within tissue extracts, individual key components can vary in concentration by seven to nine orders of magnitude (6). Furthermore, the total number of metabolites which are produced by plants vary considerably and they are in the range between 100 000 and 200 000 (38). This complexity can be used to define plants at every level of genotype, phenotype, tissue and cell. The secondary metabolites can be found and each species may content its own phenotypic expression pattern. Substantial quantitative and qualitative variation in metabolite composition is observed in plant species (19, 31). This comprises also large populations of key compound groups including 6000 different flavonoids (44) and 12 000 different alkaloids (7). The studying of the metabolomics is a major challenge to analytical chemistry and a metabolomics analysis. Currently, two complementary approaches are used for metabolomics investigations: metabolic profiling and metabolic fingerprinting (5, 27, 73). Metabolic profiling focuses on the analysis of a group of metabolites either related to a specific metabolic pathway or a class of compounds. An even more directed approach is target analysis that aims at the defining

biomarkers of disease or toxicant exposure, or substrates and products of enzymatic reactions (10). Another approach is the metabolic fingerprinting. This approach is intended to compare patterns or "fingerprints" of metabolites that change in response to disease, toxin exposure, environmental or genetic alterations. Both metabolic fingerprinting and profiling can be used in the search for new biomarkers. Having more detailed information on the biochemical composition of plant tissues has great potential value in a wide range of both scientific and applied fields (16, 17).

Evidences supporting the beneficial health effects of fruits are indisputable and largely discussed and proved (17, 19, 25, 30, 52, 72). The number of research projects aiming at determination of bioactive compounds with known plant parentage increases rapidly. Berry fruits, wild or cultivated, are proved as a traditional and rich source of bioactive compounds, possessing important biological activities (12, 13). Studies of biochemical profiles in small fruits by High Performance Liquid Chromatograpy (HPLC), revealed the presence of flavonoids (Kaempferol, Quercetin, Myricetin), phenolic acids (galic, *p*-Coumaric, Caffeic, Ferulic) and phenolic polymer (ellagic acids), (12).

Also some studies indicated that bioactive berry compounds could be regarded as new type antimicrobials, which control wide range of pathogens and may overcome problems with antibiotic resistance (43, 55). Among them, raspberry, strawberry, blueberry, bilberry, cloudberry and cranberry demonstrated high antimicrobial activity against human pathogens *Salmonella, Bacillus cereus* and *Staphylococcus* (34, 35).

Metabolomics studies of small fruits and especially the modern metabolic profiling approaches can be used for evaluation the level of beneficial polyphenolics in different fruit breeding populations and how the level of these components are genetically controlled and influenced by environmental conditions (48).

This review discusses the dynamically developing studies for registration, evaluation of antioxidant compounds in small fruits germplasm, their antimicrobial properties and influence on human health and also the development of analytical technologies for metabolomics application.

Phenolic content in small berry fruits

In recent years many research projects were targeted at studying of the bioactive berry compounds, their characterization and utilization in functional foods and clinical assessment of antimicrobial properties for human health (34, 36, 41, 42, 43, 44). Aside with that the number of reports contributing the beneficial biological activity to the fruit phenolics increased (1, 18, 22, 25, 40, 42, 43). In recent documents, the World Health Organization (WHO) emphasized on the importance of antioxidants activity of flavonoids, especially from fruits, for prevention of most important health problems as cardiovascular and diabetes diseases (2). As a matter of fact phenolic acids and flavonoids are widely distributed in higher plants and form part of the human diet.

