Luke
NATURAL RESOURCES INSTITUTE FINLAND

Jukuri, open repository of the Natural Resources Institute Finland (Luke)

This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.

Author(s): Hannu Ahokas

Title: Staining of RNA and DNA on electrophoretic gels and in cytology with juice of Vaccinium myrtillus berries

Year: 2019

Version: Published version

Copyright: The Author(s) 2019

Rights: CC BY-NC-ND 4.0

Rights url: https://creativecommons.org/licenses/by-nc-nd/4.0/

Please cite the original version:

Ahokas H. (2019). Staining of RNA and DNA on electrophoretic gels and in cytology with juice of Vaccinium myrtillus berries. Heliyon Volume 5, Issue 10, October 2019, e02666. https://doi.org/10.1016/j.heliyon.2019.e02666.

All material supplied via *Jukuri* is protected by copyright and other intellectual property rights. Duplication or sale, in electronic or print form, of any part of the repository collections is prohibited. Making electronic or print copies of the material is permitted only for your own personal use or for educational purposes. For other purposes, this article may be used in accordance with the publisher's terms. There may be differences between this version and the publisher's version. You are advised to cite the publisher's version.

Heliyon 5 (2019) e02666

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Heliyon

Research article

Staining of RNA and DNA on electrophoretic gels and in cytology with juice of *Vaccinium myrtillus* berries

Hannu Ahokas *

MTT-Agrifood Research Finland, Biotechnology and Food Research, FI-31600, Jokioinen, Finland

ARTICLE INFO

Keywords:
Biochemistry
Molecular biology
Anthocyanin
Barley caryopse
Cytological stain
Doublediffusion test
Intercalating agent
Protein polymerization

ABSTRACT

Background: My early results of cytological chromosome staining with berry juice of blueberry or bilberry (*Vaccinium myrtillus*) was re-evaluated with staining of electrophoretic agarose and polyacrylamide gels, fractionating DNA, RNA, and proteins.

Results: Electrophoretic gels were stained with juice from berries of *V. myrtillus*, only filtered, or the diluted filtrate was mixed with acetic acid and 2-propanol. The staining starts in 2 min, with the highest intensity over the background usually appearing in about 30 min. The berry juice stains RNA, DNA, and an unidentified contaminant of molar mass 200–700 g. After differentiation of the gel background, the stained zones appear purple or black. The berry juice staining with or without acetic 2-propanol shows sharp RNA and DNA zones on gels in Vis (visible) light, and the berry juice displaces a preceding staining with ethidium bromide. A secondary staining with ethidium bromide is not able to displace the berry juice in nucleic acids. Cytological staining of sectioned mature barley (*Hordeum vulgare*) grains with *Vaccinium myrtillus* juice followed by differentiation in dilute acetic acid shows nuclei and apparent RNA storing compartments in the aleurone cells and stains suberin-containing chalazal cells of the grain crease deeply red, which keeps long. The anthocyanins faintly stain some proteins on gels. The preparation of polymers from cytochrome c and myoglobin was described.

Conclusions: The staining molecules of *V. myrtillus* anthocyanins apparently intercalate in the nucleic acids competing for the sites, which could be intercalated by ethidium bromide. The juice is suggested to have use in the exchange of intercalating toxic molecules from DNA and RNA, in the inactivation of viruses, and phytotherapy. The juice may have a use as a nontoxic stain in cytology.

1. Introduction

The blueberry (*V. myrtillus*) is a common wild berry species native to coniferous forests in the Nordic countries with a wide Eurasian distribution. The blueberry is an important berry gathered in the forests of Finland, where the mean annual production of its berries was calculated to be 168.4×10^{-6} kg [1]. Its berry production varies from year to year. Its matured, thoroughly blue berries and staining capacity are familiar to people in the area of blueberry distribution. A variation of the contents and types of anthocyanins occur within the species *V. myrtillus,* which has one of the highest contents among species with blue berries. *V. myrtillus* from Northern Europe were shown to genetically vary in their anthocyanin contents, the northern proveniences having high contents [2] also within the territory of Finland [3]. The total anthocyanin content in *V. myrtillus* berries is several times higher than that in the domesticated *V. corymbosum* on dry weight basis [4].

As a university student, I made my first cytological staining of chromosomes for light microscopy with acetic *V. myrtillus* juice in 1968. I could see plant chromosomes through the microscope, though the resolution against the stained cytoplasm was not good enough in these preparations and was perhaps due to the presence of RNA in the cytoplasm. The spread preparations of chromosomes with only little cytoplasm around might be better stained with *V. myrtillus* juice. The present study is to confirm the staining of nucleic acids, both DNA and RNA, and possibly of proteins with *V. myrtillus* juice using electrophoretic gels carrying these macromolecules. My early studies on the *Vaccinium* species also resulted in a few publications in the 1970s [5, 6, 7, 8].

In the past, blueberries have been used or tried to stain garn or cloth in black and blue colors in Finland, Estonia, and Livonia [9]. Furthermore, blueberry anthocyanins have many therapeutic effects to human beings [10, 11, 12, 13] and supposedly to animals of many berry-eating species as well. The *V. corymbosum* extract also showed antimicrobial

* Corresponding author.
E-mail address: hannu.ahokas0@saunalahti.fi.

https://doi.org/10.1016/j.heliyon.2019.e02666
Received 17 April 2019; Received in revised form 29 September 2019; Accepted 11 October 2019
2405-8440/© 2019 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

