1 **Anthocyanin management in fruits by fertilization**

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13 **Abstract**

14 Anthocyanins are water-soluble vacuolar plant pigments that are mainly synthesized

15 in epidermal layers and the flesh of fruits such as apples, cherries, grapes, and other

16 berries. Because of their attractive red to purple coloration and their health-promoting

17 potential, anthocyanins are significant determinants for the quality and market value of

18 fruits and fruit-derived products. In crops, anthocyanin accumulation in leaves can be

19 caused by nutrient deficiency which is usually ascribed to insufficient nitrogen or

20 phosphorous fertilization. However, it is a little-known fact that the plant's nutrient

21 status also impacts anthocyanin synthesis in fruits. Hence, strategic nutrient supply

22 can be a powerful tool to modify the anthocyanin content and consequently the quality

23 and market value of important agricultural commodities. Here we summarize the

24 current knowledge of the influence of plant nutrients on anthocyanin synthesis in fruits

25 of major global market value, and discuss the underlying cellular processes that

26 integrate nutrient signaling with fruit anthocyanin formation. It is highlighted that

27 fertilization that is finely tuned in amount and timing has the potential to positively

28 influence the fruit quality by regulating anthocyanin levels. We outline new approaches

29 to enrich plant based foods with health-promoting anthocyanins.

30

31 Keywords: anthocyanins, plant nutrition, fruit quality, *Vitis vinifera*, *Malus domestica*,

32 *Fragaria × ananassa*

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Anthocyanins in fruits: Importance for producer and consumer

Anthocyanins are hydrophilic secondary metabolites that confer orange, red, or blue coloration to a range of fruits such as grapes and apples. The primary ecophysiological function of anthocyanins in fruits is to attract seed dispersal agents and to protect plant tissues against abiotic and biotic stressors such as high radiation or pathogens. Anthocyanins are, thus, important attributes that increase the plant fitness in demanding environments.

The anthocyanin-mediated fruit coloration determines the harvest point and raises the market value of several agricultural products such as apples, grapes, and diverse other berry fruits.[1] It has frequently been indicated that the dietary intake of anthocyanins has profound health-promoting effects in humans, owing to their antioxidant and anti-inflammatory properties [2-9]. Profound evidence suggests that the regular intake of fruits rich in anthocyanins lowers the risk of cardio-vascular diseases and diabetes.[10-14] Moreover, the consumption of anthocyanins has been reported to have positive effects on brain functioning.[15] Thus, an attractive coloration does not only increase the consumer appreciation and the market value of fruits, but most importantly, enriching fruits and vegetables with these bioactive compounds contributes to their wholesomeness. Additionally, anthocyanins derived from plant cell cultures are used as additives in processed food industry[3, 16-19], and growing concern about the use of artificial food colorants has increased the demand for anthocyanins extracted from fruit tissue as a natural alternative with higher consumer acceptance.[16, 17, 20, 21]

It is of utmost importance for producers to understand how environmental factors influence anthocyanin synthesis in fruits. Based on this knowledge cultivation strategies can be developed with the aim to produce anthocyanin-rich commodities with appealing color and human health-promoting properties. The effects of abiotic

4

70 factors such as low temperature, high light, and UV irradiation on anthocyanin

71 synthesis have been thoroughly reviewed elsewhere.[22-27] This review will discuss the

72 impact of plant nutrient availability on the coloration of fruits. After providing a brief

73 overview of the biosynthesis and biochemistry of anthocyanins in general, we highlight

74 that anthocyanin levels in fruits can be optimized via strategic fertilization in the

75 following sections.

76 This work focusses on apples, grapes and strawberries since they are the anthocyanin-

77 containing fruit varieties with the highest share of global export value[28] and are among

78 the fruits with the highest global gross production value (50,820 million US$, 68,941

79 million US$ and 14,894 million US$, respectively).[29] Previous research has mainly

80 focused on these crops, but a number of studies with other species can be found as

81 well and are summarized in Table 2.

82 **Biochemistry and biosynthesis of anthocyanins**

83 Anthocyanins are products of the phenylpropanoid pathway, and their general

84 structure consists of a flavylium ion backbone substituted with variable numbers of

85 hydroxyl and methoxy groups (Fig. 1). These functional groups as well as conjugations

86 with different sugar side branches and sugar acylation with organic acids affect

87 different properties such as color, stability, and bioactivity in humans.[3, 12, 30-34] The latter

88 is, presumably, attributed to electron-donating substituents that are attached to the

89 characteristic C6-C3-C6 carbon skeleton conferring antioxidant properties[9]. However,

90 complex mechanisms other than a direct antioxidant activity have been proposed to

91 explain the health-promoting mechanisms, as well[9].

92 The general pathway of anthocyanin synthesis is shown in Figure 2 and detailed

93 descriptions of the single biochemical steps can be found elsewhere[35, 36]. The

94   structural genes involved are well described in *Arabidopsis* as is the genetic

95   background of regulatory factors governing anthocyanin production. A complex

96   hierarchical network of diverse transcription factors is known to determine the class

97   and localization of anthocyanins in this model plant [37-40]. Together, these so-called

98   MBW-complexes (named after the transcription factor families MYB, bHLH and WD40)

99   differentially activate the expression of structural anthocyanin genes in response to

100   environmental cues including the nutritional state of the plant, and control not only the

101   total level but also the molecular diversity of anthocyanins [39].

Metabolic integration of anthocyanin synthesis with nutrient signaling

103   The question how nutrient signaling influences anthocyanin biosynthesis is only poorly

104   resolved and tenuously investigated on a genetic and molecular level especially in

105   crops. The vast majority of findings that describe the details of anthocyanin synthesis

106   and accumulation in response to nutrient imbalances are based on *Arabidopsis*. For

107   example, a detailed overview of the effects of N and P deficiency on the expression

108   levels of all major enzymes of the anthocyanin biosynthesis pathway in leaves and

109   roots of *Arabidopsis* was presented by Lillo, et al. [41]. The results of this study together

110   with other publications of the recent years [38, 39, 42-52] indicate that anthocyanins are

111   produced in a highly regulated, fine-tuned, and nutrient-specific manner as a result of

112   a metabolic adaptation to nutrient stress. Especially anthocyanin-specific genes,

113   namely dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS), as well

114   as genes involved in anthocyanidin glycosylation and sequestration into the vacuole

115   are highly expressed in response to nutrient deficiency, indicating that the observed

116   anthocyanin accumulation is not simply the result of an up-regulation of the general

117   phenylpropanoids pathway, which also yields other secondary metabolites such as

118   flavonols and lignin.

