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Research into nutritive value and anticancer properties of blueberry and raspberry fruit from biodynamic, organic and conventional production

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Background

Demand for organic and biodynamic foods is strongly driven by consumers' perception that they are more nutritious and can help them to maintain good health[1]. However, scientific opinion is divided on whether there are significant nutritional differences between organic and non-organic foods[2,3]. Moreover, available research results on the health impacts of the organic foods are limited[4], and there are hardly any studies undertaking the topic of the health-related quality of biodynamic compared to the organic or/and conventional products[5].

Composition of organic vs. conventional foods

Over the last 25 years, a significant number of research studies have compared the concentrations of nutritionally relevant minerals, toxic metals, pesticide residues, macronutrients and bioactive compounds in crops from organic and conventional production systems[2,6]. Several systematic literature reviews have recently analysed the available published information, with the aim of identifying the potential effects of organic and conventional production protocols on the nutritional quality of crops[3,7,8], and came to contrasting conclusions. As a result, there is still considerable controversy as to whether the use of organic production standards results in significant and consistent changes in the composition of crops and crop-based foods[3,7-9]. Authors of the most recent and comprehensive meta-analysis summarizing results of 343 peer-reviewed comparison studies of organic and biodynamic *vs*. conventional foods have concluded that organic crops are, on average, characterized by significantly higher concentrations of antioxidants (i.e. phenolic compounds), lower concentrations of cadmium and at least four times lower incidence of pesticide residues than their conventional comparators. In case of fruit, the reported incidence of detectable pesticide contamination was near 7 times lower in organic *vs*. conventional samples[2].

However, authors of the mentioned meta-analysis pointed out huge between-studies heterogeneity (different experimental designs, different locations with contrasting agronomic and pedo-climatic background) increasing the amount of published data required to understand the variation in composition parameters resulting from the use of contrasting crop production methods.

Anticancer properties of plant bioactive compounds

There is strong scientific evidence for health benefits associated with the increased consumption of crops rich in phenolics and other plant secondary metabolites with antioxidant activity (e.g. carotenoids and vitamins C and E)[10-12]. Results of epidemiological studies have shown that high consumption of fruit and vegetables is associated with the prevention of chronic non-communicable diseases and certain cancers[13]. Various fruit have been reported to exert antimutagenic and anticancerogenic effects based on their ability to induce cell cycle arrest and apoptosis[14,15] Litchi fruit pericarp extract was shown to exhibit a powerful anticancer activity on both ER positive and negative breast cancer, which can be attributed to DNA damage effect, inhibition of proliferation and apoptosis induction[16]. Recent reports on the relationship between food bioactive compounds and their anticancer effect in humans have been published by Faria *et al*. (2006)[17], McDougall *et al*. (2008)[18], Zhang *et al*. (2008)[19] and Ford *et al*. (2011)[20]. Analyzing the health benefits of foods researchers mainly focused on products derived from conventional farming. Results of these studies indicate that vitamins and certain phytochemical antioxidants including flavonoids (flavones, flavonols, flavanones, catechins, anthocyanidins) and carotenoids are effective against the proliferation of human colorectal, breast and stomach cancer cells[21-24]. The growth of various cancer cell lines, including those of the stomach, prostate, colon and breast, was found to be strongly inhibited by raspberry, blueberry, blackberry, cranberry, apples, strawberries, grapes[17,19,22,24-26], whole grains (wheat) and soybean[27-29].

Organic foods and cancer

As already mentioned, many of the bioactive compounds found often in higher concentrations in organic foods[6] have previously been linked to a reduced risk of chronic diseases, including certain cancers. Negative (i.e. carcinogenic) effects of cadmium and pesticide residues found more frequently in conventional products are also well proven[30-32]. On the basis of the above statements one could expect beneficial health impacts (i.e. potent anticancer properties) of organic and biodynamic compared to the conventional foods. However, the available research addressing this topic is very limited. Study of Olsson *et al.* (2006)[33] indicated stronger *in vitro* anticancer effect (antiproliferative activity against colon and breast cancer cells) of organic strawberry extracts in comparison with extracts from conventional farming. Kazimierczak *et al*. (2014)[34] demonstrated higher levels of late apoptosis and necrosis of AGS human stomach cancer cells induced by organic compared to the conventional fermented beetroot juices. Whether there is a clear correlation between *in vitro* antioxidant and anticancer effects of foods and their *in vivo* activity, it undoubtedly depends on many factors (e.g. composition of diet, food processing conditions, gut microbiota, metabolism). However, recently published results of the first big scale human cohort study to examine the association between the consumption of organic food and the risk of cancer have shown lower incidence of non-Hodgkin lymphoma in the population of organic *vs*. conventional food consumers[35]. Even though the above mentioned study did not find significant relation between organic diet and other types of cancers, it undoubtedly sheds new light on the topic of the health effects of organic foods.

Biodynamic foods

Biodynamic farming, employing a holistic understanding of agricultural processes, has been found to be more sustainable and more resilient to global environmental challenges (e.g. climate change and energy scarcity) when compared to the intensive, non-organic production[36]. Moreover, some studies indicated better soil quality measured by an increased diversity of soil microorganisms and greater soil biomass in biodynamic over organic and conventional systems[36]. At the same time, there are only a few studies into the nutritional quality of biodynamic crops. Research results of Jayasree and Annamma (2006)[37] show improved chili fruit quality (i.e. highest ascorbic acid content) in biodynamic system in the effect of application of organic manure. Maciel *et al*. (2011)[5] found higher antioxidant activity and higher concentrations of phenolic compounds in biodynamic and organic compared to the conventional mangoes (with highest contents of phenolics in organically produced fruit). The results of Tassoni *et al*. (2013)[38] indicated no significant differences in the chemical profile (anthocyanins, polyphenols and antioxidant activity) of Sangiovese and Pignoletto grape berries and wines coming from conventional, organic and biodynamic agricultural and winemaking practices. Bavec *et al.* (2011)[39] found a tendency towards higher content of sugars, phenolics and higher antioxidant activity of biodynamic compared to the organic beet roots. Angelopoulou *et al.* (2013) found significantly bigger size of biodynamic apples with a tendency towards higher flesh firmness and significantly more sugars in biodynamic wine grapes compared to the organic fruit.[40] Heimler *et al*. reported no differences in polyphenol content between biodynamic and conventionally grown chicory[41], but higher contents of polyphenols in biodynamic and organic compared to the conventionally produced Batavia lettuce.[42] Heimler underlines that the environmental sustainability of biodynamic agriculture is achieved with no disadvantage (or even with advantage) to food quality. Following the anthroposophic, holistic approach, not necessarily the concentrations of individual chemical compounds (or groups of compounds), but product as a whole, determines the health-related quality of food. Such concept has been presented by Steiner[43] and developed by Bloksma *et al.* (2007)[44] into the inner quality concept and growth-differentiation balance hypothesis. The systemic approach assumes that it is not possible to distinguish the separate factors responsible for the nutritive quality of crops, because many aspects of the agricultural management are influencing together. It is true for every farming system; in case of biodynamic system special anthroposophic practices may have additional effect enhancing soil and crop quality.[45]

So far no studies into the anticancer effects of biodynamic foods have been published.

Blueberries and raspberries as study objects

Blueberries and raspberries are amongst the most commonly consumed berries worldwide. They are known as a rich source of phenolic compounds[46]. Moreover, as previously mentioned, evidence from *in vitro*, *in vivo* and a few clinical studies suggest that both of these berry fruit and their active constituents are effective anticancer agents. Some of the mechanisms by which berries have been shown to prevent carcinogenesis include inhibition of the production of pro-inflammatory molecules, oxidative stress, DNA damage, inhibition of cancer cell proliferation and increased apoptosis[46,47].

Most of the berry crops are currently grown with conventional and integrated pest management practices. In 2012 total area of blueberry production exceeded 85,000 ha with a global yield of more than 380,000 MT, while raspberries are currently cultivated on the area of nearly 100,000 ha globally, giving a yield of 580,000 MT per year. As there is an increasing market demand for organically produced berries, organic acreage is also increasing[48]. Organic berries are produced nowadays on more than 26,000 ha in Europe. Poland became the largest producer of raspberries in the world and is the world's third largest producer of blueberry after the U.S. and Canada. Total production of raspberries and blueberries in 2013 in Poland accounted for 121,000 and 12,000 MT respectively (on the area of 28,000 ha and 7,000 ha).[49]

Consumption level as well as growers' interest in production of soft fruit (mainly blueberries and raspberries) in Sweden has increased in recent years. In addition, Swedish consumers are demanding more locally produced, high quality (in that also organic) berries[50]. The results of the planned project may significantly contribute to the overall interest and development of this area of the horticultural sector in Sweden.