High antioxidant content in foods provides potential health benefits such as reduction of coronary heart disease, anti-viral and anti-cancer activity. The last reports have listed standard cultivars of dark fruited berries (raspberry, blueberry, gooseberries, blackberries) having high natural antioxidant content relative to vegetables or other foods (24, 52, 65, 74, 75). In addition to vitamins and minerals, extracts of raspberries are also rich of anthocyanins, other flavonoids and phenolic acids. The determination of the range of anthocyanin content, phenolic content and antioxidant capacity in wild species (*Rubus* L. and *Ribes* L.) and cultivar germplasm of dark fruits is very impressive (40, 57, 59). The berry species belonging to the *Rubus* and *Ribes* genus have very high amounts of antioxidant compounds. For example, the quantity of anthocyanin content in black current cultivars (*Ribes nigrum* L.) range between 128 and 420 mg/100 g fruit; for blackberries (*Rubus hybrid*) to 250 mg/100 g fruit and for black raspberry to 630 mg/100 g fruit. The antioxidant capacity of anthocyanidin is one of their most significant biological and human health properties (67). The last studies confirm that the antioxidant ability of raspberry fruit is derived from the contribution of phenolic compounds in raspberries (67, 76). The antioxidant capacity of raspberry fruit is of course not determined by a single component. Different growing conditions influence the flavonoid content and antioxidant activity of strawberry and raspberry cultivars (65, 66). The dominant antioxidants in raspberry could be classified as being vitamin C, several anthocyanins, ellagitannins and some minor proanthocyanidins-like tannins. Vitamin C is quite abundant in many fruit and vegetable species. It is not specific for raspberry but nevertheless the fruit provides about 20 to 30 mg vitamin C per 100 g fruits. Vitamin C can make up about 20% of the total antioxidant capacity of raspberry fruits (1). Anthocyanins contribute about 25% to the antioxidant capacity of red raspberry fruit. They are flavonoids and are often involved in the pigmentation of fruits and flowers. As in other red fruits, the average content of anthocyanins in raspberry is 200 to 300 mg per 100 g dry weight. Some other berries, like bilberries (*Vaccinium myrtillus*) accumulate even more – between 2 and 3g anthocyanin per 100 g. (4). The biggest contribution to the antioxidant capacity in raspberry have ellagitannins. Among berry species ellagitannins are only represented in cloudberry and raspberry (between 1 or 2 g per 100g dry weight and to a minor extent in strawberry (around 100 mg per 100g) (8, 25).

No differences in antioxidant capacity have been found between berry cultivars. For most raspberry cultivars defined by HPLC analysis in Europe were detected nine different anthocyanin peaks. Significant differences were reported within the cultivars with respect to the relative amounts of each of these individual components. The ellagitannins were always the dominant antioxidants in all cultivars. The value of pink fruit, compared to fully ripe, red fruit, can be up to 50% lower. These differences would seem to be very relevant for determining the best harvest time. This indicates that

growth conditions, including stress, may affect the raspberry antioxidants and thus might be used in the future to manipulate antioxidant levels at the time of harvest (4, 24).

Comparison of the phenolic content of different berries is difficult because of the varying of used analytical methods. Berries, especially of family *Rosaceous*, genus *Rubus* (strawberry, red raspberry and cloudberry), are rich in ellagitannins (51 to 88%), (13, 21, 29). (**Table 1, Fig. 1)**.

TABLE 1

Berry Phenolics Content, taken from (37)

Ellagitannins	1000 – 4000 mg/kg
Anthocyanins	3000 – 8000 mg/kg
Hydroxybenzoic acids	100 – 300 mg/kg
Hydroxycinnamic acids	20 – 70 mg/kg
Flavonols	300 – 400 mg/ kg
Proanthocyanidins	500 – 3000 mg/kg
Stilbenes	1 – 7 mg/kg
Lignans	1.6 – 16mg/kg

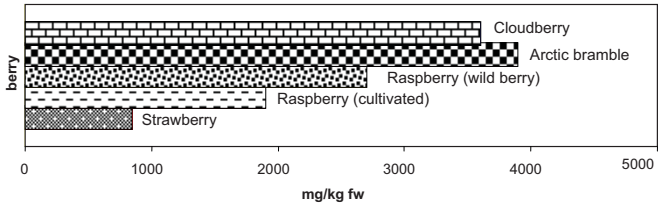

Fig. 1. Ellagitannins content of berries

The chromatographic analysis confirmed the presence of phenolic acids and further studies revealed the presence of Quercetin, Kaempferol and Ellagic acid. Ellagic acid is a naturally occurring phenolic constituent of many plant species (5) and has shown promising antimutagenic and anticarcinogenic activity against chemical - induced cancers (32, 23).

The analyses of raspberry, strawberry, and blueberry, indicate that they are rich source of flavonoids, ellagic acid and tannins which may be used for the quality assessment of *Rubus* species leaves and may suggest that some leaves could be of equal value to those which have been characterized as having medicinal properties (11).

