Food Research International 107 (2018) 297–305

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Plant cells as food – A concept taking shape

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ARTICLE INFO

Keywords:
Cellular agriculture
Plant cell culture
Nutritional quality
Sensory profiling
Vegetable food

ABSTRACT

Plant cell cultures from cloudberry, lingonberry and stoneberry were studied in terms of their nutritional properties as food. Carbohydrate, lipid and protein composition, in vitro protein digestibility and sensory properties were investigated. Dietary fibre content varied between 21.2 and 36.7%, starch content between 0.3 and 1.3% and free sugar content between 17.6 and 33.6%. Glucose and fructose were the most abundant sugars. High protein contents between 13.7 and 18.9% were recorded and all samples had a balanced amino acid profile. In vitro protein digestion assay showed hydrolysis by digestive enzymes in fresh cells but only limited hydrolysis in freeze-dried samples. The lipid analysis indicated that the berry cells were rich sources of essential, polyunsaturated fatty acids. In sensory evaluation, all fresh berry cells showed fresh odour and flavour. Fresh cell cultures displayed a rather sandy, coarse mouthfeel, whereas freeze-dried cells melted quickly in the mouth. All in all the potential of plant cells as food was confirmed.

1. Introduction

The dietary intake of plant-based food is generally considered healthier, more sustainable and safer than animal-derived food. However, it will be increasingly difficult to provide such food in sufficient amounts and quality to supply the global population, which will, according to current estimates, require altogether 60% more food by 2050 than produced today (Alexandratos & Bruinsma, 2012). Projections reveal that agricultural land can only be increased by 2% from the current 38% of the total land area. High intensity agriculture is already a huge environmental burden as it accounts for approximately 20–25% of global emissions (Olivier, Janssens-Maenhout, Muntean, & Peters, 2013) and relies on environmentally detrimental fertilizer and pesticides derived from fossil resources.

Based on these facts it becomes apparent that new technologies for diverse and healthy plant-based food production need to be developed to reduce the negative environmental impacts of agriculture including greenhouse gas emissions and soil degradation, and to protect the already dwindling water supplies and biodiversity. In addition, a large part of the world population is urban and the share of people living in cities will further increase, which calls for serious consideration of food production in the built environment, too.

Plant cell culture (PCC) technology is a new approach to consider for plant-based food production. In analogy to the radical invention of "cultured meat" (Edelman, McFarland, Mironov, & Matheny, 2005), but to an even greater extent, bioreactor-grown PCCs could be exploited as an entirely new food biomass for human consumption. Since the foundation of plant biotechnology and the concept of cellular totipotency in 1902 by Haberlandt (Krikorian & Berquam, 1969), an enormous development has taken place and PCCs have influenced food production at many different levels already. On one hand, plant biotechnology methods have been enabling the green revolution i.e. a massive yield increase of crops through breeding varieties with e.g. improved agronomic traits, nutritive value and disease resistance (Pingali, 2012). On the other hand, PCCs, particularly from undomesticated plant species, have been directly exploited for commercial production of phytochemicals (Nosov, 2012) to be used as pharmaceuticals, pigments and ingredients for cosmetics and food. In the latter case the cultivation of plant cells takes place in bioreactors rather than on the field and facilitates fully controlled, aseptic and year-round production. So far, however, the biomass generated by this method has usually been subjected to extraction and the value of the whole material as foodstuff has not been considered and investigated. To the best of our knowledge there are only very few scientific studies suggesting the use of PCCs or their extracts as food. The published reports either focus on utilizing the PCCs for extraction of specific ingredients (Fu, Singh, & Curtis, 1999), or approach the topic from a rather theoretical point of view without providing reviewable scientific data (Davies & Deroles,

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https://doi.org/10.1016/j.foodres.2018.02.045
Received 22 December 2017; Received in revised form 15 February 2018; Accepted 16 February 2018
Available online 19 February 2018

2014).

The aim of this work was to study the potential of using PCCs for food exemplified with three cell lines of berry species. By analysing the chemical composition and in vitro protein digestibility, and thus, evaluating the nutritional quality, and by performing sensory evaluation of the berry cell cultures, the target was to provide basic data helping to evaluate the applicability of PCC material as a whole for food use.

2. Materials and methods

2.1. Cultivation of the PCCs

PCCs of cloudberry (*Rubus chamaemorus*), stoneberry (*Rubus saxatilis*) and lingonberry (*Vaccinium vitis-idaea*) established earlier (Nohynek et al., 2014; Suvanto et al., 2017) were examined. The cultures were either maintained by regular subculture on solid medium or cryo-preserved in liquid nitrogen (http://culturecollection.vtt.fi/). Cell suspensions were grown in 250 ml Erlenmeyer flasks containing 60 ml of culture on an orbital shaker at 110 rpm, 24 ± 1 °C and a day-night illumination regime (photoperiod 16:8 h; irradiation 40 μmol $m^{-2} s^{-1}$). The *Rubus* species were cultivated in MS medium (Murashige & Skoog, 1962) (Duchefa Biochemie, The Netherlands) containing 3% (w/v) sucrose, 0.1 mg l^{-1} kinetin (Sigma, Munich, Germany) and 1 mg l^{-1} NAA (α-naphthaleneacetic acid; Sigma). Lingonberry cells were grown in Woody Plant Medium (Lloyd & McCown, 1981) (Duchefa Biochemie, The Netherlands) containing 3% (w/v) sucrose, 2.2 mg l^{-1} TDZ (Thidiazuron; Duchefa Biochemie, The Netherlands) and 1.95 mg l^{-1} NAA (α-naphthaleneacetic acid; Sigma, Munich, Germany). For analysis, the plant cells were separated from the medium by vacuum filtration using a Büchner funnel and Miracloth (Calbiochem, San Diego, USA) filtration tissue. Cells were washed twice with sterile MilliQ water and either used fresh or lyophilized.

2.2. Colour

The colour of the fresh cell suspensions and freeze-dried cell powders was measured with a Minolta chroma meter (CR-200). A small Petri dish was filled with sample. The colour was measured from the bottom of 2–3 Petri dishes/cell culture at 5 different points (in total 10–15 individual measurements/cell culture). The colour was recorded as coordinates in the CIE Lab colour space. L* represents the lightness of the colour (L* = 0 yields black and L* = 100 indicates diffuse white), a* its position between red/magenta (positive values) and green (negative values) and b* its position between yellow (positive values) and blue (negative values).

