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RESEARCH ARTICLE

Kinetic Degradation and Colour Stability in an Aqueous System of Spray-Dried Metal-Stabilised Amaranth Powder Encapsulated in Different Wall Materials

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Abstract The present study aims to determine the kinetics of chlorophyll degradation and green colour stability of encapsulated copper (Cu)- and zinc (Zn)-amaranth powders stored under accelerated (45°C) and room (25°C) temperature for 16 weeks and six months, respectively. The powders were spray-dried using 10% maltodextrin (DE 10), resistant maltodextrin, N-Octenyl Succinate Anhydride starches (HI-CAP® 100 and CAPSUL®), and gum Arabic (GA). The effects of pH (4 to 8, in the absence of light at 25°C for 24 hours), temperature (40 to 100°C for 90 mins), and light exposure (fluorescent light at 25°C for five days) on the chlorophyll retention and colour stability of the powders in an aqueous system were also explored. The chlorophyll degradation kinetics of the powders followed a first-order reaction model. The Zn-amaranth powder encapsulated with CAPSUL® exhibited the lowest rate constant ($k = 2.7 \times 10^{-3}$ weeks^{-1}) and the highest half-life ($t_{1/2}$ = 256.72 weeks) under storage at 25°C. The Cu-amaranth powder encapsulated with GA demonstrated the highest greenness value during storage at both 25°C (six months) and 45°C (16 weeks). In the aqueous system, the Cu-amaranth powder encapsulated with GA possessed the highest chlorophyll retention (> 90%) in acidic conditions. Meanwhile, maltodextrin-encapsulated Cu-amaranth powder exhibited better chlorophyll retention and colour stability under light exposure and high temperatures.

Keywords: Amaranth powder, degradation kinetics, chlorophyll retention, storage stability.

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Introduction

Green amaranth (*Amaranthus viridis* L.) is the largest commodity contributing to agricultural production and has the lowest price compared to other vegetables [1]. It has a mild spinach-like flavour [2], rich in nutrients, and contains several phytochemicals, including vitamins (C, A, B1, B6, B9, and E) and phytopigments, such as betacyanins, betaxanthins, chlorophylls, carotenoids, flavonoids, and phenolics [3, 4]. Green amaranth has a total chlorophyll content of 1504 mg/kg [5]. Furthermore, the flavonoid contents and antioxidant activities of leafy Amaranthus species are higher than spinach, 0.70 mg CE/g FW and 16 µmol TE/g FW, respectively [6, 7]. Amaranth also contains three times more calcium and higher levels of phosphorus, potassium, zinc (Zn), copper (Cu), and vitamin C [8] than spinach (*Spinacia oleracea*). Furthermore, amaranth contains nine and seven times more vitamin A and iron than Chinese cabbage [8].

The drawbacks of fresh vegetables are the instability of the chlorophyll components and limited shelf life due to their high moisture content of about 80 % [9]. The chlorophyll components tend to degrade during extraction and processing in unfavourable temperatures and pH and contact with oxygen or exposure to

light [10]. Degradation of chlorophylls to form chlorophyll derivatives results in discolouration and reduced antioxidant activity. These days, the demand for a food product with health-promoting functional ingredients and high-quality appearance, texture and flavour aspects has driven a substantial market increase in manufacturing of fruits and vegetables in powdered forms [11]. The growing public awareness of healthy eating increases the potential for opportunities for spray-dried, nutrient-rich juice obtained from the green leaf to be commercially produced [12]. Microencapsulation through spray drying is widely utilised in commercial fruit and vegetable powders. The approach integrates bioactive ingredients with a coating material to improve the microencapsulation efficiency and storage stability of the obtained powders [13]. Microencapsulation via spray drying also reduces food component interactions with environmental factors, such as temperature, light, moisture, oxygen [14, 15, 16], and pH [17].

Ideally, an encapsulant should possess good emulsifying activity, film forming properties, highly water soluble and low viscosity [18]. In this study, gum Arabic (GA), hydrolysed starches (maltodextrin, resistant maltodextrin), and N-Octenyl Succinate Anhydride (OSA) modified starches (HI-CAP® 100 and CAPSUL®) were utilised for encapsulation. The GA is highly soluble with low viscosity, good emulsifying attributes, and high oxidative stability [19]. Hydrolysed starches, such as maltodextrins, are water-soluble and possess low viscosity in high solid concentrations [20]. However, maltodextrins has a low emulsifying capacity [21]. Modified starches, including OSA-starch, consist of hydrophobic and hydrophilic groups. The hydrophobic and hydrophilic groups render modified starches suitable for retaining and encapsulating pigments, proteins, and flavours [22]. The OSA starch also has a low viscosity in aqueous solutions, aiding the spray drying process.

Several vegetable compounds are susceptible to processing conditions, such as pH, temperature, heat, and light [23]. Consequently, stabilising chlorophyll compounds before spray drying is critical to ensure maximum retention of functional values, particularly chlorophyll contents and antioxidant activities. In the food industry, metallo-chlorophyll derivatives can preserve the fresh green colour of vegetables during processing and storage.

Stabilising chlorophyll with metal ions, including Zn and Cu, has improved the colour of *Gliricidia sepium* leaves, avocados, pandan, pennywort, *Sauropus androgynus* leaves, and grapes [24, 25, 26, 27, 28, 29, 30]. The food-grade copper sulphate and zinc chloride powders employed in the current study are generally recognised as safe (GRAS) [31]. The substances are also commercially accessible for Cu and Zn applications, widening possible metallo-chlorophyll complex applications in vegetables.

