D. Häge, M. Reichenbacher, M. Bernhard, B. Krause, M. Zarnkow and B. Lindemann

Technological possibilities for transferring the colour of dark barley (*Hordeum vulgare* L.) into mash and wort to produce beers with a reddish hue

In some barley (*Hordeum vulgare* L.) varieties, reddish-purple anthocyanins and black allomelanins are found in addition to the carotenoids. While melanins are absolutely insoluble in water as well as alcohol and therefore cannot be used in beverage production, anthocyanins are known to provide the characteristic colour of numerous juices and red wines. One focus of this study was to assess the technological possibilities of producing a red coloured beer from coloured barley. The biggest obstacles in conventional production processes are the high pH value of the unfermented mash and wort as well as the immense thermal stress caused by wort boiling. The influence of mash pH, wort boiling and hop addition were investigated in several trials on an experimental level in the lab. No classic brewing trials were conducted. A classic congress mashing process followed by immediate acidification was able to achieve a red colouring of the mash at pH values suitable for consumption. However, delaying acidification by just one day ensured that the anthocyanins contained remained in colourless form. Even a 15-minute wort boiling significantly and irreversibly affected the colour. The addition of iso-α-acid to an acidified extract resulted in a weak but significant decrease in anthocyanin colouration. While the results of the study do not provide guidance for brewing a red beer, this does not seem impossible and further research may allow the production of coloured beers within the framework of the purity law.

Descriptors: coloured barley, anthocyanins, beer, mash, wort, colour stabilisation

1 Introduction

Over the centuries, numerous historical sources have repeatedly mentioned a "red beer" without going into detail about its production method. It therefore remains unclear whether the beer was always produced using the same method: Nowadays, beers are called "red" that do not have a completely dark colour but a reddish-brown colour due to a moderate use of malts containing melanoidin. This is not a "real" red colour, but the interaction of yellow - carotenoids and low-molecular Maillard products - and brown - caramel and melanoidins - colouring agents. This hue is found, for example, in beer styles such as Red Ale or Nürnberger Rotbier [1].

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Authors

Doris Häge (https://orcid.org/0009-0006-8775-8413), Bernd Lindemann, Hochschule Geisenheim University, Department of Food Safety, Geisenheim, Germany; Maximilian Reichenbacher, Justus Liebig University Giessen, Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management, Giessen, Germany; Marco Bernhard, Hochschule Fresenius University of Applied Science, Faculty of Chemistry and Biology, Idstein, Germany; Birgit Krause, Hochschule Geisenheim University, Department of Soil Science and Plant Nutrition, Geisenheim, Germany; Martin Zarnkow, Technical University of Munich, Research Center Weihenstephan for Brewing and Food Quality, Freising, Germany; corresponding author: bernd.lindemann@hs-gm.de

Red beers seem to have been a more common beer style in the past, because all light variants were called "white beer" to distinguish them from red beers. It should be emphasised here that this by no means meant exclusively wheat beers, as is the case today. It is conceivable that the term "red beer" was used in a similar sense to today. However, anthocyanin-containing barley was already known in the early days of agriculture and beer production. For example, the wild barley native to the Middle East, which is considered the ancestral form of our present-day malting barley, contains anthocyanins in its stalks and ears to protect it from the sun and desiccation [2]. It can therefore be assumed that the first barley deliberately cultivated by early man was a variety containing anthocyanins. With the cold mashing methods common at the time [3], it is highly likely that the beers also took on a reddish colour.

It is also interesting to note that there are reports of beers possessing an undesirable red colouring into the 20th century [4]. A publication on the use of blue barley in brewing explicitly mentions the disturbing red tinge in the beer, which had to be removed by a filtration step that is not specified in detail [5, 6]. There are also reports about the "shortage beers" at the time of the First World War, which refer to a red tinge [7].

Singh et al. (2023) showed in brewing experiments with dark wheat varieties that the beers produced from them did not have an intense red, but rather an orange-reddish colour. In contrast, the beers made from light wheat had a pale yellow colouration [8].

Singh et al. measured the colour using the one-dimensional EBC scale, which does not allow direct conclusions to be drawn about the concentration and hue of the anthocyanins [9].