The polyphenols found in fruits *in vivo*, generally can be attributed to several distinct base structures. These encompass the anthocyanins, flavonols, flavanals, isoflavones, phenolic acids, catechins and ellagitannins. Ellagic acid is a compound that is known to have significant contribution to human health. It is formed by oxidation and dimerization of gallic acid. Ellagic acid is known to occur in strawberry, and significant variation is anticipated.

To guarantee a sufficient scientific challenge, for both health aspects and flavor properties, two groups of compounds are interesting target to study with respect to their biosynthetic pathway.

Lactones are very important for the flavour of many fruits, including strawberries, peaches and coconut. The γ – decalactone, which is quite abundant in strawberry has as an isolated compound, a peach-like flavour, and thereby contributes considerably to the flavour of the strawberry fruit. Among the major flavour compounds in strawberry (terpenes, furaneol, esters, lactones, aldehydes and alcohols), the biosynthesis of lactones is the least understood. Due to their structure, lactones must be derived from long chain fatty acid and alcohols, but no enzyme has a yet been identified that is involved in the cyclization process leading to lactone formation. Strawberry varieties are known to have strong variation in lactone content, ranging from dominant presence to undetectable. Hence this is very suitable goal for research by determining the pathway and identifying the enzymes and genes involved.

There are fundamental differences between intervention studies and dietary assessment. These observations may indicate that only particular antioxidants, like specific flavonoids have a beneficial effect.

Current scientific evidence does not allow ascribing strong protective effects to specific compounds. For more detailed study, it is required not only to determine total antioxidant capacity of foods, but also to identify the antioxidants involved (5, 53) (**Fig. 2)**.

Berry phenolics and human health

Last studies indicate that bioactive berry compounds may act as a new type of antimicrobials which may control the wild spectre of pathogens and may overcome the problems with antibiotic resistance.

The modern consumers are increasingly interested in their personal health, and expect the foods to be tasty, attractive and also safe and healthy. Phenolic compounds are one of the most diverse groups of secondary metabolites in edible plants. Plant phenols have many potential biology properties and extensive studies are being carried out at present on their effects on human health (45, 62). Interest has been focused on two large groups of phenolics- flavonoids ant phytoestrogens. Flavonoids are found in many food products with plant origin such as vegetables, fruits, berries, tea and wine. Flavonols (quercetin and kaempferol) and flavones (apigenin and luteolin) are abundantly found in various plant-based foods. In addition, flavonoids exhibit various physiological activities including anti- allergic, anticarcinogenic, antiarthritic and antimicrobial activities. It is known that, the leaves from raspberry (*R. idaeus* L.) have been commonly used in traditional medicine to treat a variety of ailments including diseases of the alimentary canal, air-passage, heart and the cardiovascular system (11, 38, 69). They may also be applied externally as antibacterial, anti-inflammatory, sudorific, diuretic and choleretic agents (4, 33,64). Raspberry leaf extracts has been reported to have relaxant effect, particularly on uterine muscles (3, 39, 41). Beneficial effects of using raspberry leaves during pregnancy