119 The transferability of knowledge about anthocyanin synthesis in vegetative tissue of

120 thale cress onto crops, however, is questionable, especially regarding the lower

121 nutrient requirement of *Arabidopsis* compared to crop plants that are bred for high

122 biomass generation and yield formation. Opposing effects have been shown, for

123 example, for ethylene induced anthocyanin synthesis. This gaseous plant hormone

124 promotes pigmentation in grapes [53], but suppresses anthocyanin formation in

125 *Arabidopsis* leaves [54]. An up-regulation of several key enzymes and transcription

126 factors of the flavonoid metabolism has been documented in leaves of tomato and

127 oilseed rape in response to N limitation [55-57], but little is known about the genetic and

128 molecular mechanisms that integrate the nutritional plant status and anthocyanin

129 synthesis in generative organs such as fruits. However, the advent of next-generation

130 sequencing has increased the genomic resources and uncovered the identity of

131 anthocyanin synthesis-related genes in selected crops such as tomato, maize and rice

132 [36, 58], apple [59, 60], grape [61-63], which opens the way for elucidating the molecular cross-

133 talk between anthocyanins and specific plant nutrients. Thus far, the molecular

134 regulation of anthocyanin biosynthesis in response to nutrient application has,

135 presumably, been best described for grapevine by Soubeyrand, et al. [64]. The authors

136 provide a simplified diagram of the anthocyanin biosynthetic pathway and its regulation

137 by MYB genes, and analyze the expression level of multiple anthocyanin-related genes

138 in response to N fertilization.

139 Hopefully, the recently published functional characterization of the apple bZIP protein

140 MdHY5[65] that represents a vital player in the cross-talk between anthocyanin

141 accumulation and N metabolism might fuel further investigations in this so-far

142 neglected area of research. In *Arabidopsis* the transcription factor (TF) AtHY5

143 integrates signals from diverse metabolic pathways such as light, temperature, and

144 hormone signaling[66, 67]. An, Qu, Yao, Wang, You, Wang and Hao [65] cloned and

145 described the TF MdHY5 in apple based on its homology with AtHY5. Their results

146 indicate that MdHY5 promotes anthocyanin synthesis by directly binding to the

147 promoter region of MdMYB10, a TF that regulates the expression of structural genes

148 involved in anthocyanin synthesis. Above that, apple calli over-expressing MdHY5 had

149 higher expression levels of N acquisition-related genes, increased activity of nitrate

150 reductase and elevated nitrate contents. This study is exceptional in so far as it

151 describes a molecular component at the interface of nutrient signaling and anthocyanin

152 synthesis in a non-model species, and it hopefully is a stepping stone for further

153 investigations also in other crops.

154 Additionally, sugars such as sucrose might be relevant signaling entities in the

155 metabolic pathway of nutrient-induced anthocyanin synthesis via induction of the

156 phenylpropanoid pathway. The linkage between nutrient status, sugar accumulation,

157 and anthocyanin production has been proposed for autumnal foliar reddening [68].

158 Increased levels of anthocyanins in leaves during autumn presumably function as light

159 attenuators and/or antioxidants to protect the vulnerable photosynthetic apparatus

160 from over-excitation for example during high light stress.[24, 69]. Schaberg, Van den Berg,

161 Murakami, Shane and Donnelly [68] found that the timing and extent of red leaf coloration

162 was consistently correlated with both foliar N concentrations and starch or sugar

163 concentrations, which also varied with N status. Cross-talk between these metabolic

164 pathways seems therefore conceivable. Induction of anthocyanin accumulation by

165 sugars has been shown both in grape berries and apple [70-72], and it is tempting to

166 speculate that regulatory mechanisms exist that dovetail cellular levels of certain plant

167 nutrients, anthocyanins, and saccharides. Experimental evidence for this hypothesis

168 is, however, limited. For example, the Ser/Thr protein kinase MdSnRK1.1 was recently

169 described as a "metabolic sensor" that perceives the status of cellular carbohydrates

170 and energy, and regulates sucrose-induced anthocyanin accumulation in apple.[73]

171 SnRK-related protein kinases have also been shown to control enzymatic steps in

172 nitrogen assimilation [74], and this kinase family might therefore be involved in the

173 metabolic integration of the crop's nutrient status and anthocyanin synthesis. Further

174 investigation of the complex integration of the individual metabolic pathways is highly

175 appreciated.

Anthocyanin enrichment in fruits by optimizing nutrient supply

177 Modulation of anthocyanin levels in crops can be achieved by conventional breeding

178 or genetic engineering.[32, 35, 58, 75] Besides this, we emphasize that adjusting controllable

179 environmental factors such as nutrient availability has the potential to shift the plant's

180 metabolism towards an increased production of these health-promoting polyphenols.

181 Likewise, the expression level of anthocyanins in plant cell cultures for the production

182 of food additives can easily be optimized by adjusting the nutrient concentrations in the

183 growing medium. Especially nitrogen (N) and phosphorus (P) supply is highly important

184 for controlling anthocyanin synthesis, and limitation of both nutrients has been shown

185 to promote anthocyanin synthesis.[76-79] Therefore, a nutrient supply that is tightly

186 controlled and well-managed in amount and timing has the potential to induce

187 anthocyanin production without decreasing the yield, as will be outlined in the following

188 sections.