Aims of the project

The aim of the project was to investigate if there are significant differences in the nutritional quality (i.e. concentrations of certain bioactive compounds), sensory values and anticancer properties of fruit (raspberries blueberries and black currants) produced in biodynamic, organic and conventional agricultural systems. Additionally, we aim to determine if there are any significant correlations between the measured parameters of fruit nutritional value and their anticancer effects.

Human colon carcinoma cell line (Caco-2) was purchased to be used in the study of direct anticancer activity of fruit extracts. Colorectal cancer is the fourth most common cause of cancer related mortality globally and is proven to be strongly associated with diet[51]. Moreover, it is known that many of strong natural antioxidants, such as phenolic compounds abundant in berries, are not efficiently absorbed in the small intestine, thus the effects of their direct action in the colon can be expected[47]. As the anticancer impact of diet compounds is generally considered as protective or potentiating the therapeutic effect of anticancer drugs, we aim to examine the ability of the extracts to potentiate the effect of such drugs in inhibiting the proliferation and/or inducing apoptosis of cancer cells[52,53].

Apart from the mentioned direct effect in the gastrointestinal tract, the important aspect of anticancer properties of diet compounds is related to their potential to modulate the activity of the immune system. Since the activity of the immune system strongly depends on the cells, which induce the progression from innate to acquired immunity, we decided to address the question whether the activity of macrophages can be modulated by fruit extracts. The dual role of macrophages in cancer has been recognized. Depending on their phenotype, they can play a role as activators of lymphocytes, triggering the anti-cancer activity, or create pro-carcinogenic microenvironment[54,55]. Thus, the expression of THP-1 human macrophage surface markers involved in the activation of T helper and cytotoxic T lymphocytes as well as the production of cytokines known to create local, tumour surrounding microenvironment was planned to be tested.

Extra assessments (not planned in the Project Application): The initial plan was to focus on 2 fruit: raspberry and blueberry. However, due to some savings in the budget, we were able to include additionally the analyses on black currant from organic, biodynamic and conventional production. Moreover, in addition to the chemical composition analyses, the sensory properties of all fruit samples have been evaluated in the accredited sensory laboratory of WULS (see 'preliminary results' section for more details).

Research hypotheses

It is expected that:

1. Organic fruit produced to high quality standards, with the use of natural agricultural methods, are characterized by beneficial composition, better sensory properties and higher *in vitro* anticancer potential (both direct and indirect) compared to the fruit from conventional agricultural systems relying on artificial fertilizers and chemical pesticides.
2. Biodynamic fruit are characterized by higher nutritional value (i.e. higher concentrations of bioactive compounds), better sensory profiles and higher anticancer potential (both direct and indirect) compared to the organic fruit.
3. There is a relation between the concentration of certain groups of bioactive compounds (and/or total antioxidant activity) in fruits and (a) their sensory properties and (b) their *in vitro* anticancer properties.

Materials and methods

Fruit samples

Fruit (raspberry, blueberry and black currant) samples (each sample = 1.5 kg) were collected from biodynamic, organic and conventional plots matched for location in July-September 2016. Fruits were collected in the full maturity phase when they are known to contain the highest level of bio compounds. The collected fruit samples were immediately refrigerated & transported to the laboratory of the Chair of Organic Food (Warsaw University of Life Sciences). Part of each sample (0.5 kg) was immediately used for sensory analyses (fresh fruit). All the rest (1.0 kg) was freeze-dried, ground in a laboratory mill and stored in -80^{o}C before further analyses, to prevent loss of biologically active compounds. See the below Table 1 for more details about fruit varieties, dates of sampling, number of plots (samples) and their location. Location of the farms/plots has also been presented on the maps below.

Table 1. Information about fruit varieties, dates of sampling, number of plots (samples) and their location.

Species	**Variety**	**Date of sampling**	**Number of farms/plots (samples collected)**	**Location of farms/plots**
Raspberry	Polka	7.09.2016	10 (3 CONV, 4 ORG, 3 BIOD)	On the border of 3 voivodeships of Poland: Zachodniopomorskie, Pomorskie & Wielkopolskie (in Juchowo, Drzonowo, Biernatka, Skoki, Ostrowąsy, Wardyń Górny, Barankowo)
Blueberry	Bluecrop	27.07.2016	9 (3 CONV, 3 ORG, 3 BIOD)	On the border of 3 voivodeships of Poland: Łódzkie & Mazowieckie (in Baranów, Kazimierzów, Wałowice, Budy Grzybek, Nowy Kawęczyn)
Black currant	Ojebyn	14.07.2016	9 (3 CONV, 3 ORG, 3 BIOD)	On the border of 3 voivodeships of Poland: Zachodniopomorskie, Pomorskie & Wielkopolskie (in Janowo, Okonek, Ogartowo, Żytelkowo, Ostre Bardo, Wardyń Górny)

All farmers providing fruit samples were additionally interviewed & completed questionnaires about the soil, agronomic practices (i.e. planting, harvesting dates & methods, weed-, pest- and disease control methods, crop rotation, fertility management, problems with pests and diseases on their farm etc.).

In 2017 fruit samples will be collected from the same farms, following similar procedures.

