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Ritala, Anneli; Heiniö, Raija-liisa; Häkkinen, Suvi T.; Lille, Martina; Hyytiäinen-Pabst, Tiina; Rischer, Heiko

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Tailoring sensory properties of plant cell cultures for food use

Anneli Ritala *, Raija-Liisa Heiniö, Suvi T. Häkkinen, Martina Lille, Tiina Hyytiäinen-Pabst, Heiko Rischer

VTT Technical Research Centre of Finland Ltd, P.O.Box 1000, FI-02044 VTT, Espoo, Finland

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ABSTRACT

The nutritional value of Rowan (*Sorbus aucuparia* L.) and Arctic bramble (*Rubus arcticus* L.) plant cell cultures in terms of protein and dietary fibre contents is very good, ~ 18–22% and ~ 28–29% on dry matter basis, respectively. The aim of this study was to evaluate various processing methods and formulation to modulate sensory profiles of these plant cell cultures for food purposes. For fresh unprocessed plant cell cultures, treatment with sugar or sugar in combination with citric acid significantly improved the mouthfeel and flavour. The sugar and sugar + citric acid treated plant cell culture samples were perceived more moist, softer, less sandy and they had a less coarse mouthfeel when compared to untreated plant cell cultures. Freeze-drying produced sweet, intense, berry-like flavour and resulted in most promising sensory attributes for the studied plant cell cultures. When freeze-dried Rowan plant cell culture was further processed, the most balanced sweetness/sourness ratio was reached by using 9.5 % (w/w) sucrose and 0.1 % (w/w) citric acid or 4.8 % w/w fructose and 0.1 % w/w citric acid. We conclude that formulation and processing can greatly improve the performance of plant cell cultures for food use.

1. Introduction

Cellular agriculture, i.e. the production of agricultural commodities from cell cultures of microbes, animals and plants is currently experiencing widespread interest (Rischer et al., 2020)(Ercili-Cura & Barth, 2020). The technology holds great promise when tackling the grand challenges related to food production ranging from ethics to sustainability extensively reviewed elsewhere (Barzee et al., 2022).

Throughout farming history, the efficiency of plant production has constantly increased by breeding and cultivation practices. The green revolution facilitated by plant biotechnology accounts for a significant step up (Pingali, 2012). Nowadays, novel greenhouse technologies and vertical farming enable highly controlled food production in urban areas closer to the consumers (SharathKumar et al., 2020). Plant cell cultures as food are considered a consequent extension of this concept because not only parts or fractions of the organism are harvested and used, but rather the whole biomass leaving less waste. Scalability is based on solid commercial technology established for pharmaceutical and cosmetics products (Gubser et al., 2021). The fully contained production under aseptic conditions eliminates various problematic issues, such as food contamination with environmental pollutants and it is seasonally and geographically independent. However, cell cultures require technical process optimisation on a case-by-case basis in order to reach economic profitability and increased sustainability (Kobayashi et al., 2022). Cell cultures are prone to genetic drift when continuously maintained (Pucker et al., 2019), which is why robust industrial production involves cryopreservation to maintain the original character of the cell lines (Schmale et al., 2006). Again, such protocols are species or even cultivar specific and therefore establishment requires additional efforts in the development of commercial scenarios. Another obstacle arises from the regulatory approval requirements for Novel Foods (Murthy et al., 2015), which is based on data concerning chemical composition and toxicology. Indeed, safety assessment of novel foods such as plant cell cultures is a key step when bringing them to market. This safety assessment is a multifaceted matter and several items need to be addressed including compositional analyses, digestibility, stability, toxicity, metabolism, allergenicity to mention a few. The safety assessment methodologies and regulations are very thoroughly reviewed in (Blaauboer et al., 2016)(Angelov & Gotcheva, 2018). In case of plant cell cultures, the safety assessment data still remains scarce but is beginning to emerge in the literature (Nordlund et al., 2018)(Häkkinen et al., 2020).

The successful integration of plant cell culture biomass as a drop-in ingredient has been demonstrated for chocolate. Freeze-dried cocoa

* Corresponding author.
E-mail address: anneli.ritala@vtt.fi (A. Ritala).

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cells substituted conventionally used cocoa powder without compromising but rather enhancing the quality of the product (Eibl et al., 2018). In other applications, e.g. when the raw material is the sole or major ingredient, several sensory properties play a combined role for the attractiveness of the food and the questions are similar to those relevant for the development of plant-based meat alternatives (Webb et al., 2021). Although there is an ongoing debate in how far food processing as such affects human health (Gibney et al., 2017), there is also a need for the food industry to create a new generation of processed plant-based foods that are desirable, tasty, inexpensive, and convenient, but that are also healthy and sustainable (McClements, 2020). Currently, processing of plant cell cultures as food ingredients is a largely unexplored area with enormous scientific and application potential, as we have previously shown (Nordlund et al., 2018)(Häkkinen et al., 2020). In general, flavour and texture are the main attributes defining success of food ingredients. In this work, we provide further data on the nutritional properties, incl. protein digestibility, of two specific plant cell cultures, i.e. a tree and an herbaceous species, but focus mainly on the evaluation of various processing methods targeted at sensory profile modulation.