Anthocyanins in grapes

190 Grapevines (*Vitis vinifera*) are cultivated worldwide to produce fresh fruit, wine, and

191 other fruit-derived products. Red grapes contain anthocyanins and they endure the

192 fermentation process during winemaking. Therefore, red wine is rich in anthocyanins

193 and their oxidation products,[80] conferring beneficial properties by contributing to the

194 prevention of a number of human diseases.[81-83] Furthermore, anthocyanins are

195 responsible for major characteristics of this beverage such as hue, color stability, and

196 taste.[84-86] Their biosynthesis depends on the berry development, starting at the onset

197 of ripening (véraison) and reaching maximum levels at berry maturity.[87] Malvidin

198 derivatives (Fig. 2A) are often the major anthocyanins in grapevines[88] but each variety

199 has got a unique set of anthocyanins (Table 1).[87] Anthocyanins accumulate mainly in

200 the epidermal layers of berries but these pigments are also present in the flesh (pulp)

201 of the berry in some cultivars.[81] Anthocyanin patterns in grapes are of economic value

202 because they impact grape and wine quality and are, thus, target for strategic

203 enrichment by breeding or by agricultural management. Cell cultures represent

204 controllable systems for anthocyanin production and can be used for studying the

205 underlying regulatory cellular processes that lead to the pigment's accumulation.[89, 90]

206 For example, cell suspensions derived from the red-colored *Vitis vinifera* variety

207 Gamay Fréaux contain all genes for a cell suspension-autonomous anthocyanin

208 biosynthesis [20, 91]. Since cell cultures have been suggested for the production of natural

209 food-colorings, systematic studies have been carried out to increase the efficiency of

210 anthocyanin production by improving the composition of the culture medium.[76]

211 Especially low external nitrate or phosphate concentrations in the growth medium

212 stimulate the anthocyanin synthesis.[76, 78, 79] It is suggested that these metabolic

213 changes result from growth inhibition of cells and hence an antagonistic relationship

214 between the primary and secondary metabolism.[76, 79] Furthermore, Dedaldechamp,

215 Uhel and Macheix [79] found a good correlation between anthocyanin synthesis and the

216 activity of dihydroflavonol reductase that catalyzes the reduction of dihydrokaempferol

217 to leucoanthocyanidin in grape cells cultured in medium with low P levels. Moreover,

218 Sinilal, et al. [92] revealed that magnesium (Mg) plays a role in increasing anthocyanins

219 stability especially in cell suspension culture. A Mg supplementation increased the

220   anthocyanin concentration produced by red grape cells. However, no substantial

221   induction of the biosynthetic genes was measured, but Mg inhibited pigment

222   degradation, especially that of anthocyanin species with low stability. Consequently,

223   the ratio between different anthocyanins and the color of the cultured cells changed.

224   These studies in cell cultures demonstrate that the availability of several plant nutrients

225   directly influences the anthocyanin level in grapes, and they indicate that an adequate

226   reduction of N or P fertilization could represent a cultivation approach with the aim to

227   enrich the anthocyanin concentration in grapes and consequently modify the wine

228   quality in terms of sensory perception and health benefits. For example, it has been

229   shown in field-grown grapes that high N supply promotes excessive vegetation, delays

230   fruit maturation and impairs both the quantitative and the qualitative biosynthesis of

231   anthocyanins. Moreover, excessive N fertilization enhances anthocyanins degradation

232   during the final steps of berry maturation.[93, 94] However, balanced potassium (K) supply

233   appears to lessen the negative effects of excessive N fertilization, as was shown in

234   Tempranillo grapes.[94] Delgado, Gonzalez and Martin [94] showed that fertilization with

235   maximum N resulted in higher polyphenol and anthocyanin concentration in grapes

236   when it was combined with high K nutrition. Vice versa, a high K application alone

237   reduced anthocyanins, but when high K was applied with sufficient N, no reduction in

238   anthocyanins was detected. Therefore, a balance between N and K seems to be crucial

239   for the synthesis of anthocyanins.[94] Furthermore, low N supply in the form of NH_4NO_3

240   stimulated the anthocyanin synthesis in Cabernet-Sauvignon berries at two ripening

241   stages (26 days post-véraison and maturity); delphinidin and petunidin derivatives

242   were the most affected compounds in this study.[64] This might be caused by a

243   competition between protein and phenylpropanoid synthesis since both pathways

244   compete for the common, limiting precursor phenylalanine. The protein competition

245   hypothesis of phenolic allocation[95] states that under conditions of reduced N

availability, the enzyme phenylalanine ammonia lyase deaminates the amino acid phenylalanine to yield ammonia and trans-cinnamic acid. The released ammonia is thought to be recycled to generate amino acids required for the biosynthesis of proteins while the trans-cinnamic acid - the key substrate for phenylalanine-derived phenolics such as anthocyanins - is shunted into the phenylpropanoid pathway.[44, 95] The analysis of transcript levels of both structural and regulatory genes involved in anthocyanin synthesis confirmed the stimulation of the phenylpropanoid pathway in response to reduced N nutrition in Cabernet-Sauvignon berries.[64] Keller and Hrazdina [86] found that the N availability at bloom changes the anthocyanin profile in Cabernet-Sauvignon grapes at maturity: Among the five measured anthocyanins, the accumulation of cyadinin-3-glucoside was most strongly reduced by high N nutrition, in contrast to malvidin-3-glucoside, whose percentage of the total anthocyanin content increased with high rates of N fertilization. This is of high importance for the vinification process, because the composition of individual anthocyanins determines the hue, taste and color stability of the wine. In general, the redness increases with the degree of anthocyanin methylation, while the blueness increases with the number of anthocyanin hydroxylation.[86] Therefore, the authors conclude that a low N nutrition of vine plants results in the highest wine quality, leading to a deeply colored Cabernet Sauvignon wine showing a well-balanced, crimson to purple hue. Furthermore, not only the quantity of available N but also the fertilizer type is considered to be important for wine production. The application of organic manures can be favorable for wine quality because N is released and taken up more slowly. A study by Malusa, et al. [96] showed that the overall polyphenol concentration was higher in grapes that were cultivated with organic instead of mineral fertilizers. Therefore, the regulation of the N fertilization can be a means to increase the formation of anthocyanins and polyphenols in grapes. Importantly, anthocyanins and flavonols are accumulated in grapes after véraison[97]

272 and late N application can thus result in an impairment of anthocyanin synthesis in the

273 skin and pulp of grapes. Consequently, finely orchestrating N fertilization with the plant

274 developmental stage and its changing N demand throughout the growing period can

275 be a strategy for anthocyanin enrichment in grapes without negatively impacting the

276 overall plant growth and grape yield.