2.3. Microscopy

Fresh cell suspensions were diluted with water and examined as such (without staining) with a light microscope (Olympus BX40F, Japan) in phase contrast mode. A drop of cell suspension was placed on a glass slide and a cover glass was placed on top of the sample. Micrographs were obtained using a DS Camera Head DS-Fi1 (Nikon, Japan) and DS Camera Control Unit DS-L2 (Nikon, Japan).

2.4. Dry matter content

The dry matter content of the fresh PCCs (n = 4) was determined after filtration by oven drying of about 3 g material over night at 105 °C.

2.5. Elemental composition

C, H, N and S were determined using a FLASH 2000 series analyzer. The samples (n = 3) were weighted in tin/silver capsules, placed inside the Thermo Scientific MAS 200R auto-sampler at a pre-set time, and then dropped into an oxidation/reduction reactor kept at a temperature of 960 °C. The exact amount of oxygen required for optimum combustion of the sample is delivered into the combustion reactor at a precise time. The reaction of Oxygen with the Tin capsule at elevated temperature generates an exothermic reaction which raises the temperature to 1800 °C for a few seconds. At this high temperature, both organic and inorganic substances are converted into elemental gases which, after further reduction, are separated in a chromatographic column and finally detected by a highly sensitive thermal conductivity detector (TCD). Results were calculated using Certified Elemental Microanalysis standards using K factor (CHNS/CHNS-O Standards Kit: Cystine, Sulphanilamide, Methionine and BBOT: Catalogue Code.Thermo: 33840010).

2.6. Carbohydrate analysis

To determine the total carbohydrate composition, the freeze-dried PCC samples (n = 3) were hydrolysed with sulphuric acid and the resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS-5000 equipped with CarboPac PA20 column) according to NREL method (Hausalo, 1995; Sluiter et al., 2008). The free sugars were analysed with the same HPAEC method (with CarboPac PA1 column), but using water extraction (5 °C for 25 min with constant mixing) as a pre-treatment instead of the acid hydrolysis. Total, insoluble and soluble dietary fibre content of the dried cells was analysed by enzymatic-gravimetric method (AOAC method 991.43), and the total starch content was analysed by the AOAC Method 996.11.

2.7. Amino acid analysis

The total amino acid content of the freeze-dried PCC samples (5 mg, accurately weighted; n = 3) was determined as described earlier (Saarela et al., 2017). Briefly, the samples were oxidized with fresh performic acid and hydrolysed in acidic conditions (6 N HCl). For determination of tryptophan a separate alkaline hydrolysis was performed. Subsequently, the samples were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) reagent and UPLC analysis was performed on an Acquity UPLC system (Waters, Milford, MA, USA) with an Acquity UPLCTM BEH C18 column (2.1 × 100 mm, 1.7 μm). Signal was detected at 260 nm. His, Ser, Arg Gly, Asp, Glu, Thr, Ala, Pro, Cys, Lys, Tyr, Met, Val, Ile, Leu, Phe and Trp were quantified in the samples. In acid hydrolysis Asn is converted to Asp and Gln to Glu. Cysteine and methionine were determined as cysteic acid and methionine sulfone after the oxidation procedure.

2.8. Fatty acid and sterol analysis

The fatty acid composition of the PCC samples (n = 3) was determined from 30 mg of freeze-dried material (Seppänen-Laakso, Nygren, & Rischer, 2017), which was spiked with free (26.8 μg) and esterified (105.5 μg) heptadecanoic acid (C17:0 FFA and TAG). Fatty acids were transesterified with sodium methoxide (0.5 M), the samples were acidified with 15% sodium hydrogen sulphate and the methyl esters (FAME) and free fatty acids (FFA) were extracted with petroleum ether before analysis by GC–MS (Agilent 7890A GC combined with a 5975C MSD). After running on an FFAP column, the samples were trimethylsilylated with MSTFA and run on an Rtx-5ms column and the samples were further studied for α-tocopherol, free fatty acids and sterols. The total amount of fatty acids and sterols was used as a measure of fat content. The repeatability of the method for FAME was 4.7% (CV) varying from 2.5 to 9.8% for individual fatty acids, and respectively for FFA 3.5% (CV) varying from 2.4 to 9.5% (n = 6).

2.9. HS-SPME analysis of volatile compounds

Volatile compounds in freeze-dried and re-hydrated freeze-dried cells were analysed by using headspace solid-phase microextraction (HS-SPME) technique (Ritala et al., 2014) combined with GC–MS (Agilent 5973 N Mass Selective Detector and 6890 gas chromatograph). The samples were incubated with a 50/30 µm DVB/CAR/PDMS Stableflex (2 cm) fibre at 60 °C for 30 min. The injector and interface temperatures were 250 and 260 °C, respectively, and helium was used as carrier gas. The desorption time was 10 min and a splitless injection mode was used. The chromatographic column was a 25 m HP-FFAP silica capillary column with an internal diameter of 0.20 mm and a stationary-phase film thickness of 0.30 µm. The oven temperature increased from 40 °C (3 min) to 240 °C (2 min) at a rate of 10 °C/min. The MS detector was operated in scan mode with 70 eV electron impact and the data was collected at a mass range of *m/z* 20–500. Data analyses and library comparisons were performed by using MSD ChemStation Data Analysis Application and NIST08 library.

2.10. Protein digestibility by in vitro analysis

The upper intestinal model was performed with fresh and freeze-dried PCCs as described by Minekus et al. (2014) with enzyme concentrations as described by Aura et al. (2015) with the exception that half the bile extract concentration was added. Briefly, samples (5 g fresh cells and 0.50 g freeze-dried cells), 0.50 g of casein reference or only water (enzyme control) were dosed to incubation tubes in triplicates, which went through oral, gastric and duodenal stages consecutively in the same tubes. Simulated salivary fluid (SSF (pH 7); 3.9 ml), 25 µl 0.3 M calcium chloride solution and distilled water to adjust the total volume to 10 ml were incubated for 2 min to solubilize the ingredients at 37 °C in a water bath with shaking. Oral stage was ended by adding simulated gastric fluid (SGF (pH 3); 7.5 ml), which lowered the pH, 5 µl 0.3 M calcium chloride solution and 1.6 ml pepsin (2 mg/ml; P-7012; Sigma). Volume was adjusted to 20 ml with distilled water and samples were incubated for 120 min as described above.

Gastric stage was ended by adding 11 ml of simulated intestinal fluid (SIF; pH 7), which increased the pH to that of the duodenal stage. In addition 2.5 ml porcine bile extract 75 mg/ml (B-8631; Sigma), 40 µl 0.3 M calcium chloride solution and 5.0 ml pancreatin (18.75 mg/ml; P-3292; Sigma-Aldrich) were added. Finally, distilled water adjusted the volume to 40 ml, and the duodenal stage was incubated for 120 min. All samples were incubated as triplicates. The protein digestion was ended in each sample by increasing the pH to neutral (particularly for the gastric stage) using protease inhibitor (see details below).