It is also interesting to note the reference to the term “Rotbier” (red beer), which has been used again and again in various contexts over the centuries [4, 7]. Although this could simply mean dark or reddish-brown beer styles, anthocyanin-containing barley has been known since the beginning of agriculture and was cultivated in many parts of the world until the advent of modern cultivars [10]. Due to the green revolution and the sharp increase in fertiliser applications, ancient varieties lost their importance as their culms were not strong enough to support the heavy spikes of fertilised plants, which lead to lodging [11]. Breeding programmes not only focused on phenotypic traits, but also on improved disease resistance [12] and higher yields, which led to modern varieties replacing the ancient ones. Nowadays, none of the commercially grown barley varieties contain pigments other than carotenoids [13].

In addition to the yellow barley (*Hordeum vulgare* L.) that is predominantly cultivated today, there are also varieties with purple, blue or black colouring. As shown by *Häge* et. al. (2023), these ancient grains get their colour from two different types of pigments: The red to bluish coloured anthocyanins and the black allomelanins [14].

Like all known melanins, the allomelanin detected in barley is completely insoluble in water and organic solvents [15, 16]. The use of these black pigments in the beverage sector is therefore ruled out. However, it would be possible to use barley containing melanin to produce dark-coloured baked goods. In the Mediterranean region, bread and pasta specialities have traditionally been made with animal melanin from cuttlefish (*Sepia officinalis*) for centuries [17].

Anthocyanins are among the most abundant secondary plant metabolites and are responsible for the characteristic colour of numerous fruits and vegetables, as well as the autumn colour of numerous deciduous tree species [5, 16, 18, 19]. In the beverage sector, they play an important role primarily in red wine production, but also in red juices, since an attractive and stable colour is one of the valuable quality characteristics for these products [20].

Due to their antioxidant and presumably even anticarcinogenic as well as anti-inflammatory effects, anthocyanins are considered to be beneficial to health and have increasingly been the focus of scientific and medical investigations in recent years [21]. Their use as natural colourants for food is also a conceivable way of replacing synthetic food colourings.

The use of coloured barley as a natural colouring agent for beer requires a good understanding of the underlying processes. To shed more light on this, this study was conducted.

Its first focus was on reviewing how anthocyanins are synthetised and which aspects influence their colour. We further review the mechanisms relevant for transferring the reddish colour into wort and beer. A second focus was studying, on an experimental level, the technological possibilities of transferring them to mash and beer and thus having them contribute to the colour of the product, by using model wort. This study was not aiming at the development of a recipe for brewing a beer with a decent red colour and sensory profile. This experimental study will solely provide a better understanding of the various stages within the brewing process that could influence or prevent the transfer of a reddish colour into wort and beer.

This would be particularly interesting, for example, as a coloured beer produced according to the German Purity Law would be a complete product innovation. As *Carvalho* et al. (2017) showed, the colour of a beer has a strong influence on consumer expectations in terms of taste as well as price [22]. Producing red coloured beers from ancient barley varieties might be predominantly interesting for the craft brewing industry, a fast growing market, mainly driven by younger consumers with changing demand and expectations regarding more diversified beer styles. Craft brewers are trying to meet these demands by using local or historic ingredients with high quality as well as non-traditional ingredients to create a unique sensory profile, using specific recipes and brewing techniques [23–25].

1.1 Synthesis pathway

During synthesis within plant cells, anthocyanidins are formed from colourless precursors, the leucoanthocyanidins (syn. flavan-3,4-diols), which in turn belong to the group of proanthocyanidins. This reaction can also be induced artificially by the influence of acid and heat [26]. The term anthocyanogens is often used in brewing science jargon, although it is somewhat vaguely defined: for the most part, the term is used synonymously for proanthocyanidins [27–29], but sometimes the term is also used to mean their glycosides [30] or only the flavan-3,4-ols in a narrower sense [31]. In terms of the importance of these precursors to colour malt, *Kunze* (2009) reports that the proanthocyanidins found in the raw grain can be converted to red anthocyanidins by heating and oxidation by air during the malting process, and this is exploited to produce special malts for red beers [32]. However, no further literature or examples from malting practice could be found to support this statement.