277 **Anthocyanins in apples**

278 The color of apples is the combination of several plant pigments, including chlorophylls,

279 anthocyanins, and carotenes,[98] with cyanidin 3-galactoside (Fig. 2B) being the main

280 anthocyanin representative.[99] The coloration of red or bicolored apples (*Malus*

281 *domestica*) such as 'Delicious', 'Gala' and 'Jonagold' is an important qualitative trait for

282 farmers and consumers. The extent and intensity of fruit coloration determines the time

283 of fruit harvest and, moreover, the red skin color together with the fruit size define the

284 basis for the grading standards that influence the commercial value.[100] Apples with an

285 intense coloration attain the highest visual consumer acceptance and command better

286 prices.[101] Moreover, the content of anthocyanins in the fruit skin influences the

287 antioxidant activity and is critical for the fruit's nutritional value and – owing to their

288 ecophysiological properties – the fitness of the plant. Attaining the optimal coloration

289 in apples at harvest is often a serious problem in southern European countries because

290 the prevalent climatic conditions with hot dry summers do not favor fruit color

291 development.[100] Consequently, the harvest is delayed in order to attain a certain

292 degree of color, but this has a negative effect on other quality parameters such as

293 firmness and storability and increases the incidence of fruit cracking, for example, in

294 'Gala' apples.[100] Therefore, the potential for directed anthocyanin management by

295 strategic fertilization is highly relevant in apple orchards, because it would enable

296 achieving both, the aesthetic product appearance favored by the consumer and

297 intrinsic qualitative-associated fruit properties at the ideal harvest time.

298   It has been shown that $CaCl_2$ foliar application can improve the fruit skin redness in

299   'Jonathan', 'Red Delicious' and 'Golden Delicious' apples.[102, 103] However, antagonistic

300   and synergistic uptake effects between the nutrients could be responsible for these

301   observations.[102]

302   In orchards, N uptake has been linked to fruit skin color, and excessive N fertilization

303   is commonly known as an inhibitory factor for the red pigmentation of apples.[104]

304   Increasing N supply decreases both, anthocyanin synthesis and chlorophyll

305   degradation in the fruit skin, but accelerates pulp starch degradation in 'Gala' apples.[105]

306   In green apple cultivars such as 'Granny Smith' where red blush is undesirable, N

307   fertilization may be used for suppressing anthocyanin formation.[106] Awad and de Jager

308   [107] found that the fruit N concentration was the most important variable in predictive

309   models for the anthocyanin concentration in 'Elstar' apples, which is in accordance

310   with other studies that found negative effects of high N fertilization on fruit coloration in

311   the apple cultivars 'Aroma', 'Fuji', 'Goldrush', 'Cox's Orange Pippin' and 'Gala'.[98, 108-

312   112] The skin anthocyanin concentration can either be directly or indirectly influenced by

313   the N status of the tree. An indirect relationship is given because higher N fertilization

314   increases leaf growth and consequently shading of apples. Consequently, the light-

315   induced anthocyanin production in the skin is thought to decrease with increasing N

316   status. Furthermore, high N can lead to higher chlorophyll concentration in the fruit skin

317   and increased greenness of fruits. For example, Reay, Fletcher and Thomas [98] showed

318   that foliar application of urea to 'Gala' apple trees increases the chlorophyll

319   concentration on the shaded back of the fruit and lessened the increase of

320   anthocyanins in the sun-exposed blush side skin. Consequently, the harvest was

321   delayed for two weeks because of greener color scores, which are used as indicators

322   for fruit picking and grading. However, Tahir, Johansson and Olsson [110] found that N

323   application was positively correlated with fruit color. They used a demand-oriented

324 fertigation system based on soil N_{min} content and the part of the growth season, and

325 the authors state that negative effects of N supply on fruit coloration could be avoided

326 by fertigation according to the moderate model which was applied in this study. Thus,

327 as aforementioned for grapes, not only the amount but also the timing of N fertilization

328 has to be taken into account in order to attain the desired fruit attractiveness. High N

329 levels are most detrimental to color formation in apples if maintained late in the

330 season.[106] Nitrogen fertilization on demand (estimated by soil and plant tissue

331 analysis) at a critical time point during fruit development could be an effective method

332 to improve fruit coloration.[110] By this means, other qualitative fruit parameters such as

333 fruit firmness and sugar concentration can simultaneously be enhanced.[105, 107, 108] In

334 summary, the current literature clearly indicates that the consumer acceptance and

335 marketability of apples can be increased by optimizing fertilization especially that of N

336 since the N status influences apple skin coloration and consequently the fruit

337 attractiveness.[107]

338 **Anthocyanins in strawberries**

339 Strawberries (*Fragaria × ananassa*) are one of the most popular fruit crops from a

340 commercial point of view, and the color of the berries is an important quality

341 parameter.[113] Increasing demand for strawberries due to their flavor and nutritional

342 value continuously increases the economic impact of strawberries.[114] Owing to their

343 high content of compounds with oxygen radical absorbance activity, strawberries are

344 highly nutritious fruits with profound health benefits.[2, 4, 13, 32, 115] Pelargonidin 3-

345 glucoside (Fig. 2C) is the predominant anthocyanin type in strawberries,[113, 116-118]

346 imparting their attractive redness and contributing to their antioxidant potential.[116] A

347 number of studies showed that the antioxidant activity and hence the nutritional value

348 of fresh or processed strawberries increases concomitant with the fruit anthocyanin

349 content.[113, 117, 119-121] Fertilization of strawberry plants is likely to influence the

350 anthocyanin content in the fruits and adjusting the level of nutrient application can be

351 an effective means to produce high-quality strawberries with the desired amount of

352 bioactive compounds such as anthocyanins.[113, 119] However, contradictory results

353 about the effects of nutrient supply on the color and anthocyanin concentration in

354 strawberries have been reported.[122] We here summarize some key findings that

355 describe the ongoing dispute in the literature.