Samples were drawn from the end of oral stage (2 min, Oral), from gastric and duodenal stages at 0 min, 30 min, 60 min and 120 min after starting each incubation. A 0.5 ml sample was drawn for analysis of amino termini as indicator of protein digestion. The sample for protein digestion was pipetted into SIF (adjusting the pH to neutral) with 40 µl of protease inhibitor (cOmplete; Roche REF 04693132001; 1 tablet/2 ml of SIF-stock). Since 0.50 ml of sample was added to 0.50 ml of SIF and 0.040 ml of protease inhibitor, all samples were multiplied by factor 1.040/0.50. Calculation of total released leucine equivalents was performed from released amino termini reacting with OPA reagent and analysed spectrophotometrically (Avila Ruiz et al., 2016). The measured absorbance was correlated to a leucine standard curve as leucine equivalents (µmol/g dry weight (dw)), from which relative digestibility of proteins was calculated in respect to amino acid content of the sample as follows:

$$\text{Digestibility of protein}_{(\text{mol-}\%)} = \frac{(\text{Digested}_{\text{Leu eq t/min}} - \text{Oral}_{\text{Leu eq,Initial}})_{(\text{mg/g dw})} * 100\%}{\text{MW}_{\text{Leu(mg/mmol)}} * \text{AA}_{\text{Tot (mg/g dw)}}}$$

in which

$\text{Digested}_{\text{Leu eq, t/min}}$: Released leucine equivalent after gastric or duodenal digestion at each time point (0–120 min).
$\text{Oral}_{\text{Leu eq}}$: Initial released leucine equivalent after oral digestion (2 min).
MW_{Leu}: Molecular weight of leucine: 131.2 mg/mmol.
AA_{Tot}: Total amino acids, which were analysed using the method described above (Section 2.7) and expressed as mg/g dw of cells and calculated as a sum of individual amino acids on molar basis. (See supplementary material Table S1 for calculations).

Results are expressed as averages of the three measurements and standard deviations are visualized as error bars (Fig. 2). The extent of protein digestion is expressed similarly in the end of the duodenal stage (120 min) in the Table S1.

2.11. Sensory characteristics

The descriptive panel consisted of ten trained assessors with proven skills. All sensory work of the plant cells (both as fresh and freeze-dried) was carried out at the sensory laboratory of VTT Technical Research Centre of Finland Ltd., which fulfils the requirements of the ISO standards (ISO 6658, 2017 and ISO 8589, 2007). All assessors on the internal sensory panel have passed the basic taste test, the odour test and the colour vision test. They have been trained in sensory methods at numerous sessions over several years, and their evaluation ability is routinely checked. The panel was particularly familiarized with the sensory descriptors and the attribute intensities of various plant-based materials, including berries, in several sessions prior the evaluations. The method in sensory profiling was descriptive analysis (Lawless & Heymann, 2010). The attributes were carefully defined and described verbally, and the ends of the intensity scales of the attributes were anchored verbally, the evaluated attributes being fresh odour, berry-like odour, sandy mouthfeel, coarse mouthfeel, fresh flavour, berry-like flavour, bitter and astringent. The attribute intensities (0 − 10) were rated on continuous graphical intensity scales, verbally anchored from both ends, where 0 = attribute not existing, 10 = attribute very clear (Supplementary Table S2). The samples were coded with three-digit numbers and served to the assessors in random order in two replicate sessions. The scores were recorded and collected using a computerized Compusense Five data system, Ver. 5.4 (Compusense, Guelph, Canada). The means of the raw data obtained from both sensory sessions were calculated. The significance of each descriptive attribute in discriminating between the samples was analysed using analysis of variance (ANOVA) and Tukey's honestly significant difference test (significance of differences at $p < 0.05$). A two-way ANOVA was applied for the samples using the Compusense software, and a two-way ANOVA was applied as the general linear model (GLM) procedure for the samples by using the IBM SPSS Statistics software, Ver. 22 (IBM Corporation, New York, USA). ANOVA was used to test statistical differences in sensory attributes between the samples, and the statistical difference between the two sessions and the assessors. When the difference in ANOVA among the samples was statistically significant ($p < 0.05$), pairwise comparisons of these samples were analysed using Tukey's test. The intensities of the sensory attributes and areas of the volatile compounds of the plant cells were related statistically by PLS (partial least squares) regression using an Unscrambler software package, Ver. 10.4 (Camo Software AS). Both the sensory and analytical data were standardized before the multivariate analysis by Unscrambler. The model was validated by cross-validation. PLS regression is specifically designed to determine relationships existing between blocks of dependent (Y sensory) and independent (X instrumental) variables by seeking underlying factors common to both sets of variables.