Determining the shelf lives of food products is crucial, considering that they provide information on how long the products will retain their quality during storage [32]. Degradation kinetics, including reaction order and rate constant, are also necessary to estimate product stability during thermal processing and storage. The chlorophyll degradation and green colour stability kinetics of various vegetables have been studied, including green beans [33], broccoli [34], mint leaves [35], green peas [36], coriander leaves [37], and asparagus [38]. The reports revealed that degradation of chlorophylls followed the first-order kinetics model.

Kang *et al*. [39] investigated the storage stability of chlorophylls microencapsulated with various combinations of GA and maltodextrin for 10 days under different temperatures (4, 20, and 40°C). Resultantly, maltodextrin was the most effective encapsulant in maximising storage stability, encapsulation efficiency, and chlorophyll contents. In another study, Comunian *et al*. [40] discovered that maltodextrin and GA-encapsulated chlorophyllide powders provided acceptable protection from environmental conditions after 90 days of storage at room temperature.

Limited information exists on the effects of wall materials and metal ions of spray-dried amaranth powders on the colour, total chlorophyll stability, and antioxidant activities during storage and in aqueous systems. Consequently, this study evaluated the stability of spray-dried amaranth powders at different storage temperatures. It also examined the powder response to pH, light, and temperature in model aqueous systems.

Materials and Methods

The encapsulating agents, maltodextrin DE 10 (MD) and resistant maltodextrin (Fibresol®-2) (RMD) employed in this study were obtained from San Soon Seng Food Industry (Malaysia) and Archer Daniels Midland (ADM) Company [United States of America (USA)], respectively. The GA was purchased from RandM Chemicals, while OSA starches, HI-CAP® 100 (HICAP), and CAPSUL® (CAP) were bought from

Ingredion Malaysia Sdn. Bhd. Acetone, sodium phosphate dibasic dehydrate, monobasic sodium phosphate and potassium sorbate were procured from R&M Chemicals [Essex, United Kingdom (UK)].

Preparing the Cu- and Zn-Amaranth Powders

In this study, fresh amaranths were cleaned, drained, and chopped, with the roots discarded. The cleaned amaranth leaves and stalks were blended in a laboratory blender (2-speed, Waring MS 153-5, Torrington, CT, USA) for 5 minutes to make amaranth purees. Subsequently, the purees were stabilised with copper sulphate and zinc chloride to form Cu- and Zn-amaranth purees [41].

The metal-stabilised purees were individually pre-treated with Viscozyme L (1% v/w) enzyme at pH 5 and 45°C before being incubated for 3 hrs. The enzyme-treated Cu- and Zn-amaranth purees were strained through a 0.4 mm mesh sieve to remove undigested coarse fibres that might clog the spray-drying atomiser nozzle [42].

The Cu- and Zn-amaranth filtrates were encapsulated in 10% MD, RMD, CAP, HICAP, and GA before filtering through a 150 µm mesh filter screen. The feed mixtures were subsequently spray-dried using a pilot-scale spray drier (Niro A/S, GEA, Germany) with inlet and outlet temperatures set at 150 and 85°C, respectively. The spray drying was conducted at a 10 mL/min feed rate with the rotary atomiser operating at 15000 rpm [42]. The powders were collected, filled in aluminium laminated polyethylene pouches, sealed, and stored at 4°C until further analysis. Henceforth, the powders were labelled CuMD, CuRMD, CuCAP, CuHICAP, and CuGA for the amaranth stabilised with Cu, while ZnMD, ZnRMD, ZnCAP, ZnHICAP, and ZnGA represent the amaranth stabilised with Zn. The present study also prepared a control powder of fresh amaranth puree (non-enzymatic and non-metal stabilised) spray-dried with 10% MD under identical conditions.

Extraction and Determination of Total Chlorophyll Content

The total chlorophyll (TC) contents of the powders employed in this study were measured according to Dere *et al*. [43]. Firstly, the Cu- and Zn-amaranth powders (0.5 g) were diluted in 10 mL of distilled water and vortexed for 15 mins to dissolve the powders completely. The mixtures were centrifuged at 3000 × *g* for 10 mins before transferring the supernatants into centrifuge tubes. The supernatants were diluted to 50 mL with 100% acetone and centrifuged at 3500 × *g* for 10 mins.

The extracted chlorophyll in the supernatant was measured against 100% acetone as the blank at 662 and 645 nm for chlorophylls *a* and *b*, respectively. An ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, Japan) was employed for the evaluation. Subsequently, the chlorophylls *a* and *b* and TC contents (mg/g) of the powders were calculated according to Equations 1, 2, and 3 [44].

$$\text{Chlorophyll } a = 11.75A_{662} - 2.35A_{645} \quad (1)$$

$$\text{Chlorophyll } b = 18.61A_{645} - 3.96A_{662} \quad (2)$$

$$\text{TC content (mg/g)} = (16.26A_{645} + 7.79A_{662}) \times \text{Dilution factor}/1000 \quad (3)$$

In the current study, the percentage of TC retention obtained was utilised to assess the stability of the encapsulated powder according to Equation 4 [39]. The TC retention percentage denotes the ratio between the TC content retained in a powder after selected times and its initial TC content.

$$\text{TC retention (\%)} = \frac{(\text{The TC content remaining in control samples or powder})}{(\text{The initial TC content in control samples or powder})} \times 100 \quad (4)$$

Storage Stability of the Powder Samples

The accelerated storage stability evaluations were carried out according to Ee *et al*. [45]. Approximately 150 g of the spray-dried enzyme-liquefied Cu- and Zn-amaranth and control powder (10% MD spray-dried non-enzymatic and non-metal stabilised) samples were packed in opaque aluminium pouches (750 mm ×100 mm ×10 mm). The bags were heat-sealed and stored in controlled conditions at 45 ± 2°C (60% RH) for 16 weeks. Sampling for the lot was conducted every fortnight. Another sample group was kept at room temperature (25 ± 2°C, 50–70% RH) and sampled monthly for six months (24 weeks). The storage stability of the samples was determined based on their TC retention from the initial (day 0) and during storage. All analyses in this study were performed in triplicates.