2. Material and methods

2.1. Plant cell culture propagation, cryopreservation and freeze-drying

Rowan (*Sorbus aucuparia* L.; VTTCC P-120009) cell culture was established as described in (Suvanto et al., 2017) and cultivated in MS medium (pH 5.8) with kinetin (0.1 mg/l) and NAA (1 mg/l). Subculturing was performed every 7th day by inoculating 20 mL into 50 mL fresh culture medium (250 mL Erlenmeyer). Arctic bramble (*Rubus arcticus* L.; VTTCC P-120088) cell culture was established as described in (Suvanto et al., 2017) and cultivated in modified MS medium (pH 4.9) with kinetin (0.1 mg/l) and NAA (1 mg/l). Subculturing was performed every 10th day by inoculating 20 mL into 50 mL fresh culture medium (250 mL Erlenmeyer). Flasks with cell suspension cultures were incubated in rotary shakers (110 rpm) in continuous light. Rowan cell biomass needed for sensory evaluations was generated in Wave bioreactors. Inoculum was prepared by cultivation in 250 mL shake flasks for 7 days. The biomass was harvested and 35 g/l were used to inoculate 10 l Wave bags with 3.5 l working volume (Cultibag RM 10 l basic, Sartorius). The cultivation parameters were as follows: air = 0,3 l/min; angle = 8; rpm = 22; T = 24 °C. Cultivation was continued for 7 days before harvesting by filtration.

Filtration was performed by using sterile Buchner funnels with sterile Miracloth filters and by washing the cells with 4 × 200 mL sterile water. Separated cells were placed in sterile sealable glass beakers. Fresh cells used for sensory evaluation were prepared at the same day. For freeze-drying, the cells were placed on sterile trays and freeze-dried in food grade facilities (Epsilon 2–25 freeze-dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) with the standard program for 72 h, until completely dry.

For cryopreservation of Rowan cell culture, a slow-freezing procedure described by Ogawa and co-workers (2012) was used. Before cryopreservation, the culture was pre-cultivated for five days. Packed cell volume (PVC) was adjusted to 30 % by centrifugation (150 *g*, 5 min) and adding the LSP solution (2 M glycerol, 0.4 M sucrose, 86.9 mM proline) in order to reach the 30 % PCV. The obtained suspension was incubated in normal growth conditions for one hour, after which the cell suspension was divided in 1.8 mL cryo-vials in 1 mL aliquots. The samples in cryovials were frozen with a controllable freezer (Planer Kryo 560–16, PLD Finland). The freezing profile was adjusted as follows: − 5 °C/min into 0 °C, hold 5 min in 0 °C, − 0.5 °C/min into −35 °C, and hold 30 min in −35 °C. After freezing, the samples were dipped into liquid nitrogen. The samples were stored in liquid nitrogen for 6 months, after which they were placed in a container filled with sterile water in + 40 °C water bath. The vials were thawed for 2 min so that all visible ice had melted and then the vials were let to stand for a couple of minutes. When the cells had settled down in the vial, the excess LSP-solution was pipetted off and discarded. The cell slurry was placed onto a solid growth medium with two filter papers. The plates were sealed with parafilm and cultivated at 24 °C in darkness. Next day, the filter paper with the cells was transferred to a fresh plate and the incubation was continued under a 16 h/8h light/dark regime.

2.2. Microbiological assays

Samples for microbiological analyses were taken from plant cell cultures which were used in sensory evaluations prior to treatments e.g. freeze-drying. Culture samples were pipetted to plates with media to cultivate a broad range of yeasts, molds and bacteria, i.e. PDA (potato dextrose agar, Sigma), LB (Luria broth, Sigma) and ½TSA (tryptic soy agar, Sigma). In addition, the absence of the most critical food pathogenic bacteria was confirmed by cultivating the plant cell culture samples on plates used for detecting aerobic heterotrophic bacteria, *Enterobacteriaceae*, *Bacillus cereus*, aerobic spore forming bacteria, coliformic bacteria, *Staphylococcus aureus, Salmonella* sp. and *Listeria* sp. In order to approve the plant cell culture samples for sensory evaluation, the limit of microbial cell count was set to < 10 CFU/g or < 1 CFU/ml.

2.3. Processing of cell cultures for sensory evaluations

Samples for four separate sensory evaluation sessions were prepared from untreated, frozen or freeze-dried cell cultures with various ingredients as shown in Table 1.

In the first and second sensory evaluation sessions the effects of sugar and/or citric acid addition on untreated Rowan and Arctic bramble cell cultures was studied. The sugar used was crystalline sucrose, which was mixed as such to the cells. Citric acid was used as a 50% (w/w) solution in water and added to the cells right after sugar addition. The samples were prepared 1 h before starting the sensory evaluation session.

In the third sensory evaluation session the effects of freezing and freeze-drying on Rowan and Arctic bramble cell cultures was studied. Fresh cells were frozen over night at –23 °C and allowed to thaw at room temperature 2 h before the sensory evaluation. The freeze-dried samples were evaluated as such (dry powder).

In the fourth sensory evaluation session, freeze-dried Rowan cell culture was used. Sucrose, fructose or xylitol were used as sweeteners and citric acid as acidifier. The sugars and the acid were dissolved in water and the solution mixed with the freeze-dried cells 1 h before the sensory evaluation.

2.4. Sensory evaluation and statistical data analysis

Sensory evaluations followed the procedure described previously (Nordlund et al., 2018). The descriptive panel consisted of 11 or 12 trained assessors with proven skills. All sensory work of the plant cells 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 of 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. In accordance with EU General Data Protection Regulation GDPR (2016/679), necessary individual information of the members of the panel is collected in the Data protection registry, and the panelists have also given their consent for this. The protocol for performing the sensory evaluation has been accepted by the Ethical Committee of VTT (**Supplementary Appendix 1**).

The method in sensory profiling was descriptive analysis (Lawless & Heymann, 2010a). The attributes were carefully defined and described

Table 1
Description of samples analysed in sensory evaluation sessions 1–4.

