

Compounds Necessary for Plant Growth and Their External Supply

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Introduction

Chlorophylls are oil-soluble, and the most widely distributed pigments responsible for the characteristic green colour of rice plants. The structure of chlorophylls is porphyrins or closed ring tetra-pyrroles chelated with a centrally located magnesium atom. There are five classes of chlorophylls that have been found in rice plants and photosynthetic organisms. This difference leads to difference in colour, chlorophyll a appears blue green, while chlorophyll b possesses yellow green colour [1]. Chlorophylls are highly sensitive to heat, light, oxygen, acids, and enzymes, leading to their easy degradation and colour change. Acids and Mg-dechelataase, which is an enzyme found in algae and rice plants, are the main causes of conversion of chlorophyll's colour from green to olive brown. These changes occur due to the loss of central magnesium atom in the chlorophyll's structure, with the replacement by hydrogen ions, leading to the transformation of chlorophyll's structure from native chlorophyll to pheophytin, which exhibits olive brown colour. Other enzymes, such as chlorophylls and oxidative enzymes are also related to chlorophyll's degradation and cause change in colours. Thus, inhibition of enzyme activity and avoidance of acidic condition must be exercised to prevent changes in chlorophyll's colour. Heat is another important factor that indirectly causes conversion in chlorophyll's structure, which subsequently leads to colour changes [2]. Many researchers have investigated the effect of thermal processes on change in chlorophyll's colour. For example, Lau studied the colour change of green asparagus during thermal treatment and found that the colour of the sample changed to olive colour due to degradation of chlorophylls by heat. In fact, heat simply induces breaking up or disruption of plant cell walls; intracellular acids are thus released from the cells, resulting in the change of pH to an acidic condition. For these reasons, the structure of native chlorophylls changes to pheophytin, and hence the colour changes from green to olive brown as mentioned earlier. Carotenoids are lipid-soluble pigments responsible for many of the brilliant red, orange, and yellow colours of fruits, vegetables, and flowers.

Discussion

Based on their structure, carotenoids can be divided into two groups, which are carotenes and xanthophyll. Carotenes are constituted by carbon and hydrogen, whereas xanthophyll is constituted by carbon, hydrogen, and oxygen. Carotenoids can be divided based on their functionality into primary [3]. Basic structure of chlorophylls pigment, secondary carotenoids. Primary carotenoids are the pigments required for photosynthetic process, whereas secondary carotenoids are the pigments that are not directly related with plant survival. Degradation of carotenoids is mainly caused by the reactions of oxidation and isomerization, which lead to a decrease in the redness and yellowness of plant material. There are several factors affecting the occurrence of oxidation and isomerization of carotenoids. Oxygen naturally causes oxidation of carotenoids; the reaction can also be stimulated by light, heat, peroxide, metal ions, and enzymes. Most carotenoids in rice plants are trans-isomers, and the isomerization of trans to cisomers occurs during food processing. Heat and light as well as acids are the main factors that can promote isomerization of carotenoids from trans- to cisomers. However, isomerization of carotenoids leads to slightly reduced biological activities and colour saturation, whereas oxidation

leads to complete loss in the activities and colour of carotenoids. Several precautions have therefore been proposed to prevent oxidation reaction. Betalains are N-heterocyclic water-soluble pigments, which exhibit red-purple or yellow colour depending on the pigment structure. These pigments are found in many fruits, vegetables, and flowers. Betalains can be classified into two groups based on their structure. Instability of betalains to light, heat, alkaline, oxygen, and metal ions restricts them from extensive applications in food. Among various factors, heat is the most critical factor affecting the degradation of betalains, while other factors affect the heat sensitivity of betalains. Heat mostly induces oxidation, aldimine bond hydrolysis, and decarboxylation of betalains, which results in colour change to orange yellow. Since betalains are susceptible to heat, it is most important to cool down immediately the treated plant material after thermal treatment. Besides heat, stability of betalains is also related to pH. These pigments are most stable in a pH range. Betacyanins are more resistant to acidic condition, while betaxanthins are most stable at neutral pH. The colour of betalains does not change within the pH range [4]. When the pH is below 3, the structure of betalains is converted from red anionic to violet cationic, resulting in colour change from red to blue violet shade. On the other hand, alkaline condition brings about aldimine bond hydrolysis, which results in rapid degradation of betalains into betalamic acid and cyclodopa-5-O-glucoside and the colour change to yellow-brown. Metal ions, which may contaminate a plant, can accelerate oxidation of betalains, resulting in loss in colours. However, contamination of metal ions can be minimized by washing. Light does not significantly cause degradation of this group of pigments. Anthocyanins are water-soluble pigments found in many flowers, fruits, and rice leaves with various shades. The basic structure of anthocyanins is glycosides of anthocyanidins, which is known as flavylium cation. The term anthocyanidins is referred to anthocyanins without sugar molecules. The most abundant anthocyanins in nature are based on six anthocyanidins, which are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin [5]. Differences in the number of hydroxyl and methoxyl groups in the structure affect the colour variation of pigments. Pigments that have a larger number of hydroxyl groups exhibit more bluish shade, while those having a larger number of methoxyl groups exhibit more redness. The colour stability of anthocyanins is influenced not only by structural features but also by pH, temperature, light, presence of co-pigments, enzymes, oxygen, and