Kinetic Analysis of Total Chlorophyll (TC) Degradation

The total chlorophyll (TC) retention of the amaranth powders in the current study was expressed in terms of degradation rate constant (*k*) and half-life value (t½). The half-life value corresponds to the time the

chlorophyll content is reduced by 50% with respect to zero time. This study applied the first-order reaction model, as reported by Tonon *et al*. [46] (Equation 5). Concurrently, the half-life values for a first-order reaction were determined at a specific temperature based on Equation 6. The degradation rate constants (k) were obtained from the slope of the natural log of TC retention versus time plotted.

$$\ln\ (C_t/C_0) = -kt \quad (5)$$

$$t_{1/2} = -(ln\ 0.5/k) \quad (6)$$

Where C_0 denotes the initial TC content (day 0), and C_t represents the TC content at any storage time, *t*.

Colour Changes During Storage

Colour lightness (L*) and a* (+a* = red, -a* = green) and b* (+b* = yellow, -b* = blue) values of the powders were determined with a Chroma Meter CR-410 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). The a*-to-b* ratio (a*/b*) was employed as a green colour variation [47]. A negative a*/b* value indicated a greener and less yellow amaranth powder. All colour parameters in this study were plotted against time to evaluate the colour changes of the spray-dried powders subjected to accelerated and room storage temperatures.

pH, Temperature, and Light Stability Analyses of the Reconstituted Cu- and Zn-Amaranth Powders

pH Stability Analysis

The effects of pH on the stability of chlorophyll content and colour of the reconstituted Cu- and Zn-amaranth powder samples were evaluated on a wide range of pH values: 4.0, 5.0, 6.0, 7.0, and 8.0. The procedures employed as described by Selim *et al*. [48]. Approximately 400 mg of the amaranth powder was mixed with 30 mL of 1 M sodium phosphate buffer of the desired pH value in screw-capped test tubes. Subsequently, the tubes were wrapped in aluminium foil to provide complete darkness and kept at room temperature (25 ± 2°C) for 24 hrs. The TC retention (%) and greenness (-a*/b*) values of the powders were determined in triplicates.

Temperature Stability Analysis

Reconstituted Cu- and Zn-amaranth powders (40 mg/L) were kept in a temperature-controlled water bath (40, 60, 80, and 100°C) for 0, 15, 30, 60, and 90 mins. The present study applied the procedures Cai *et al*. [49] outlined with slight modifications. The sample solutions were distributed into 50 mL glass tubes sealed with screw caps and wrapped with aluminium foil (dark environment). The tubes were immediately cooled in an ice bath before determining TC retention (%) and greenness (-a*/b*) values.

Light Stability Analysis

The light stability of the reconstituted amaranth powder samples in the current study was determined according to Shaaruddin *et al*. [50] with minor alterations. Each powder (0.4 g) was added to distilled water (20 mL) containing 0.5% potassium sorbate and stirred. The solution was distributed in three 10 mL test tubes with screw caps. The solutions were exposed to white fluorescent light (18 W) elevated 50 cm above the samples or left in the dark at room temperature (25 ± 1°C) for five days. The TC retention (%) and greenness (-a*/b*) of the powders were assessed in triplicates.

Results and Discussion

Storage Degradation Kinetics of Total Chlorophyll Content in Amaranth Powders

Table 1 summarises the rate constant (*k*) and half-life (t½) values of TC retention of the amaranth powder samples. The samples were subjected to room and accelerated temperature conditions during the assessment. The spray-dried powder with the highest half-life value at a 25°C storage temperature was ZnCAP (256.72 weeks), followed by ZnHICAP and CuHICAP powders (239.02 weeks). Meanwhile, at 45°C, the ZnGA powder exhibited the highest half-life value (48.14 weeks), followed by the CuGA (38.51 weeks) encapsulated powders. Cai and Corke [51] suggested that the negative charges on the OSA-modified starch molecules could contribute to the high pigment retention, thereby enhancing the stability and extending the shelf life of amaranth powders encapsulated with HI-CAP® 100 and CAPSUL®. Additionally, Ramakrishnan *et al*. [52] found that tamarillo powders prepared with GA had the highest retention of carotenoids, attributed to the thermo-protective effect provided by GA during exposure to the high temperature of spray drying.

The results of this study indicated that the CuRMD powders had the highest rate constant value (k = 22.4 x 10^{-3} $week^{-1}$) and the lowest half-life value (t½ = 30.94 weeks) at 45°C. The CuRMD powders recorded a more significant chlorophyll degradation due to the high-temperature storage [53]. The results coincided with Koca *et al*. [54], in which the half-life of chlorophyll in green peas decreased with rising blanching temperature. In another study, Weemaes *et al*. [34] demonstrated that the kinetics of chlorophyll degradation in broccoli during heat processing at 80 to 120ºC was temperature-dependent. The study results revealed that the OSA-modified starch (HI-CAP® 100 and CAPSUL®) and GA powders had higher storage stability and lower chlorophyll degradation than the other wall materials employed at a 25°C storage temperature.