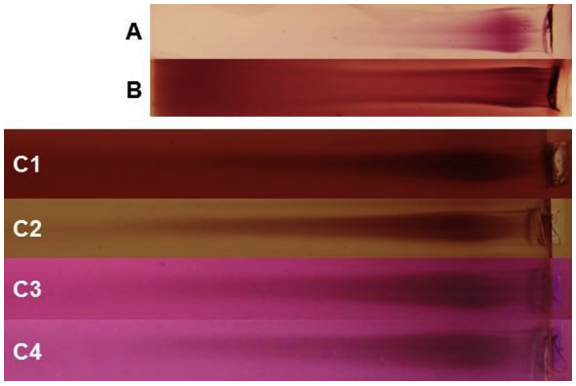

Fig. 1. *V. m.* juice stains DNA on horizontal agarose gels. Wells loaded with about 150 μg ctDNA, tracks stained in various ways, photographed with Vis or UV. – Tracks A and B. The gel cut in two and stained with different solutions, ultimately dried between Gel Drying Films after soaking in 2 % glycerol in deionized water: Vis. (A) MQ for 1 h → *V. m.* juice, AcA, 2-PrOH for 12 h 20 min → MQ for 48 h. – (B) MQ for 1 h → *V. m.* juice for 12 h 20 min → MQ for 48 h, with the DNA staining stronger than in A. – Tracks C1–C4. A single track treated sequentially. C1. MQ for 9 h 30 min → *V. m.* juice for 12 h → MQ for 12 h: Vis. – C2. MQ for 9 h 30 min → *V. m.* juice for 12 h → MQ for 15 h 30 min → EtBr for 12 h: Vis. – C3. The same as C2: UV. – C4. MQ for 9 h 30 min → *V. m.* juice for 12 h → MQ for 15 h 30 min → EtBr for 36 h: UV. Even an incubation of 36 h in EtBr does not appear to displace the *V. m.* juice molecules in DNA, while the agarose gel with EtBr turns fluorescent in UV, hence, the earlier treatment with *V. m.* juice does not quench EtBr.

activity [14]. It may be of interest, if any of the beneficial effects are regulated on the nucleic acids level. A direct interaction with DNA may also raise concern about the mutagenic or teratogenic effects of anthocyanins. On the other hand, the anthocyanins may compete with harmful molecules able to intercalate into DNA and may have a detoxifying effect as the competitor.

2. Materials and methods

2.1. Berries and staining with their juice

I collected mature, qualified berries of blueberry (*V. myrtillus*) free from berries of other species in natural forest stands in Southern Finland, froze them in zip-lock polyethene bags in 320 g (about half-liter) lots, and stored at -18 °C. Usually, 320 g of frozen berries were partly melted in a microwave oven at 360 W in a glass vial in 1 min lasting pulses, mixing the berries after each pulse. The melted but cold berry mass was filtered through Nylon gauze in glass funnels, gently pressing the berries with a glass test-tube as a pestle. The *V. m.* (*Vaccinium myrtillus*) berry juice filtrate (about 100 ml) was gathered in 500 ml glass bottles standing in an ice bath. Either used as such or the filtrate (about 100 ml) was made up to 195 ml with MQ (Milli-Q water), and 75 ml 2-PrOH (2-propanol) was vigorously mixed, and rapidly 30 ml AcA (acetic acid) was mixed successively; the freshly made mixture was poured on the gel to be stained. The gel was gently shaken first by hands and then horizontally in a shaker, usually 25 rpm for various lengths, from minutes to several hours.

2.2. Gel electrophoresis

Protein samples of barely grain and commercial purified proteins and part of the ystRNA (yeast soluble RNA) samples were extracted with Tris-buffered [tris(hydroxymethyl)aminomethane, 40 mM, pH 8] 2-PrOH (50 % v/v) and βME (β-mercaptoethanol) (5 % v/v) followed by a precipitation step with 2.5 vols of MetOH (methanol) [15, 16] or with the evaporation of the extraction supernatant with a vacuum centrifuge. The methanolic supernatant usually also evaporated with the vacuum centrifuge. The extraction steps were repeated once. The samples were

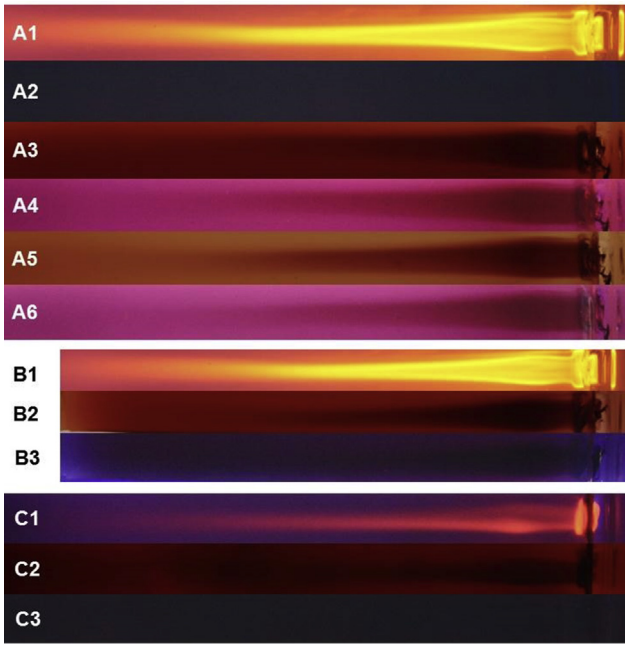

Fig. 2. *V. m.* juice displaces EtBr, which cannot backwards displace *V. m.* juice. Tracks on horizontal agarose gels. Wells loaded with 150 μg ctDNA, stained in various ways including *V. m.* berry juice and destained. – Tracks A1–A6. A single track treated sequentially. A1. EtBr for 9 h 30 min: UV. – A2. EtBr for 9 h 30 min → *V. m.* juice for 12 h → MQ for 30 min: UV. – A3. EtBr for 9 h 30 min → *V. m.* juice for 12 h → MQ for 12 h 30 min: Vis. – A4. EtBr for 9 h 30 min → *V. m.* juice for 12 h → MQ for 15 h 30 min → EtBr for 24 h 15 min: UV. – A5. The same as in A4: Vis. – A6. EtBr for 9 h 30 min → *V. m.* juice for 12 h → MQ for 15 h 30 min → EtBr for 48 h 15 min: UV. – Tracks B1–B3. A single track treated sequentially. B1. EtBr for 9 h 30 min: UV. – B2. EtBr for 25 h → *V. m.* juice for 12 h → MQ for 12 h: Vis. – B3. The same as in B2: UV. – Tracks C1–C3. A single track treated sequentially. C1. *V. m.* juice for 9 h → EtBr for 24 h: UV. – C2. *V. m.* juice for 9 h → EtBr for 27 h → *V. m.* juice for 12 h → MQ for 12 h: Vis. – C3. The same as in C2: UV. Without a preceding soaking in water, the gel does not allow *V. m.* juice to diffuse but slowly, while EtBr penetrates faster. *V. m.* juice does not quench EtBr.