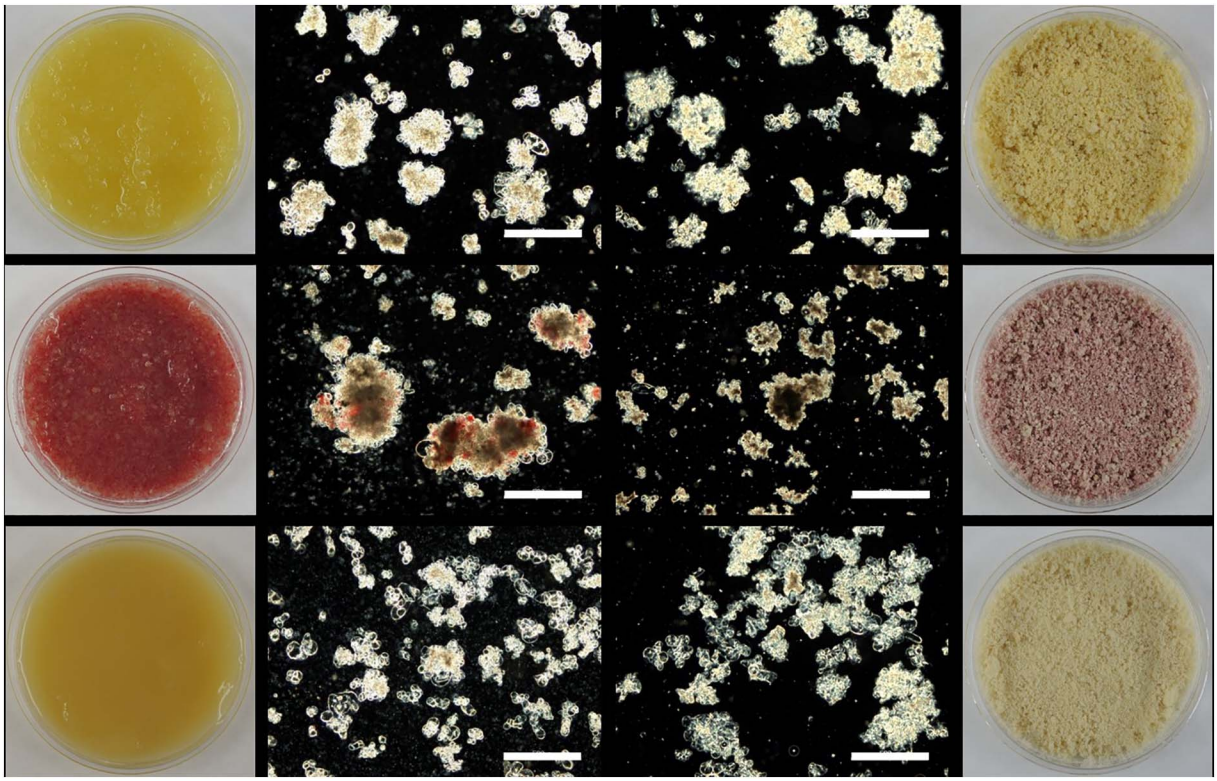

Fig. 1. Photographs of fresh cell suspensions and freeze-dried cell powders and phase contrast microscopy images of the same material suspended in water (scale bar 500 μm).

3. Results

3.1. Appearance of plant cell materials

Fresh cells from suspensions were brightly coloured as shown in Fig. 1. Lingonberry cells were red and cloudberry and stoneberry cells yellowish. The colours of the freeze-dried powdered materials were less intense. The measured colour values (L*, a* and b*) are presented in Table 1.

Fresh cells had a rather coarse structure. Stoneberry cells appeared less coarse than the other ones. Phase contrast microscopy (Fig. 1) revealed that the suspensions consisted of large cell clusters. The cluster size was clearly larger for lingonberry and cloudberry cultures than for stoneberry. The largest cell clusters were > 500 μm in diameter (e.g. in lingonberry cultures). In the micrographs of the freeze-dried material (Fig. 1) cells appeared less intact than in the fresh materials. The freeze-drying process did not seem to reduce the particle size of the material, as large cell clusters were still visible after suspending the material in water.

3.2. Dry matter content

The dry matter contents of the filtered cells varied considerably. Lingonberry cells exhibited the highest percentage (4.1 ± 0.1%) followed by cloudberry (3.1 ± 0.1%), and stoneberry (2.8 ± 0.0%).

Table 1
Colour of fresh cell suspensions and freeze-dried cell powders. The values are expressed as coordinates in the CIE Lab colour space (average ± SD).

	L*	a*	b*
Cloudberry, fresh	45.5 ± 0.3	−4.1 ± 0.1	13.6 ± 0.3
Lingonberry, fresh	36.3 ± 0.5	14.4 ± 0.6	3.1 ± 0.2
Stoneberry, fresh	44.6 ± 0.4	−5.2 ± 0.1	18.9 ± 0.4
Cloudberry, freeze-dried	72.9 ± 0.7	−2.5 ± 0.2	31.0 ± 0.4
Lingonberry, freeze-dried	59.6 ± 0.3	12.5 ± 0.3	7.5 ± 0.1
Stoneberry, freeze-dried	76.1 ± 0.8	−0.7 ± 0.2	23.4 ± 0.4

3.3. Organic elemental analysis

Elemental analysis including carbon, hydrogen, nitrogen and sulphur did not reveal any major differences between the PCC samples (Table 2). The major element in all samples was carbon (452.8–460.6 mg/g dw). Cloudberry cells contained more sulphur (8.5 mg/g) and nitrogen (72 mg/g) than the other two samples (1.2–3.5 mg/g sulphur, 38–42 mg/g nitrogen). Hydrogen content was rather similar in all three PCC samples (58.5–61.6 mg/g).

3.4. Carbohydrate, dietary fibre and sugar composition

Starch content of the samples ranged between 0.3 and 1.3%: cloudberry cells had the lowest and stoneberry cells the highest starch content (Table 3). The total free sugar content varied between 17.6 and 33.6%, stoneberry cell sample accumulated the lowest and cloudberry the highest amount of free sugars. The most abundant sugars in cloudberry and stoneberry cells were glucose, fructose and sucrose, whereas in lingonberry cells the quantitative order of sugars was fructose, glucose and sucrose. The content of xylose, arabinose, galactose, mannose and rhamnose were clearly higher after the acid hydrolysis (data on the total sugar composition in Table 3), indicating that they

Table 2
Elemental composition of PCC samples. The values are expressed as mg/g dw ± SD (n = 3).

	Cloudberry	Lingonberry	Stoneberry
Nitrogen [mg/g] dw	72.0 ± 0.8	38.0 ± 0.4	42.0 ± 0.5
Carbon [mg/g] dw	458.0 ± 0.3	460.6 ± 0.3	452.8 ± 0.3
Hydrogen [mg/g] dw	61.6 ± 0.2	58.5 ± 0.2	58.7 ± 0.2
Sulphur [mg/g] dw	8.5 ± 1.4	3.5 ± 0.6	1.2 ± 0.2

Table 3
Starch, dietary fibre (DF, including insoluble (ISDF) and soluble (SDF) dietary fibre, and acid insoluble and soluble material) and sugar composition of cloudberry, lingonberry and stoneberry cell cultures. The results are expressed as % or mg/g dw ± SD (n = 3).