Table 1. Degradation kinetics of total chlorophyll retention in Cu- and Zn-amaranth powders encapsulated in different wall materials

Powder	Temperature (˚C)	Rate constant ($k \times 10^{-3}$ $week^{-1}$)	Linear regression (R^2)	t½ (week)
Control	25	4.2	0.9398	165.04
	45	17.6	0.8537	39.38
CuMD	25	4.4	0.9599	157.53
	45	20.2	0.9002	34.31
CuRMD	25	4.4	0.9236	157.53
	45	22.4	0.9019	30.94
CuHICAP	25	2.9	0.9072	239.02
	45	18.7	0.9247	37.07
CuCAP	25	4.2	0.8986	165.04
	45	18.3	0.9031	37.88
CuGA	25	3.6	0.9308	192.54
	45	18.0	0.9058	38.51
ZnMD	25	4.0	0.9322	173.29
	45	19.9	0.901	34.83
ZnRMD	25	4.4	0.9489	157.53
	45	19.6	0.9494	35.36
ZnHICAP	25	2.9	0.9233	239.02
	45	18.5	0.9305	37.47
ZnCAP	25	2.7	0.9459	256.72
	45	18.8	0.9556	36.87
ZnGA	25	3.7	0.9128	187.34
	45	14.4	0.9400	48.14

(Note: Cu: Copper, Zn: Zinc, MD: Maltodextrin, RMD: Resistant maltodextrin, HICAP: HI-CAP 100, CAP: CAPSUL, GA: Gum Arabic)

Figures 1 and 2 illustrate the chlorophyll degradation plots of the amaranth powders at 25 and 45°C storage temperatures. The results indicated that the samples followed the first-order reaction kinetics at room (25°C) and accelerated (45°C) storage temperatures. The first-order kinetics model also describes the degradation of chlorophyll derivatives in pandan leaves [55, 26], broccoli [34], and spinach [56]. The plotted graphs also recorded a linear regression (R2 > 0.85) of [ln (C_t/C_0)] with a negative slope. The finding coincided with several reports on pigment retention, including red pitaya [45], spray-dried Amaranthus powder [51], and roselle calyces [14].

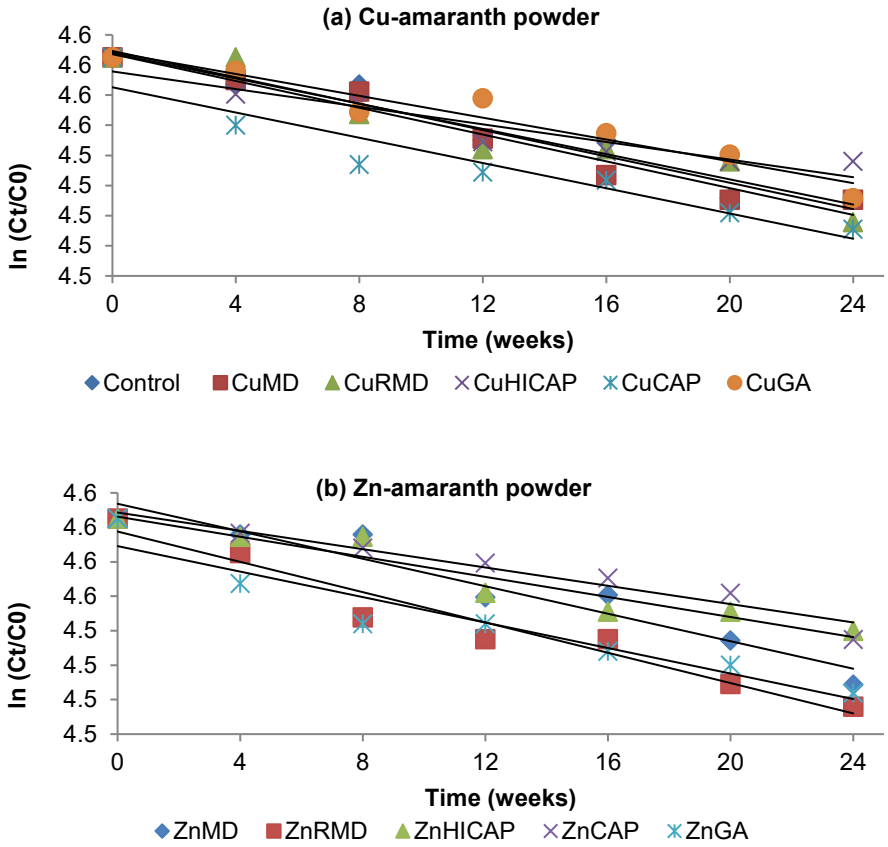

Figure 1. The first-order kinetic plots of the chlorophyll retention in (a) Cu-amaranth, control, and (b) Zn-amaranth powders encapsulated in varying wall materials during storage at 25°C

Colour Changes in the Amaranth Powders During Storage

Figures 3 and 4 illustrate the effects of storage period and temperature on the greenness (-a*/b*) and lightness (L*) values of the Cu-, Zn-amaranth, and control powders. Based on Figure 3, the encapsulating agent significantly impacted the greenness values of the samples. At both storage temperatures, the highest greenness value was recorded by the CuGA, followed by CuCAP and CuHICAP. Nevertheless, storage temperature and time had minimal effects on the amaranth powders.

The amaranth powder samples revealed minimal colour changes at 25 and 45°C throughout the storage period, indicating enhanced amaranth stabilisation with Cu and Zn against high temperatures. The observations supported by Idham *et al*. [14] concluded that storage temperatures at 4, 25, and 37°C did not influence the colour of spray-dried encapsulated anthocyanins from *Hibiscus sabdariffa* L. in 105 days. Nonetheless, they revealed that wall material types (MD and GA) affected the colour variations of the powder. Comunian *et al*. [40] also demonstrated that GA-encapsulated chlorophyllide powder denoted the highest stability during 90-day storage at 10 and 25°C.