Sensory evaluation session	Sample code	Type of plant cell culture used				Sample ingredients (% w/w)					
		Cell culture	Untreated	Frozen	Freeze-dried	Cells	Sucrose	Fructose	Xylitol	Citric acid	Water
1	**R1**	Rowan	x			100					
1	**R2**	Rowan	x			83.3	16.7				
1	**R3**	Rowan	x			82.9	16.7			0.3	0.3
2	**A1**	Arctic bramble	x			100					
2	**A2**	Arctic bramble	x			83.3	16.7				
2	**A3**	Arctic bramble	x			82.9	16.7			0.3	0.3
3	**R4**	Rowan	x			100					
3	**R5**	Rowan		x		100					
3	**R6**	Rowan			x	100					
3	**A4**	Arctic bramble	x			100					
3	**A5**	Arctic bramble		x		100					
3	**A6**	Arctic bramble			x	100					
4	**R7**	Rowan			x	5	9.5			0.1	85.4
4	**R8**	Rowan			x	5		9.5		0.1	85.4
4	**R9**	Rowan			x	5			9.5	0.1	85.4
4	**R10**	Rowan			x	5		4.8		0.1	90.1
4	**R11**	Rowan			x	5		2.4		0.1	92.5

verbally, and the ends of the intensity scales of the attributes were anchored verbally. 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 S1**). The evaluated attributes of the Rowan and Arctic bramble cell cultures after freezing or freeze-drying were odour intensity, odour freshness, berry-like odour, moisture of texture, softness of texture, sandiness of mouthfeel, coarseness of mouthfeel, berry-like flavour, bitterness, sourness, sweetness and flavour intensity. The evaluated attributes of the Rowan cell culture after addition of sugars and citric acid were odour intensity, sandiness of mouthfeel, berry-like flavour, bitterness, sourness, sweetness, taste balance and flavour intensity. The samples were coded with three-digit numbers and served to the assessors in random order as such from odourless disposables covered by a lid accompanied with a spoon, both in two replicate sessions. The scores were recorded and collected using a computerized Compusense Five data system, Ver. 5.6 (Compusense, Guelph, Canada).

The means of the raw data obtained from the two replicate sessions of both sensory evaluations were calculated by using the Compusense software (Compusense Five data system Ver. 5.6, Canada), respectively. 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, 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. 25 (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 sensory results of the descriptive profiling were analysed by PCA (principal component analysis) by the Unscrambler software package (Unscrambler, Ver. X10.5.1, CAMO ASA).

2.5. *In vitro digestibility*

In vitro upper intestinal model for protein digestibility analyses was performed as previously described by (Minekus et al., 2014). Enzyme concentrations according to (Aura et al., 1999) were used, except two times higher bile acid concentration. *In vitro* model was performed with 5.0 g of fresh Rowan and Arctic bramble cells, 0.5 g of freeze-dried cells and 5.0 g of processed Rowan sample (5.0 % dry cells, 7.5 % fructose + 0.25 % citric acid). For preparing 5.0 g ingredient suspension, 4.5 g water was added to freeze-dried samples. Casein was used as a reference material and water as negative control (enzyme control). Samples were dosed into centrifuge tubes in three replicates. Oral phase (pH 7) was performed in a volume of 10 mL; simulated salivary fluid (SSF) 3.9 mL, 0.3 M calcium chloride solution ($CaCl_2$) 25 μL and water 1.075 mL was added to ingredient suspension. In the oral phase, samples were digested for 2 min in a 37 °C incubator with mild shaking. After oral phase, gastric phase (pH 3) was started by adding 7.5 mL of simulated gastric fluid (SGF), 5 μL 0.3 M $CaCl_2$, 0.695 mL water and 1.6 mL pepsin (2 mg/mL, P-7012, Sigma) as a digestive enzyme to oral digesta. pH was adjusted to 3 with hydrochloric acid solution (HCl). Gastric digestion (20 mL) was incubated for 120 min at + 37 °C in mild shaking. After gastric phase, duodenal phase (pH 7) was started by adding 11 mL simulated intestinal fluid (SIF), 2.5 mL porcine bile extract (75 mg/mL, B-8631, Sigma), 40 μL 0.3 M $CaCl_2$, 5.0 mL porcine pancreatin solution (18.75 mg/mL, P-3292, Sigma) and 1.31 mL water. The pH was adjusted using sodium hydroxide solution (NaOH).

Aliquots for protein digestibility analyses were drawn as follows: oral phase 2 min, gastric phase 0 min, 30 min, 60 min, 120 min and duodenal phase 0 min, 30 min, 60 min and 120 min. Enzyme activity was inhibited adding 40 μL of cOmplete protease inhibitor (04 693 132 001, Roche). Samples were stored at −20 °C until the analysis. Protein digestibility was spectrophotometrically measured from digesta as previously described by Nordlund et al. (2018). The method is based on o-phthaldialdehyde (OPA) reagent, which binds to released amino termini of digested proteins. Degree of hydrolysis was calculated from released amino termini and released leucine equivalents. Degree of hydrolysis results are shown as averages of triplicates as a function of the digestion stage and time. Standard deviations of results are indicated as error bars.

2.6. *Nutritional value*

The compostion of Rowan and Arctic bramble cell cultures was determined as described in detail previously (Nordlund et al., 2018). Shortly, carbohydrates were analysed by high-performance anion-exchange chromatography (HPAEC) with pulse amperometric detection, fibres by an enzymatic–gravimetric method, proteins by UPLC-UV and lipids by GC–Ms.