Figure 1. Location of raspberry and black currant farms

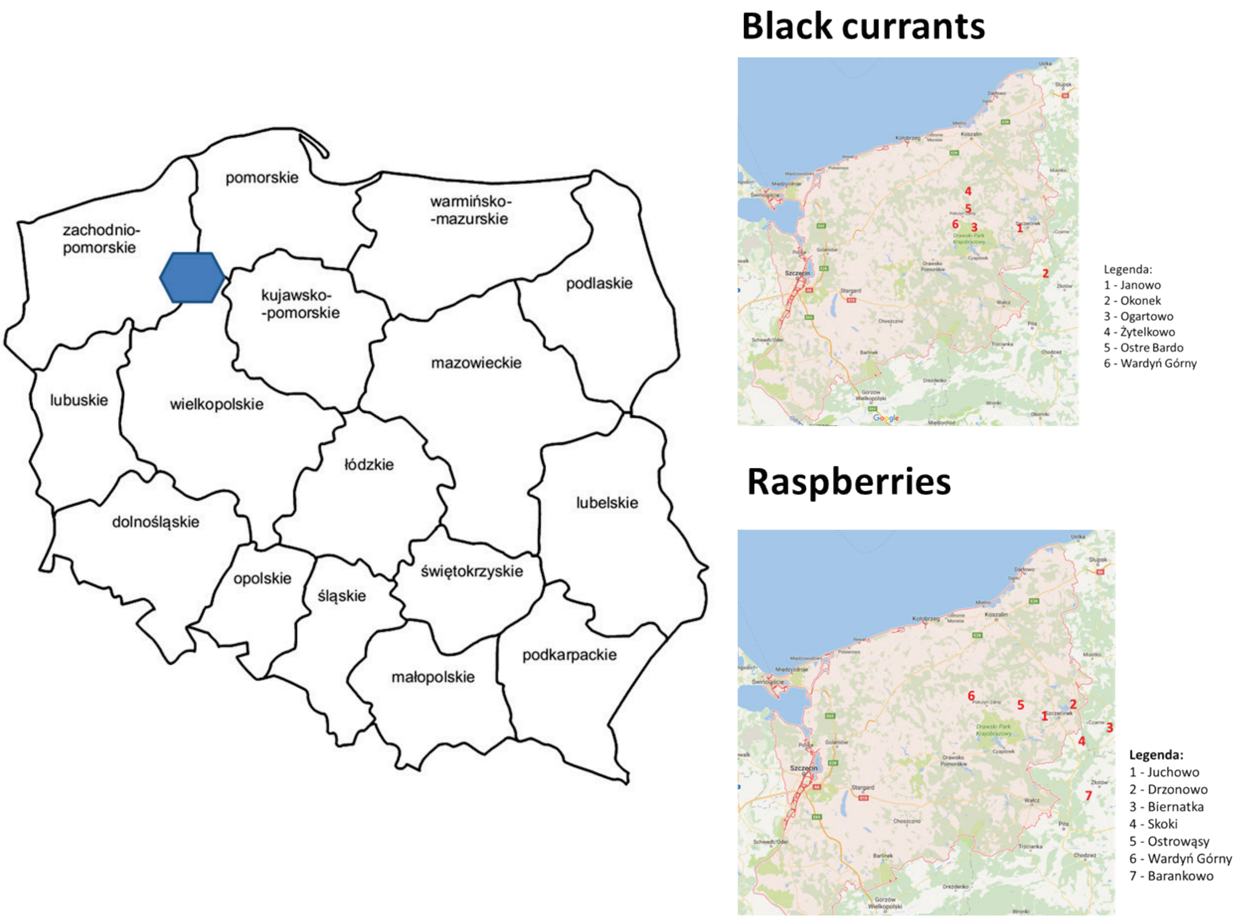
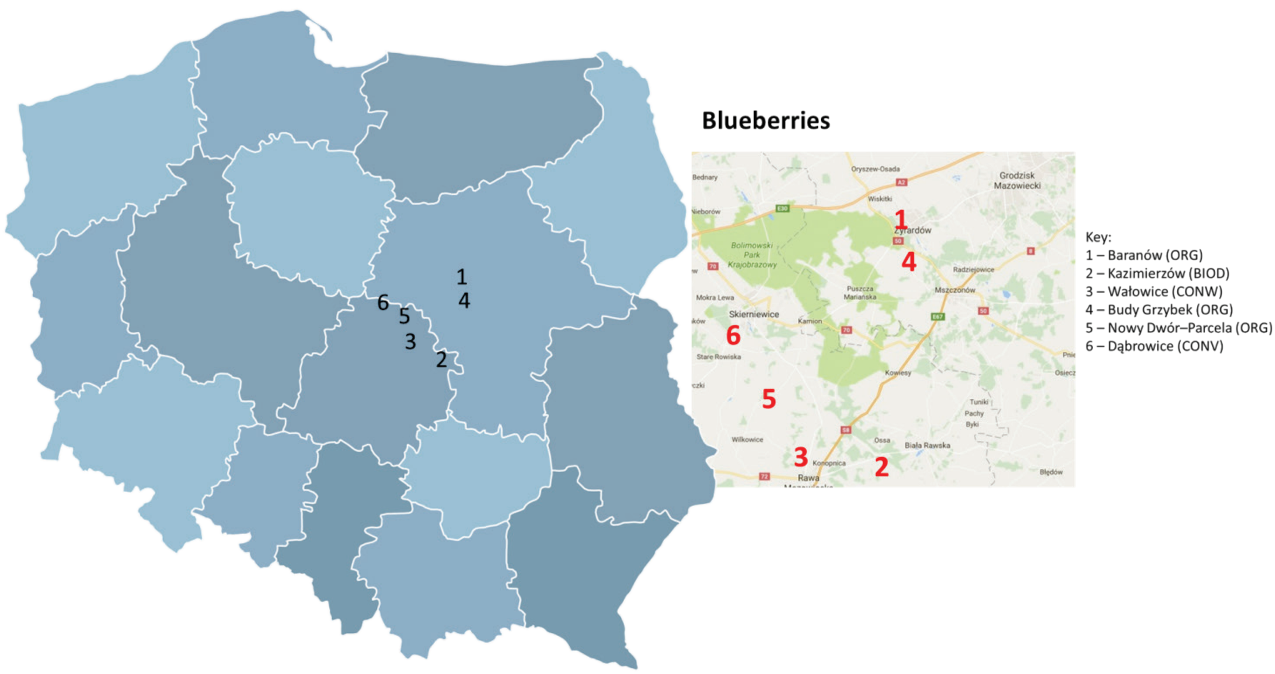


Figure 2. Location of blueberry farms



Fruit quality measurements

Chemical composition

The fruit samples collected in 2016 were analyzed in terms of selected important parameters of their nutritional value as well as sensory properties. The following analyses were carried out in the analytical laboratory of the Chair of Organic Food using validated published protocols and methods: concentration of total and reducing sugars (by Luff-Schoorl method)[56], vitamin C (by spectrophotometric method)[57], phenolic acids (by HPLC)[58], flavonoids (anthocyanins, flavanols, flavonols) (by HPLC)[58], proanthocyanidins (by HPLC)[59], carotenoids (by HPLC)[60], antioxidant activity (with a spectrophotometric method using ABTS synthetic cation radicals)[61], dry matter content (by gravimetric method)[62], acidity (by titration)[63]. Each sample was analyzed in 3 replications. In addition, all fruit samples have been sent to the Institute of Plant Protection (National Research Institute), Department of Pesticide Residue Research in Poznań, Poland to be tested for pesticide residues (using multi-residue method based on gas chromatography with ECD/NP/MS detectors). We are awaiting results of the pesticide residue analyses.

Sensory properties

In addition, all fruit samples undertook sensory assessment with the Quantitative Descriptive Analysis (QDA®) method in the accredited sensory laboratory of the Warsaw University of Life Sciences. QDA is one of main descriptive analysis techniques in sensory evaluation. In the QDA® methodology, multiple product evaluations are suggested to capitalize on panelists' skill in making relative judgments with a high degree of precision. Humans are good at judging relative sensory differences but poor at evaluating absolute differences. Similarly to other descriptive methods, subjects are screened based on their performance on discrimination tests and verbalization in the QDA® methodology. Standards for subject qualification are arbitrary and may vary depending on the project. A panel of ten to twelve is recommended in QDA®. Line scales are employed for panel training and data collection in QDA®. This line scale is designed as 6-inch in length with sensory intensities word anchors located 0.5 inch from each end. The scale direction goes from left to right with increasing intensities, e.g., weak to strong, little to much. During data collection, panelists measure sensory intensities independently at individual booth without reference served as intensities standards. Panelists are allowed to use different parts of the scale to determine the sensory intensities by themselves. As a result, the difference among products produced by QDA® will be a relative measurement; the importance of absolute scale value has been neglected. Subjects' reliability is evaluated by their repeated measurements on product attributes. The results from QDA® are informative for statistical practices to meet project goal. Panel performance can be examined by interaction of product and panelist; product difference can be diagnosed by means of a one-way ANOVA based on attributes. Statistical procedures, such as multivariate analysis of variance, principle component analysis, factor analysis, cluster analysis can be widely applied to QDA® dataset; means of attributes in the same sensory category can be graphically presented by a "spider web".

Analysis of anticancer properties

Freeze-dried fruit powder (from samples collected in 2016) was used to prepare extracts (solvent: 80% ethanol) for *in vitro* experiments. The analyses of anticancer properties have just been initiated in the last months, with a delay compared to the initial project time schedule, due to procedural obstacles & procedures necessary to purchase biological materials and equipment needed for this part of the study. However, as planned in the project proposal, both direct and indirect anticancer properties of selected berries are currently being tested. Direct activity of fruit extracts is estimated based on proliferation and apoptosis *vs*. necrosis level of Caco-2 Human Colon Carcinoma Cells exposed to extracts applied alone or with anticancer drugs. To assess the level of cell apoptosis and necrosis flow cytometry technique is being used. Annexin V conjugated with fluorescein and propidium iodide cell staining is used to examine the percentage of apoptotic and necrotic cells in cell cultures[64]. To examine the level of cell proliferation the CFSE technique is used according to the method described by Lyons (2000).[65] The percentage of proliferating cells is analyzed using flow cytometry. Indirect (systemic) anticancer activity is being tested based on the effect of extracts on the modulation of phenotype of human macrophage cell line (THP-1 Human Monoblastic Leukemia Cell Line). We are preparing to examine the expression of macrophage surface markers involved in the activation of T helper and cytotoxic T lymphocytes (MHC I, MHC II, CD80, CD86) as well as production of cytokines known to create local, tumour surrounding microenvironment: (IL-10, IL-12, TNF-alpha, IL-1, IL-6). The expression of mentioned surface markers on THP-1 Human Monoblastic Leukemia cells treated with fruit extracts alone or with anticancer drugs will be estimated using flow cytometry technique. Cells will be stained with monoclonal antibodies labelled with different fluorochromes. Concentrations of cytokines released by THP-1 cells will be assessed in CBA test using flow cytometry technique. Each extract is being tested in 2 replicates, and two independent experiments are conducted.