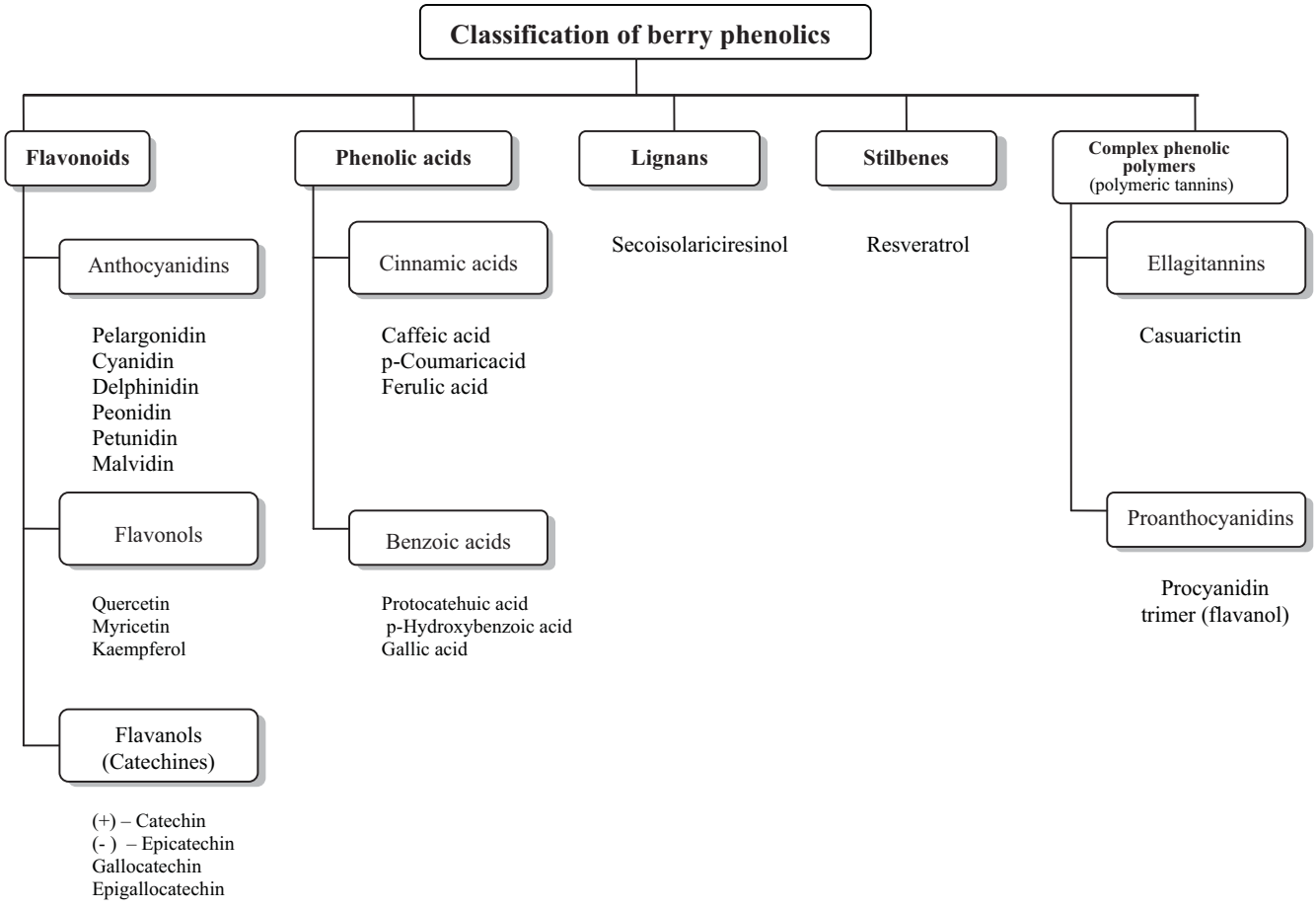

Fig. 2. Classification of berry phenolics

have been noticed (47, 70, 71). Generally, berries are good sources of various phenolic compounds, especially flavonoids. Strawberry, raspberry and cloudberry contain few flavonols, but they are rich in ellagitannins which are polymers of ellagic acid. Ellagitannins are not found in any other common foods, so these berries remain the most important sources of them. The antimicrobial activities of the pure phenolic compounds are widely studied. However, there is very little information about antimicrobial activity of the berries, which contain a very complex mixture of phenolics compounds, specific for each berry species (48). The studying of antibacterial activities of berry phenolics compounds proven the widest bactericidical activity of berries belong to genus *Rubus* (raspberry and cloudberry) (17). In general, berry extracts inhibited the growth of Gram-negative bacterial species but not Gram-positive lactobacillus species. The experiments show that raspberry and strawberry extracts are strong inhibitors of *Salmonella, Escherichia and Staphylococcus strains.* It can be hypothesized that ellagitannins could be one of the components in cloudberries, raspberries and strawberries causing the inhibition against *Salmonella. Escherichia coli* strain 50 was sensitive to all small berry phenolic extracts except blackcurrant.

Berry phenolics seem to affect on the growth of different bacterial species in different mechanisms, yet it is not well understood. There seems to be complex interactions between pH of the growth media and antimicrobial effects of the berry phenolics varying in different bacterial species and in different phenolic compounds (46, 47).