usually loaded in Laemmli's [17] sample buffer. If a reducing agent, βME or DTT (DL-dithiothreitol) was included, it was added after the heat treatment of the samples (95 °C, 2 min). In some trials, fresh *V. m.* juice was additionally loaded in the sample wells before the start of running. The vertical SDS-PA (sodium dodecyl sulphate polyacrylamide) gels, with 5 % stacking gel and 10.8 % fractionating gel, were prepared and run as described [15]. The gels were stained with the *V. m.* juice as above or sometimes post-stained with CBB (Coomassie Brilliant Blue R 250), as described [15]. The horizontal 11 mm thick agarose gels (0.9 %) were run in 1×TBE (tris, borate, ethylenediamine tetraacetic acid disodium salt) buffer, pH 8. Primary staining with EtBr (ethidium bromide) 5 μg ml^{-1} was done in 1×TBE buffer, in later stages in MQ.

2.3. Gel photography

The stained gels were photographed with a Canon EOS 1000D digital camera. The gels were transilluminated with UV light of wave length 312 nm (BI–O-Vision, Spectroline) or with visible light, usually fluorescent tubes as the source. Kodak UV barrier filters, though changed the image, did not improve the fluorescence resolution with this camera, and the UV illumination set-up and exposures were made without UV filters. The gel Figures are unedited records with the camera system.

2.4. Double-diffusion test

For double-diffusion tests introduced for the purpose, 10.8 % SDS-PA

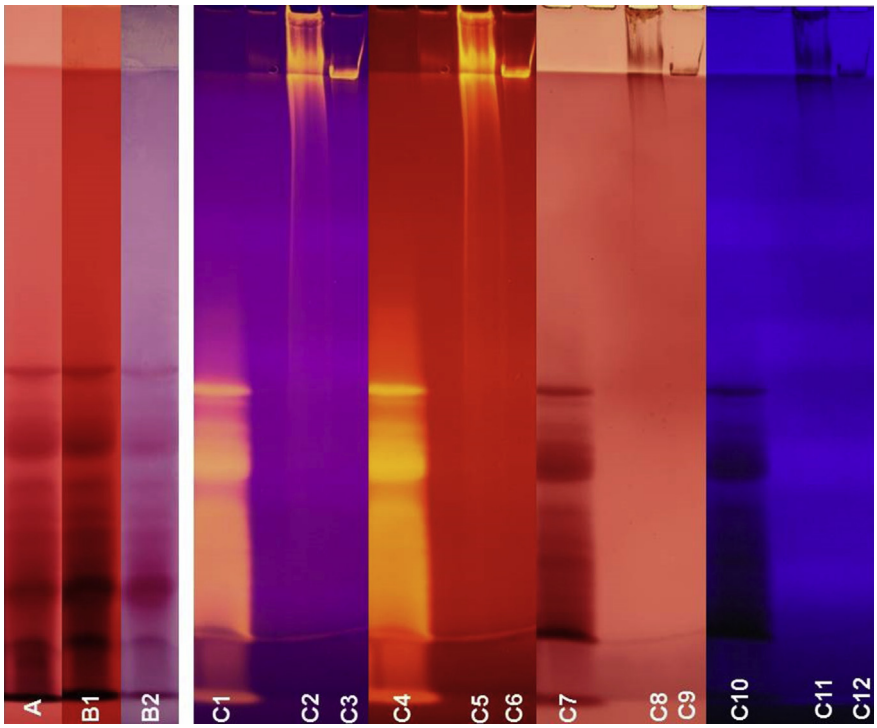

Fig. 3. Vertical SDS-PA gels fractionating ystRNA and DNA and stained alone with *V. m.* juice or EtBr or post-stained with *V. m.* juice or CBB. – Tracks A, B1, and B2. RNA loaded 184 μg per well; photographed in Vis. – A. Stained with *V. m.* juice in AcA + 2-PrOH solution for 11 h and washed with MQ, AcA, and 2-PrOH for 2 h. – B1. Stained with *V. m.* juice solution alone for 11 h and washed with MQ, AcA, and 2-PrOH for 2 h. – B2. B1 post-stained with CBB over night and washed with lowering proportions of 2-PrOH in 10 % AcA. – Tracks C1–C12. ystRNA (C1, C4, C7, and C10), ctDNA (C2, C5, C8, and C11), and linearized plasmid DNA, pCaMVCN (C3, C6, C9, and C12) were loaded 140 μg, 140 μg, and 9 μg per well, respectively, in 14 μl followed by 26 μl of *V. m.* juice in each sample well. The DNAs mainly travelled only in the 5 % stacking gel. – C1–C3. Stained with EtBr for 2 h; UV without a filter, and C4–C6 using UV filters which did not improve the image. – C7–C9. C1–C3 post-stained with *V. m.* juice solution for 17 h → MQ for 3 h 15 min: Vis. This staining gives sharper images of the bands than in the fluorescence with EtBr and UV illumination. *V. m.* juice in the sample well partly precipitates DNA in the entry into the gel (C8). – C10–C12. C7–C9 photographed in UV transillumination, showing a total lack of fluorescence of the nucleic acids and the gel. – The *V. m.* juice included in the sample well (C1 – C12) changes the mobility of the ystRNA zones from those without the juice in the sample wells (A, B1, and B2).

similar to that of electrophoresis separation gel was poured in Petri dishes covered with a home-made Teflon lid (thickness 1 mm) with tight holes, adopting two gel combs side-by-side. The Teflon lid lining the upper level of the gel in the dish was additionally covered with water to reduce oxygen in the polymerizing acrylamide to make a smooth surface. The sample solutions to be tested were pipetted in one of the well rows, *V. m.* juice in the other.