1.2 Anthocyanins as pH indicators

The highly water-soluble anthocyanins act as a pH indicator in a specific way: they change colour not only at a single transition point but have several different colours that they produce across the pH spectrum. In addition, they are sensitive to thermal stress and can irreversibly lose their colour if stored for a long time under excessively high pH values. The typical red colouration only appears in the positively charged flavylium cation form (see Fig I supplementary material), which is found at very low pH values. In this state, the molecule has seven conjugated double bonds, resulting in a reflection that humans perceive as red. A rapid increase in pH leads to a neutralisation of the positively charged oxygen atom and subsequently, to a deprotonation of the hydroxy group (Fig. II – III supplementary material). These changes are perceptible as a colour shift into the violet to blue range [33].

Another significant effect on the colour stability of food occurs when the anthocyanin remains in a slightly acidic to neutral pH range for a longer period of time. This environment provides the necessary conditions for hydroxylation of the C2 atom to the hemiacetal form (Fig. IV supplementary material), which in turn is the precursor for the irreversible opening of the C ring. In the resulting open chalcone form (Fig. V supplementary material), the anthocyanin is only very pale yellow. Table I (supplementary material) provides an overview of the anthocyanin configurations that occur in different pH ranges.

The colouring of natural products containing anthocyanins cannot be explained without the effect of copigmentation. This is the complexation of anthocyanins with other substances (copigments), which contributes to the stabilisation of the electron system. Due to the presence of the copigment, the anthocyanin is shielded against the attacking OH^- and can retain its cationic form inside the complex longer than would actually be expected. In this way, the flavylium form can still appear at significantly higher pH values which, in the case of monomeric anthocyanins, would already favour the weakly coloured hemiacetal and chalcone forms. The copigments can also act as chromophores, but this is the exception rather than the rule [34–36]. Colour stabilisation (hyperchromism) is usually accompanied by a blue shift (bathochromism) [5].

Important naturally occurring copigments are almost exclusively phenolic compounds. These are mainly phenolic acids (including rosmarinic, caffeic, gallic and chlorogenic acids), flavanols (catechin, epicatechin) and flavonols (rutin, quercetin) [34, 37–39].

Differentiation is made between three main forms of copigmentation: intramolecular and intermolecular copigmentation, and self-association. In all variants, anthocyanin and copigment are present in stacked π-π interactions. While in intramolecular copigmentation the copigment is covalently bound to the anthocyanin via the C3 sugar residue. In the intermolecular variant the units are only loosely stacked and coalesce via hydrogen bonds. Self-association is an intermolecular copigmentation in which several anthocyanins act as copigments for each other. Due to covalent bonding, intramolecular copigmentation provides the most stable anthocyanin colouration. Which type of copigmentation is formed depends on the molecular structure of the copigments: While the intermolecular form comes from extended conjugated electron systems that favour π-π-stacking, intramolecular bonding requires functional groups that form stable covalent bonds on the glycoside [40].

In addition to copigmentation, the formation of pyranoanthocyanins also known as vitisins, plays a major role in winemaking. These are condensates from anthocyanins and by-products of alcoholic fermentation, namely pyruvate and ethanal. Both are, in principle, intermediate stages of the fermentation of sugar to alcohol, but some of them also leave the yeast cell directly. After leaving the yeast cell, it is possible for the anthocyanins to attack, resulting in the formation of the complexes. The compound ACN pyruvate is called type A vitisin, ACN ethanal is called type B vitisin. Both compounds are primarily formed during the main fermentation – i.e. when most of the precursors are excreted from the yeast cell – and are characterised by a slight hypsochromic shift with increased colour stability. The insensitivity of vitisins to oxidative degradation is especially noticeable during extended storage, whereas the concentration of monomeric anthocyanins starts to decrease significantly during fermentation [36, 41–43].

The amount of vitisins formed can also be influenced by the yeast strain selection: For example, when Tempranillo must was fermented by *Schizosaccharomyces pombe* versus a conventional wine yeast, an increased amount of vitisin by a factor of 4 was observed, resulting in a stronger colour [42, 43].

These simple pyranoanthocyanins can now be derivatised into more complex molecules. Particular mention should be made of the covalent bonding with hydroxycinnamic acids, which leads to the formation of vinylphenol pyranoanthocyanins, also known as pinotins. These show a high stability especially over years of storage and it is possible to use them to determine the age of decades-old red wines due to the very slow degradation. The most important binding partners are caffeic, ferulic, mustardic and *p*-coumaric acids [44].