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sugars. Change in pH causes reversible transformation in anthocyanins structure and significantly affects shift in the colour of anthocyanins. In an aqueous solution, the molecular species of anthocyanins, which are flavylium cation, carbinol pseudobase, quinoidal base, and chalcone, are in equilibrium. Flavylium cation, which exhibits red colour, dominates at pH, but when the pH increases, the concentration of carbinol pseudobase, which is colourless, increases at an expense of flavylium cation. At pH 6, hydroxyl groups present in the structure and the concentration of quinoidal base increase, yielding unstable blue or violet colour. Quinoidal base continuously increases until the pH rises; quinoidal base would then transform into chalcone, which exhibits yellow colour [6]. Heat is well known as an important factor affecting the colour and stability of natural pigments. However, anthocyanins are more stable to heat than the aforementioned pigments. Degradation of anthocyanins occurs when temperature approaches or even higher. The first step of thermal degradation involves the formation of colourless. Due to the instability of natural pigments, various pre-treatment methods have been suggested to enhance stability and modify the structure of plant materials. Pre-treatment methods may be divided into three groups, which are physical, chemical, and biological pre-treatments. Chemical pre-treatments are proposed to enhance the stability of pigments. On the other hand, the main purpose of physical and biological pre-treatments is plant structural modification to enhance subsequent extraction [7]. The use of physical and biological pre-treatments in combination with extraction will be discussed in the later section. Since the stability depends on the type of pigments, a suitable method of pre-treatment that should be used to increase the stability also depends on pigment-types. Stability enhancement of chlorophylls Enzymes and acids are the major factors affecting the degradation of chlorophylls as mentioned in the section Chlorophylls [8]. Thus, enhancing the stability of chlorophyll pigments may be done by inactivating the enzymes and/or preventing an acidic condition. Many pre-treatment methods have been proposed for such purposes. Blanching is a short-time thermal treatment, which is usually performed by subjecting a sample to hot water or steam, with the aim to inactivate enzymes involved in the change of colour of plant materials. Since peroxidase is one of the highly thermal resistant enzymes and its activity is easy to measure, it is generally used as an indicator of adequate blanching, for example, reported that blanched Brussels sprouts exhibited more greenness than the unbalanced sample after storage for months. However, after eight months of frozen storage, the greenness values of both blanched and unbalanced samples decreased. Blanching nevertheless leads to changes in the cellular structure of plant, sometimes resulting in undesirable softening of plant tissues. In addition, blanching cannot solve the problem of acidic condition that may occur in further processing steps. Alkaline treatment is proposed to prevent acidic condition. Alkalinizing agents in combination with blanching are usually used to enhance the stability of green colour. Alkalinizing agents raise the pH of a blanched sample, thus enabling the retention of green colour of chlorophylls. Commercial application of alkaline treatment is still not too effective, as alkalinizing agents do not have the ability to neutralize interior tissue acids over a long period of time, leading, for instance, to the change in the colour of canned green peas after less than two months of storage at room temperature. Complexation of chlorophylls into metal complexes of chlorophyll derivatives has been proposed to alleviate the aforementioned problems. Chlorophylls can be transformed into metallo-chlorophyll derivatives, which exhibit green colour similar to native chlorophylls but are more stable to acids and heat. Although both copper and zinc ions can be utilized for metallo-chlorophyll derivatives formation, zinc ions are of greater interest due to the toxic nature of copper ions. To create metallo-

chlorophyll derivatives, chlorophylls are first acidified to change the structure of chlorophylls to pheophytin prior to blanching in a medium containing metal ions, such as zinc or copper ions, to form metallo-chlorophyll derivatives. Many researchers have indeed investigated the thermal stability of these metallo-chlorophyll derivatives and reported improved results [9]. Degradation of carotenoids with their highly conjugated double-bond structure is mainly due to oxidation reaction. Inactivation of oxidative enzymes is among the possible means of solving the problem of carotenoids oxidation. Hot water or steam blanching is a simple pre-treatment method that can be used to inactivate enzymes such as lipoxygenase, which catalyses oxidative decomposition of carotenoids. However, although blanching has a benefit of enzyme inactivation, many investigators have reported that blanching in some cases led to the degradation of carotenoid pigments. Marx and Lavelli who studied the effect of blanching on the carotenoids degradation of carrot juice and dehydrated carrot, respectively, reported that carrot juice and freeze-dried carrot produced from blanched carrot suffered more extensive degradation of carotenoids than those produced from un-blanched carrot; the degradation led to the fading colour of products. This observation might be caused by the change in carotenoid structure from *trans*- to *cis*-isomers due to heat upon blanching. Chemical pre-treatments are another alternative method to prevent the oxidation of carotenoids. Antioxidant agents are proposed to retard pigment degradation by oxidation. Hackett reported that addition of antioxidants helped decrease the degradation rate of lycopene in tomato. Hiranvarachat showed that *b*-carotene content of carrot soaked in citric acid tended to be unchanged during drying, while *b*-carotene content of untreated carrot decreased continuously. However, adding acid-based antioxidants leads to a decrease in the pH value of a sample; when the pH of a sample becomes less, carotenoids would start to degrade. Note that carotenoids would significantly degrade at pH, whereas these become more stable. Under acidic condition, carotenoids are protonated, resulting in the isomerization of their structure from *trans*- to *cis*-isomers.

Conclusion

Thus, the pH value of a sample should be carefully controlled when acid-based antioxidants, such as organic acids, are used. To improve the stability of betalains, blanching is usually required to inactivate the betacyanin decolouring enzyme, which leads to colour fading. Nevertheless, heat is the most crucial factor affecting the stability of betalains. As mentioned in the section Betalains, the colour loss due to the degradation of betalains is mainly caused by heat and alkalinity, which induce aldimine bond hydrolysis, leading to the separation of betalains structure into BA and CDG.

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Conflict of Interest

None

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