2.5. Cytology with berry juice staining

For microscopy, hand-cut sections of mature, dry barley (*Hordeum vulgare*) caryopses, cv. 'Adorra' and landrace Line 269 were pretreated for 1 or 3 h with MetOH or mixture of 1 volume of Tris-buffered (40 mM, pH 8) 2-PrOH (50 % v/v) and βME (5 % v/v) plus 2.5 volumes of MetOH. Subsequently, the sections were soaked in a mixture of *V. m.* berry juice: AcA: 2-PrOH (65 : 10: 25 % v/v, respectively) for about 8 h. The stain was differentiated with changes and mounted on slides in 10 % AcA, which was displaced with glycerol. The light microscopic views (Zeiss Axioskop) were photographed with Kodak Ektar 100 or Agfaphoto CT Precisa 100 film using a 40× Plan objective.

2.6. Sources of used laboratory chemicals

Acetic acid ca. 100% (Bang & Co.) or Emprove (Merck). Acrylamide (99.9%) and N,N′-methylene-bis-acrylamide (electrophoresis purity) (Bio-Rad). Agarose (GTG Agarose, Biometra). Ammonium persulfate (electrophoresis purity) (Bio-Rad). β-Mercaptoethanol puriss (Fluka). Boric acid p.a. (Merck). Bromophenol blue (Merck). Coomassie Brilliant Blue R 250 (Serva). Cytochrome-C, type III (Sigma C-2506). DL-dithiothreitol (Sigma D-0632). DNA from Calf Thymus Type I, Na salt, highly polymerized, (Sigma D-1501). Ethidium bromide (Sigma E8751). Ethylenediamine tetraacetic acid disodium salt p.a. (Merck). Gel Drying Films (Promega). Glycine p.a. (Riedel-deHaën 33226). Methanol p.a. or SeccoSolv (Merck). Myoglobin from Horse Heart (Sigma M-9267). Plasmid pCaMVCN (Pharmacia) 4177 bp, linearized with Cla I (NEB). Precision Protein Standards (Bio-Rad 161-0362) with given molar masses of 250, 150, 100, 75, 50, 37, 25, 15, & 10 kDa. 2-propanol, p.a. Emsure or SeccoSolv (Merck). RNA, soluble from Yeast, Type III (Sigma R-7125). Sodium dodecyl sulphate (BDH 44215). TEMED (electrophoresis purity) (Bio-Rad). Tris, Trizma preset pH, base and HCl (Sigma).

3. Results

3.1. Staining gels with Vaccinium myrtillus berry juice

The melting and filtering of the *V. m.* juice from berries were done at a chilled temperature to keep the anthocyanins. The staining solution usually had low pH due to AcA, the berry juice also being naturally acidic. Genomic ctDNA (calf thymus DNA) was used as the test material for the staining with *V. m.* berry juice on agarose gels. After a 1 h rinse in MQ water, the DNA and gels stain in different ways depending on the components of the stain. The *V. m.* berry juice alone stains efficiently, but the gel itself retains more the stain than, if AcA and 2-PrOH were added to the stain solution (Fig. 1, tracks A and B). After soaking in water, if the gel was stained with *V. m.* berry juice, EtBr staining is inefficient, even after a prolonged EtBr soaking (Fig. 1, tracks C1 – C4, the single track treated sequentially). In Figure legends, an arrow (→) indicates the change to a next-step solution at gel staining or destaining, the duration of which is given in hours (h) and minutes (min). Even a prolonged (>48 h) soaking in EtBr is not capable of replacing *V. m.* juice color from DNA (Fig. 1, track C4), though this happens in the gel itself, which starts to show the typical fluorescence in UV after a prolonged incubation in EtBr (Fig. 2, tracks A4, A6). EtBr is not quenched due the presence of *V. m.* juice (Fig. 2, track C series), but *V. m.* juice competes with EtBr for the staining sites in DNA (Fig. 2, track A and B series).

V. m. juice stains RNA on gels with high specificity. Adding *V. m.* juice (26 μl) in the gel wells of the buffered sample (14 μl) before the start of running affects the relative mobility of the RNA bands (Fig. 3, tracks A and B vs. C series) and partly precipitates DNA in the sample well (Fig. 3, track C8). The *V. m.* juice reacts directly with nucleic acids.

Some bands which stain blue-black fast and strongly with the *V. m.* juice on gels showed M_r of about 200–700 g M mass and proved to be a contaminant in the MetOH poured in a laboratory bench flask and used to prepare the sample. If the sample was not treated with βME before

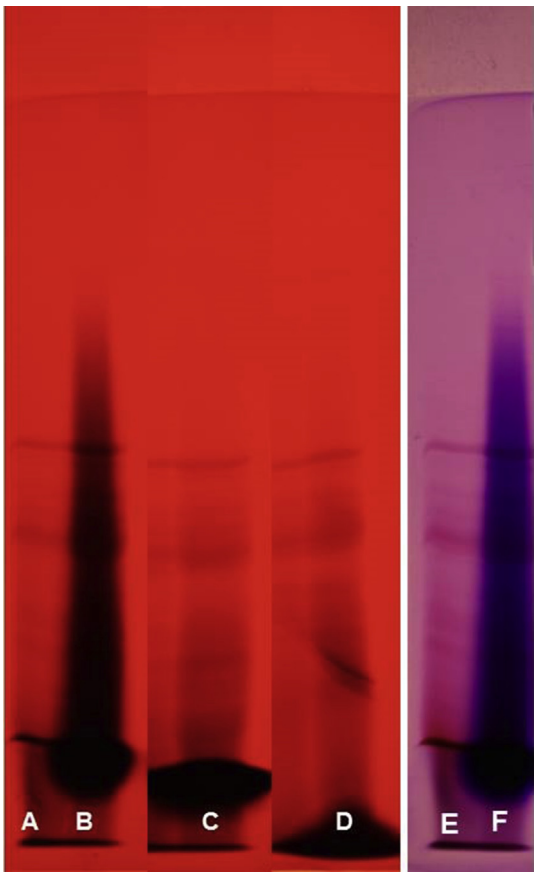

Fig. 4. Vertical SDS-PA gel fractionating ystRNA with or without a sulfhydryl-reductant affecting the mobility of the black-staining contaminant caused by the MetOH treatment. – A–F. Tracks on the same gel. The wells were loaded with 600 μg of ystRNA. The gel was stained with *V. m.* juice alone. Samples without a reductant (A and B), with the contaminated MetOH treatment in B, causing the association of the contaminant with ystRNA. – C. Like B, but with 5 % v/v of βME, which dissociated the contaminant. – D. Like C, but with 0.03 % w/v of DTT instead of βME, which caused faster movement of the dissociated contaminant than βME. – E and F. A & B stained for 9 h 30 min with *V. m.* juice and post-staining with CBB, washed with 10 % AcA, and lowering proportion 2-PrOH in water. CBB also stains the contaminant in the ystRNA complex.