	Cloudberry	Lingonberry	Stoneberry
Starch [%]	0.5 ± 0.0	0.3 ± 0.0	1.3 ± 0.1
DF total [%] (sum ISDF + SDF)	36.7	34.5	21.2
DF insoluble [%]	31.9 ± 1.3	30.0 ± 0.2	18.3 ± 0.2
DF soluble [%]	4.8 ± 1.2	4.5 ± 0.5	2.8 ± 0.6
Acid insoluble material [%]	3.5 ± 0.3	17.3 ± 1.4	2.1 ± 0.2
Acid soluble material [%]	7.6 ± 0.6	5 ± 0.4	3.8 ± 0.3
Free sugars [%] (Sum of Glu, Xyl, Ara, Gal, Man, Rha, Fru, Suc)	33.6	23.3	17.6
Free sugar composition [mg/g]			
Glucose	124.5 ± 2.8	53.9 ± 2.7	153.4 ± 1.7
Xylose	< 0.4	0.9 ± 0.0	< 0.4
Arabinose	< 0.4	< 0.4	< 0.4
Galactose	1.3 ± 0.0	0.8 ± 0.0	1.5 ± 0.1
Mannose	1.2 ± 0.0	< 0.4	0.4 ± 0.0
Rhamnose	< 0.4	< 0.4	< 0.4
Fructose	110.4 ± 3.3	100.0 ± 2.8	21.0 ± 0.2
Sucrose	72.5 ± 4.8	16.3 ± 0.4	1.65 ± 0.2
Total sugar composition [mg/g]			
Glucose[a]	142.2 ± 2.1	153.2 ± 2.3	268.3 ± 4.0
Xylose	11.9 ± 0.2	11.4 ± 0.2	6.5 ± 0.1
Arabinose	29.5 ± 0.4	19.9 ± 0.3	6.7 ± 0.1
Galactose	21.8 ± 0.3	27.6 ± 0.4	14.8 ± 0.2
Mannose	5.2 ± 0.1	3.3 ± 0.0	2.4 ± 0.0
Rhamnose	4.9 ± 0.1	3.4 ± 0.1	2.3 ± 0.0
Fructose[b]	nd	nd	nd

[a] Contains also glucose from sucrose (sucrose degraded in the acid hydrolysis used in the analysis).
[b] Fructose was not detectable by the method used.

are mostly present in bound form in the PCC samples. The total sugar composition of the plant cell samples analysed after acid hydrolysis is only indicative, since fructose was not detectable by the method used.

Total dietary fibre (DF) content of the PCC samples varied between 21.2 and 36.7% (stoneberry cells contained the lowest amount, cloudberry cells the highest) (Table 3). Insoluble DF (ranging from 18.3 to 31.9%) was clearly dominating in all of the samples. The acid insoluble material content was clearly highest, i.e. 17.3%, in lingonberry cell samples, whereas in the cloudberry and stoneberry cells the corresponding value was 3.5 and 2.1%, respectively. Acid soluble material content of the samples varied between 3.8 and 7.6%.

3.5. Amino acid composition

Amino acid composition was analysed to derive the protein content and the amino acid profile of the PCC samples (Table 4). In line with the elemental analysis results, cloudberry cells had the highest (18.9%) and lingonberry cells the lowest (13.7%) protein content calculated based on the amino acid contents. For all the samples, glutamic acid was the most abundant amino acid whereas tryptophan accumulated the least. Cloudberry cells contained the highest amount of all amino acids except tryptophan, which was most abundant in lingonberry cells. On the other hand, lingonberry cells produced the lowest amount of all amino acids except cysteine, methionine and tryptophan, which were least abundant in stoneberry cells. There were, however, clear differences in the ratios of the amino acids in the cells of the different species. For example aspartic acid, alanine and arginine had larger ratios in cloudberry and stoneberry than in lingonberry cells. On the other hand, lingonberry cells had the highest ratios of lysine, leucine, tryptophan, cysteine and methionine out of the three studied species.

3.6. Fatty acids and sterols

PCCs contained fat from 16.3 to 22.9 mg/g freeze-dried material corresponding to a relative proportion of 1.6–2.3% of dw, which is well

Table 4
Amino acid composition of PCC samples. The results are expressed as mg/g dw ± SD (n = 3). The calculated sum of the amino acids is considered as the total protein content of the sample.

Amino acid content [mg/g] dw	Cloudberry	Lingonberry	Stoneberry
His	5.0 ± 0.2	3.4 ± 0.5	4.5 ± 0.2
Ser	13.2 ± 0.2	9.9 ± 0.4	12.0 ± 0.8
Arg	13.6 ± 0.6	8.4 ± 0,6	11.3 ± 0,7
Gly	8.5 ± 0.1	6.5 ± 0,2	7.3 ± 0,4
Asp	22.5 ± 0.5	13.7 ± 0,5	17.2 ± 0,8
Glu	22.8 ± 0.6	18.4 ± 0,5	21.9 ± 1,4
Thr	8.8 ± 0.4	6.0 ± 0.7	7.0 ± 0.7
Ala	12.1 ± 0.2	7.4 ± 0.3	9.7 ± 0.5
Pro	7.9 ± 0.3	6.3 ± 0.5	6.3 ± 0.5
Cys	3.0 ± 0.1	2.8 ± 0.3	2.7 ± 0.2
Lys	15.3 ± 0.4	11.8 ± 0.6	12.2 ± 0.3
Tyr	5.4 ± 0.1	4.1 ± 0.3	4.3 ± 0.3
Met	4.9 ± 0.2	3.9 ± 0.3	3.7 ± 0.3
Val	10.4 ± 0.1	7.4 ± 0.2	8.3 ± 0.6
Ile	9.4 ± 0.4	6.8 ± 0.6	7.6 ± 0.6
Leu	15.2 ± 0.2	11.7 ± 0.3	12.1 ± 0.6
Phe	9.3 ± 0.6	6.6 ± 0.5	7.3 ± 0.6
Trp	1.4 ± 0.0	1.5 ± 0.1	1.4 ± 0.0
SUM as %	18.9	13.7	15.7

Table 5
Lipid compounds in berry cells. The results are expressed as mg/g dw ± SD (n = 3). Abbreviations: SaFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FFA, free fatty acids.

Lipid	Cloudberry	Lingonberry	Stoneberry
Class/compound	[mg/g] dw	[mg/g] dw	[mg/g] dw
SaFA	6.68 ± 0.31	4.95 ± 0.23	4.45 ± 0.21
MUFA	1.49 ± 0.04	1.01 ± 0.03	0.98 ± 0.03
PUFA	11.80 ± 0.59	7.03 ± 0.35	8.35 ± 0.42
FFA	0.63 ± 0.03	0.49 ± 0.03	0.79 ± 0.04
Campesterol	0.05 ± 0.01	0.16 ± 0.01	0.03 ± 0.00
Stigmasterol	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
β-Sitosterol	1.78 ± 0.12	0.86 ± 0.09	0.87 ± 0.04
Other Sterols	0.39 ± 0.03	1.25 ± 0.20	0.73 ± 0.14
Squalene	0.05 ± 0.01	0.28 ± 0.04	0.06 ± 0.00
Oleanolic acid	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
Amyrins (α + β)	nd	0.47 ± 0.11	nd

nd, not detected.

in line with earlier larger scale cultivations of cloudberry cells (Nohynek et al., 2014). The investigated PCCs are rich sources of polyunsaturated fatty acids (Table 5) including both essential fatty acids, linoleic (LA) and α-linolenic acids (LLA). The LA/LLA ratio was 0.7 in lingonberry cells and 1.1 and 1.2 in cloudberry and stoneberry cells, respectively. Among saturated fatty acids, palmitic (C16:0), stearic (C18:0) and arachidic (C20:0) acids were most abundant. The highest fat content in cloudberry cells was reflected at the level of β-sitosterol, too. In lingonberry cells, however, the total content of other sterols or sterol type compounds as well as squalene and the characteristic terpenoids, amyrins, were highest. In contrast, α-tocopherol level was lowest in lingonberry cells.