3. Results

3.1. *Propagation, cryopreservation and growth behaviour of plant cell cultures*

Fully established, homogenous and vigorously growing Rowan (*Sorbus aucuparia* L.) and Arctic bramble (*Rubus arcticus* L.) plant cell

cultures were used. Rowan cells are shown in Fig. 1. In shake flasks 200 g/l fresh weight (FW) corresponding to 9.5 g/l dry weight (DW) of cell biomass accumulated in 7 days. The growth behaviour of Rowan cell culture measured as fresh weight remained basically unaltered after cryopreservation (Fig. 1**A**). Dry weight biomass of cryopreserved cells was approximately 20 % higher at day 7 compared to non-cryopreserved cells, however the difference was decreasing at later time points (Fig. 1**B**). The appearance and growth pattern of Rowan cell culture was comparable to Arctic bramble cell culture (data not reported). In Wave bioreactor cultivations ca. 75 g/l (FW) biomass accumulated in 7 days for Rowan (corresponding to 3.6 g/l (DW)) whereas Arctic bramble, when cultivated in 2 l volume, reached ca. 100 g/l (FW) (corresponding to 6.0 g/l (DW)) in 10 days.

3.2. Sensory evaluation of plant cell cultures

For sensory evaluations, Rowan and Arctic bramble cell culture biomass was generated in a Wave bioreactor, harvested aseptically and freeze-dried in food-grade conditions. The microbiological quality of the aseptic cell cultures was evaluated and the approved material was subsequently subjected to sensory evaluations (**Supplementary Table S2**).

The effect of sucrose and citric acid addition on sensory characteristics of Rowan and Arctic bramble cell cultures were evaluated in sensory sessions 1 and 2. The samples containing sucrose and citric acid differed statistically significantly from untreated samples of the respective species in all evaluated attributes except for odour and bitterness (Table 2). The addition of sucrose or sucrose + citric acid significantly improved the mouthfeel and flavour of the plant cell cultures, resulting in more moist, softer, less sandy and less coarse texture / mouthfeel. In case of Arctic bramble cell culture, the improvement in mouthfeel was substantial (4–6-fold) whereas with Rowan cell culture a 2-fold change was observed. The sucrose and sucrose + citric acid additions caused clearly higher sweetness sensation (ca. 6-fold increase) as well as higher flavour intensity in both cell cultures when compared to untreated samples. Plant cell cultures with sucrose + citric acid were also evaluated as more sour than the other samples (Table 2).

In sensory session 3, the sensory characteristics of fresh (untreated), frozen and freeze-dried Rowan and Arctic bramble cell cultures were compared, and the PCA plot of these treated cell cultures is presented in Fig. 2**.** The two first principal components explained in total 98% of the variation (PC1 70%, PC2 28%). There were statistically significant differences between the samples in all evaluated attributes except in odour intensity and berry-like odour (**Supplementary Table S3**). An interesting finding was that the treatment of the plant cell culture material seemed to have a much more significant impact on the sensory characteristics and perception than the cell culture species: Freeze-drying produced sweet, intense, berry-like flavour, whereas moist texture was linked to frozen and melted samples, and freshRowan cell culture had a coarse and sandy texture. Thus, freeze-drying resulted in most promising sensory attributes for plant cell cultures. Freezing and melting alone did not have very crucial effects on sensory properties when compared to untreated cells, and thus it might be worth combining freezing with sugar and/or acid treatments (Fig. 2, **Supplementary Table S3**).

The effects of various sweeteners (sucrose, fructose and xylitol) together with citric acid on sensory characteristics of freeze-dried Rowan cell cultures were studied in sensory session 4. The PCA plot of this sensory evaluation is presented in Fig. 3**.** The two first principal components explained in total 98% of the variation (PC1 92%, PC2 6%). The samples deviated statistically significant in sweetness, taste balance and flavour intensity (**Supplementary Table S4**). Addition of 2.4 % w/w fructose and 0.1 % w/w citric acid to lyophilized Rowan plant cells deviated from the others, and the sample was perceived not sweet but rather sour, mild and unbalanced in flavour. The most balanced sweetness/ sourness ratio was reached by adding 9.5 % (w/w) sucrose and 0.1 % (w/w) citric acid or 4.8 % w/w fructose and 0.1 % w/w citric acid to the plant cell cultures (Fig. 3).

3.3. Nutritional value of plant cell cultures

The nutritional compositions of Rowan and Arctic bramble cell cultures are shown in **Supplementary Table S5**. Based on these figures a portion of 100 g freeze-dried cell culture would cover 19.6, 18.3 and 23.9 % of the daily value of energy, total fat and carbohydrates, respectively. Moreover, the 100 g freeze-dried proportion would correspond to 43.4 % of the daily protein need. The analysed vitamin contents