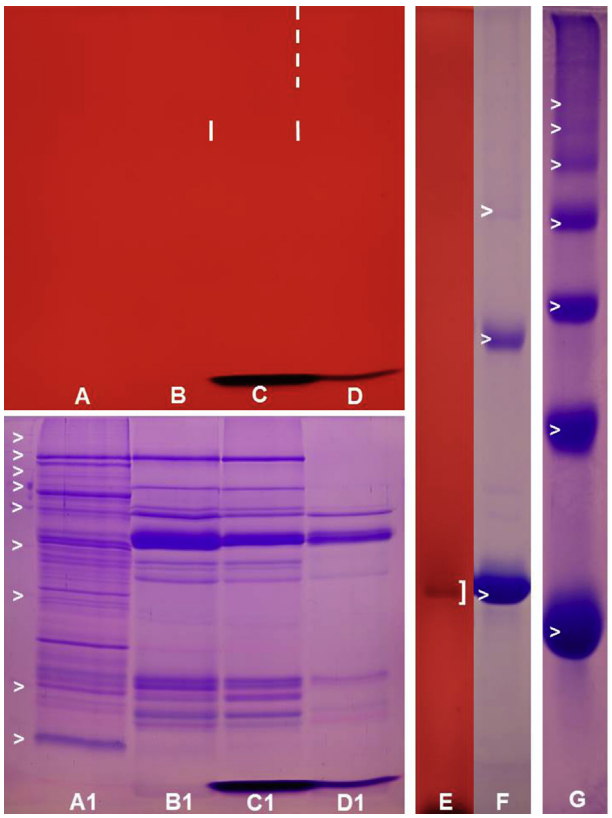

Fig. 5. Vertical SDS-PA gels fractionating proteins. Photography in Vis. – Tracks A–D. Proteins from embryo-less barley grains prepared as described [15] and stained with *V. m.* juice in AcA + 2-PrOH solution. – A. Residual proteins from extraction in B. – B. Proteins solved by the Tris, 2-PrOH, βME solution and evaporated to dryness. – C. Proteins solved by the Tris, 2-PrOH, βME solution and precipitated with 2.5 volumes of the contaminated MetOH. – D. Proteins in the evaporated, contaminated MetOH supernatant from C. The contaminant stains black in the lower edge of the gel. A faint staining of the major protein zones and the upper smear (white bars). – Tracks A1–D1. The gel with A – D was post-stained with CBB to show the proteins. The angles show the position of the nine Precision Protein Standards. – Track E. Myoglobin from a MetOH supernatant evaporated and electrophoresed on a gel pre-run for 2 h before the samples were loaded. The gel was directly stained with *V. m.* juice for 12 h 30 min → MQ for 30 min. Bract shows myoglobin band stained with *V. m.* juice. – Track F. Myoglobin polymers up to trimer (angles) from a MetOH precipitate, the gel stained with CBB. – Track G. Cyt c polymers at least up to heptamer (angles) from a MetOH precipitate, the gel stained with CBB.

running, the contaminant moved associated with the RNA (Fig. 4, tracks B and F), moved dissociated from RNA, when βME was included (Fig. 4, track C), but moved dissociated faster, when βME was displaced with DTT (Fig. 4, track D). The contaminant was also stained with CBB and further when stained after *V. m.* juice (Fig. 4, track F). The staining of the contaminant was not observed, when 2-PrOH and Tris-buffer (pH 8.0) were omitted from the preparations. The tracking dye, bromophenol blue [17], does not complex with *V. m.* juice.

V. m. berry juice alone, without added AcA and 2-PrOH, also slightly stains proteins, as seen in transmitted visible light. The protein zones may be observed for a short time before the background staining of the gel intensifies (Fig. 5, tracks B, C, and E). The protein staining possibly requires some helical sections, which, after the denaturing running conditions, might be reassumed in the gel in the presence of the acidic berry juice. Anthocyanins of *V. m.* blueberries stain human skin upon handling. The stain on fingers may reveal different tissue types, some of them lasting days.

With the analytical quality of AcA and 2-PrOH as the components of the *V. m.* juice stain, the mixed stain keeps several hours at room temperature, and the stained gels could at times be taken in 10 % AcA on a glass plate wiped with cotton for the photography, after which, the staining in *V. m.* juice with AcA and 2-PrOH could be continued. Contaminants to 2-PrOH from polyethene plastic containers were found to cause precipitation in the *V. m.* juice when staining gels. Solvents stored in glass bottles worked better. The amount of the contaminated MetOH stored in the 100 ml lab bottle was so small, that the suspected contaminant could not be chemically identified.

The MetOH precipitation of solved proteins with Tris-buffered 2-PrOH [15, 16] was observed to promote the polymerization of cyt c (cytochrome c) up to heptamer and that of myoglobin to trimer (Fig. 5, tracks F and G). The precipitation with MetOH was not observed to increase protein zones, i.e., to cause polymerization in barley grain proteins (Fig. 5, tracks C1 and D1 vs. B1). The addition of MetOH at the precipitation was measured to lower the pH of the extraction solution by about 2 units. The fast-running stainable band was also observed where proteins were precipitated with the contaminated MetOH lot (Fig. 5, tracks C, C1, D, and D1). Such contaminated samples in the double-diffusion test in gel formed staining bands between the *V. m.* juice and the contaminant (Fig. 6), which behaved as could be expected from results with the SDS-PA electrophoresis of the same samples. The meeting of the