The inhibitory effects of berry extracts may not be due to simple phenolics but to more complex phenolic polymers such as ellagitannins, tannins and proanthocyanidins. The antimicrobial activity of berry extracts is evidently a synergistic effect of various phenolic compounds, many of which are still unidentified. Also other bioactive compounds in plant extracts, alone or in combination with phenols, might be responsible for antimicrobial effects (44, 45, 60, 78).

In recent few years the knowledge about bioactive compounds, especially for the phenolic compounds have increased a lot. The utilization of antimicrobial activity of berry phenolic compounds as natural antimicrobial agents may offer many new applications for food industry and medicine. Natural food preservatives targeted to foods which are easily contaminated by bacteria, such as *Salmonella* and *Staphylococcus* are desired.

Development of new approaches using berry compounds for the prevention and control of infections caused by bacteria resistance to antibiotics will be very important issue for future investigations concerning safety, toxicology and combined use with traditional medicines (28, 48, 49, 50).

Metabolic profiling - approaches towards metabolomics

Metabolic profiling or the quantative analysisis of a selected number of metabolites involved in the same biochemical pathway is a widespread tool to study different aspects of metabolism (6). Many technical reviews and reports have already been written on the different strategies available for metabolomics data. Success can be considered to be dependent on a few key aspects- production of the biological material/ sample preparation and sample extraction/metabolite detection. Comparative analyses in particular depend from the detection of statistically significant differences between samples and chosen approaches for both aspects (53). A very useful review on appropriate strategies for the design of metabolomics experiments has been written by Gullberg et al., (19) and could be used as an effective starting point. Pilot experiments always required before planning a full-scale metabolomics analysis and employing the help of a statistician experienced in experimental design is to be strongly recommended. All samples for comparison should be grown and harvested together under identical conditions as this will allow maximum biological relevance to be linked to the conclusions.

Large-scale of preparation sample can mean significant time differences between the moment of extraction and measurement of different samples and, even with - 80^0C storage, this can be reflected in obtained fingerprint. This adds an extra complication during analysis of data. Plant extracts generally have a more complicated biochemical composition and choice of suitable analytical technologies for detection of secondary metabolites is quite important.

Numerous of analytical technologies have been used for metabolomics applications, such as Mass Spectrometry (MS). It is the primary detection method for plant metabolomics due to its sensitivity, speed and broad application. Depending of the type of plant extract, Gas Chromatography (GC) or Liquid Chromatography (LC) is most routinely used for metabolite separation before the samples pass into the mass spectrometer. Gas chromatography-mass spectrometry (GC-MS) is currently proving to be the most popular global analysis method. The technology is more broadly applicable to groups of non-volatile metabolites, by converting these into volatile and thermostable compounds through chemical derivatization. (6, 19, 20, 65). Another versatile technology for analysis of many large groups of secondary metabolites present in plant tissue is Liquid chromatography-MS (61, 63, 68). Advances in chromatographic technologies in combination with advances in column chemistry are significantly improved separation potentials. The high analytical precision of modern LC techniques combined with the high sensitivity and mass accuracy and resolution of MS systems is proving very useful in the analysis of complex metabolite mixtures typified by plant extracts.

The improvements of instrumentation design will lead to increasing popularity of these approaches. The application of metabolomics technologies for genotyping/phenotyping studies increase and it will continue in the future.

Conclusions

The accumulated research experience, knowledge and practical methodology applications during the last years concerning bioactive berry compounds, in particular phenolic compounds has increased a lot. Future work is supposed to be focused on treatments of fruit promoting bioavailability and also on more determined confirmation of the effects of antioxidant compounds from berries on consumer health.

The biosynthetic capacity of the whole plants (berry fruit, leaves and cell culture) will be evaluated and used. The potential value of secondary metabolite profiling in the field of berry quality is in relation with the development of breeding strategies for plant improvement.

Several studies indicate that berry compounds inhibit the grown of human pathogenic bacteria, such as *Salmonella*, *Staphylococcus*, *Helicobacter* and *E .coli*.

The utilization of antimicrobial activity of berry phenolic compounds as natural antimicrobial agents may offer many opportunities for use in food industry and medicine. The metabolic profiling approaches are highly relevant to study the interface between plant breeding for food and human nutrition.