3.7. Volatiles

When considering the peak areas of volatile compounds adsorbed and released from the fibre in HS-SPME analyses, alcohols, hydrocarbons and aldehydes were the major groups (Supplementary Fig. S1). Among numerous compounds, pentanol, hexanol, octanol, dodecane and hexanal were consistently found in all PCC samples. Several studies have shown that the aroma intensity is decreased after lyophilization while the volatile compound profile is not affected. Re-hydration of freeze-dried samples can recover the aroma intensity (Palacios,

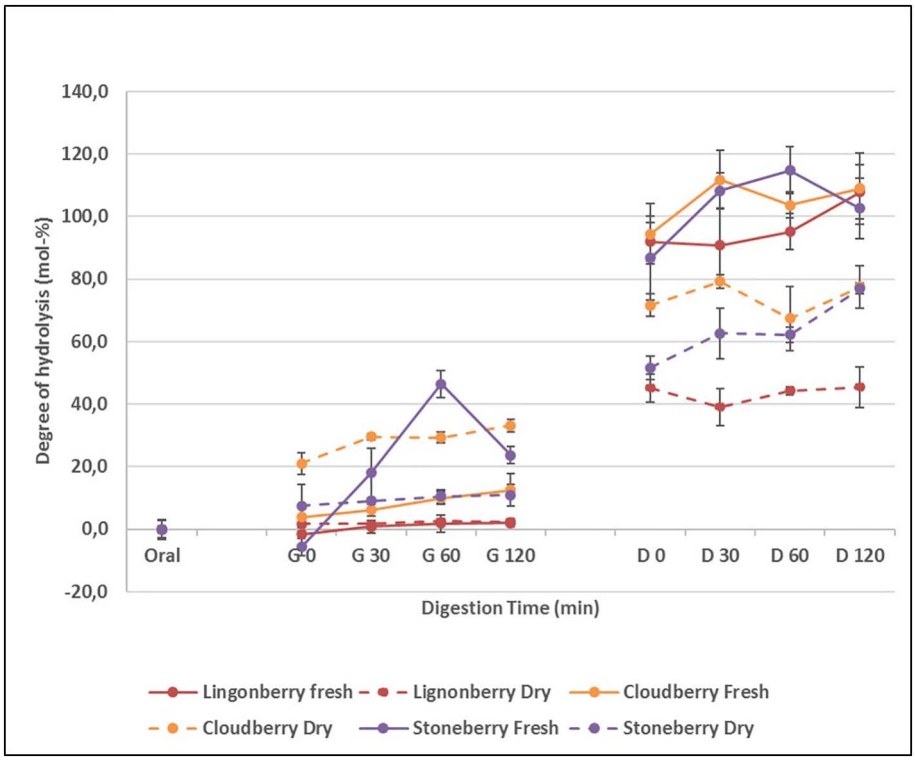

Fig. 2. In vitro protein digestion of fresh cell suspensions and freeze-dried cell powders. The results (averages of triplicate measurements with error bars indicating standard deviation) are expressed as degree of protein hydrolysis (mol-%) in respect to total amino acid content. Oral: Oral digestion (2 min); G: Gastric digestion (0–120 min); D: duodenal digestion (0–120 min).

Guillamón, García-Lafuente, & Villares, 2014), but here the volatile compound intensities and profiles were similar in both dry and re-hydrated cells.

3.8. Digestibility of proteins

In vitro protein digestion assay showed relative digestibility of proteins in respect to the total amino acid content of the sample. When proteins are hydrolysed to peptides and amino acids, amino termini calculated as leucine equivalents increase. The relative digestibility of fresh and freeze-dried PCC samples depended on the in vitro digestion phase and the species (Fig. 2). At the acidic gastric stage, fresh and dry lingonberry cells did not release much amino acids (2.0 ± 1.4 and 2.5 ± 0.3 mol-%, respectively) in the end, whereas both fresh and dried cloudberry cells (12.6 ± 5.1 and to 33.1 ± 2.0 mol-%) and fresh and dry stoneberry cells (23.7 ± 2.8 and 10.9 ± 3.5 mol-%, respectively) were clearly more digested.

At the end of the duodenal stage protein digestion of fresh PCCs was completed, because the degree of hydrolysis in respect to amino acid content reached the maximum extent (108 ± 9, 109 ± 11 and 103 ± 10 mol-% for lingonberry, cloudberry and stoneberry, respectively). The final levels of protein digestibility of the dry PCCs cells were lower (45 ± 6, 77 ± 7 and 77 ± 2 mol-% for lingonberry, cloudberry and stoneberry cells, respectively) (See Supplementary Table S3). Standard deviations of all duodenal stage measurements were slightly higher than those of the gastric stage.

3.9. Sensory profiling of plant cells

The plant cell samples deviated significantly ($p < 0.001$) from each other regarding all evaluated sensory attributes (Table 6). Fresh odour was significantly higher in the fresh cell materials than in the dried samples. Berry-like odour was slightly more intense in fresh samples, but only the berry-like odour of lingonberry cell samples was significantly more intense than the dried samples. In the corresponding flavours, the differences between fresh and dried samples were not straight forward. Regarding the berry-like flavour intensity, the cloudberry and stoneberry cell samples were slightly higher in the dried samples, which is opposite to the odour results where the berry-odour intensity was higher in fresh samples. Freeze-dried lingonberry cells were very bitter and astringent, and generally, the drying seemed to increase the intensity of bitterness and astringency. When the mouthfeel was evaluated, fresh samples were significantly more sandy and coarse in the mouth, when compared to the corresponding dried berry cell samples. Among the fresh PCCs, stoneberry was perceived less coarse than cloudberry and lingonberry, which is in accordance with the smaller cell cluster size of stoneberry as observed by microscopy (Fig. 1).