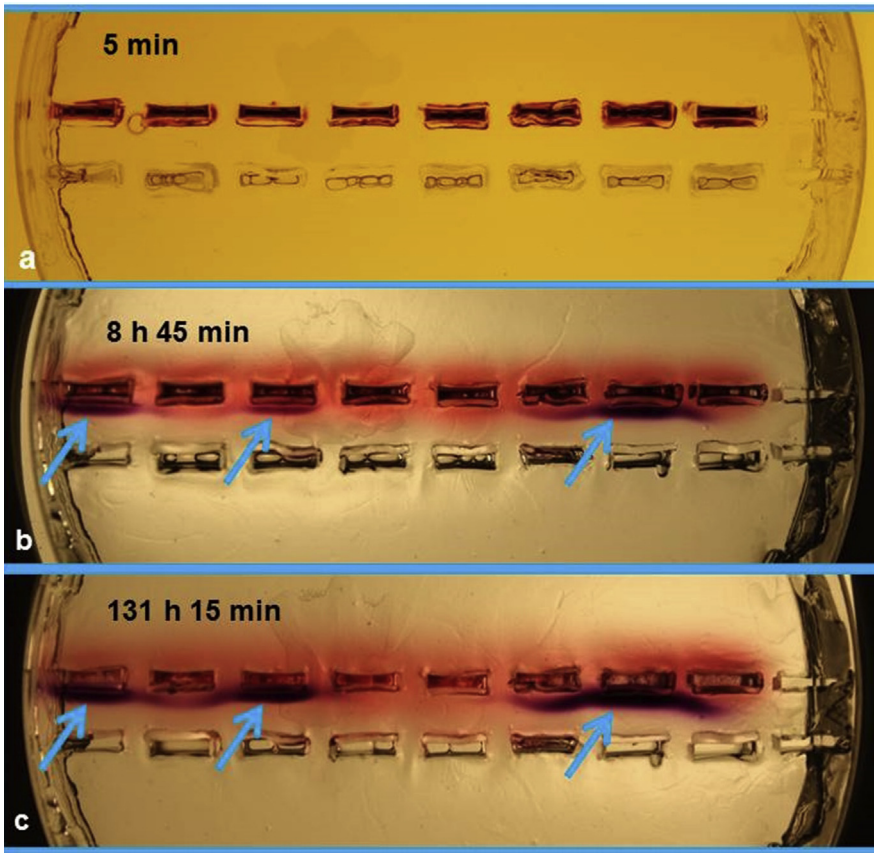

Fig. 6. a–c. Double-diffusion on SDS-PA gel cast on a Petri dish (Ø 90 mm) with parallel rows of wells for samples after incubation for 5 min (a), 8 h 45 min (b), and 131 h 15 min (c). The wells in the upper row were loaded with 30 μl of *V. m.* juice, and in the lower row, with 30 μl of the samples in Laemmli's [17] loading buffer. The samples contain barley endosperm extracts, three of which included the precipitation step with the contaminated MetOH. The contaminant shows the staining zones close to the *V. m.* juice wells (blue arrows), therefore, diffusing faster than the stain of the juice.

V. m. juice and the contaminant in the gel also stopped further diffusion. When the Petri dish was sealed with Parafilm against drying out, the double-diffusion could continue for days, intensifying the stained zones and, thus, showing good stability of the anthocyanins in such an environment with SDS (sodium dodecyl sulphate) and buffer (Fig. 6).

3.2. Staining with *Vaccinium myrtillus* berry juice in cytology

In mature barley grains, *V. m.* juice with acetic 2-PrOH principally stains the nuclei in the caryopses after differentiation of the background in 10 % acetic acid (Fig. 7a). In the aleurone cells, the cytoplasm also retains the stain, which is probably due to the stored RNA in the cytoplasm. The degraded nuclei in the mature, undividable starch endosperm also stain, while all the starch grains remain unstained (Fig. 7b). In barley caryopses, strong staining was observed in the crease (Fig. 7c), where the staining was strong in the chalazal cells and has remained so for over five years in dry-out preparation in the dark. The chalazal cells are known to contain suberin [18], which may thus be highly reactive with the *V. m.* juice.

4. Discussion

The earlier tried and observed cytological stainability with the *V. m.* juice of metaphase chromosomes and interphase nuclei appears to be explainable as the staining of DNA because the juice also stains genomic and plasmid DNA and additionally RNA on gels. The *V. m.* juice staining of proteins, e.g. those associated with chromatin in the cells, is much weaker than that of the nucleic acids and could be less responsible for the staining of chromatin in cells.

The *V. m.* juice staining is strongly competitive with EtBr and displaces EtBr from nucleic acids on electrophoretic gels (Fig. 1, tracks C1–C4, and Fig. 2). Molecular similarity of some anthocyanins and EtBr suggests that many of the anthocyanins are also intercalating dyes in nucleic acids, which was also supported by sequential staining of a single gel with EtBr and *V. m.* juice in various orders with or without washes in between. The observed competing interaction of blueberry anthocyanins with the binding of EtBr suggests an intercalative reaction of one or some of them into DNA. The intercalation of anthocyanins in DNA was also reviewed [19]. The interaction of the flavonoids hesperitin and naringenin with DNA was shown to be intercalative [20]. Naringenin occurs as an intermediate in the flavonoid pathway leading to the anthocyanins in *V. myrtillus* fruits [21].

It is apparent that *V. m.* juice competes with EtBr. Therefore, *V. m.* juice might be used to detoxify accidental exposure of mouth and upper alimentary tract to EtBr or perhaps to other compounds intercalating in nucleic acids. A commercialized preparation of *V. myrtillus* anthocyanins was not found to be mutagenic or teratogenic [10]. On the contrary, anthocyanins instead belong to the colorants causing curing apoptosis in cancer tissue as reviewed [22]. A related flavonol, quercetin occurs in *V. myrtillus* flowers and fruits [21, 23]. There has been concern about the mutagenic or teratogenic effects of quercetin. Evidence for such *in vivo* toxicity of quercetin is, however, lacking [24]. The inactivation of viruses with *V. m.* juice thought to occur in human cures is conceivable through the affinity of one or more components of the juice with viral nucleic acids. The upregulation of genes directly by an anthocyanin interference with DNA might occur; *V. m.* juice anthocyanins are known to upregulate the heme-oxygenase-1 gene in human retinal pigment epithelial cells [25]. In rats with diabetes-induced oxidative stress and inflammation in retinas, blueberry anthocyanin feeding increased the mRNA levels of the heme-oxygenase-1 and its regulator, the nuclear factor-erythroid 2 in retinas [12]. It is to be seen, if the blueberry anthocyanins directly interfere with DNA in the regulation of the heme-oxygenase-1 gene or other genes *in vivo*. Anthocyanin-DNA copigment protects from OH radical damages and was suggested to also operate in the nuclei of plant cells [26].