All the analyses (chemical, sensory, anticancer) carried out and initiated in the 2016/2017 season will be repeated in the year 2017/2018, in accordance with the project plan.

Experimental design

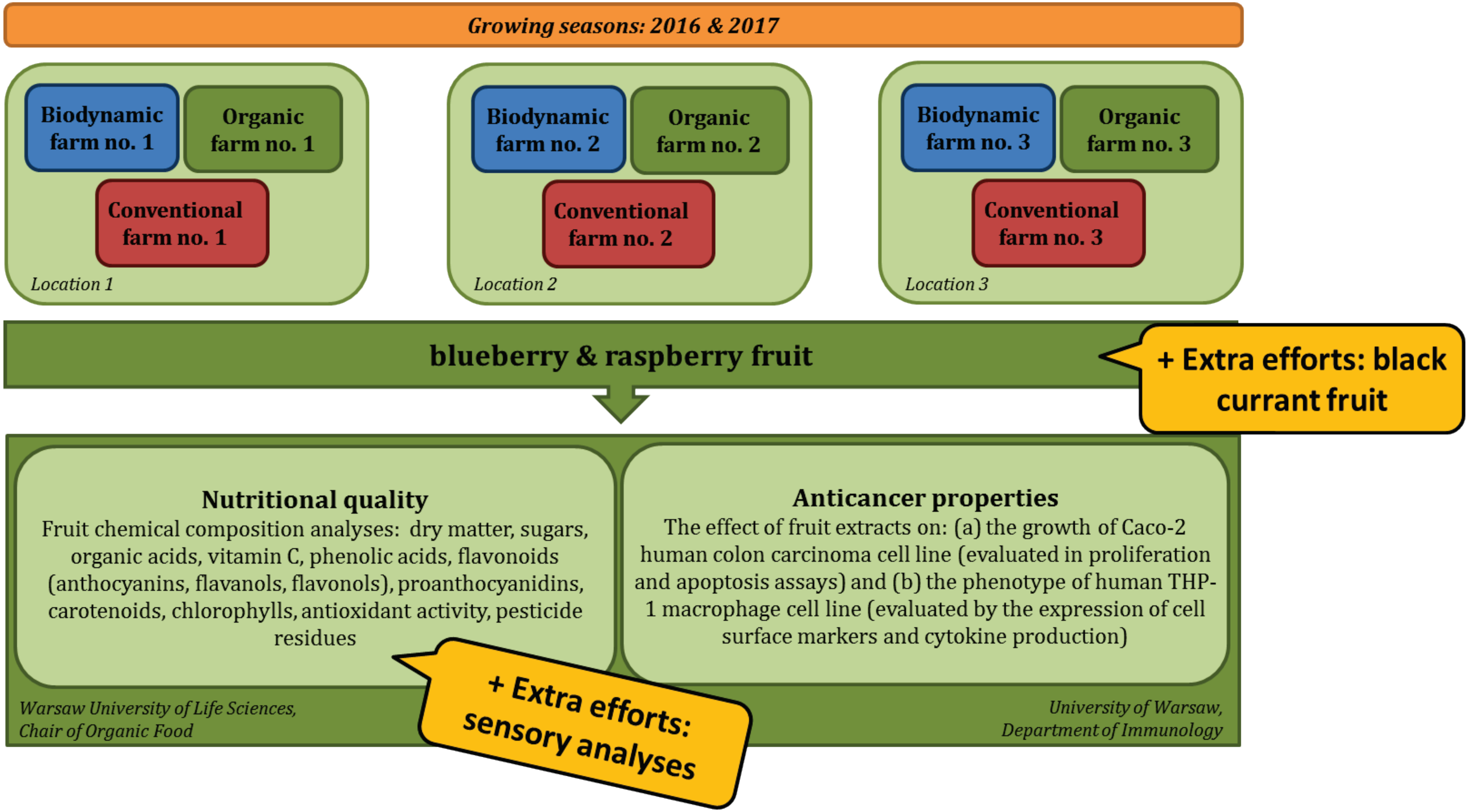


Figure 3. General outline of the project 'Research into nutritive value and anticancer properties of blueberry and raspberry fruit from biodynamic, organic and conventional production'. Some extra efforts undertaken by the project team (not planned in the initial project proposal) are shown in the yellow boxes.

What has been done in the last year

The project has started on the 1st of April 2016. During the first months (Apr-Jun 2016) project team made an effort to establish cooperation with biodynamic, organic and conventional farmers. We have also purchased most of the laboratory equipment, materials and reagents necessary to carry out the planned chemical composition analyses in these first months. In July-September fruit samples were collected from the farmers, used for the sensory evaluation and prepared for further analyses of chemical composition and anticancer properties.

As already described in previous sections of the Report, all sensory evaluations and most of the planned fruit chemical composition analyses have been completed for the raspberry, blueberry and black currant fruit samples collected in the 1st growing season (2016). Preliminary results of the mentioned analyses can be found in the next section of the Report. Ethanol extracts of fruit have been also prepared for the anticancer effects analyses.

Due to procedural obstacles, the experiment on fruit anticancer effects, initially planned for Oct 2016-Feb 2017 has been delayed. It is now ongoing and is planned to be finished in July-September 2017. As now there are no more limits for this experiment, all the materials, cell cultures and reagents have already been successfully purchased, and the pilot anticancer studies have been initiated, we do not expect further delays in the second season/year of the project (the second round of the anticancer properties analyses will start in late 2017, as soon as the fruit samples are collected).

The first year of the project was also rich in the dissemination activities. Our Ekhagastiftelsen project has been mentioned at more than 10 conferences, workshops, international meetings; information about the project has been spread within big international and national research and education networks such as e.g. Organic Food System Programme, Forum of Organic Agriculture in Poland, within a group of IFOAM members, during research visit of team member at Newcastle University in the UK; during a number of activities organised at the Warsaw University of Life Sciences and during many other events. Moreover, we have submitted three research articles based on the project for the 19th Organic World Congress which will be organised in New Delhi, India, on the 9-11 Nov 2017, and we are planning to widely disseminate the first project results during this event.

Preliminary results

Chemical composition of fruit

Blueberry

Table 2. Chemical composition of blueberry fruit from biodynamic, organic and conventional agriculture.

Parameter		Biodynamic		Organic		Conventional	
		Mean	SD	Mean	SD	Mean	SD
Dry matter	g/100 g f.w.	13,1	0,62	13,44	1,08	12,33	1,00
Vitamin C	mg/100 g f.w.	10,42	3,36	7,47	3,67	6,13	0,27
Dehydroxyascorbic acid (DHA)	mg/100 g f.w.	10,28	3,38	7,22	3,56	6,01	0,28
L-ascorbic acid (ASC)	mg/100 g f.w.	0,14	0,02	0,26	0,13	0,17	0,02
Sugars (total)	g/100 g f.w.	5,56	0,52	5,09	0,63	4,50	0,58
Glucose	g/100 g f.w.	2,5	0,24	2,33	0,30	2,04	0,25
Fructose	g/100 g f.w.	3,06	0,28	2,76	0,33	2,46	0,33
Polyphenols (total)	mg/100 g f.w.	100,83	3,88	126,55	14,07	106,75	10,43
Phenolic acids (total)	mg/100 g f.w.	8,03	0,62	15,00	2,55	16,71	1,52
Gallic acid	mg/100 g f.w.	4,3	0,92	12,07	2,44	13,42	1,37
caffeic acid	mg/100 g f.w.	3,26	0,21	2,59	0,32	2,91	0,26
p-cumaric acid	mg/100 g f.w.	0,48	0,10	0,33	0,03	0,37	0,07
Flavonoids (total)	mg/100 g f.w.	11,67	0,75	11,05	2,21	9,28	1,34
3-O-Quercetin rutinoside	mg/100 g f.w.	2,14	1,15	4,30	1,59	3,87	0,39
3-O-kaempferol glycoside	mg/100 g f.w.	1,37	0,49	0,98	0,48	1,53	0,66
Myricetin	mg/100 g f.w.	0,8	0,06	0,75	0,10	0,75	0,04
Luteolin	mg/100 g f.w.	0,23	0,08	2,69	1,83	1,65	1,66
Quercetin	mg/100 g f.w.	1,88	0,06	0,91	0,47	0,55	0,30
Kaempferol	mg/100 g f.w.	5,26	1,46	1,42	0,96	0,93	0,07
Anthocyanins (total)	mg/100 g f.w.	80,73	4,56	98,79	12,92	83,14	7,33
di-O-3,5-delphinidin galactoside	mg/100 g f.w.	53,63	3,81	66,05	9,57	53,88	6,13
di-O-3,5-cyanidin galactoside	mg/100 g f.w.	21,44	0,58	22,89	2,38	21,73	1,19
di-O-3,5-malvinidin galactoside	mg/100 g f.w.	5,66	3,44	9,84	1,73	7,53	0,29

Black currant

Table 3. Chemical composition of black currant fruit from biodynamic, organic and conventional agriculture.