The role of yeast in colour development during fermentation is quite well researched using the example of red wine, since anthocyanin colouration is a critical quality characteristic in this product. In principle, the fermentation of a substrate containing anthocyanin can intensify its colour; the main reason for this is likely to be the decreasing pH value in the process [19, 45]. However, the presence of yeast has another, opposite effect: a small portion of anthocyanins adsorb on the yeast cell wall and are thus removed during racking [19, 42, 46]. The effect of this adsorption is low, but is deliberately exploited in the production of Blanc de Noir wines to prevent an undesirably strong colouration [19]. In the case of red wine, however, timely removal of the lees is important to prevent adversely affecting the colour [47]. It should be mentioned here, that vitisins and their phenolic acid complexes are adsorbed to a much lesser extent than free anthocyanins. This additionally contributes to the increased stability of these derivatised anthocyanins [48]. It is known from winemaking that sulphurisation of must and wine can lead to reductive decolourisation of anthocyanins by hydrogen sulphite ions (HSO_3^-). Although no sulphur is added directly in beer production, yeast produces SO_2 as a fermentation by-product – especially in the late phase of the main fermentation – which is also desirable to a certain extent, as it contributes to the stability of the beer. Corresponding trials could test whether the rather small amounts produced in this way (a maximum of 10 mg/kg is permissible) influence the colour to any appreciable extent. The level of SO_2 production can be satisfactorily controlled by modern cellar technology [49].

1.3 Temperature sensitivity

Anthocyanins are only stable to a limited extent at high temperatures. An exponential decline occurs above approx. 60 °C, whereby the specific speed is proportional to the temperature. Some anthocyanidins are more sensitive than others, but it is the type of copigmentation that is decisive: monomeric anthocyanins degrade particularly quickly, which is a well-known problem in the pasteurisation of strawberry juice, for example, as strawberries contain many monomeric anthocyanins [50]. In contrast, intramolecularly copigmented anthocyanins are the most stable [51–53]. Very high sugar contents (> 400 g/l) can slow down the formation of the colourless hemiacetals, which also contributes to stabilisation against thermal degradation. This is probably due to the fact that sugars, through their high water-binding capacity, lower the a_W-value and thus prevent the attack of OH^- ions [54]. Highly processed products are generally more susceptible to colour loss on heating, as naturally occurring copigment complexes can be destroyed in the course of processing. For subsequent stabilisation, it is possible to add copigments artificially and the addition of metallic cations can also have a stabilising effect [55].

1.4 Interaction with organic molecules

The effect of a pH-independent colour weakening has so far only been observed with ascorbic acid: Various experiments showed that red fruits rich in anthocyanins quickly lose their colour if the ascorbic acid content is too high during storage. This affects, for example, products made from acerola cherry (*Malpighia glabra*), which rapidly lose their colour despite the high anthocyanin content [56]. The exact mechanism behind this has not yet been clarified, but it is considered likely that ascorbic acid covalently bonds to the C4 atom of the anthocyanin to form a new, colourless molecule [57–60]. However, there is no understanding as yet of how this product is structured and which functional groups of anthocyanin and ascorbic acid are involved in the reaction.

The focus of this study was to assess the technological possibilities of producing a red coloured beer from coloured barley. The biggest obstacles in conventional production processes are the high pH value of the unfermented mash and wort as well as the immense thermal stress caused by wort boiling. The influence of mash pH, wort boiling and hop addition were investigated in several trials on an experimental level in the lab. No classic brewing trials were conducted, as this study was not aiming at the development of a recipe for brewing beer with a decent red colour and sensory profile.

2 Material and methods

2.1 Reagents

All brewing trials were carried out with fully desalinated water on a small- scale at University of Geisenheim, Department of Beverage Technology. The malt used was a mixture of different harvests of the anthocyanin-containing ancient, hull-less spring barley variety ‘Weihenstephaner Schwarze Nackte’ from cultivation areas in the federal states of North Rhine-Westphalia and Rhineland-Palatinate, vintage 2021. This variety was used, as previous research [14] showed high anthocyanin content in this variety. Malting was carried out as a modified MEBAK small malting process using a rotating germination box. Steeping times were observed according to the regulations, but pre-sorting of the barley was omitted. A kilning programme for Pilsner malt was used. The top-fermenting yeast SafAle US-05 (Fermentis, Marquette-lez-Lille, France) was used. The hops were added in the form of the isomerised extract iso-Hop 30 % α-acid from BarthHaas (Nuremberg, Germany). To adjust the pH values, HCl and NaOH were each used as a 1 N standard solution from Carl Roth (Karlsruhe, Germany).