As depicted in Figure 4, the L* values of the Cu- and Zn-amaranth powders remained stable throughout the 16 weeks and six months of storage. Conversely, noticeable differences were observed in the control powder compared to the encapsulated powders at both storage temperatures. The control powder also showed increased L* values at 25 and 45°C. The observations could indicate discolouration [57] due to the degradation of chlorophylls during storage, which resulted from fresh amaranth without Zn or Cu stabilisation. According to Schwartz and Lorenzo [58], temperature and pH are critical factors influencing chlorophyll stability in maintaining its green colour. The spray-dried powders procured in this study are demonstrated in Figure 5.

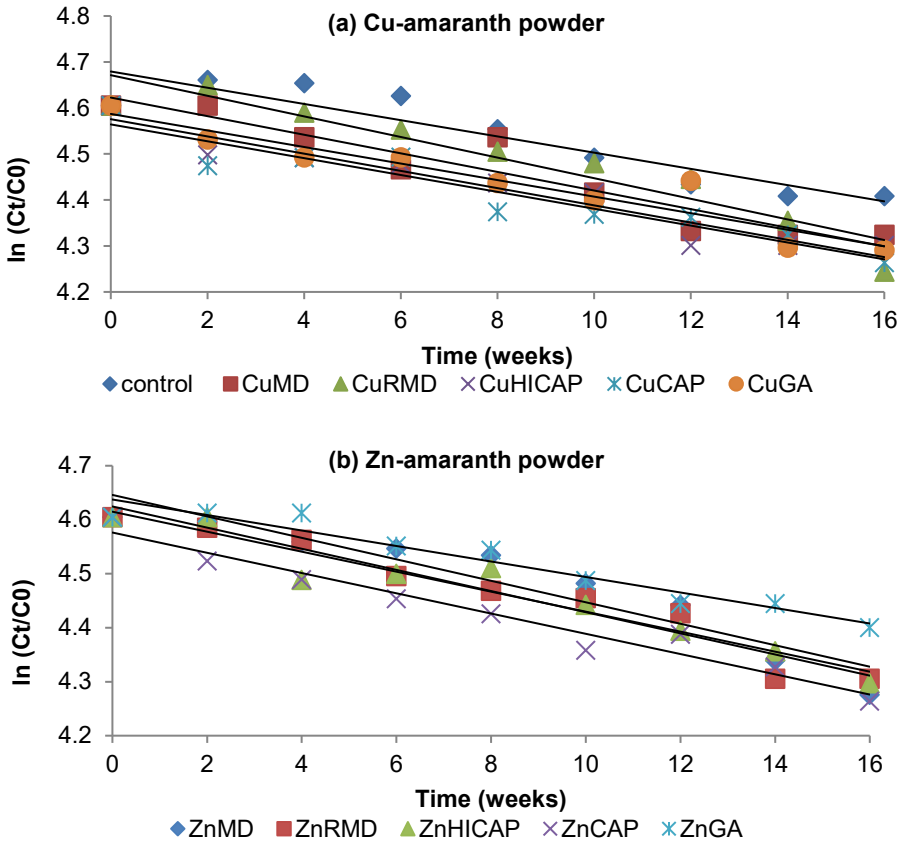

Figure 2. The first-order kinetic plots of the chlorophyll retention in (a) Cu-amaranth, control, and (b) Zn-amaranth powders encapsulated in different wall materials during storage at 45°C

Stability of the Reconstituted Cu- and Zn-Amaranth Powders Under Different pH, Temperature, and Light Conditions

Effects of pH

The highest greenness value (-a*/b*) of 0.7, 0.6, and 0.6 was observed in the CuGA solution at pH 4, followed by CuCAP and CuHICAP, respectively [Figure 6(a)]. The differences in the greenness values of the reconstituted powders could be attributed to the wall material utilised [59] and pH. Nonetheless, all Zn-amaranth powder solutions recorded lower colour stability than their Cu-amaranth counterparts.

The reconstituted control powder in the current study recorded the lowest colour stability in the pH range evaluated [Figure 6(a)] . As the control sample was produced from fresh amaranth puree without Cu or Zn addition, the discolouration occurred due to conversions of chlorophyll to pheophytin in acidic conditions [60]. Conversely, the samples reflected chlorophyll retention above 90% at pH 4, 5, and 6 before diminishing to below 80% at pH 7 [Figure 6(b)]. The results indicated that the powder solutions had a stable green colour and retained more chlorophyll under acidic conditions.

The study results paralleled the findings reported by Reshmi *et al*. [61]. Although the red colour of betacyanin solutions was stable under neutral and acidic conditions, it was unstable in alkaline solutions. Cai *et al*. [62] also reported that the betaxanthin pigment from *Celosia argentea* was significantly stable within the 4.0 to 7.0 pH range. On the other hand, Selim *et al*. [48] reported that the red colour of anthocyanins from roselle was stable under acidic conditions (pH < 3.0) when stored at room temperature.

