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A Short Review on the Stability of Chlorophylls and Metallo-Chlorophyll Complexes in Fruits and Vegetables

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ABSTRACT Chlorophylls are the primary photosynthetic pigment responsible for the green colour in fruits and vegetables and contributed as an antioxidant. Sensitivity of chlorophylls to processing conditions such as light, heat, pH changes, oxygen and chemicals affected the bright green colour and its antioxidant activity which lead to the formation of its derivatives; pheophytins and pyropheophytins. Therefore, the formation of metal complexes of chlorophyll derivatives has been suggested to exhibit green colour similar to native chlorophylls but with more stable processing conditions. This article aims to review the current research on the stabilization process of chlorophylls using divalent cations such as zinc or copper in the formation of metallo-chlorophyll complexes in fruits and vegetables.

KEYWORDS: Chlorophylls; Natural colourant; Green pigment; Vegetables; Metal ions
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Review Article

INTRODUCTION

The pigment responsible for the green colour is known as chlorophyll and it is the most abundant pigment in plants. This green pigment varies widely and depends on the type of plants and fruits, growing conditions, stage of maturity at harvest and postharvest conditions. It exhibits a green colour appearance which is used as a parameter of maturity, quality, and freshness of plants (Wang *et al.*, 2005; Liu *et al.*, 2006). Extensive reviews on the extraction techniques using various solvents, identification and quantification of chlorophylls and their derivatives have been discussed by Scotter *et al.* (2005); Aparicio-Ruiz *et al.* (2011); Harp *et al.* (2020); and Scholl *et al.* (2020). Meanwhile, the literature related to antioxidant, chemopreventive, antimutagenic, anticlastogenic, antigenotoxic and anticancer activities of chlorophylls and their derivatives are also reviewed in detail by Solymosi & Mysliwa-Kurdziel (2017); Tumolo & Lanfer-Marquez (2012); Ferruzzi & Blakeslee (2007); and Nagini *et al.* (2015). Chlorophylls are rapidly degraded by processing conditions or other factors such as enzymatic reaction, light, heat, pH, oxygen, and chemicals (Koca *et al.*, 2007) which would also affect their antioxidant activities. According to Schwartz & Lorenzo (1990), temperature and pH are the most important factors affecting the stability of chlorophylls. They reported that at pH values less than 7.0 and temperatures at 60°C or higher, chlorophyll *a* and chlorophyll *b* will be changed to a magnesium ion (Mg^{2+}) free derivative such as pheophytins (olive-green colour). Prolonged heating and strong acid condition would cause the formation of brown colour pheophorbide and pyropheophorbide (Weemaes *et al.*, 1999; Östbring *et al.*, 2014). Hence, to overcome these drawbacks, chlorophylls can be converted into derivatives of metallo-chlorophyll, which have a green colour similar to native chlorophylls but are more stable to acids and heat (Marquez *et al.*, 2007). In this stabilization process, the naturally-occurring Mg^{2+} in the porphyrin ring of the chlorophyll is substituted by a divalent cation such as zinc or copper, to form metallo-chlorophyll complex and this substitution makes the green colour reappear (Leunda *et al.*, 2000). The stabilization of chlorophylls with metals on green leafy plants will potentially increase the functional properties of the chlorophylls and thus increase the commercial value to apply as natural colourants and functional ingredients in the food industry.

Chlorophyll *a* to chlorophyll *b* ratio in higher plants is approximately 3:1 (Pocock *et al.*, 2004). The range of chlorophyll content in various fruits and vegetables is exemplified in Table 1. Naturally, chlorophyll is a stable pigment; however, it degrades during processing and extraction, because of acid, oxygen, heat, light, and enzymes (Koca *et al.*, 2007). Chlorophyll degradation in fruits and vegetables during processing has been extensively studied (Teng & Chen, 1999; Van Boekel, 1999). Thermal processing, acidification and/or enzymatic treatment caused a discolouration of vegetable tissue (Östbring *et al.*, 2014) due to the disappearance of chloroplast envelope, disruption of cytoplasmic membranes which leads to chlorophylls degradation to form, for instance, pheophytins (Van Boekel, 2000).