2.2 Implementation

The pH dependence of anthocyanin colour was investigated using an ethanolic extract. For this purpose, analogous to the method presented by *Abdel-Aal* et al. (1999) for the determination of the total anthocyanin content in cereals, the following methods were used [61]: 30 g of ground barley malt in 240 ml ethanol, was acidified with 15 % 1 N HCl, stirred for 30 min on stirring plate and then clarified through a folded filter. Subsequently, the pH was raised by dropwise addition of NaOH in 0.5 increments. The colour development was visually assessed and recorded instrumentally via the absorbance at $\lambda = 535$ nm in the photometer (DR 6000, Lange, Vienna, Austria). The same experiment was carried out analogously with beer wort (Avalon, Pilsner malt) instead of ethanol as the extraction agent, which was acidified gradually following extraction.

In addition to cold extraction, the colour intensity of a conventional heated mashing process was investigated. For this purpose, ‘Weihenstephaner Schwarze Nackte’ malt was mashed according to the MEBAK congress mashing process and then acidified by adding hydrochloric acid. The colour development was again recorded visually and photometrically. The influence of thermal stress was determined by boiling the wort on hot plate for 15 minutes. To compensate for any colour intensification due to concentration, the wort was diluted back to the original volume with water after boiling.

The temporal sensitivity of the colour stabilisation was investigated in a brewing experiment. For this purpose, a mash was produced in the same way as described in the above experiment and fermented without subsequent wort boiling. During the fermentation process, the extent to which red colouration can be induced by acidification was recorded daily.

To investigate the influence of hop addition on the colour, a congress mash was again prepared, acidified to pH 1 by adding HCl and stored for $t = 2$ weeks at $\vartheta = 8$ °C. A blank sample was compared with a hopped sample. For the latter, 200 mg/l isomerised hop extract was added to the cooled congress mash, corresponding to approx. 60 mg/l iso-α-acid. This experimental setup was specifically designed to study the interaction between iso-α-acid and anthocyanins in isolation. For this reason, storage conditions were chosen which, although far from a real product, are optimal for the stability of the anthocyanins.

3 Results and discussion

The ethanolic extract showed a strong pH dependence, which was to be expected with a solution containing anthocyanin. The red colouration was most pronounced at pH 1 and is already clearly attenuated with a slight increase in pH. Above approx. pH 3.8, it was not possible to visually discern any red colouration. After a largely colourless middle pH range, the colour changed in the alkaline milieu towards shades of yellow (pH > 8) to green (pH > 11). The wort extract resulted in a much less intense colour. Only by very strong acidification to pH 1 could any colour change be achieved and even at pH 2.5, no difference to the natural colouring could be detected visually or photometrically. The colour development of both extracts was completely reversible by adding acid and alkali.

For the congress mash, gradually lowering the pH value resulted in a differently pronounced colour change into the red range. As shown in figure 1, the local maximum characteristic of anthocyanins at $\lambda \approx 535$ nm increases steadily with decreasing pH. While the decrease to pH 3.77 can already be detected instrumentally, the colour is visually perceived as (pink) red from about pH 3.4. Figure 2 shows the colour gradient with decreasing pH value.

This result is remarkable in a way that a much stronger red colouration, especially one that is far more robust to pH changes, can be achieved in a conventional mashing process rather than by cold extraction. The latter is probably due to the fact that some kind of copigmentation is formed during mashing. This would be possible very simply by releasing a substance that can act as a copigment. The subsequent bonding (intramolecular) or cohesion (intermolecular) forms spontaneously and without delay [40]. Ferulic acid, which is released from its ester-like bond at low mashing temperatures (44 – 46 °C) and is available for reactions, comes to mind as a possible copigment [62, 63].