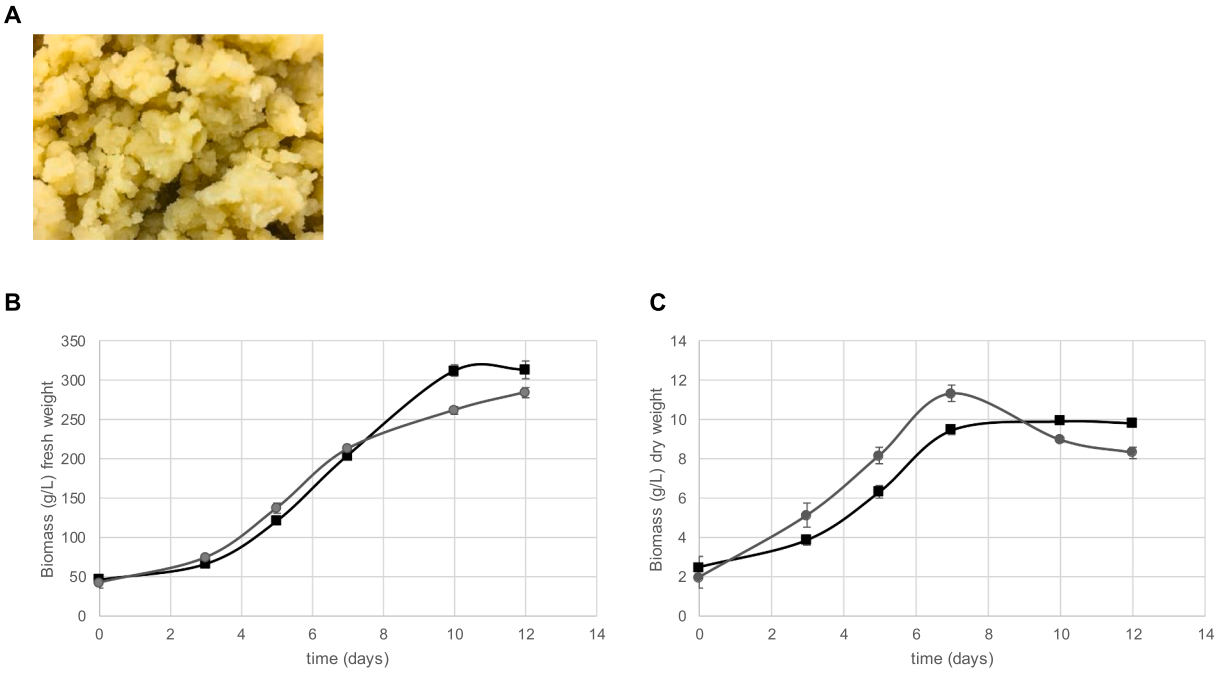

Fig. 1. Rowan (*Sorbus aucuparia* L.) cell culture harvested by filtration (A). Growth behaviour of Rowan cell culture before (square) and after (circle) cryopreservation in cultivation volume of 20 mL. Data presents the biomass mean ± stdev of three replicates. Fresh weight values shown in (B) and dry weight in (C).

Table 2
Sensory evaluation of fresh Rowan and Arctic bramble cell culture after sucrose and citric acid additions (n = 12 and 11 in the two replicate sessions). Evaluated samples were i) fresh non-treated (**R1**, **A1**), ii) fresh with 16.7 % (w/w) sucrose addition (**R2**, **A2**) and iii) fresh with 16.7 % (w/w) sucrose and 0.3 % (w/w) citric acid additions (**R3**, **A3**). Rowan and Arctic bramble samples were not cross-evaluated. 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. The values marked with different letters (a, b) indicate statistically significant differences within a column with $p < 0.05$, $p < 0.01$ or $p < 0.001$. (ns = statistically no significant differences).

Rowan cell culture												
Sample	**Odour intensity (ns)**	**Odour freshness (ns)**	**Berry-like odour (ns)**	**Moist texture ($p < 0.001$)**	**Soft texture ($p < 0.001$)**	**Sandy mouthfeel ($p < 0.05$)**	**Coarse mouthfeel ($p < 0.001$)**	**Berry-like flavour ($p < 0.001$)**	**Bitterness (ns)**	**Sourness ($p < 0.001$)**	**Sweetness ($p < 0.001$)**	**Flavour intensity ($p < 0.001$)**
R1: untreated	4.7	5.4	3.8	5.9^{b}	6.3^{b}	7.2^{a}	4.7^{a}	2.6^{b}	3.2	2.5^{b}	1.3^{b}	3.4^{b}
R2: 16.7 % sucrose	5.6	5.6	4.3	6.9^{a}	8.4^{a}	5.1^{b}	2.4^{b}	5.9^{a}	2.1	2.6^{b}	7.6^{a}	6.4^{a}
R3: 16.7 % sucrose + 0.3% citric acid	5.4	5.6	5.2	7.8^{a}	8.2^{a}	4.8^{b}	2.3^{b}	5.7^{a}	2.5	5.0^{a}	7.1^{a}	6.5^{a}
Arctic bramble cell culture												
Sample	**Odour intensity (ns)**	**Odour freshness (ns)**	**Berry-like odour (ns)**	**Moist texture ($p < 0.001$)**	**Soft texture ($p < 0.001$)**	**Sandy mouthfeel ($p < 0.001$)**	**Coarse mouthfeel ($p < 0.001$)**	**Berry-like flavour ($p < 0.001$)**	**Bitterness (ns)**	**Sourness ($p < 0.01$)**	**Sweetness ($p < 0.001$)**	**Flavour intensity ($p < 0.001$)**
A1: untreated	5.2	5.7	3.9	4.7^{b}	6.7^{b}	4.6^{a}	2.6^{a}	2.8^{b}	2.4	2.0^{b}	1.2^{b}	3.5^{b}
A2: 16.7 % sucrose	4.0	5.4	3.3	6.5^{a}	8.7^{a}	0.8^{b}	0.5^{b}	5.3^{a}	1.2	2.2^{b}	6.8^{a}	5.9^{a}
A3: 16.7 % sucrose + 0.3% citric acid	5.7	5.3	3.5	7.0^{a}	8.6^{a}	1.2^{b}	0.9^{b}	5.7^{a}	1.4	4.8^{ab}	6.2^{a}	6.5^{a}

of Rowan cell culture are minor (**Supplementary Table S5**, Table 3).

3.4. Digestibility of Rowan cell culture

In the *in vitro* digestibility study, the proteins of Rowan samples did not hydrolyze significantly in gastric stage and there was no difference between fresh, dry and processed samples. At the end of the gastric stage, the degree of hydrolysis was 8.2 ± 6.0 mol-% for fresh Rowan, 8.6 ± 0.6 mol-% for freeze-dried Rowan and 5.4 ± 5.3 mol-% for processed Rowan, respectively. Proteins were further digested in the duodenal stage, where fresh and processed Rowan samples had the highest hydrolysis rate and were completely digested (102.9 ± 5.9 mol-%. and 104.2 ± 4.4 mol-%, respectively). Protein digestion was significantly lower in freeze-dried Rowan sample at the end of the duodenal stage (68.4 ± 5.9 mol-%) (Fig. 4.).