Parameter		Biodynamic		Organic		Conventional	
		Mean	SD	Mean	SD	Mean	SD
Dry matter	g/100g f.w.	18,71	2,28	19,98	1,68	19,59	0,56
Sugars (total)	g/100g f.w.	3,98	0,63	4,14	0,91	3,97	0,51
Glucose	g/100g f.w.	1,94	0,31	2,04	0,39	2,05	0,27
Fructose	g/100g f.w.	2,03	0,33	2,11	0,52	1,92	0,25
Polyphenols (total)	mg/100g f.w.	188,81	42,72	216,23	50,28	171,74	11,48
Phenolic acids (total)	mg/100g f.w.	14,83	7,37	39,43	15,83	32,30	4,78
Gallic acid	mg/100g f.w.	11,00	4,12	34,24	12,34	28,78	3,20
Chlorogenic acid	mg/100g f.w.	3,21	3,42	4,43	3,51	2,73	1,64
caffeic acid	mg/100g f.w.	0,62	0,40	0,76	0,16	0,79	0,19
Flavonoids (total)	mg/100g f.w.	30,23	17,05	31,44	11,18	30,24	10,45
3-O-Quercetin rutinoside	mg/100g f.w.	13,58	11,80	12,15	7,32	10,09	5,73
3-O-kaempferol glycoside	mg/100g f.w.	2,78	2,00	3,85	2,64	5,51	2,48
Myricetin	mg/100g f.w.	0,78	0,12	0,99	0,28	0,82	0,01
Quercetin	mg/100g f.w.	13,09	4,24	14,46	3,53	13,82	2,35
Anthocyanins (total)	mg/100g f.w.	143,75	31,04	145,35	28,39	109,20	5,80
3,5-di-O-cyanidin rutinoside	mg/100g f.w.	16,45	3,02	16,52	3,07	13,52	0,43
3,5-di-O-delfinidin rutinoside	mg/100g f.w.	86,15	17,55	84,02	15,31	62,58	3,86
3,5-di-O-cyanidin glycoside	mg/100g f.w.	7,28	1,00	7,43	0,75	7,12	0,27
3,5-di-O-delphinidin glycoside	mg/100g f.w.	33,87	9,91	37,38	9,38	25,98	1,40

Raspberry

Table 4. Chemical composition of raspberry fruit from biodynamic, organic and conventional agriculture.

Parameter		Biodynamic		Organic		Conventional	
		Mean	SD	Mean	SD	Mean	SD
Dry matter	g/100 g f.w.	13,77	0,36	13,00	1,06	13,42	0,44
Vitamin C	mg/100 g f.w.	38,90	8,64	36,21	9,08	31,38	2,29
Dehydroxyascorbic acid (DHA)	mg/100 g f.w.	16,93	9,41	13,80	7,00	10,45	4,75
L-ascorbic acid (ASC)	mg/100 g f.w.	21,98	2,61	22,42	2,69	20,93	2,66
Sugars (total)	g/100 g f.w.	5,16	0,49	7,38	1,62	6,24	1,40
glukoza	g/100 g f.w.	2,02	0,19	2,52	0,48	2,38	0,49
Sucrose	g/100 g f.w.	0,39	0,12	1,54	0,52	0,45	0,11
Fructose	g/100 g f.w.	2,75	0,23	3,31	0,63	3,40	1,05
Polyphenols (total)	mg/100 g f.w.	95,56	7,05	97,23	9,02	79,21	1,22
Phenolic acids (total)	mg/100 g f.w.	25,26	2,98	13,14	6,01	6,12	0,71
Gallic acid	mg/100 g f.w.	0,46	0,01	0,47	0,08	0,49	0,08
Chlorogenic acid	mg/100 g f.w.	0,75	0,07	0,75	0,20	0,66	0,01
caffeic acid	mg/100 g f.w.	1,54	0,18	1,08	0,26	1,06	0,51
p-cumaric acid	mg/100 g f.w.	0,44	0,06	1,50	1,33	2,43	0,19
Ellagic acid	mg/100 g f.w.	22,07	3,27	9,34	6,85	1,48	0,20
Flavonoids (total)	mg/100 g f.w.	0,82	0,01	1,23	0,40	1,25	0,44
Luteolin	mg/100 g f.w.	0,40	0,02	0,42	0,17	0,59	0,15
Quercetin	mg/100 g f.w.	0,14	0,01	0,20	0,10	0,45	0,28
3-O-kaempferol glycoside	mg/100 g f.w.	0,23	0,00	0,46	0,31	0,16	0,03
Kaempferol	mg/100 g f.w.	0,04	0,02	0,16	0,18	0,05	0,08
Anthocyanins (total)	mg/100 g f.w.	94,28	7,05	95,53	8,84	77,47	1,17
3,5-O-di-cyanidin glycoside	mg/100 g f.w.	46,57	3,50	56,63	15,50	45,00	1,00
3,5-di-O-pelargonidin glycoside	mg/100 g f.w.	26,67	8,05	18,88	1,29	18,55	1,63
3-O-delfinidin glycoside	mg/100 g f.w.	21,04	1,85	20,03	7,75	13,91	0,45

Sensory properties of fruit

The sensory evaluation of fresh fruit was done using Quantitative Descriptive Analysis – QDA acc. to Stone & Sidel (1985). Following this procedure, 10 qualified panelists were initially involved in selection of attributes (of taste, smell, consistency, flavor) relevant for the tested fruit species. Attributes were discussed and definitions unified. There were 17 quality attributes in the analysis of blueberry and raspberry, and 19 attributes in the analysis of black currant. 2 independent sessions were organized. Individual samples (20-30 g of fresh fruit each) were presented to panelists in closed plastic containers (volume of 125 ml). The study was blinded.

Blueberry

Table 5. Quantitative Descriptive Analysis (QDA) of blueberry fruit (n=20).

Sample No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
Agricultural system	BIOD 1	BIOD 2	BIOD 3	CONV 1	CONV 2	CONV 3	ORG 1	ORG 2	ORG 2 n	ORG 3	ORG 4	ORG 5
Odour - blueberry	1,94	1,81	2,03	1,80	1,67	2,83	2,62	1,67	2,30	3,32	3,86	3,70
Odour - acidic	0,69	0,69	0,59	0,83	0,78	0,67	0,47	0,63	0,51	0,90	0,98	0,87
Odour -cut grass	1,68	1,18	1,73	1,54	1,53	1,43	1,43	1,57	1,21	1,89	2,29	2,06
Odour - sweet	0,97	1,12	0,82	0,76	0,81	0,69	0,98	0,61	0,51	1,11	1,29	1,31
Odour - other	0,07	0,08	0,06	0,11	0,08	0,11	0,08	0,07	0,10	0,18	0,42	0,33
fruit size	6,72	7,54	8,09	6,30	3,77	7,28	4,10	5,44	6,14	8,20	3,54	3,41
coating on fruits	5,69	6,28	6,34	5,49	6,75	6,20	5,86	5,72	6,20	5,19	5,53	5,77
firmness	4,43	4,93	5,43	5,12	4,89	5,28	6,49	5,76	5,19	3,99	4,98	3,76
juiciness	5,52	6,27	6,26	5,83	4,62	6,07	5,22	6,01	5,99	6,64	5,87	4,34
hardness peel	3,04	2,71	2,84	3,38	3,61	4,00	4,86	3,86	3,19	1,59	2,90	3,73
Taste - blueberry	6,20	6,63	6,99	5,52	5,24	6,71	6,76	5,92	6,92	6,19	6,16	5,77
Taste - sourness	4,01	2,78	2,48	3,79	4,39	3,82	2,57	4,80	3,41	2,73	2,53	2,33
Taste- sweetness	3,87	5,12	5,94	3,80	2,92	5,01	5,61	4,68	5,47	4,57	4,59	3,79
astringency	1,64	0,81	0,94	1,71	2,18	1,57	0,84	2,03	1,54	1,28	1,12	1,73
Taste - grassy	0,54	0,35	0,31	1,22	0,95	0,49	0,39	0,59	0,39	1,06	1,05	0,98
Taste - other	0,09	0,09	0,11	0,13	0,13	0,12	0,21	0,16	0,12	0,06	0,41	0,31
Overall quality	**5,87**	**7,36**	**7,84**	**5,85**	**4,61**	**6,64**	**6,86**	**6,17**	**6,73**	**7,39**	**5,94**	**5,68**