The relation between the sensory profiles and volatile compounds of the plant cells was revealed by statistical multivariate analysis i.e. PLS regression (Fig. 3). Volatile compounds were determined either from freeze-dried and from re-hydrated freeze-dried cell samples. The sensory attributes of the plant cells were explained by the detected volatile compounds. The closer the volatile compounds and the sensory descriptors were located to each other on the 2-dimensional map, the more they were correlating with each other. The volatile compounds explained altogether 66% (PC1 15% and PC2 51%) of the variation between the samples, and perceived sensory attributes 91% in total (PC1 62% and PC2 29%) of the variation. Perceived fresh and berry-like odour and fresh flavour were closely related to fresh cloudberry cells. Among the volatile compounds 1-heptanol and hexanal, but also dodecane showed high correlation with berry-like flavour which was typical for freeze-dried cloudberry cells. Fresh stoneberry cells were related to fresh flavour and to 3-hexen-1-ol and benzyl alcohol. Fresh lingonberry cells provoked a sandy and coarse mouthfeel, and freeze-dried lingonberry cells were related again to bitterness and astringency. Freeze-dried stoneberry cells showed very different perceived characteristics, and were therefore located far from the space of the other samples (Fig. 3, arrow in the lower left corner). Fresh and freeze-dried plant cells differed in their perception, bitterness, astringency and berry-like flavour being typical only for freeze-dried samples. All volatile compounds were located close to each other, and explained mainly the perception of the berry-like flavour of plant cells.

Table 6
Sensory profiling of the fresh and freeze-dried plant cell samples on an intensity scale 0–10 (n = 2 × 10).

	Fresh odour ($p < 0.001$)	Berry-like odour ($p < 0.001$)	Sandy mouthfeel ($p < 0.001$)	Coarse mouthfeel ($p < 0.001$)	Fresh flavour ($p < 0.001$)	Berry-like flavour ($p < 0.001$)	Bitter ($p < 0.001$)	Astringent ($p < 0.001$)
Cloudberry, fresh	6.4^{a}	4.7^{b}	8.4^{a}	8.1^{a}	4.8^{a}	2.3^{c}	2.1^{c}	1.7^{b}
Lingonberry, fresh	7.3^{a}	7.2^{a}	8.8^{a}	9.1^{a}	5.2^{a}	4.2^{ab}	3.2^{bc}	2.7^{b}
Stoneberry, fresh	6.8^{a}	5.3^{b}	6.9^{b}	4.7^{b}	4.7^{a}	2.4^{c}	2.7^{bc}	2.3^{b}
Cloudberry, freeze-dried	4.9^{b}	4.2^{b}	0.6^{c}	0.7^{c}	4.9^{a}	5.3^{a}	2.3^{c}	1.8^{b}
Lingonberry, freeze-dried	4.7^{b}	4.3^{b}	1.5^{c}	1.7^{c}	3.0^{b}	3.4^{bc}	8.5^{a}	7.8^{a}
Stoneberry, freeze-dried	4.4^{b}	4.0^{b}	0.7^{c}	0.6^{c}	4.3^{ab}	4.2^{ab}	4.0^{b}	2.9^{b}

a–c: Means in each column followed by a different letter signify that the samples are statistically significantly different in respect of that variable (Tukey's HSD test; $p < 0.05$).

4. Discussion

The cell material of all three species visually resembled partially homogenized berry fruit mash or dried fruit powder, respectively. In the case of cloudberry and lingonberry the colour of the cells even resembled that of the respective fruit tissue i.e. yellow and red. Then again, stoneberry fruits are red, and the cells exhibited a yellowish tone. Plant secondary metabolites are bountifully produced in PCCs (Suvanto et al., 2017), affecting the colour of the cells although composition is highly dependent on culture conditions (Decendit & Merillon, 1996). Concerning sensory attributes it appears that the impact of processing (fresh vs. freeze-drying) is relevant but it is dependent on the plant species, too. In comparison to berry fruits the flavour intensity of the PCCs seems to be low but still fresh and berry-like. To reflect the sensory profile of cells to berry fruits, for example, the profile of lingonberry cells has similar characteristics and comprises a range of volatile compounds that are found in lingonberry fruits, too (Viljanen, Heiniö, Juvonen, Kössö, & Puupponen-Pimiä, 2014). Since no significant off-flavour or very high flavour intensity was perceived it could lead to broader usability of plant cells in food products. Among the berry species fresh lingonberry cells appear to have most fresh and berry-like odour and flavour (Table 6). However, perceived fresh and berry-like odour and fresh flavour are closely related to fresh cloudberry cells, whereas 1-heptanol, hexanal and dodecane show high correlation with berry-like flavour typical for freeze-dried cloudberry cells. Mouthfeel of food ingredients is of course important for consumer acceptance and applicability, and is especially important when the cells are consumed as such. In this respect, it is interesting to note that simple freeze-drying could alleviate the sandy and coarse sensation of fresh cells completely, and the freeze-dried berry cells in practice melted in the mouth. Presumably, the turgor and particle size of the fresh cells is responsible for this phenomenon rather than specific cell wall constituents. Regards to processability, plant cells contain high amounts of water and the dry matter contents are relatively low as compared to typical dry matter contents of berry fruits. For cloudberry and lingonberry fruits, dry matter contents of around 14% have been reported (Ekholm et al., 2007; Piironen, Syväoja, Varo, Salminen, & Koivistoinen, 1986) in contrast to about 3–4% of the cell cultures. However, when cultured cells and fruits are compared, it must be taken into account that a large proportion of the fruits is made up of, mostly indigestible, seeds and skin.

Cloudberry fruits and the respective cell cultures differ in the elemental composition. While carbon levels are quite similar, the contents of nitrogen and sulphur are enhanced in the cell culture as compared with the fruits (Parent, Parent, Hebert-Gentile, Naess, & Lapointe, 2013). This is hardly surprising since cloudberry plants grow in nutrient limited bogs whereas cell cultures are maintained under optimal conditions. From the nutritional quality point of view, the cell cultures had high contents of lipids, dietary fibre, sugar and protein, when compared to berry fruits. Interestingly, the plant cells contain high amounts of nutritionally valuable lipids that are typically located in the storage tissue i.e. the seeds of the berry fruits. For example campesterol, β-sitosterol and oleanolic acid concentrations (3.91 µg/g dw, 11.87 µg/g dw, 6.67 µg/g dw) reported from lingonberry fruits including seeds (Klavins, Klavina, Huna, & Klavins, 2015) are orders of magnitude lower than the values recorded here from lingonberry cells (0.16 mg/g dw, 0.86 mg/g dw, 0.02 mg/g dw; Table 5). Other compounds such as for example squalene are present in a similar range in the fruit (2.04 µg/g dw vs. 0.28 mg/g dw; Table 5). Total dietary fibre content in cultured cells as compared to fruits is very high, too. For example, cloudberry fruits with seeds contain 6–8% fw dietary fibre (Jaakkola, Korpelainen, Hoppulab, & Virtanena, 2012) while cloudberry cells contain almost