356 Wang and Lin [121] tested the two strawberry cultivars 'Allstar' and 'Honeoye' and found

357 that strawberry plants that were cultivated with high nutrient levels yielded fruits with

358 the highest levels of phenolics, including anthocyanins. Likewise, a negative impact of

359 N deficiency on the anthocyanin concentration was reported in other strawberry

360 cultivars.[123] In contrast to these positive correlations between the amount of supplied

361 nutrients and anthocyanin concentrations other studies reported a negative correlation.

362 These studies have shown that a reduced nutrient application represents a proper way

363 to obtain strawberries with increased anthocyanin concentrations, which is in

364 accordance with results described for grape berries and apples. For example, Wold

365 and Opstad [124] recommend solely pre-planting fertilization and the avoidance of

366 additional nutrient application before harvest, which is in agreement with Moor, Karp,

367 Poldma and Pae [118], who conclude that the best treatment based on increased

368 anthocyanin content would be growing strawberries with only pre-planting fertilization

369 without additional application of liquid fertilizer during the growing season. Likewise, a

370 study with organic cultivation that is characterized by an abandonment of mineral

371 fertilizers and synthetic pesticides resulted in strawberry fruits that had a higher content

372 of pelargonidin-3-glucoside.[117] D'Evoli, Tarozzi, Hrelia, Lucarini, Cocchiola, Gabrielli,

373 Franco, Morroni, Cantelli-Forti and Lombardi-Boccia [117] attribute these differences

374 partly to the utilization of compost, which implies that N is not rapidly present in a plant-

375 available form, leading to the synthesis of non-nitrogen-containing compounds such

376 as anthocyanins. Similarly, several studies revealed higher anthocyanin content and

377 antioxidant activity in unfertilized or organically fertilized strawberries when compared

378 to plants that were supplied with mineral fertilizer.[120, 125] However, in most cases this

379 increase cannot exclusively be ascribed to the plants nutrition because also the

380 different management of pest control in organic farming systems might have

381 contributed to the induction of the plant stress response by causing pathogen stress,

382 as described by Liu, et al. [126]

383 Hydroponically grown strawberries cultivated under reduced P and iron nutrition

384 showed remarkably higher concentrations of fruit anthocyanins. Interestingly, both, low

385 P and low iron application, reduced the shoot growth of strawberry plants but the fruit

386 yield per plant and the average fruit number per plant increased compared to fertilized

387 control plants.[113] Both, the yield and quality parameters such as acidity, firmness, and

388 soluble solid content were unaffected. However, these results should be handled with

389 care, because sufficient P nutrition has been shown to be pivotal for the soluble solid

390 content of the fruit, which is an important parameter in determining the strawberry flavor

391 as well as the nutritive value.[127] Increasing the fruit's anthocyanin content could also

392 be achieved by applying so-called 'biostimulants' such as phosphite. Phosphite is the

393 salt of phosphorous acid and is absorbed and translocated in the plant in a similar way

394 as inorganic P. However, phosphite does not metabolically substitute inorganic P in

395 the plant.[128] In field studies conducted by Estrada-Ortiz, et al. [129] and Moor, et al. [130] it

396 was shown, that phosphite application increased the anthocyanin content in

397 strawberries without having significant effects on the yield. This increase in

398 anthocyanins in the fruits was not caused by the induction of a physiological P

399 deficiency because phosphite application did not negatively impact the P

400 concentrations in leaves. Therefore, the authors recommend the usage of phosphite

401 as a stimulator to increase the fruit quality by inducing the synthesis of compounds

402 such as anthocyanins. However, the effect of phosphite as a plant stimulant is hardly

403 understood and phosphite application has even been shown to have deleterious

404 effects on plant growth depending on the plant's P status.[131] Therefore, the reported

405 positive effects of phosphite on strawberries in terms of anthocyanin content cannot

406 be generalized at this point.

407 In conclusion, adjusting strawberry fertilization may provide a potential means to

408 increase the color and nutritional value of strawberries by altering the anthocyanin

409 content in the fruit and especially a demand-driven N fertilization corrected for soil N

410 secures fruit yield and quality.[132]

411 **Concluding remarks and perspectives**

412 Consumers are increasingly concerned about the health implications of their nutrition,

413 and the desire to eat healthy is among the five most important factors influencing

414 consumers food choice.[133] Numerable studies indicate that the consumption of

415 anthocyanins positively impacts the physical wellbeing and protects against diverse

416 diseases. Therefore, fruits containing anthocyanins with potential human health-

417 promoting activity are of high consumer appreciation. Above this, anthocyanins confer

418 an attractive coloration to fruits such as apples, grapes, and strawberries and hence

419 increase the consumer acceptance and market value of these commodities. Enriching

420 fruits with these so-called "phytonutrients" by metabolic engineering can be achieved

421 by using new breeding technologies or by developing improved crop varieties via

422 genetic modification. Applying these techniques requires detailed knowledge about the

423 identity of anthocyanin synthesis-related genes, however, which is limited to a very few

424 crop species until now. Above that, nutritionally-enhanced crops have to be accepted

425 by both farmers and consumers in order to take effect in promoting public health.

426 However, the approval of genetically-engineered functional food is impinged by the

427 prevailing skepticism among the public towards GMOs and non-established varieties,

428 and the uncertain legal status of modified crops.