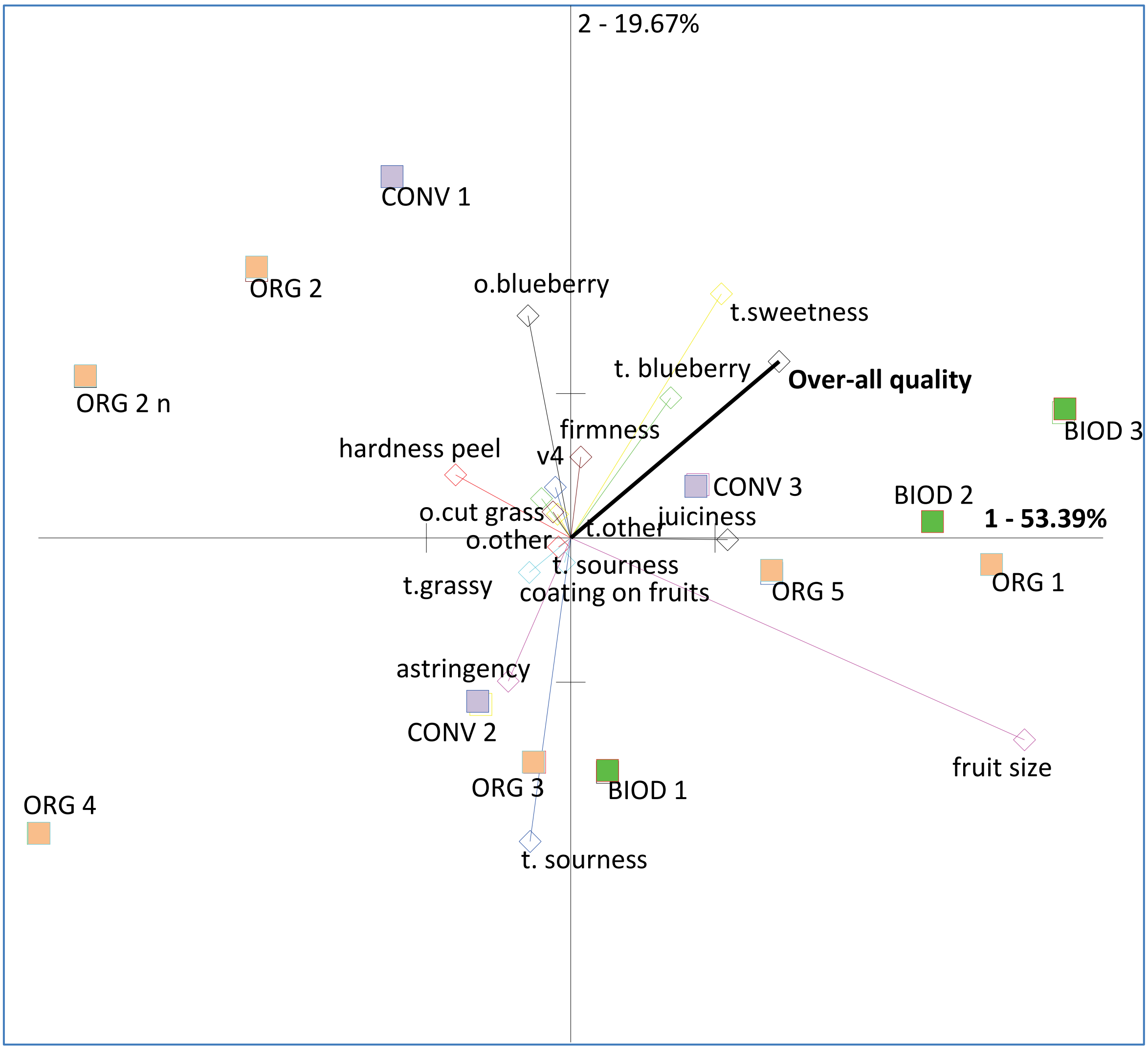


Figure 4 PCA results of Quantitative Descriptive Analysis (QDA) of blueberry fruit

Table 6. Comparison of sensory qualities of blueberry depending on the cultivation system.

	Cultivation system		
	Biodynamic	Organic	Conventional
Odour - blueberry	1,92	**2,91**	2,10
Odour - acidic	0,66	1,10	0,76
Odour - cut grass	1,53	1,62	1,50
Odour - sweet	0,97	1,10	0,75
Odour - other	0,07	0,33	0,10
fruit size	7,45	4,43	5,78
coating on fruits	6,10	6,07	6,15
firmness	4,93	5,05	5,10
juiciness	6,02	5,44	5,51
hardness peel	2,86	3,82	3,66
Taste - blueberry	6,61	5,62	5,83
Taste - sourness	3,09	3,51	4,00
Taste - sweetness	4,97	4,49	3,91
astringency	1,13	1,87	1,82
Taste - grassy	0,40	0,82	0,89
Taste - other	0,10	0,33	0,13
Overall quality	**7,03**	**5,55**	**5,70**

Black currant

Table 7. Quantitative Descriptive Analysis (QDA) of black currant fruit (n=20).

	Black currant fruit								
Sample No.	1	2	9	3	4	5	6	7	8
Agricultural system	BIOD 1	BIOD 2	BIOD 3	ORG 1	CONV 1	ORG 2	ORG 3	CONV 3	CONV 2
Odour - currant	6,53	6,06	5,86	6,77	5,14	5,47	5,75	5,17	5,36
Odour - acidic	2,71	2,29	1,95	2,59	2,09	2,14	1,84	1,78	1,91
Odour - cut grass	1,42	1,82	1,96	1,42	2,32	2,30	1,48	2,99	2,71
Odour - sweet	2,43	1,08	2,23	3,81	0,85	1,03	2,63	1,07	1,20
Odour - other	0,14	0,50	0,32	0,44	0,54	0,29	0,74	0,17	0,19
fruit color peel	8,03	7,69	7,98	8,16	7,90	7,59	7,84	8,01	7,58
Peel shine	6,87	6,18	6,47	7,33	6,48	6,14	6,36	6,18	6,05
color of fruit pulp	5,09	4,41	3,21	6,04	3,27	3,78	4,64	3,16	3,23
fruit size	5,14	5,19	6,95	4,65	4,89	6,95	5,25	4,74	4,89
firmness	4,58	5,22	4,69	6,16	6,98	4,69	4,69	6,51	6,98
juiciness	5,85	5,59	6,10	5,14	5,38	6,10	5,98	5,10	5,38
hardness peel	4,82	5,87	4,59	4,71	6,53	6,98	5,49	6,78	6,53
Taste - currant	7,14	6,67	6,61	6,61	6,30	6,74	7,05	6,66	6,73
Taste - sourness	3,77	5,10	4,99	4,54	5,68	6,02	4,47	5,57	5,84
Taste - sweetness	4,94	2,68	3,29	4,49	2,43	1,90	4,28	2,87	2,26
astringency	2,64	3,45	3,22	2,74	4,06	4,32	3,31	4,01	3,97
Taste - grassy	1,46	2,49	1,91	1,08	3,34	3,20	1,66	2,80	2,65
Taste - other	0,04	0,05	0,07	0,15	0,03	0,03	0,04	0,18	0,08
Overall quality	**7,48**	**5,71**	**6,76**	**7,41**	**5,08**	**3,98**	**7,58**	**5,83**	**5,65**