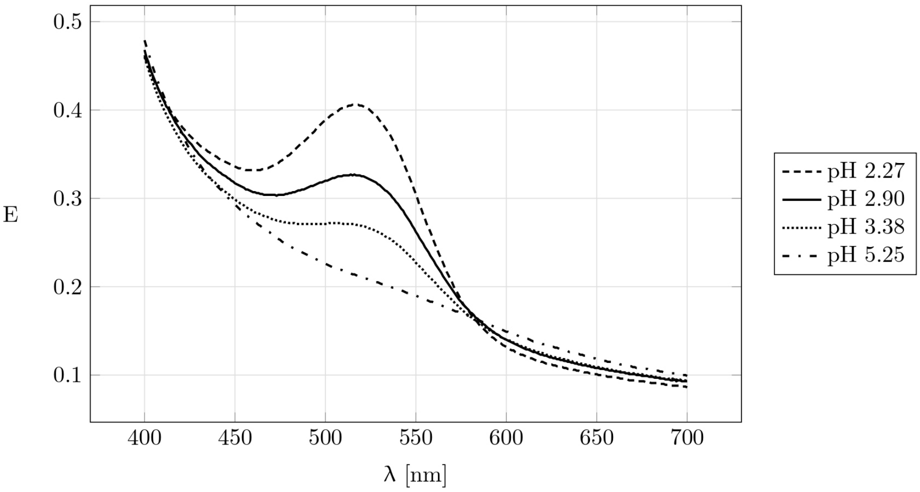

Fig. 1 **Congress mash colouration at different pH values adjusted with 1 N HCl**

In contrast, even a short wort boiling caused irreversible weakening of the anthocyanin colour: even acidification to pH 1 could no longer bring about a colour change. This can be explained by the known temperature sensitivity of anthocyanins. Here, copigmented anthocyanins would also be more stable, as they are less thermolabile [64].

When looking at the effect of adding hops, after three weeks of storage, no difference could be detected visually between the samples with and without hops. Photometrically, a very small but statistically significant ($p = 0.008$) decrease in absorbance was measured, which was limited to the local maximum at $\lambda = 535$ nm, as figure 3 illustrates. The latter is a clear indication that there is indeed an interaction with the anthocyanins here. So far, there are no concrete clues as to the reaction mechanism underlying this decolourisation. With regard to the use of hop products in beer, a long-term trial would reveal whether the effect increases over the course of longer storage or whether the decolourisation remains at such a low level that the colour change is not visually perceived.

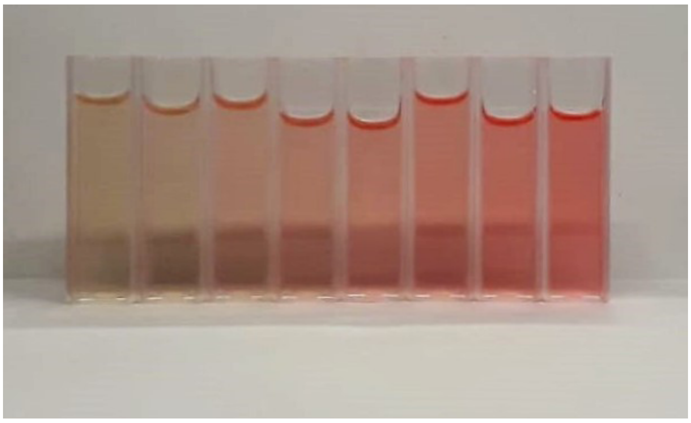

Fig. 2 **By lowering the pH value from 5.25 (left) to 2.27 (right), a drastic change in colour can be achieved. Below and around pH 3.8 the colour is perceived as reddish**

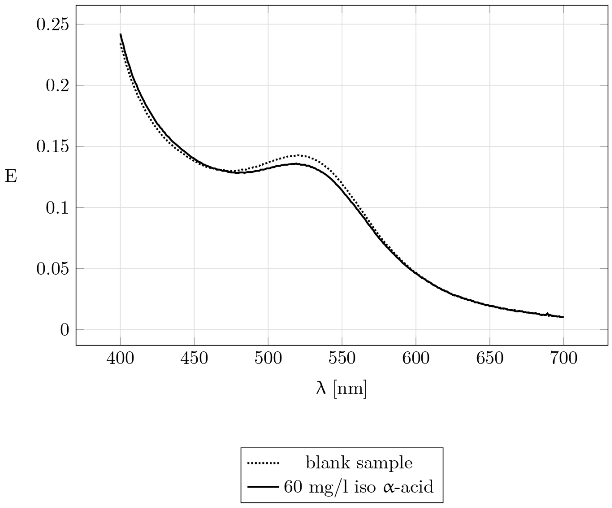

Fig. 3 **Effect of hop addition on anthocyanin colouration after three weeks of storage**