429 Our review highlights that anthocyanin levels in fruits with major global trading value

430 are influenced by the plant's nutrient availability and therefore by the agronomic

431 nutrient management. This enables the producer to control fruit anthocyanin formation

432 by adjusting the fertilization practice. Especially a restricted N fertilization can lead to

433 elevated coloration and antioxidant capacity of fruits by shifting the plants metabolism

434 towards an increased anthocyanin synthesis. However, lower N fertilization is

435 oftentimes associated with reduced yield formation, which might create a conflict of

436 interests between fruit quantity and quality for producers. Also, anthocyanin-oriented

437 growing strategies might potentially be inversely correlated with other fruit quality

438 parameters such as acidity or firmness. We present several studies indicating that

439 anthocyanin enrichment by reduced nutrient application can be achieved without

440 negatively affecting the fruit yield or other quality characteristics. This requires a careful

441 fine-tuning of timing and amount of fertilization to account for different nutrient

442 demands between vegetative and generative growth phases or fruit yield-determining

443 and fruit quality-defining stages, respectively.

444 An up-regulation of the anthocyanin synthesis pathway by environmental factors is

445 limited by species- or variety-specific genetically-determined margins, of course, and

446 might furthermore be restricted to certain tissues. For example, it has been proposed

447 that anthocyanin accumulation in the mesocarp is governed by developmental factors,

448 whereas anthocyanin synthesis in the epicarp is more under environmental control[134].

449 However, if this assumption holds true for abiotic effects other than light, remains to be

450 investigated. Hence, exploring the molecular mechanisms that mediate the effect of

451 nutrients on the biosynthesis of anthocyanins in fruits, as well as the crosstalk between

developmental signals and other environmental stimuli remains an outstanding task for the future, in order to fully exploit the potential of anthocyanin enrichment in fruits by well-orchestrated fertilization strategies. Certainly, univocal conclusions and generalizations cannot be made for complexes such as agricultural systems because of a dynamic interplay between culture, site, weather conditions, and agricultural practices including the amount and timing of different fertilizers. However a general trend pointing towards the potential to increase anthocyanins in fruits by reduced nutrient input and fine-tuning of fertilization timing can be seen across the here reviewed literature.

Acknowledgements

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860

861

862 **Tables**

863 Table 1

864 Anthocyanidins and anthocyanins in the skin of blue vine berries (Alphonse-Lavallee,

865 Muscat-Bleu) and red wine (Regent). Berries and wine were collected at the

866 Hohenheimer Schlossberg (n = 8). Data were measured by HPLC according to

867 Kammerer, et al. [135] by a Merck-Hitachi HPLC series 7000 (Merck, Darmstadt,

868 Germany); D-7000 HSM software, L-7612 degasser,

869 L-7200 ternary gradient pump, L-7450A diode array detector, and a Knauer Jetstream

870 column oven. The separation was performed with a Phenomenex, (Torrance, CA)

871 Aqua C18 column (250x4.6 mm; 5 μm particle size), with a C18 ODS guard column

872 (4.0x3.0 mm) at 25 °C. *Malvidin derivatives other than oenin (malvidin 3-glucoside).

[mg kg^{-1}]	Delphinidin	Kuromanin	Oenin	Petunidin	Peonidin	Other malvidins*	Σ
Alphonse-Lavallee	868	332	2322	620	1001		5144
Muscat-Bleu	1115	289	2777	786	869		5835
Regent (wine)	5804	1074	4932	5479	1197	7317	25803

873

874

875   Table 2

876   Effect of nutrient supply on anthocyanin concentration and yield of various fruits. Experimental

877   setup and results are stripped-down for reasons of clarity and comprehensibility. -: negative

878   effect; +: positive effect; o: no or inconclusive effect; x: not determined. *Different dosages

879   within the given range were tested. N: nitrogen, P: phosphorus, K: potassium, Mg: magnesium,

880   Ca: calcium, Fe: iron, LA: leaf application.

881

Nutrient	Nutrient range	Analytical technique	Effect of increasing fertilization on		Experimental setup	Comments	Ref.
			[Anth]	Yield			
Culture: Grape							
N	0 - 25 mM	Photometry ($A_{535\,nm}$) HPLC	-	+	Cell culture		[76]
N	1.4 - 7.2 mM	Photometry ($A_{520\,nm}$) HPLC	-	o	Glasshouse, soil		[93]
N	0 - 200 g plant^{-1}	HPLC	-	o	Field	Effect of N×K interaction detected.	[94]
K	0 - 120 g plant^{-1}		o	x			
N	0 - 120 kg ha^{-1}	HPLC	-	o	Field		[64]
N	0.9 - 1.5 ha^{-1}	HPLC	o	x	Field	N applied as phenylalanin or urea.	[97]
N	0.34 - 3.4 g plant^{-1}	HPLC	o	x	Field	Anthocyanin profile changed with N amount.	[86]
N	20 - 180 mM	Photometry ($A_{525\,nm}$)	-	o	Cell culture	Also ammonium-nitrate ratio effects tested.	[78]
P	20 - 600 mM	Photometry ($A_{525\,nm}$)	-	+			
P	0 - 1.1 mM	Photometry ($A_{530\,nm}$) HPLC	-	+	Cell culture		[79]
Mg	0 - 50 mM	Photometry ($A_{530\,nm}$) LC-MS/MS	+	o	Cell culture		[92]
Culture: Apple							
N	1% urea (LA)	Photometry ($A_{530\,nm}$)	-	x	Field		[98]
	8.8 - 105.4 g plant^{-1}	Photometry ($A_{520\,nm}$)	-	+	Field, pots		[105]
N	0 - 210 kg ha^{-1} (soil)	RP-HPLC	-	x	Field		[107]
P	0 - 101 kg ha^{-1} (LA)		o				
K	0 - 150 kg ha^{-1} (soil)		o				
Mg	0 - 20 kg ha^{-1} (LA)		o				
Ca	0 - 16.5 kg ha^{-1} (LA)		o				
N	28.4 - 170.5 kg ha^{-1}	Photometry	+	o	Field		[108]
N	0 - 200 kg ha^{-1}	Color assessment	-	x	Field		[109]
K	0 - 200 kg ha^{-1}		+				
N	0 - 90 kg ha^{-1}	Colorimeter (h°)	-	o	Field		[111]
	0 - 5% urea (LA)		-	o			
N	0 – 50 g tree^{-1} day^{-1}	Colorimeter (h°)	+	+	Field	Fertigation adjusted based on N_{min} and season.	[110]
Ca	9 kg ha^{-1}	Chromameter	+	+	Field		[102]