The development of alternative approaches, by implementing of berry compounds for the prevention and control of infections caused by bacteria resistant to antibiotics will also be very important issue for definite research priorities in the future.

REFERENCES

1. **Ancos B.De., Gonzalez E.M. and Cano M.P.** (2000) J. Agric. Food Chem., **48**, 946-952.
2. **Arts I.C., Hollman P.C**. (2005) Am. J. Clin. Nutr., **81**, 317-325.
3. **Bazzano L.A**.(2005) Background paper of the joint FAO/ WHO Workshop on Fruit and Vegetables for Health, 1-3 September 2004, Kobe, Japan.
4. **Beekwilder J., Hall R.D. and Ric de Vos C.H.** (2005) BioFactors, **23**, 197-205.
5. **Bruno E.J.J., Ziegenfuss T.N., Landis J**. (2006) Curr. Sports Med. Rep., **5**, 177-181.
6. **Bum J.H., Withell E.R.** (1941) Lancet, **5**, 1-3.
7. **Czygan F.Ch**. (1995) Z. Phytother., **16**, 366-374.
8. **Daniel E.M., Krupnick A.S., Heur Y.H., Blinzler J.A., Nims R.W., Stoner G.D.** (1989) Journal of Food Composition and Analysis, **2**, 338-349.