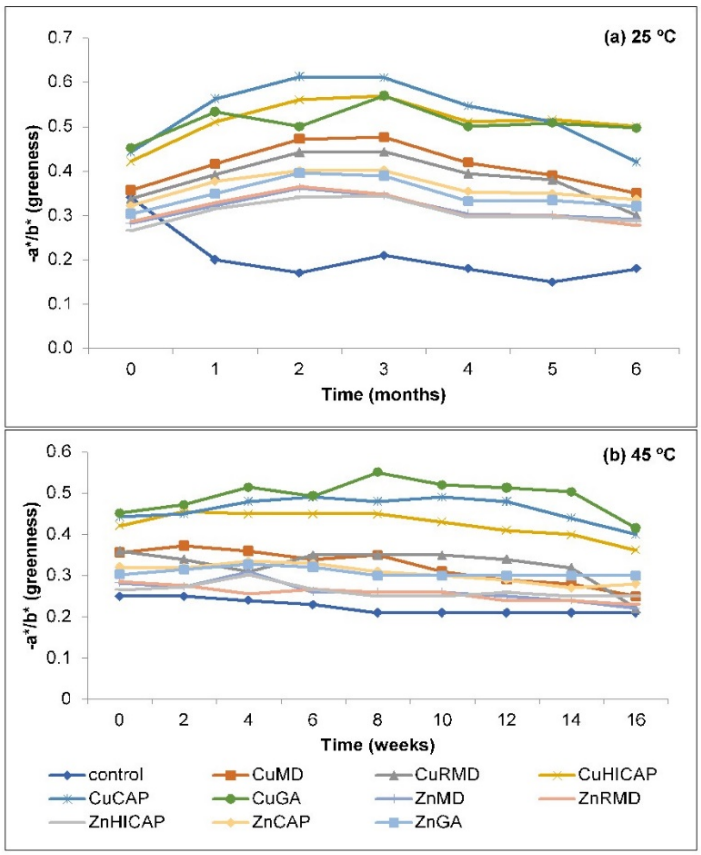

Figure 3. Changes in the greenness (-a*/b*) values of the Cu- and Zn-amaranth and control powders at (a) 25°C and (b) 45°C

Effects of Temperature

The amaranth powder solutions obtained in this study demonstrated a slight decrease in chlorophyll retention (%) during the initial 15 mins of heat treatment at 40, 60, 80, and 100°C, which persisted for 90 mins (Figure 7). Nonetheless, increases in time and temperature produced minimal effects on chlorophyll stability, thus indicating that the wall materials ensured adequate stability during heating [63]. According to Jafari *et al*. [64], wall materials act as physical barriers that reduce oxygen, light, heat, and moisture impacts on encapsulated ingredients.

Natural pigments are susceptible to degradation at higher storage temperatures [45]. In the present study, the reconstituted control amaranth and followed by ZnGA powder recorded the most significant losses during heat treatments (Figure 7). The results indicated that non-stabilised chlorophylls are more prone to losing their green colour and chlorophyll content when subjected to heat treatment above 60°C [58]. The highest chlorophyll retention was observed in reconstituted CuMD, with an initial chlorophyll content of 1.33 mg/g powder. When treated at 40, 60, 80, and 100°C for 90 mins, respectively, 95.5, 96.4, 93.1, and 91.9% chlorophyll retention were documented. The results indicated that chlorophyll stability was affected by storage temperature. Chlorophyll derivative entrapment within powders augmented their storage stability [39]. Similarly, Kang *et al*. [39] revealed that MD-coated chlorophyll powders denoted the highest storage stability (94.7–97.5%) after 10 days of storage at 4, 20, and 40°C, respectively. The lower molecular mobility of the CuMD powder in the present study might be due to its lower moisture content (4.66%) compared to the other powders.

The reconstituted ZnGA powder recorded an initial 1.35 mg/g powder chlorophyll content with lower retentions (81.5, 76.2, 66.5, and 69.8%) than the Cu-amaranth powders after 90 mins of treatment at 40, 60, 80, and 100ºC. The results might be attributable to the higher water activity (0.51) of ZnGA compared to other powders. According to Tonon *et al*. [46], water activity is critical in facilitating the physicochemical reactions of powder degradation. These findings are consistent with those reported by Ramakrishnan *et al*. [52]. The study found that tamarillo powder prepared with GA had the highest degradation rate at 25°C after 24 days due to its high of water activity.

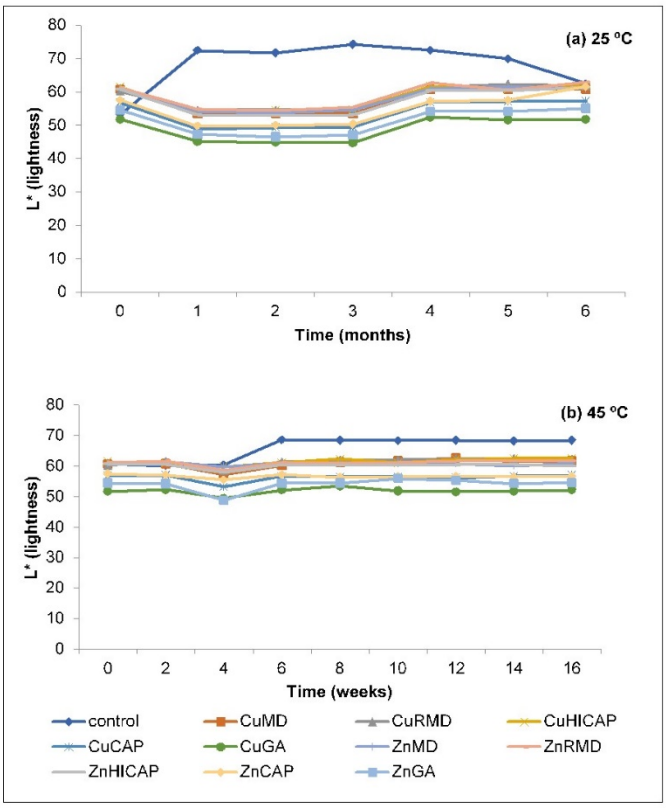

Figure 4. Changes in the L* values of the Cu- and Zn-amaranth and control powders at (a) 25°C and (b) 45°C