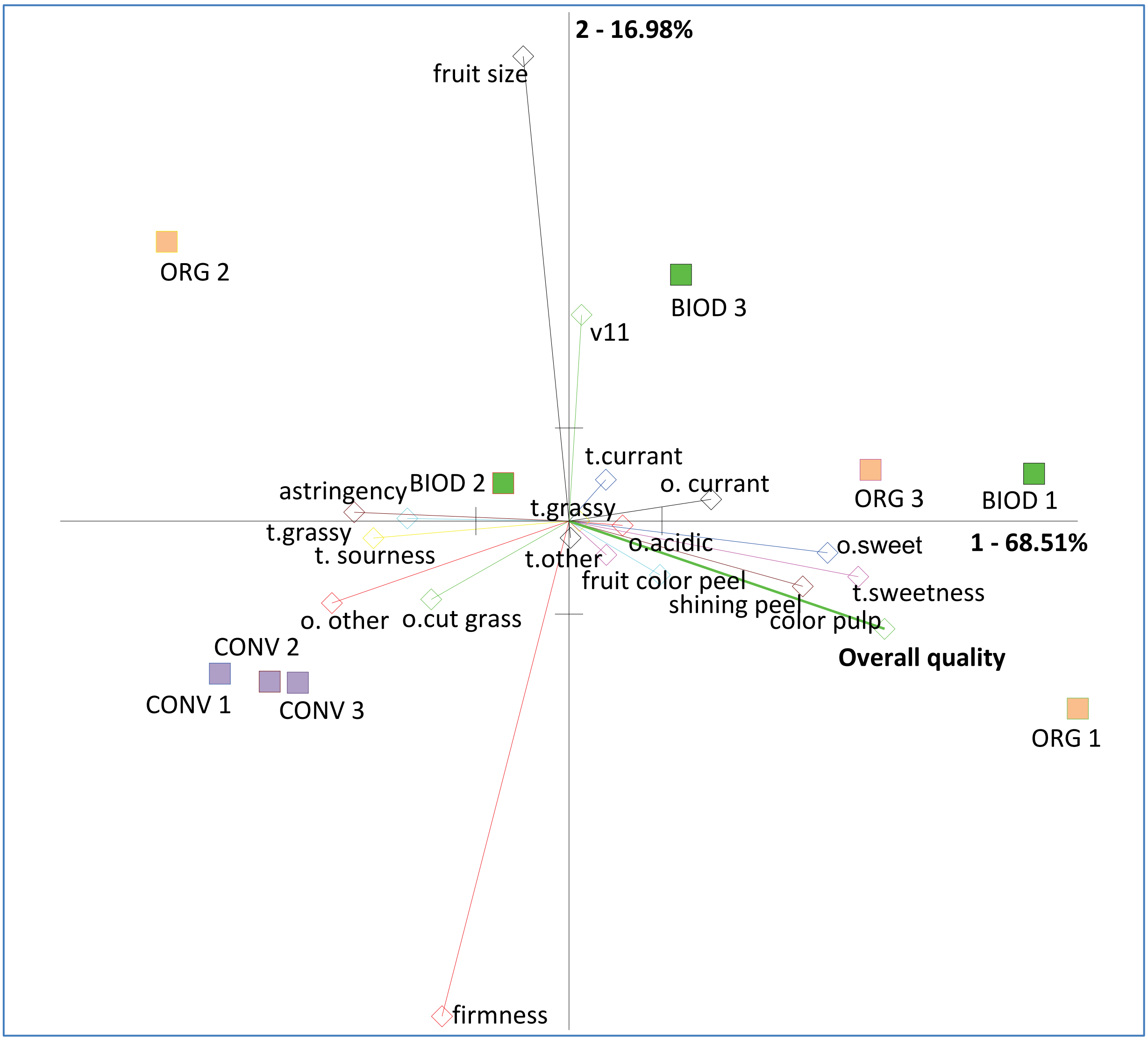


Figure 5. PCA results of Quantitative Descriptive Analysis (QDA) of black currant fruit.

Table 8. Comparison of sensory qualities of black currant fruit depending on the cultivation system.

	Cultivation system		
	Biodynamic	Organic	Conventional
o. currant *	6,15	6,00	5,22
o.acidic	2,32	2,19	1,92
o.cut grass	1,73	1,73	2,67
o.sweet	1,91	2,49	1,04
o.other	0,32	0,49	0,30
fruit color peel	7,90	7,86	7,83
shining peel	6,50	6,61	6,23
color of fruit pulp	4,24	4,82	3,22
fruit size	5,76	5,62	4,84
firmness	4,83	5,18	6,82
juiciness	5,85	5,74	5,29
hardness peel	5,09	5,72	6,61
t. currant **	6,81	6,80	6,56
t. sourness	4,62	5,01	5,70
t. sweetness	3,64	3,56	2,52
astringency	3,10	3,46	4,01
t.grassy	1,95	1,98	2,93
t.other	0,05	0,07	0,10
Over-all quality	**6,65**	**6,32**	**5,52**

* Odour; ** Taste.

Raspberry

Table 9. Quantitative Descriptive Analysis (QDA) of raspberry fruit (n=20).

	Raspberry fruit									
Sample numer	1	2	3	4	5	6	7	8	9	10
Agricultural system	BIOD 1	BIOD 2	BIOD 3	ORG 1	ORG 2	ORG 4	ORG 5	CONV 2	CONV 3	CONV 4
o.raspberry*	4,42	5,76	5,22	5,01	4,13	4,88	6,03	5,82	6,30	4,54
o.acidic	2,04	2,47	2,15	1,82	1,93	2,06	2,38	2,10	2,33	1,78
o.cut grass	1,58	1,21	1,39	1,01	1,03	0,80	1,26	0,62	0,79	0,97
o.sweet	2,11	2,79	2,77	1,98	1,63	2,02	3,28	2,40	3,63	1,99
o.other	0,32	0,41	0,34	0,32	0,71	0,81	0,05	0,22	0,11	0,58
fruit size	4,08	5,21	5,79	6,11	6,30	6,70	5,45	7,07	7,24	5,58
fruit color	5,22	6,12	6,00	6,96	6,58	7,02	6,19	7,38	6,72	6,48
firmness	5,28	4,64	5,75	5,50	5,60	5,12	5,47	5,58	5,20	5,36
juiciness	4,58	5,84	4,91	5,85	5,75	5,90	5,30	6,65	6,16	5,62
color uniformity	5,36	5,63	6,06	6,79	6,79	6,84	6,68	7,45	6,89	6,21
t.raspberry**	4,60	6,04	4,97	6,09	5,94	6,08	5,78	6,85	6,30	5,34
t. sourness	3,77	3,07	3,91	2,90	2,95	2,92	3,90	2,52	3,38	3,78
t.sweetness	2,43	3,61	2,47	3,55	3,16	3,70	3,32	4,73	4,13	2,39
Astringency	2,04	1,41	1,92	1,05	1,17	0,84	1,67	0,56	1,67	1,44
t.grassy	1,11	0,63	1,14	0,36	0,24	0,28	1,04	0,15	0,44	0,61
t.other	0,58	0,35	0,39	0,28	0,48	0,54	0,11	0,21	0,11	0,34
Over-all quality	**4,30**	**5,78**	**4,78**	**6,10**	**5,36**	**5,89**	**5,76**	**7,45**	**6,51**	**4,73**