9. **Desbrosses G.G., Kopka J., Udvardi M.K.** (2005) Plant Physiology, **137**, 1302-1318.
10. **Dettmer K., Hammock B.D**. (2004) Environ. Health Perspect., **112**, 396–397.
11. **Dettmer K., Aronov P.A. and Hammock B.D**. (2007) Mass Spectrometry Reviews, **26**, 51-78.
12. **Dunn W.B., Ellis D.I.** (2005) Trends in Analytical Chemistry, **24**, 285-294.
13. **Facchini P.J., Bird D.A., St-Pierre B**. (2004) Trends in Plant Science, **9**,116-122.
14. **Fiehn O.** (2002) Plant Mol. Biol., **48**, 155-171.
15. **Fukusaki E., Kobayashi A.** (2005) Journal of Bioscience and Bioengineering, **100**, 347-354.
16. **Gibon Y., Blaesing O.E., Hannemann J., Carillo P., Hohne M., Hendriks J.H.M., Palacios N., Cross I., Selbig J., Stitt M.** (2004) Plant Cell, **16**, 3304-3325.
17. **Goodacre R.** (2005) J. Experimental Botany, **56**, 245-254.
18. **Gudey J., Tommczyk M.** (2004) Arch. Pharm. Res., **11**, 1114-1119.
19. **Gullberg J., Jonsson P., Nordstrom A., Sjostrom M., Moritz T.** (2004) Analytical Biochemistry, **331**, 283-295.
20. **Hakkinen S.H., Heinonen M., Karenlampi S., Mykkanen H. M., Ruuskanen J., Torronen R.** (1999) Food Research International, **32**, 345-353.
21. **Hakkinen S.H., Karenlampi S., Heinonen M.I., Mykkanen H.M., Torronen R.A**. (1999) J. Agric. Food Chem., **47**, 2274-2279.
22. **Hakkinen S., Karenlampi S., Mykkanen H., Heinonen M I., Torronen R.** (2000) Eur. Food Res. Technol., **212**, 75-80.
23. **Hall R.D.** (2005) New Phytologist., **169,** 453-468.
24. **Hall R.D., Vos C.H.R., Verhoeven H.A., Bino R.J.** (2005) In: Metabolome analyses: strategies for systems biology. (Vaidyanathan S., Harrigan G.G., Goodaece R., Eds.) Springer, NY, USA.
25. **Hancock R.D.,Viola R.** (2005) J. Agric. Food. Chem., **53,** 5248-5257.
26. **Hung H.C., Joshipura K.J., Jiang R., Hu F.B, Hunter D., Smith-Warner S.A., Colditz G.A., Rosner B., Spiegelman D., Willett W.C**. (2006) J. Natl. Cancer Inst., **96**, 1577-1584.
27. **Jansen R.C.,Vreugdenhil D.,Koornneef M**. (2006) Nature Genetics, **38**, 842-849.
28. **Jenkins D.A., Popovich D.G., Kendallb C.W.C.,Vidgenb E., Tariqb N., Ransomb T.P.P., Woleverb T.M.S., Vuksanb V., Mehling C.C., Boctorb D.L., Bolognesib C., Huang J., Patten R.** (1997) Metabolism, **46**, 530-537.
29. **Joshipura K.J., Ascherio A., Manson J.E., Stampfer M.J., Rimm E.B., Speizer F.E., Hennekens C.H., Spiegelman D., Willett W.C.** (1999) JAMA, **282**, 1233-1239.
30. **Kahkonen M.P., Hopia A.I., Heinonen M.** (2001) J. Agric. Food Chem., **49**, 4076-4082.
31. **Keurentjes J.B., Jingyuan F., Ric de Vos C.H., Lommen A., Hall R.D., Bino R.J., Linus H.W., van der Plass., Jansen R.C.,Vreugdenhil D., Koornneef M.** (2006) Nature Genetics, **38**(7), 842-849.
32. **Kikuchi J., Shinozaki K., Hirayama T.** (2004) Plant and Cell Physiology, **45**, 1099-1104.
33. **Kresty L.A, Frankel W.L., Hammond C.D., Baird M.E., Mele J.M., Stoner G.D, Fromkes J.J.** (2006) Nutr. Cancer, **54**, 148-156.
34. **Lee K.W., Lee H. J.** (2006) Biofactors, **26**,105-121.
35. **Lee J.E., Giovannucci E., Smith-Warner S.A., Spiegelman D., Willett W.C., Curhan G.C.** (2006) Cancer Epidemiol. Biomarkers Prev., **15**, 2445-2452.
36. **Maas J.L., Galletta G.J., Stoner G.D.** (1991) Hort. Science, **26**, 10-14.
37. **Määttä-Rįįhįnen et al.** (2004) J. Agric. Food Chem., **52**, 6178-6187.
38. **Mazza G., Miniati E.** (1993) CRC Press. Boca Raton, FL. p.362.
39. **McDougall G.J., Stewart D.** (2005) Biofactors, **23**, 189-195.
40. **Memelink J.** (2004) Trends in Plant Science, **7**, 305-307.
41. **Michels K.B., Giovannucci E., Chan A.T., Singhania R., Fuchs C.S., Willett W.C.** (2006) Cancer Res., **66**, 3942-3253.
42. **Misciagna G., Cisternino A.M., Freudenheim J.** (2000) Digestive and Liver Disease, **32**, 468-472.
43. **Moyer R., Hummer K., Wrolstad R.E**. (2002) Acta Hort., **585**, 501-505.
44. **Mulen W., Stewart A.J., Lean M.E.J., Gardner P., Duthie G.G., Crozier A.** (2002). J. Agric Food Chem, **50,** 5197-5201**.**
45. **Nakaishi H., Matsumato H., Tominaga S., Hirayam M.** (2000) Alternative Medicine Review, **5**, 553-562.
46. **Oksman-Caldentey K-M., Inze D.** (2004) Trends in Plant Science, **9**, 433-440.
47. **Okuda T., YoshidaT., Hatano T.** (1989) Planta Medicine, **55**, 117-122.
48. **Ozarowski A. and Jaroniewski W**. (1989) IWZZ, Warszawa, **184**, p. 243.
49. **Puupponen-Pimiä R., Nohynek L., Meier C., Kahkonen M., Heinonen M., Hopia A., Oksman-Caldenteyy K.M.** (2001) Journal of Applied Microbiology, **90,** 494-507.
50. **Puupponen-Pimiä R., Aura A.M., Oksman-Caldentey K.M., Myllärinen P., Saarela M., Mattila-Sandholm T., Poutanen K.** (2002) Trends in Food Science & Technology, **13**, 3-11.