4 Conclusion

The results of the experiments demonstrate firstly that it is possible that the term red beer had a different meaning in the past, namely for the reddish coloured beers made from coloured barley varieties that were more common at that time. Secondly, there may be possibilities to produce a pink beer according to the German Purity Law through innovative brewing methods, which would be a complete novelty on today's market.

The experiments carried out clearly show that, in principle, it is possible to use the anthocyanins present in some barley varieties to give mash, wort and beer a pink or red colour. There are some obstacles to this. In particular, wort boiling is a major thermal stress that leads to irreversible weakening of the colour. In addition, the pH value of a standard beer is too high for the expression of a reddish colour. Further experiments are necessary to find out to what extent the colour can be stabilised so that the pH drop that occurs during fermentation is sufficient for the colour to develop.

It may be possible to stabilise the anthocyanins by increasing the ferulic acid content. In future studies, attention should also be paid to the formation of vitisins and pinotins during fermentation and storage. The development of colour during storage of the bottled product for several months should also be investigated.

Ultimately, the new findings provide an exciting basis for further research: First of all, it would be necessary to verify the thesis that copigmentation by ferulic acid is the reason for the increased colour expression in mash compared to cold extract. This could be investigated by adding this substance and other copigments such as rosmarinic acid and chlorogenic acid to allow for comparison.

Subsequently, brewing experiments that optimise copigment availability and bring about a rapid pH drop through the selection of raw materials and technologies (e.g. wheat content, long ferulic acid rest) could pave the way to a beer with anthocyanin colouration. Several technological parameters should be taken into account when selecting a suitable beer style: The availability of the possible copigment ferulic acid can be controlled both through the selection of raw materials – specifically through a high barley content in the pour – and through a mashing process with a long ferulic acid hydrolase rest [62]. A brewing method with the addition of hops in the lauter tun and the omission of wort boiling, as is traditionally used for Berliner Weisse [1], for example, reduces the thermal stress on the anthocyanins and thus maintains their colour strength. The most important factor is certainly a sufficiently low pH value, at which the anthocyanin colouring comes to its own. For this reason, sour beer styles would be ideal for further experiments.

Also further research must be carried out, with focus on the sensory properties of the resulting red coloured beer. The latter will be of great importance due to the low pH value needed. The work presented here was solely on an experimental level and did not take into account the products sensory profile. This work was not aiming at the development of a recipe for beer with red or pink hue together with appealing aroma and taste. The results of the experiments should only provide insights into which parameters, within the brewing process, might influence the colour of the final beer. Further research is required for instance into whether a slightly more intensive mashing process with higher enzyme activity might facilitate pigment release and how a decent sensory profile can be achieved.

Using ancient coloured spring barley for brewing beer with a red or pink hue might be mainly of interest for craft brewers. Transferring the colour into a final beer requires specific malting and brewing techniques which might be more time and resource consuming. This could be compensated through higher prices which can be achieved when selling craft beer, compared to beer produced on an industrial scale. Working with those ancient spring barley varieties might also create a competitive advantage to industrial breweries, especially if the raw material can be sourced regionally.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary Material

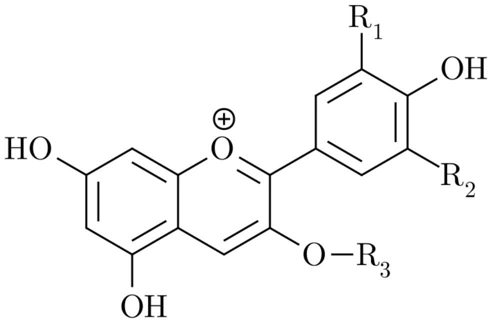

Fig. I **The flavylium cation is the positively charged state of the anthocyanin, which appears red, depending on the substituents R 1,2**