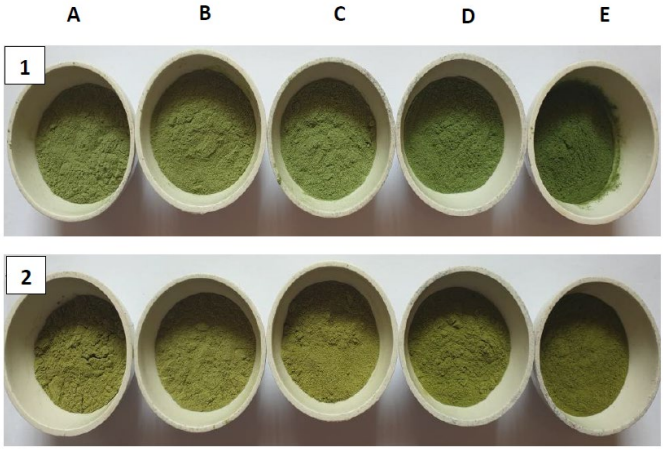

Figure 5. The (1) Cu- and (2) Zn-amaranth powders encapsulated in 10% (w/w) (A) MD, (B) RMD, (C) HICAP, (D) CAP, and (E) GA

Effects of Light

The colour and chlorophyll stability evaluations of the reconstituted Cu- and Zn-amaranth powders were performed at 25°C for five days. The samples were assessed in the presence and absence of light. The results are illustrated in Figure 8.

Generally, the reconstituted powders in this study exhibited better colour and chlorophyll stability when stored in the dark compared to under light conditions. Nonetheless, the greenness values of the Cu-amaranth samples were higher than their Zn counterparts [Figure 8(a)]. The CuMD conferred better light protection for chlorophyll retention than the other samples. After being stored for five days under

fluorescent light at 25°C, the MD-encapsulated Cu-amaranth retained approximately 54.1% of its initial chlorophyll content. The sample also exhibited the highest chlorophyll retention, approximately 79.5%, when stored in the dark.

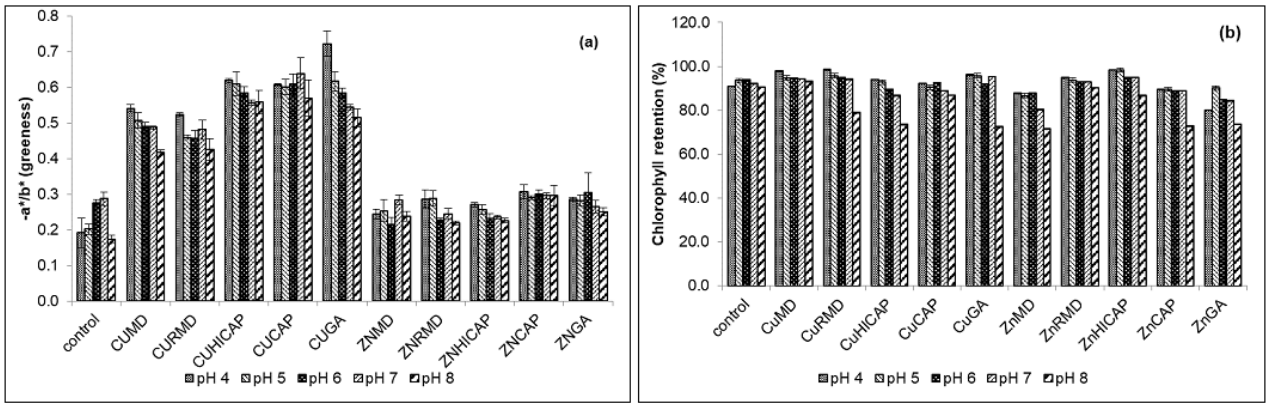

Figure 6. The effects of pH on the (a) greenness (-a*/b*) values and (b) chlorophyll retentions (%) of the reconstituted Cu- and Zn-amaranth and control powders