Table 1. Chlorophylls a and b contents of plants

Plants	Chlorophyll *a* (mg kg^{-1} fresh weight)	Chlrophyll *b* (mg kg^{-1} fresh weight)	Total chlorophylls (mg kg^{-1} fresh weight)	Reference
Amaranth (*Amaranthus viridis*)	1147	357	1504	Vivek *et al.* (2013)
Broccoli (*Brassica oleracea*)	103	51	154	Kmiecik *et al.* (2008)
Kale (*Brassica oleracea var. sabellica*)	1898	406	2304	Belitz *et al.* (2009)
Cucumber (*Cucumis sativus*)	72.1	45	-	Costache *et al.* (2012)
Cassava leaves (*Manihot esculenta*)	-	-	26	Gunathilake & Ranaweera, (2016)
Lettuce (*Lactuca sativa*)	283	70	353	Bohn & Walczyk, (2004)
Water spinach (*Ipomoea aquatica*)	-	-	25.01	Gunathilake & Ranaweera, (2016)

A study by Weemaes *et al.* (1999) has indicated that chlorophyll started to degrade in heated broccoli juices at a temperature range of 80°C to 120°C to form pheophytins. Ahmed *et al*. (2000) recorded that up to 60% loss of chlorophyll was noted in green chillies after 15 minutes of heating at 100°C. At low pH, chlorophylls green colour would change to pheophytin (olive green colour) (Humphrey, 2004) by the pheophytinization reaction, while under the strong acidic form, the pheophytin can convert to pheophorbide which has a brown colour (Schwartz & Lorenzo, 1990). The conversion of chlorophylls *a* and *b* to form Mg^{2+} free derivatives such as pheophytin, pheophorbide, pyropheophytin, pyropheophorbide a and b occurred at pH values less than 7.0 and temperature at 60°C or higher (Weemaes *et al.*, 1999) where the Mg^{2+} is substituted by hydrogen ion (2H), in the porphyrin ring of chlorophylls. Chlorophyll pigments in the thylakoid membranes are surrounded by carotenoid, protein, and lipid to protect from light destruction during photosynthesis (Rontani *et al.*, 1995). When this protection is lost during plant senescence, pigment extraction and tissue disruption during food processing, chlorophylls are susceptible to photodegradation (Von Elbe, 2000).

STABILIZATION OF CHLOROPHYLLS

Despite all the information on the chlorophylls and their derivatives, still a limited of published literature on the regreening or metallo-chlorophylls process have been discussed. Stabilization of chlorophyll using metal ions such as zinc and copper was reported to successfully improve the colour in plants as simplified in Table 2. Furthermore, these metallo-chlorophyll complexes behaved more effectively as antioxidants than their natural forms (Ngo & Zhao, 2007). Previous studies concluded that the development of metallo-chlorophyll derivatives relies on metal concentration, pigment concentration, pH (LaBorde & Von Elbe, 1990), temperature (Lin & Schyyens, 1995), and duration of contact between the metal solution and plant tissue (Canjura *et al*., 1999). Thus, the present review targets are to summarize the recent research on the formation of metal–chlorophyll derivatives in fruits and vegetables.