Table I **Colour impression of the anthocyanins depending on the pH value**

Abbreviation	Form	pH	Colour impression
AH^+	Flavylium cation	< 1	red
A	Neutral quinoidal base	6 – 7.5	purple
A	Anionic quinoidal base	7.5 – 10	blue
B	Hemiacetal	4 – 5	colourless
C	Chalcone	> 3.5	yellowish

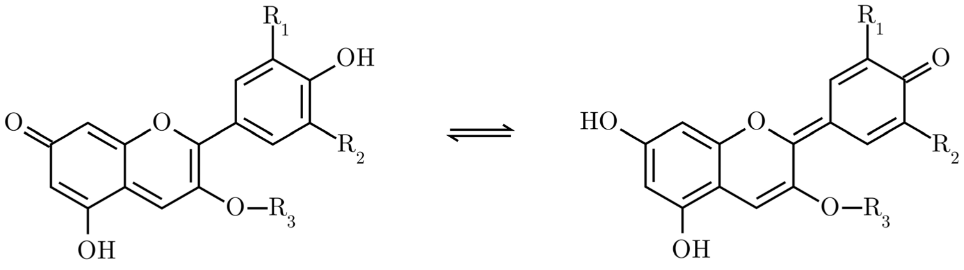

Fig. II **A rapid increase in pH initially leads to a neutralisation of the positively charged oxygen atom and a deprotonation at the A or B ring. The anthocyanin appears purple**

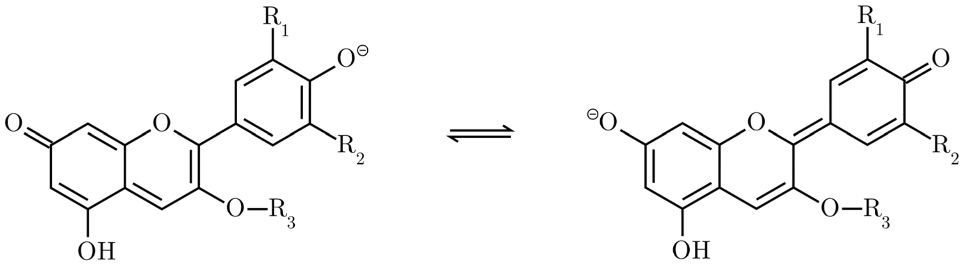

Fig. III At very high pH values, both outer oxygen atoms are deprotonated. The molecule is simply negatively charged and appears blue

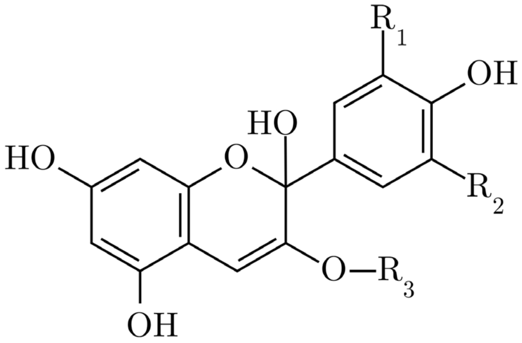

Fig. IV When the pH value is gradually increased, there is no deprotonation, but hydroxylation of the C2 atom occurs. This results in a complete loss of colour of the molecule

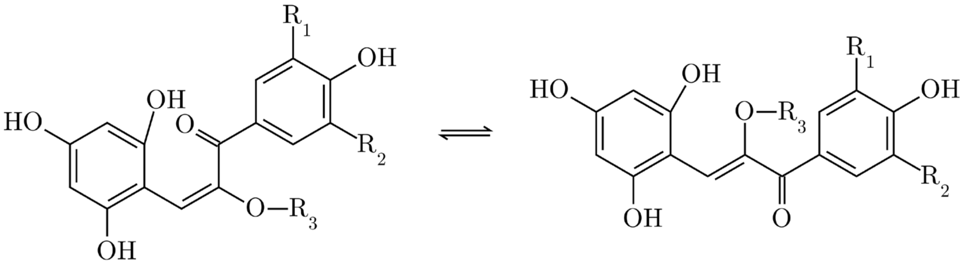

Fig. V After a longer standing time, the hemiacetal changes to the chalcone (in E- or Z-configuration, from left). Here, the C-ring opens irreversibly. Anthocyanins in chalcone form are pale yellow in colour