4. Discussion

Good growth and efficient biomass production capacity are prerequisities of plant cell cultures for any application. In addition, for commercial and industrial use, the master plant cell lines need to be cryopreserved to make sure that the cell line can be recovered if the production cell line doesn't perform (Reed et al., 2001)(Schmale et al., 2006)(Häkkinen et al., 2018). Both cell lines, Rowan and Arctic bramble were successfully cryopreserved. Their biomass productivities were ca. 10 g/l/d (FW) in 7 to 10 days, i.e. rather low when compared to the very fast-growing tobacco BY-2 cell line that can reach a yield of ca. 90 g/l/d (FW) in 6 days showing the potential of plant cell culture technology (Reuter et al., 2014)(Kobayashi et al., 2022). Thus, both plant cell lines need further culture optimisation to reach economic potential in industrial food applications. However, the current scale and productivity would already suffice for small scale applications e.g. in home bioreactors (Eibl et al., 2018).

Flavour and texture are the main factors influencing sensory properties of food ingredients. Flavour is composed of odour (nasal olfaction) and taste that covers four basic oral perceptions: sweet, salty, sour, and bitter (Lawless & Heymann, 2010b). Flavour is one of the most important, although not the only factor in consumer food choice (Clark, 1998) (Nasser, 2001). Thus, improvement of flavour and texture characteristics formed the basis in our work when tailoring Rowan and Arctic plant cell cultures for food use.

In suspension cultures, plant cells typically grow in clusters. The size of the cell cluster is dependent on the plant species and culture conditions. The mouthfeel of plant cell mass is highly dependent on the size and mechanical properties of the individual cells or cell clusters. For the mechanical properties the turgor pressure within the cells has an important role (L. Wang et al., 2006). The cells can be considered as thin-walled, liquid-filled spheres, in which a turgor pressure is formed as osmotic swelling is mechanically restricted by the cell wall (C. X. Wang et al., 2004)(L. Wang et al., 2006). The magnitude of the turgor pressure can be considerable; typically 5–10 times higher than atmospheric pressure (Tomos, 2000). Therefore, fresh plant cells might be difficult to break down by oral processing.

In our previous study, cell clusters larger than 500 μm were observed for lingonberry, and the fresh cell suspension was reported to have a very coarse mouthfeel (Nordlund et al., 2018). A similar coarse and sandy mouthfeel was in the present study observed especially with the fresh Rowan cells. The coarse mouthfeel of fresh Rowan and Arctic bramble could, however, significantly be reduced by the addition of sucrose to fresh plant cells, by freezing or by freeze-drying the cells. Mixing sucrose (in solid form) with the fresh cells, resulted in release of water from the cells due to osmosis, and a subsequent loss of cell turgor pressure. During freezing, the formation of ice crystals cause damage to the cell membrane, which results in water release and collapse of the native cell structure upon thawing. All these changes are expected to "soften" the cells and thereby reduce the coarse mouthfeel.

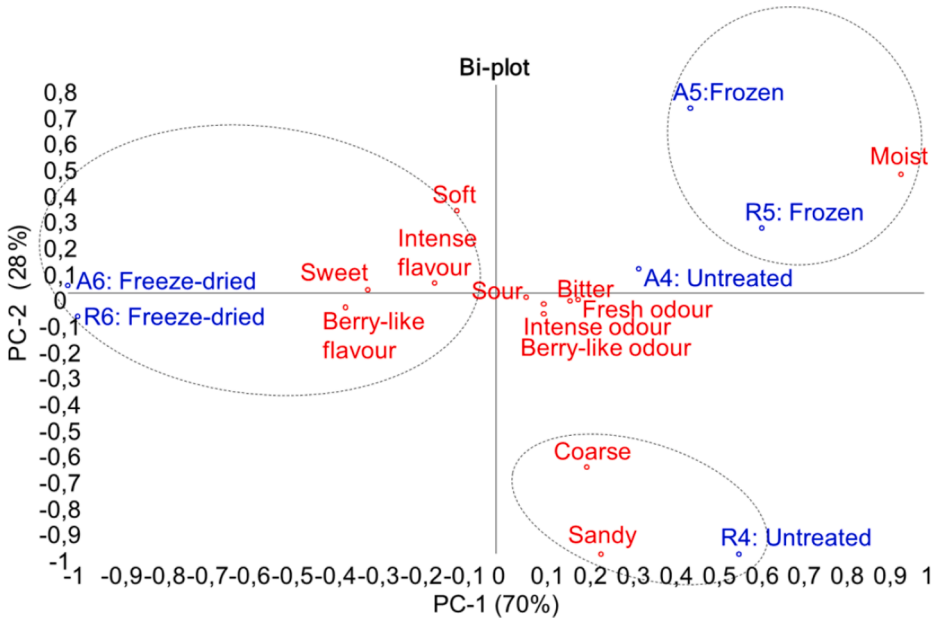

Fig. 2. PCA of the sensory evaluation outcome of the Rowan and Arctic bramble cell cultures after freezing or freeze-drying based on the data presented in **Supplementary Table S3**. Evaluated samples were **R4 & A4)** untreated; **R5 & R5**) fresh-frozen, melted and **R6 & A6**) freeze-dried.