| Nutrient	Treatment	Method			Location	Comments	Ref
	(LA, up to 8x)						
Ca	leaf application, diverse rates + times		+	o	Field		[103]
Culture: Strawberry							
P	0 - 0.25 mM	HPLC	-	o	Chamber,		[113]
Fe	0 - 20 µM		-	o	hydroponics		
	0/0.5x/1x multinutrients	HPLC	+	x			[121]
	0/50/100% compost		+	x			
N	96 - 112 kg ha^{-1}	HPLC	+	x	Field	General effect of plastic mulch tested. No information about nutrient amount.	[116]
	Organic vs. conventional cultivation	HPLC	+	x	Field	Higher anthocyanin content with organic cultivation. No information about nutrient amount.	[117]
	White vs. brown mulch	Photometry (A$_{515 nm}$)	+	x	Field	Higher anthocyanin content with brown mulch. No information about nutrient amount.	[119]
	Organic vs. conventional cultivation	Photometry (A$_{510nm}$)	+	x	Field	Higher anthocyanin content with organic cultivation.	[120]
	Organic vs. conventional cultivation	HPLC	+	x	Field	Higher anthocyanin content with organic cultivation.	[125]
N	0 - 6.75 mM	Colorimeter	-/o	x	Glasshouse,	Nutrient depletion was started after flowering. No effects of K and P; effects of N nutrition on	[123]
P	0 - 0.75 mM	HPLC			soil	anthocyanins was cultivar- and time-dependent.	
K	0 - 3 mM						
Culture: Black chokeberry							
NPK	Low rate vs. high rate	Photometry	-	+	Field	Low rate: 30 kg N ha^{-1}, 45 kg P ha^{-1}, 150 kg K ha^{-1}; high rate: 90 kg N ha^{-1}, 135 kg P ha^{-1}, 450 kg K ha^{-1}.	[21]
Culture: Blackberry							
NK	Low rate vs. high rate	HPLC	+	x	Glasshouse,	Low rate: 60 kg N ha^{-1}; 66.4 kg K ha^{-1}; high rate: 100 kg N ha^{-1}; 104 kg K ha^{-1}.	[136]
					soil		
Culture: Blueberry							
	Organic vs. conventional cultivation	Photometry (A$_{510 nm}$)	+	x	Field	Higher anthocyanin content with organic cultivation.	[137]
	Organic vs. conventional cultivation	Photometry (A$_{515 nm}$) HPLC	o	x	Field		[138]
Culture: Cranberry							
N	0 - 44 kg ha^{-1}	Photometry (A$_{535 nm}$)	o	o	Field	Different timings of fertilization tested in several varieties and locations.	[139]

Culture: Mango						
N	0 - 300 g plant^{-1} (soil)	Colorimeter (h°)	-	+/-	Field	[140]
	0 - 50 g plant^{-1} (LA)		-	-		