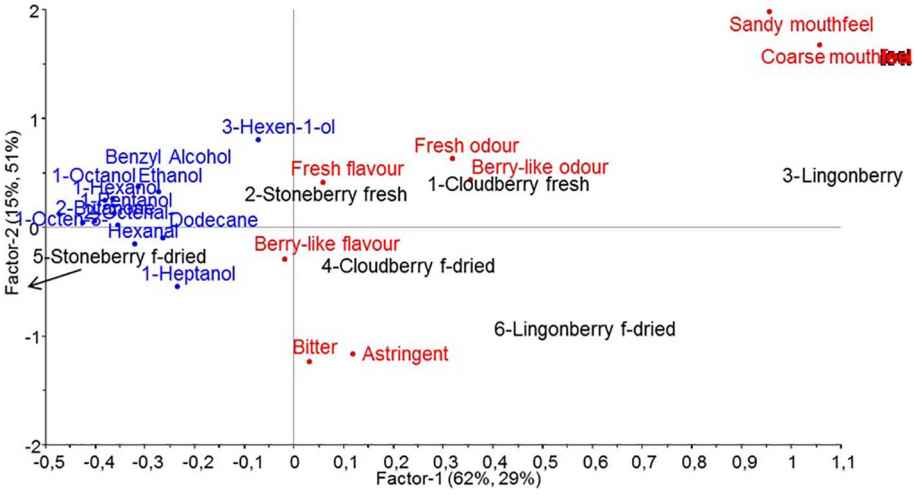

Fig. 3. PLS regression plot of sensory profiles (in red) and volatile compounds (in blue) of the plant cells. The arrow indicates that the freeze-dried stoneberry sample was located far from the space of the other samples. Volatile compounds are partly overlapping because they are rather highly related and thus located close to each other on the 2-dimensional PLS regression plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

37% dw (Table 3). Interestingly, both the amount of free sugar and the ratios of the various sugars varied a lot between the three investigated species although all cultures were grown in media with equal sucrose concentrations (3%). The ratios are different from those in the corresponding fruits, too. Lingonberry fruits, for example, contain more glucose than fructose and only very little sucrose i.e. 38 g/kg fw, 29 g/kg fw fructose and 4 g/kg fw, respectively (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012) while the cell cultures contain almost the double amount of fructose than glucose (100 mg/g dw versus 54 mg/g dw) and 16 mg/g dw sucrose (Table 3).

In terms of total protein contents the cultured cells contain 14–19% protein dw (Table 4) and are therefore on par with macroalgae (15–23% dw) widely considered as "superfood" (Wells et al., 2016). In comparison, cloudberry fruits, again with seeds, only contain on average 16 g/kg proteins based on fresh weight (Jaakkola et al., 2012) i.e. about 8% dw. Regarding nutritionally essential amino acids (histidine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine and tryptophan) all the samples showed balanced amino acid profiles and the values (mg/g protein) exceeded the amino acid requirements for adults determined by WHO (2007). The contents of essential amino acids were close to or higher than those reported for soy protein isolates and concentrates (Han, Chee, & Cho, 2015; Hughes, Ryan, Mukherjea, & Schasteen, 2011). Therefore, it appears that despite the differences in the ratios of the amino acids in the investigated species all samples are nutritionally relevant. However, to determine the nutritional value of proteins their digestibility and bioavailability in the intestinal tract must be examined. Due to the novelty of the topic, reference literature on nutritional properties of cell cultures is absent. Berry fruits on the other hand do not qualify as reference either since their protein content is much lower and mostly located in the seeds. The observations of the current digestibility study offer first insights and subsequent research is needed to identify causalities. Various plant secondary metabolites and especially phenolics can interfere with protein digestibility by influencing protease activity and protein substrate accessibility (Cirkovic Velickovic & Stanic-Vucinic, 2017). Cell cultures including the species studied here contain tannins and other polyphenols that are known to exert health-promoting effects (Gerlach, 2016) but accumulate relative low amounts (Suvanto et al., 2017). Strikingly, the least digestible samples not releasing any leucine equivalents at all in acidic gastric conditions, lingonberry cells, exhibited the highest acid insoluble material content. The analytical method employed here is the same as used for wood to detect acid insoluble lignin, which is a phenolic polymer precipitating in acidic conditions and is soluble in alkaline conditions (Wang & Chen, 2013). However, in berry fruits such as bilberry, the fraction of acid insoluble material is mainly derived from the waxy skin, and not lignin (Aura et al., 2015). Regardless of the chemistry of the acid insoluble material in the cell cultures, it is possible that proteins precipitate on the surface of acid insoluble material under acid conditions, which may lower their digestibility. The change from acidic to neutral duodenal conditions may cause partial solubilisation of the fresh cell matrix and increased susceptibility of proteins to digestive proteases, and finally, allows complete digestion.

Apparently drying of the cell matrix strongly affected the release of leucine equivalents after the duodenal phase in relation to the total amino acids. It is possible that in dried cell cultures protein was tightly attached to the insoluble dietary fibre matrix and protein was only slowly degraded. This is supported by results of dried stoneberry cells, which had the lowest DF and acid insoluble material contents and highest protein digestibility rate both in gastric and duodenal stages. Therefore, it appears that DF, acid insoluble material contents and drying constitute important factors affecting the nutritional value of the berry cells.

5. Conclusions

The current study validates the great potential of plant cell cultures for food purposes. All tested cultures exhibit colours resembling the corresponding fresh berries in their visual appearance and mild but fresh and berry-like sensory characteristics. Nutritionally valuable compounds are produced in qualitative ratios and quantitative amounts that are comparable or better than reference material such as fruits even though the cultures were not specifically optimised for the purpose. Simple processing such as freeze-drying was found to alter sensory and digestibility properties of PCCs and thus opens up multiple industrial applications. A challenge for the establishment of plant cells for food is the regulatory hurdle. Safety assessments are generally required to prevent unwanted metabolites (Murthy, Georgiev, Park, Dandin, & Paek, 2015). Prior to commercialisation, the material must be registered via applicable authorisation procedures. In Europe, the Novel Food Regulation (EU) 2015/2283 is applicable (EFSA NDA Panel, 2016). In order to provide basic information to support the regulatory path, further studies on the production, composition, ADME (absorption, distribution, metabolism, excretion), allergenicity and toxicity are needed.

Acknowledgements

The excellent technical assistance of Airi Hyrkäs, Anna-Liisa Ruskeepää, Natalia Maiorova, Joonas Simonen, Siv Matomaa, Leila Kostamo in the analysis and cultivation of PCCs are acknowledged.

Funding: This research did not receive any specific grant from external funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.02.045.

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