Diluted *V. m.* juice might be used as a vital staining as an ultrafiltrate

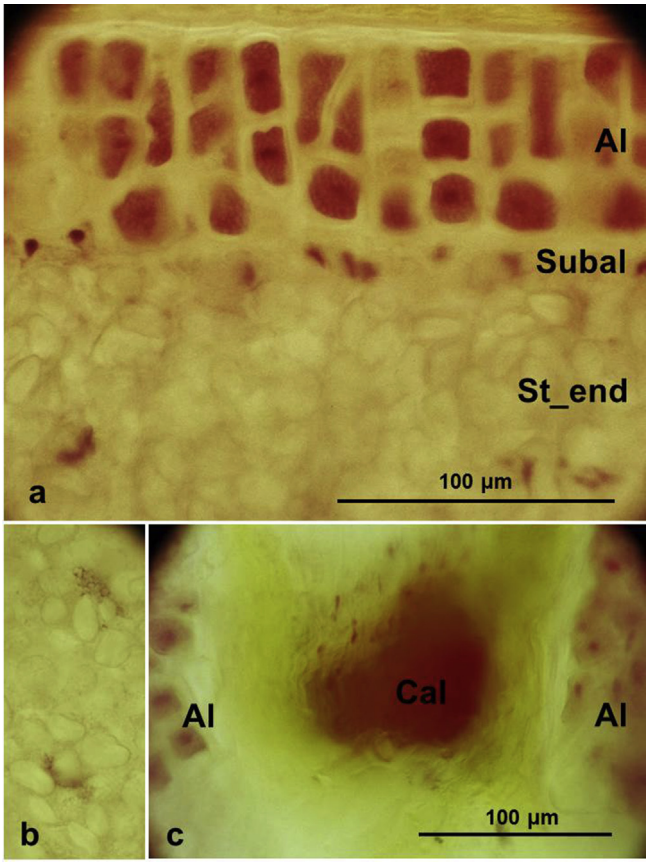

Fig. 7. a–c. Dry sections of mature barley grains pretreated with MetOH (a & b) or Tris-bufferred 2-PrOH + MetOH (c) stained with *V. m.* juice in AcA and 2-PrOH, and destained in 10 % AcA. – a. Caryopses has an aleurone (Al) with three cell-layers, showing interphase nuclei stained purple and cytoplasmic staining, probably by RNA. The subaleurone (Subal) cell-layer has nuclei with less intact appearance. The starch endosperm (St_end) has remains of nuclei. – b. Degraded chromatin in the starch endosperm tissue with plenty of unstained starch grains. – c. In the transversally cut crease, the chalazal (Cal) cells stain strongly, apparently due to their suberin. The aleurone (Al) layers border the crease of the grain on two sides. Scales 100 μm, in a common for b.

in sterile cell cultures as well. Anthocyanins are known to be more stable in acidic pH, low temperature [27, 28], and in the presence of positively charged surfactants like SDS [29]. In the methods used here, low pH, in the beginning, the chilled conditions, and in the double-diffusion tests, SDS were to support the stability of the juice.

The MetOH precipitation after the extraction with Tris-buffered, βME-reduced 2-PrOH solution [15, 16] was observed to promote the polymerization of cyt c at least up to a heptamer and that of myoglobin to trimer. A purified polymeric series of cyt c might serve as a protein calibration standard in electrophoresis or chromatography. Ethanol and trichloroacetic acid treatments are capable of inducing polymers of cyt c, which may be reversed by chaotropic treatments [30]. Methionyl 80 of cyt c was found to be responsible for the polymerization to di-, tri-, and tetrameres [31].

5. Conclusion

The anthocyanins from *V. myrtillus* berries efficiently and fast stain DNA and RNA fractionated on electrophoretic gels. The staining molecules apparently intercalate in the nucleic acids competing for the sites, which could be intercalated by EtBr. The nucleic acids can be seen in visible light without exposure to UV. The juice is suggested to have a use to inactivate viruses and to exchange intercalating toxic molecules from DNA and RNA *in vitro* and *in vivo*. The juice may have a use as a nontoxic stain in cytology and could show an otherwise unnoticed, unidentified contaminant in a laboratory chemical. The suitability of the *V. m.* juice-stained RNA and DNA to enzymatic modifications and the possibility to remove the stain from nucleic acids is to be studied.

Declarations

Author contribution statement

Hannu Ahokas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

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

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

I am grateful for the opportunity to use the lab facilities of MTT-Agrifood Research Finland while doing this study after my retirement.