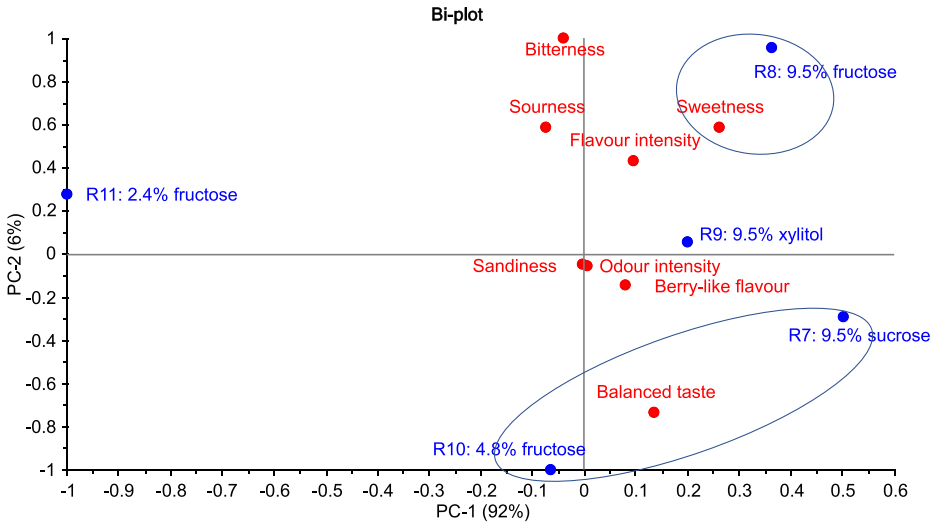

Fig. 3. Principal component analysis (PCA) of the sensory evaluation outcome of the Rowan cell culture after addition of sugars and citric acid based on the data presented in **Supplementary Table S4**. The freeze-dried Rowan cell culture was treated with **R7**) 9.5 % w/w sucrose and 0.1% w/w citric acid; **R8**) 9.5 % w/w fructose and 0.1 % w/w citric acid; **R9**) 9.5 % w/w xylitol and 0.1 % w/w citric acid; **R10**) 4.8 % w/w fructose and 0.1 % w/w citric acid; **R11**) 2.4 % w/w fructose and 0.1 % w/w citric acid. The details of treatments can be found from Table 1.

The addition of citric acid to the sucrose containing plant cell mixture did not significantly change the evaluated sensory attributes, except for sourness. Citric acid is widely used in processed fruits and vegetables to inhibit polyphenoloxidase-induced browning (Abd-Elhady, 2014), and as a preservation agent (Vipan et al., 2018). Citric acid would most likely also inhibit colour changes and microbiological spoilage of processed plant cells, but this aspect was not addressed in this study as the samples were evaluated immediately after processing.

Storage and shelf life features of food ingredients greatly influence on their usability and acceptability (Gould, 1996). Eventhough, the shelf-life was not in the focus of our current study we wanted to evaluate what kind of effect freezing and freeze-drying would have on the sensory characteristics of Rowan and Arctic bramble plant cell cultures. In the evaluations, freezing and thawing was linked to moist texture of fresh Rowan cell culture, whereas, freeze-drying produced sweet, intense, berry-like flavour particularly for the Rowan cell culture. In general, freeze-drying is a powerful way to generate food powders that in dehydrated form have extended shelf-life and superior quality. Of course it is an energy intensive and expensive technology. However, freeze-drying might have beneficial effect on energy consumption in grinding as the time required for achieving the wanted particle size distribution is reduced (Oyinloye & Yoon, 2020).

All the tested types of sweeteners (sucrose, fructose and xylitol) were perceived equally sweet when added to freeze-dried Rowan cells at a concentration of 9.5% w/w together with citric acid. No difference in sandiness perception was found between the different sweeteners. A clear drop in sweetness perception was observed with decreasing fructose concentration, but the taste balance (sweetness/sourness) was only hampered at the lowest fructose concentration of 2.4%. In our experiments, the sweetener and acid additions were made after freeze-drying, however in fruit and berry processing the additions are usually done before freeze-drying to obtain better sensory characteristics (Sette et al., 2016). Adopting the industrial order of treatments might further improve the sensory characteristics and perception of plant cell cultures.

Our data show that the nutritional value of Rowan and Arctic bramble plant cell cultures is very good. In terms of protein and dietary

Table 3
Nutritional value of the freeze-dried Rowan cell culture (see **Supplementary Table S5**). The values, determined according (Nordlund et al., 2018), shown against white background are mandatory (Regulation (EU) No 1169/2011) and against light grey are voluntary (Regulation (EU) No 1169/2011) to be indicated in a food product package.

	Unit	Per 100 g	Percent Daily Value (DV)
Energy[a]	**kcal**	**392.4**	**19.6 %**
Total fat[b]	**g**	**12.8**	**18.3 %**
of which saturates	g	4	20.0 %
of which mono-unsaturates	g	0.3	–
of which polyunsaturates	g	8.5	–
Total Carbohydrates[c]	**g**	**62.1**	**23.9 %**
of which sugars[d]	g	26.2	29.1 %
of which polyols	g	–	–
of which starch	g	6.9	–
Fibre	**g**	**29**	–
Protein	**g**	**21.7**	**43.4 %**
Salt	**g**	–	–
Vitamin E[e]	**mg**	**0.2**	–
Vitamin C[f]	**mg**	**3.4**	–

[a] Calculated based on starch, fibre, protein and fat content.
[b] Sum of saturated, mono-unsaturated and poly-unsaturated fatty acids.
[c] Sum of starch, sugars and fibre.
[d] Free sugars.
[e] Can be declared if the product contains significant amounts, at least 15 % of the nutrient reference value i.e. 1.8 mg is required.
[f] Can be declared if the product contains significant amounts, at least 15 % of the nutrient reference value i.e. 12 mg is required.