*Odour; ** Taste

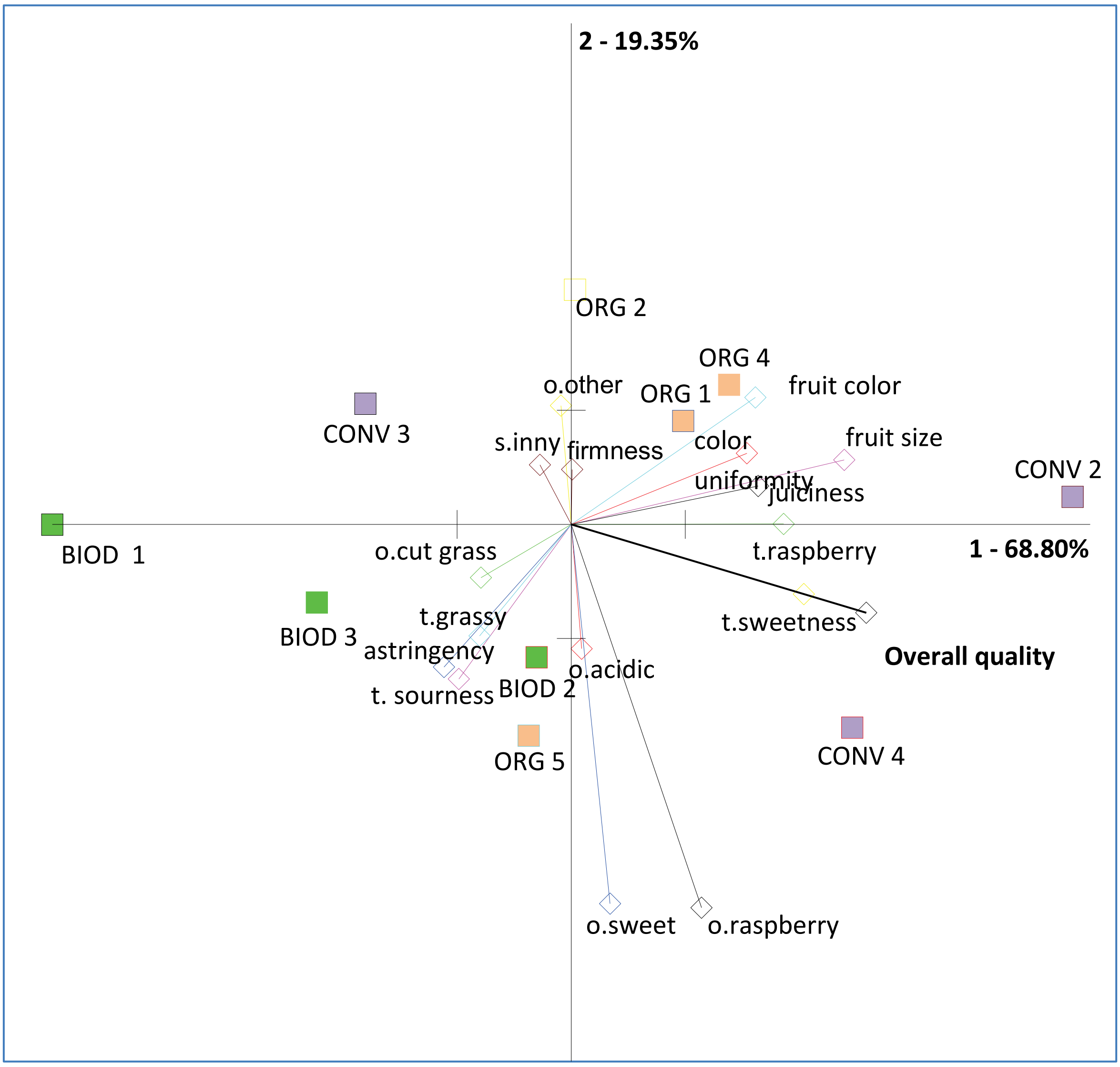


Figure 6. PCA results of Quantitative Descriptive Analysis (QDA) of raspberry fruit.

Table 10. Comparison of sensory qualities of raspberry fruit depending on the cultivation system.

	Cultivation system		
	Biodynamic	Organic	Conventional
o.raspberry*	5,13	5,01	5,55
o.acidic	2,22	2,05	2,07
o.cut grass	1,39	1,02	0,79
o.sweet	2,55	2,23	2,67
o.other	0,35	0,47	0,30
fruit size	5,03	6,14	6,63
fruit color	5,78	6,69	6,86
firmness	5,22	5,42	5,38
juiciness	5,11	5,70	6,14
color uniformity	5,68	6,77	6,85
t.raspberry**	5,20	5,97	6,16
t. sourness	3,58	3,16	3,23
t.sweetness	2,83	3,43	3,75
astringency	1,79	1,18	1,22
t.grassy	0,96	0,48	0,40
t.other	0,44	0,35	0,22
Overall quality	4,95	5,77	6,23

Fruit anticancer properties – actions taken so far

The aim of the first part of the 'anticancer study' is to evaluate the biological activity of blueberries and raspberries extracts to inhibit the proliferation and to induce cell death of human colon carcinoma cell line Caco-2. A wide range of fruit or plant compounds are investigated for their anti-cancer properties. Based on the results of our previous studies, we can postulate that usually the impact of extracts alone demonstrate a moderate anti-cancer activity. Thus, in this study we aim to examine the ability of the extracts to potentiate the effect of commonly used anti-cancer drug, 5-FU (5-fluorouracil) in inhibiting the proliferation and/or inducing apoptosis of Caco-2 cells. 5-FU is a chemotherapeutic agent classified as an antimetabolite S-phase specific that acts on actively proliferating cells.

We have established optimal conditions for Caco-2 cells growth in RPMI-1640 or DMEM supplemented with all recommended agents (Natoli M et al. Good Caco-2 cell culture practices, Toxicology in vitro, 2012, 26: 1243-1246). For preliminary experiments 24 hours incubation with different concentrations of 5-FU has been used. Caco-2 cells were seeded onto 24-well plates in 0.5 ml of complete medium. The drug was added after 6 hours of incubation. The following concentrations of 5-FU were used for cell treatment: 1; 5; 10; 20; 50 and 100 mM. Two concentrations have been chosen for further experiments: 1 and 20 mM causing respectively about 80 and 60% of cell count reduction. Cells were counted in a hemocytometer after 24 hours of drug treatment (after 30 hours of total incubation).

The next step of the study will be performed according to the following schedule:

- Fruit extracts addition to the Caco-2 cells at time 0h, supplementation with 5-FU at 6h incubation, proliferation and apoptosis measurement at 30h incubation
- Fruit extracts and 5-FU simultaneously added at time 0h, proliferation and apoptosis measurement at 24h incubation
- Controls: (a) without extracts nor 5-FU; (b) with 5-FU alone; (c)with fruit extracts alone.

The level of Caco-2 proliferation will be estimated by CFSE staining method. The percentage of apoptotic and necrotic cells will be analyzed based on AnnexinV/PE and 7-AAD staining. CFSE staining of cancer cells will be performed before seeding onto culture plate. Staining of cells for the estimation of apoptosis vs necrosis will be performed after appropriate incubation time. FACSVerse (Becton-Dickinson) will be used for cell acquisition, and Diva software for cell analysis.

We have established the optimal conditions for co-culture of THP-1 cell line with CaCo-2 to examine the ability of the fruit extracts to influence the cancer-macrophage interaction in the culture with anti-cancer drug, 5-FU (5-fluorouracil). Macrophages are known as a central players in anticancer activity of immune system. According to the literature, in colon cancer, the presence of inflammatory macrophages is associated with better prognosis, thus establishing the macrophages status in our experimental system could be beneficial.

Aims and plan for the coming year

In the coming year we are planning to repeat the experiment following exactly the same procedures, to be able to assess the impact of year-to-year variation on the quality and anticancer effect of fruit coming from different agricultural systems. We are already cooperating with the same farmers who are willing to provide us with fruit samples in the coming season, as soon as they are ripe & ready for harvest.

As already mentioned, due to procedural obstacles, the experiment on fruit anticancer effects has been delayed. Therefore our priority now is to finish this part of the study. As mentioned, we do not expect further delays in the second season/year of the project (the second round of the anticancer properties analyses will start in late 2017, as soon as the fruit samples are collected). Once we collect all the results, we are planning to run statistical analyses including the results from 2 years of the experiment & start the final interpretation and writing phase of the project & prepare at least drafts of 2 quality research papers by the end date of the project.

Moreover, as previously mentioned, we have submitted three research articles based on the project for the 19th Organic World Congress which will be organised in New Delhi, India, on the 9-11 Nov 2017, and we are planning to widely disseminate the first project results during this coming important event.

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