References

[1] M. Turtiainen, K. Salo, O. Saastamoinen, Model-based estimates of regional and national bilberry and lingonberry yields on mineral soils in Finland, Univ. Joensuu, Fac. For., Res. Notes. 167 (2005), 44 p.
[2] A. Åkerström, L. Jaakola, U. Bång, A. Jäderlund, Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of Vaccinium myrtillus L. (bilberries), J. Agric. Food Chem. 58 (2010) 11939–11945.
[3] A.K. Lätti, K.R. Riihinen, P.S. Kainulainen, Analysis of anthocyanin variation in wild populations of bilberry (Vaccinium myrtillus L.) in Finland, J. Agric. Food Chem. 56 (2008) 190–196.
[4] D. Müller, M. Schantz, E. Richling, High performance liquid chromatography analysis of anthocyanins in bilberries (Vaccinium myrtillus L.), blueberries (Vaccinium corymbosum L.), and corresponding juices, J. Food Sci. 77 (2012) C340–C345.
[5] H. Ahokas, Cytology of hexaploid cranberry with special reference to chromosomal fibres, Hereditas 68 (1971) 123–136.
[6] H. Ahokas, Notes on polyploidy and hybridity in Vaccinium species, Ann. Bot. Fenn. 8 (1971) 254–256. https://www.jstor.org/stable/i23724628.
[7] H. Ahokas, Geneettisiä Ja Sytologisia Havaintoja Sekä Kokeita Vaccinium-Lajeilla, Ms. Thesis, Dept. of Genet, Univ. of Helsinki, 1972, 80 p., append.
[8] H. Ahokas, Artificial, reciprocal hybrids between Vaccinium microcarpum and V. vitis-idaea, Ann. Bot. Fenn. 16 (1979) 3–6. https://www.jstor.org/stable/i23725168.
[9] E. Pakarinen, Sinisen, harmaan ja mustan painamisesta kasviaineksilla Suomessa, Virossa ja Liivissä, Sanakirjasäätiön Toim. 1 (1931) 261–269.
[10] P. Morazzoni, E. Bombardelli, *Vaccinium myrtillus* L. Fitoter. 67 (1996) 3–29.
[11] Y. Liu, X. Song, D. Zhang, F. Zhou, D. Wang, Y. Wei, F. Gao, L. Xie, G. Jia, W. Wu, B. Ji, Blueberry anthocyanins: protection against ageing and light-induced damage in retinal pigment epithelial cells, Br. J. Nutr. 108 (2012) 16–27.
[12] Y. Song, L. Huang, J. Yu, Effects of blueberry anthocyanins on retinal oxidative stress and inflammation in diabetes through Nrf2/HO-1 signaling, J. Neuroimmunol. 301 (2016) 1–6.
[13] L. Ma, Z. Sun, Y. Zeng, M. Luo, J. Yang, Molecular mechanism and health role of functional ingredients in blueberry for chronic disease in human beings, Int. J. Mol. Sci. 19 (2018) 2785–2804.
[14] S. Silva, E.M. Costa, M. Mendes, R.M. Morais, C. Calhau, M.M. Pintado, Antimicrobial, antiadhesive and antibiofilm activity of an ethanolic, anthocyanin-rich blueberry extract purified by solid phase extraction, J. Appl. Microbiol. 121 (2016) 693–703.

[15] H. Ahokas, Methanol precipitation using universal protein fractionation method for cultivar identification in cereal, pea and Brassica seeds, Seed Sci. Technol. 30 (2002) 437–449.
[16] H. Ahokas, M.-L. Manninen, Retrospecting genetic variation of Finnish oat (Avena sativa) landraces and observations on revived lines grown prior to 1957, Genet. Resour. Crop Evol. 47 (2000) 345–352.
[17] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[18] M.P. Cochrane, L. Paterson, E. Gould, Changes in chalazal cell walls and in the peroxidase enzymes of the crease region during grain development in barley, J. Exp. Bot. 51 (2000) 507–520.
[19] S. Jaldappagari, N. Motohashi, M.P. Gangeenahalli, J.H. Naismith, Bioactive mechanism of interaction between anthocyanins and macromolecules like DNA and proteins, in: N. Motohashi (Ed.), Topics in Heterocyclic Chemistry, 15, Springer, Berlin, Heidelberg, 2008, pp. 49–65.
[20] A.H. Hegde, S.N. Prashanth, J. Seetharamappa, Interaction of antioxidant flavonoids with calf thymus DNA analyzed by spectroscopic and electrochemical methods, J. Pharm. Biomed. Anal. 63 (2012) 40–46.
[21] L. Jaakola, K. Määttä, A.M. Pirttilä, R. Törrönen, S. Kärenlampi, A. Hohtola, Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, protoanthocyanidin, and flavonol levels during bilberry fruit development, Plant Physiol. 130 (2002) 729–739.
[22] A. Anantharaman, B. Subramanian, R. Chandrasekaran, R. Seenivasan, R. Siva, Colorants and cancer: a review, Ind. Crops Prod. 53 (2014) 167–186.
[23] S.H. Häkkinen, S.O. Kärenlampi, I.M. Heinonen, H.M. Mykkänen, A.R. Törrönen, Content of flavonols quercetin, myricetin, and kaempferol in 25 edible berries, J. Agric. Food Chem. 47 (1999) 2274–2279.
[24] M. Harwood, B. Danielewska-Nikiel, J.F. Borzelleca, G.W. Flamm, G.M. Williams, T.C. Lines, A critical review of the data related to the safety of quercetin and lack of evidence of the in vivo toxicity, including lack of genotoxic/carcinogenic properties, Food Chem. Toxicol. 45 (2007) 2179–2205.
[25] P.E. Milbury, B. Graf, J.M. Curran-Celentano, J.B. Blumberg, Bilberry (Vaccinium myrtillus) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells, Investig. Ophthalmol. Vis. Sci. 48 (2007) 2343–2349.
[26] A.D. Sarma, R. Sharma, Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage, Phytochemistry (Oxf.) 52 (1999) 1313–1318.
[27] X. Sui, Impact of Food Processing on Anthocyanins, Springer Thesis, Springer Science+Business Media, Singapore, 2016, 129 p.
[28] Y. Liu, Y. Liu, C. Tao, M. Liu, Y. Pan, Z. Lv, Effect of temperature and pH on stability of anthocyanin obtained from blueberry, J. Food Meas. Charact. 12 (2018) 1744–1753.
[29] S. Liu, Y. Fu, S. Nian, Buffering colour fluctuation of purple sweet potato anthocyanins to acidity variation by surfactants, Food Chem. 162 (2014) 16–21.
[30] E. Margoliash, J. Lustgarten, Interconversion of horse heart cytochrome c monomer and polymers, J. Biol. Chem. 237 (1962) 3397–3405.
[31] S. Hirota, Y. Hattori, S. Nagao, M. Taketa, H. Komori, H. Kamikubo, Z. Wang, I. Takahashi, S. Negi, Y. Sugiura, M. Kataoka, Y. Higuchi, Cytochrome c polymerization by successive domain swapping at the C-terminal helix, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 12854–12859.