51. **Puupponen-Pimiä R., Nohynek L., Alakomi H.L., Oksman-Caldentey K.M.** (2004) Applied Microbiology and Biotechnology, **23**, 11-24.
52. **Puupponen-Pimiä R., Nohynek L., Schmidlin S., Kähkőnen M., Heinonen M., Oksman-Caldentey K-M.** (2004b) J. Appl. Microbiol. and Biotechnology (in press).
53. **Puupponen-Pimiä R., Nohynek L., Alakomi HL.,Oksman-Caldentey K.** (2005) BioFactors, **23**, 243-251.
54. **Patel A.V., Rojas-Vera J., Dacke C.G.** (2004) Curr. Med. Chem., **11**, 1501-1512.
55. **Robbers J.E., and Tyler. V.E**. (1999) In: Tyler's Herbs of choice: the therapeutic use of phytomedicinals, The Haworth Herbal Press, New York-London, **63-64**, 194-195.
56. **Ross H.A., McDougall G.J., Stewart D**. (2007) Journal of the Science of Food and Agriculture, **70**, 133-150.
57. **Rojas-Vera J., Patel A.V., Dacke C.G.** (2002) Photother. Res., **16**, 665-668.
58. **Saito K., Dixon R., Willmitzer L.** (2006) Plant Metabolomics. Heidelberg, Germany, Springer Verlag. (in press).
59. **Scalbert A., Johnson I.T., Saltmarsh M. (**2005) Am J. Clin. Nutr, **81**, 215-217.
60. **Schaffer S., Eckert GP., Schmitt-Schillig S., Muller WE.** (2006). Forum Nutr. **59**, 86-115.
61. **Schijlen E.G.W.M., deVos C.H.R., vanTunen A.J., Bovy A.G.** (2004) Phytochemistry, **65**, 2631-2648.
62. **Shanmuganayagam D., Warner T.F., Krueger C.G, Reed J.D., Folts J.D.** (2007) Atherosclerosis, **190**, 135-142.
63. **Shepherd T., Dobson G., Verrall S.R., Conner S., Griffiths D.W., McNicol J.W., Davies H.V., Stewart D**. (2007) GC-MS potato metabolomics: What are the limiting factors, (in press).
64. **Simpson M., Parsons M., Greenwood J., Wade K.** (2001) J. Midwifery Women's Health, **46**, 51-59.
65. **Stewart D., McDougall G.L., Sungurtas J., Verrall S., Graham J. and Martinussen I.** (2007) Molecular Nutrition and Food Research (in press).
66. **Trethewey R.N.** (2004) Curr. Opin. Plant Biol., **7**, 196-201.
67. **Tsiotou A.G., Sakorafas G., Anagnostopoulos G. and Bramis J.** (2005) Medical Science Monittor, **11**, 76-85.
68. **Tohge T., Nishiyyamaa Y., Hirai M.Y., Yano M., Nakajima L., Awazuhara M., Inoue E., Takahashi H., Goodenowe D.B., Kitayama M., Noji M., Yamazaki M., Saito K.** (2005) Plant Journal, **42**, 218-235.
69. **Torronen R., Hakkinen S., Karenlampi S., Mykkanen H.** (1997) Cancer Lett., **114**, 191-192.
70. **Valsta L.M.** (1999) British Journal of Nutrition, **81**(2), 49-55.
71. **Viberg U., Sjoholm I.** (1996) Livsmedesteknik, **38**, 38-39.
72. **Verhoeven H.A., de Vos C.H.R., Bino R.J, Hall R.D.** (2006) Plant Metabolomics, Heidelberg, Germany, Springer Verlag 9 (in press).
73. **von Roepenack–Lahaye E., Degenkolb T., Zerjeski M., Franz M., Roth U.,Wessjohann L., Schmidt J., Scheel D., Clemens S.** (2004) Plant Physiology, **134**, 548-559.
74. **Wang H., Cao G., Prior R.L.** (1996) J. Agric. Food Chem., **44**, 701-705.
75. **Wang H., Cao G., Prior R.L.** (1997) J. Agric. Food Chem., **45**, 304-309.
76. **Wang S.Y., Zheng W., Galletta G.J.** (2002) J. Agric. Food Chem., **50**, 6534-6542.
77. **Weckwerth W., Ehlers-Loureiro M., Wenzel K., Fiehn O**. (2004) Proceedings of the Natinal Academy of Sciences, USA, **101**, 7809-7814.
78. **Yang Y., Gallaher D.D**. (2005) Nutr. Cancer., **53**, 117-125.