fibre content they can be indeed considered as super foods. Diets rich in dietary fibre have been shown to decrease a number of cardiovascular diseases and other common non-communicable diseases like type 2 diabetes. The dietary fibre content of both Rowan and Arctic bramble cell lines was far higher (28–29 %) than what is found in foods that are typically measured to have high fibre conetent; whole grain wheat (13 %), soy (15 %) and flaxseed (22 %) (Dhingra et al., 2012). Consuming 100 g of freeze-dried Rowan plant cell culture would easily meet the Finnish daily dietary fibre intake recommendation of 25–35 g (Mikael Fogelholm; Paula Hakala; Raija Kara, 2014). The protein content of Rowan and Arctic bramble cell cultures was 18–22 %, which is comparable to cereal protein contents: e.g. oats (21%) and wheat (22%). The methionine and lysine contents are known to be lower in plant-based proteins when compared to animal proteins and this was also true for plant cell cultures as the methionine and lysine contents were 0.4 % and 1.5–1.7 %, respectively (Gorissen et al., 2018).

Protein digestion depends on the accessibility of proteins in the sample matrix; if they are located outside the matrix (solubilized or on the surface of the berry matrix), hydrolysis with proteolytic enzymes can start already in the gastric phase, but if the proteins are bound inside the cells they won't be that easily accessible to the digestive enzymes. The digestibility of Rowan proteins in all samples was quite low at the low pH prevailing in the gastric phase. The reason for that is not known, but it is possible that the solubility of the proteins was lower in the gastric phase than in the duodenal phase. In the duodenal phase, the proteins in fresh cells were quickly fully digested. Freeze-drying clearly hampered the digestibility of the proteins. Dehydration by freeze-drying makes the plant cell wall material more rigid and compact and may even cause protein crosslinking (Lewicki, 1998). These changes are not fully reversible upon rehydration and may limit the accessibility of proteins to digestive enzymes. Interestingly, the protein digestibility in the freeze-dried material could be improved by the addition of fructose and citric acid to the rehydration media, which may have caused more damage to the cells than rehdyration in pure water. The berry matrix may be closed still in the oral phase and only change of pH from 3 to 7 causes swelling of the berry matrix and enables the release of proteins to the solution and thus make them available for the digestive enzymes in the duodenal stage. Nevertheless, the digestion is possible only if the proteins are released from the berry matrix to the solution. Difference in digestibility between fresh and freeze-dried berry cells in duodenal stage was similar to the digestibility of lingonberry, cloudberry and stoneberry as previously described (Nordlund et al., 2018).

5. Conclusions

Fresh, untreated Rowan and Arctic bramble plant cell cultures had mild flavour and rather coarse textures. Various processing treatments like addition of sugars, citric acid, and freeze-drying affected favorably sensory properties of plant cell cultures. However, *in vitro* protein digestibility was not as efficient in freeze-dried cells than in fresh cells. Thus, food technology and processing can greatly influence the performance, nutritional quality and overall acceptability of plant cell cultures

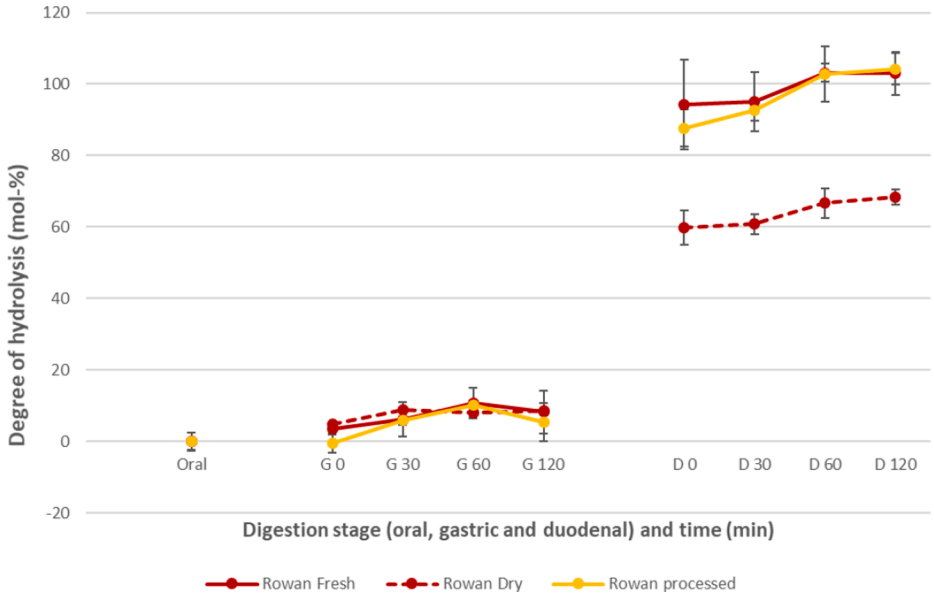

Fig. 4. Protein hydrolysis (mol-%) based on total amino acids Protein digestibility of Rowan cells evaluated with the *in vitro* upper intestinal model. The degree of protein hydrolysis is shown as a function of the digestion stage (oral, gastric (G) and duodenal (D)) and time (min). The solid line represents untreated cells, dashed line freeze-dried cells and dotted line an aqueous suspension containing 5% freeze-dried cells, 7.5 % fructose and 0.3 % citric acid (w/w).

in food applications. All plant-based raw materials need processing to be applicable as food ingredients. We have shown here that the very same food processing technologies can be applied for improving plant cell cultures for food use. Further development of the Rowan and Arctic bramble plant cell cultures is necessary prior to initiating the Novel Food application process. Particularly, the composition of the culture medium should be addressed as well as the scaling to industrial volumes. Nevertheless, the study presented here verifies the potential Rowan and Arctic bramble cell cultures hold in food use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of Competing Interest

The other authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111440.

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