Table 2. Stabilization of chlorophylls and chlorophyll derivatives in plants

Source	Stabilization conditions				Reference
	Metal solution (ppm)	Temperature (°C)	Time (min)	pH	
Spinach	300 ppm Zn^{2+}	110°C	15	3-9	Özkan & Bilek (2015)
Spinach	300 ppm Zn^{2+}	115°C	30	-	Femat-Castañeda *et al.* (2019)
Pandan leaf	300 ppm $ZnCl_2$	110°C	15	5	Senklang & Anprung, (2010)
Pennywort	75 ppm $ZnCl_2$	95-100°C	0-10	4.5	Chaiwanichsiri *et al.* (2000)
Suji Leaves	700 ppm $ZnCl_2$	85°C	-	7	Rahayuningsih *et al.* (2018)

The principle of metallo-chlorophylls derivatives process is based on the reactions between Mg-free chlorophyll derivatives, such as pheophytin or pyropheophytins, with zinc or copper ions to form Zn-pheophytin or Zn-pyropheophytins. The effect of different metal concentrations was studied by Von Elbe & Schwartz (1996). They found that the 2 hydrogen atoms within the tetrapyrrole of chlorophyll derivatives are easily replaced by zinc or copper ions during heat treatment. Zheng *et al*. (2014) found that the addition of Cu^{2+} in the grape puree with pH 4 had higher chlorophyll pigment retention as compared to Mg^{2+}, Zn^{2+}, and K^{+} at 20°C for 30 min. Senklang and Anprung (2010) stated that treatment of pandan leaves with 300 ppm $ZnCl_2$ at pH 5 and 110°C for 15 min lead to the formation of Zn-chlorophyll derivative in pandan leaves such as Zn-pheophytin and Zn-pyropheophytin. These derivatives are green in colour, like native chlorophyll, but more stable to heat and acid and act as antioxidants (Tonucci & Von Elbe, 1992).

The effect of pH on the stabilized chlorophyll from spinach was studied by Özkan & Bilek (2015). Spinach pulp was treated at 121°C for 15 min with 300 ppm of $ZnCl_2$ and the pH was adjusted to different pH values from 3 to 9. Meanwhile, the effect of pH 3 to 8 on the colour changes of broccoli during blanching was investigated by Gunawan & Barringer (2000). Although the colour of broccoli became undesirable at pH 3 within 20 min, there was no substantial difference in the colour change between pH 7 and 8. These results demonstrate that at a pH range of 6-8, the green colour is protected. It was also observed that pH affects the chlorophyll content as Rahayuningsih *et al.* (2018) found that the highest total chlorophyll content was obtained at pH 7, $ZnCl_2$ concentration of 700 ppm and temperature of 85°C.

The thermal treatment has been reported to be critical for the retention of green colour when treated with metal because of the heat involved in the removal of carbomethoxyl at the C10 carbon location on the isocyclic chlorophyll ring. This makes the pyrrole nucleus become a cation (Canjura & Schwartz, 1991) and, thus improved the diffusion of divalent metal ions (Zn^{2+} or Cu^{2+}) to dislodge Mg^{2+} into vegetable tissue to form metal-complexes with chlorophyll (Ngo & Zhao, 2005). Guzmán *et al.* (2002) demonstrated that avocado puree treated with zinc or copper at 87 – 89°C had a greener colour than untreated samples. Ngo & Zhao (2007) found that after treatment with 2600 ppm of zinc solution for 12 min at 94°C, Zn-pheophytin would be the main compound contributing to the green colour of pear peels. Plant tissue degradation products can react with the Zn^{2+} when it is added to the medium, to form Zn–pheophytin complexes which have a stable green colour (Canjura *et al*., 1999; Tonucci & Von Elbe, 1992). However, chlorophyll *b* was more stable than chlorophyll *a* at 70°C, but both were highly degraded at 117°C (Rodriguez-Amaya, 2016).

CONCLUSION

The present review had revealed that formation of metallo-chlorophyll derivatives in fruits and vegetables depend on the pH, temperature, and metal concentration. These processing conditions are optimized to increase the green colour, chlorophyll contents and antioxidant activity of the plants. However, there is still limited study conducted for wild edible vegetables in Borneo and thus, there is an opportunity for researchers to explore the potential of these plants especially their chlorophyll contents and antioxidant activity.

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