882
883

884 **Figures**

885 Figure 1
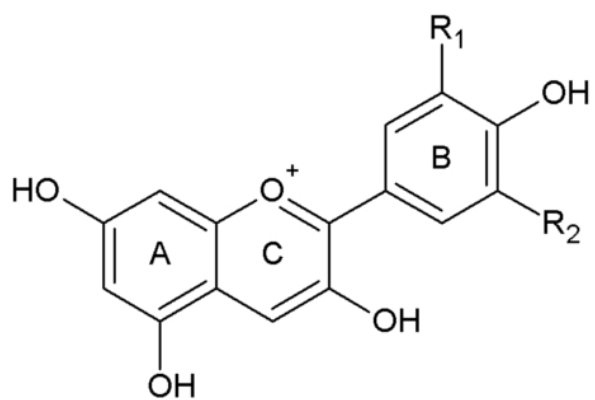
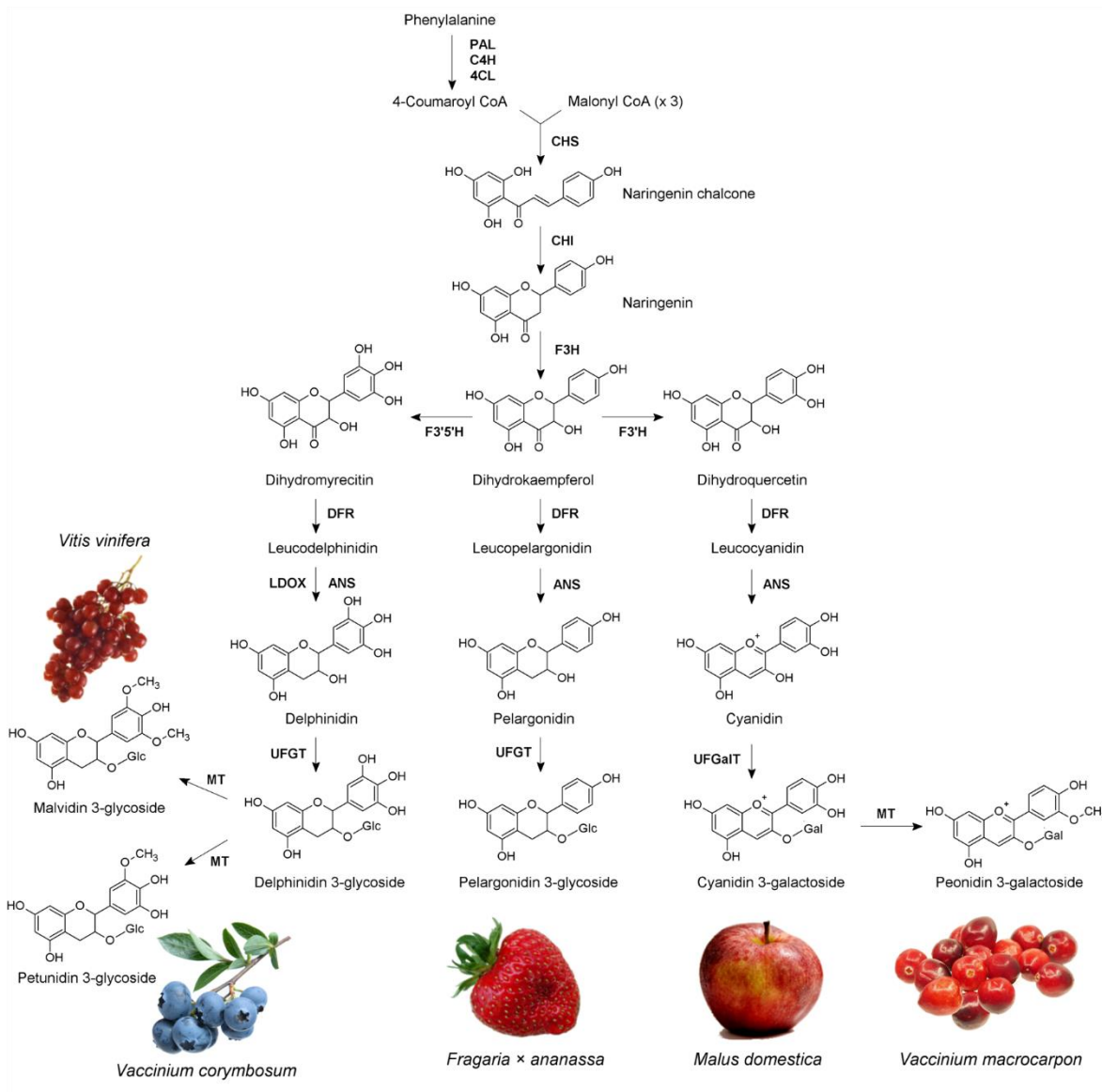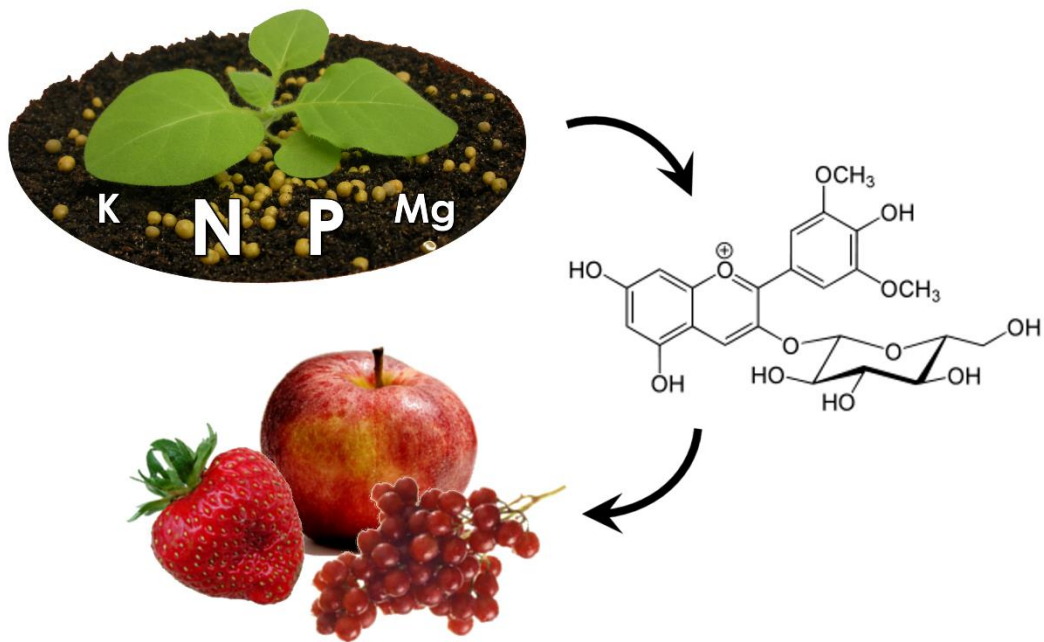|	R$_1$	R$_2$
Pelargonidin	-H	-H
Cyanidin	-OH	-H
Peonidin	-OCH$_3$	-H
Delphinidin	-OH	-OH
Petunidin	-OCH$_3$	-OH

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889

890 Figure 2

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892

893

894   Table of contents graphic (TOC)

895

896

Figure captions

898 Figure 1

899 Basic chemical structure of the main anthocyanidins in nature with variable hydroxyl (-

900 OH) and methoxy (-OCH$_3$) groups including ring nomenclature of the flavylium core

901 molecule (A, B, C).

902

903 Figure 2

904 Simplified schematic of the biosynthetic pathway of the major anthocyanins that are

905 found in grapes (*Vitis vinifera*; malvidin 3-glucoside), blueberry (*Vaccinium*

906 *corymbosum*; petunidin 3-glucoside), strawberry (*Fragaria x ananassa*; pelargonidin 3-

907 glucoside), apple (*Malus domestica*; cyaniding 3-galactoside), and cranberry

908 (*Vaccinium macrocarpon*; peonidin 3-galactoside). The structural enzymes catalyzing

909 the steps along the pathway are phenylalanine ammonia lyase (PAL), cinnamate 4-

910 hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS),

911 chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase

912 (F3'H), flavonoid 3′-5'-hydroxylase (F3'5'H), dihydroflavonol 4-reductase (DFR),

913 anthocyanidin synthase (ANS), leucoanthocyanidin dioxagenase (LDOX), UDP-

914 glucose:flavonoid 3-*O*-glucosyltransferase (UFGT), UDP-galactose:flavonoid 3-*O*-

915 galactosyltransferase (UFGalT), and methyltransferase (MT). Image sources (left to

916 right): commons.wikimedia.org, pixabay.com, public-domain-image.com,

917 maxpixel.freegreatpicture.com.