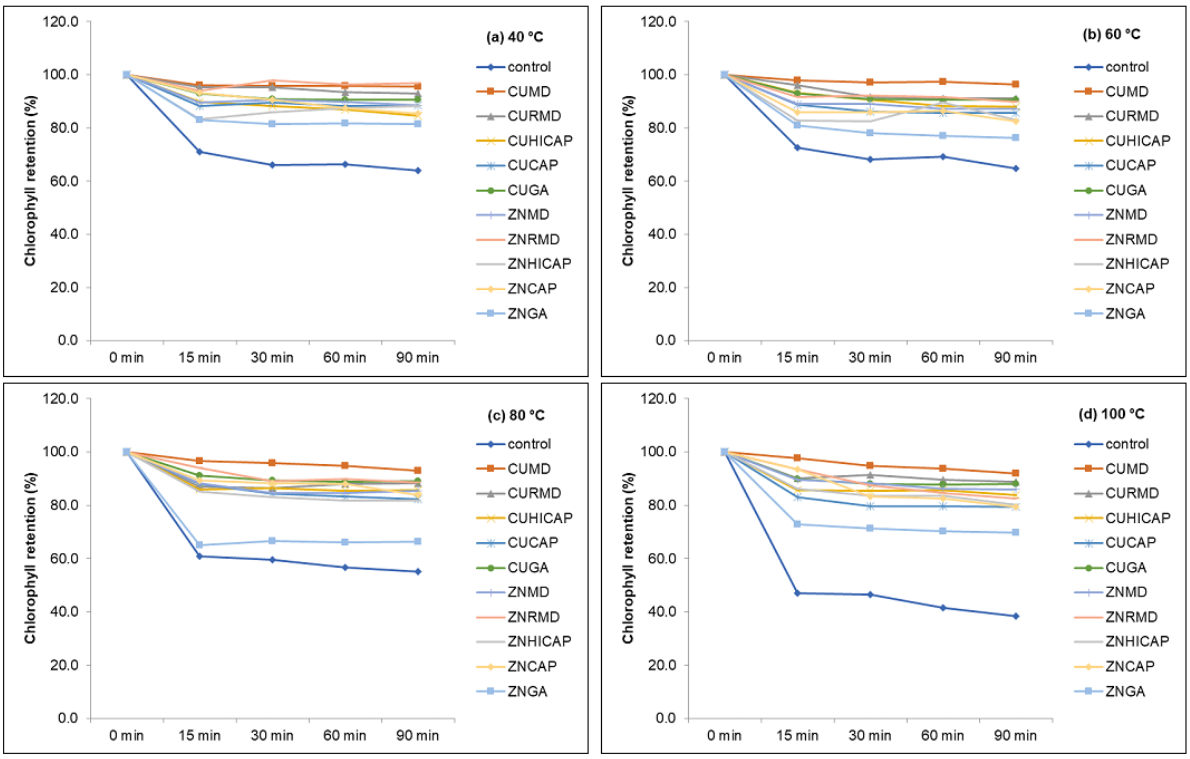

Figure 7. Temperature influences on the chlorophyll retention (%) in the reconstituted Cu- and Zn-amaranth and control powders encapsulated with 10% (w/w) of different wall materials

Chlorophyll degradation in the Cu- and Zn-amaranth powders might be due to the absorption of visible radiant energy from the fluorescent light, resulting in unstable and excited molecule formations [65]. The excited molecules were converted into excited triplet-state molecules, which directly interact with electron acceptors, such as quinones and phenol compounds, forming free-radical ions [65, 66]. Further conversions of the ions could break the porphyrin structure of chlorophylls and leads to the loss of green colour [67].

Lee *et al*. [68] reported a similar finding that chlorophyll content decreased faster under light than in the dark. Similarly, Acosta Murillo [69] observed a decrease in the chlorophyll content by 20.7% after 60 min exposure to UV-light. In another study, Sanja Petrović *et al*. [70] reported that chlorophyll had 50% decrease in 30 min when exposed to UV light.

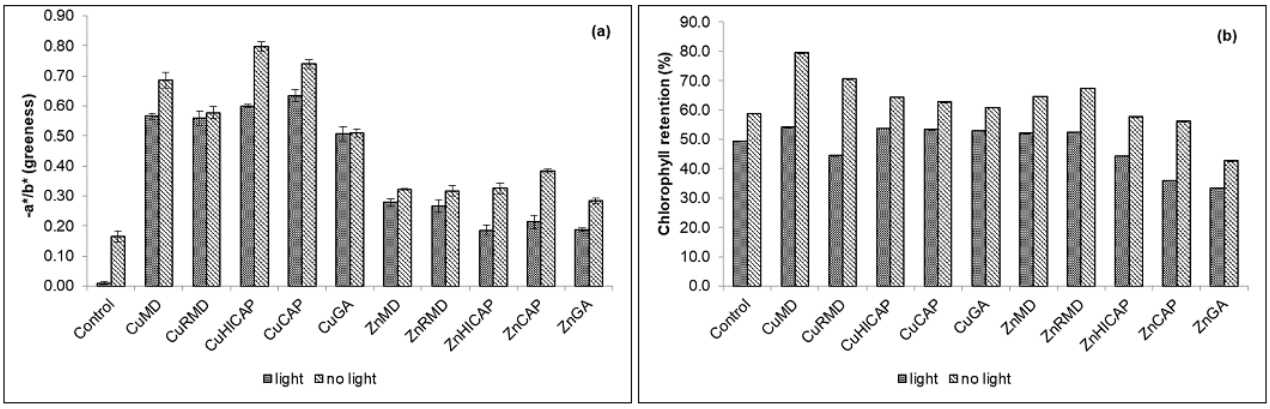

Figure 8. The influences of light on the (a) greenness (-a*/b*) values and (b) chlorophyll retentions (%) of the reconstituted Cu- and Zn-amaranth and control powders stored under fluorescent light (15 W) and in the dark at room temperature (25 ± 1˚C) for five days

Conclusions

The findings of this study indicated that the thermal degradation of chlorophylls in the Cu- and Zn-amaranth powders exhibited first-order kinetics during storage at accelerated (45°C) and room (25°C) temperatures. The rate constants of the samples also increased with rising temperature, leading to enhanced chlorophyll degradation. The spray-dried powder with the lowest degradation rate constant (k) and the most prolonged half-life (t1/2) at 25°C storage was the ZnCAP powder at 256.72 weeks, while ZnHICAP and CuHICAP both recorded 239.02 weeks.

The present study results suggested that storage at 25°C and encapsulation with OSA-modified starch could prolong the shelf life of amaranth powders. Although the types of wall materials protected the colour of the powders, storage temperatures negatively influenced it. The reconstituted powders in the study also demonstrated better green colour and chlorophyll retention in acidic conditions. Over 90% of chlorophyll derivatives in the powders were retained at pH 4. The CuMD powder recorded better heat and light stability. The powder retained over 90% and 50% chlorophyll after being heated at 40 to 100°C for 90 minutes under light conditions for five days.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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References

[1] FAMA. (2020). *Laporan Harga Purata Terkini Peringkat Runcit Komoditi Harian*. Retrieved from http://www.fama.gov.my/web/pub/harga-pasaran-terkini

[2] Topwal, M. (2019). A review on amaranth: Nutraceutical and virtual plant for providing food security and nutrients. *Acta Scientific Agriculture, 3*(1), 09–15.

[3] Venskutonis, P. R., & Kraujalis, P. (2013). Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. *Comprehensive Reviews in Food Science and Food Safety, 12*, 381–412.

[4] Repo-Carrasco-Valencia, R., Hellstrom, J. K., Pihlava, J. M., & Mattila, P. H. (2010). Flavonoids and other

phenolic compounds in Andean indigenous grains: Quinoa (Chenopodium quinoa), kaniwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus). *Food Chemistry, 120*, 128–133.

[5] Vivek, P., Prabhakaran, S., & Shankar, S. R. (2013). Assessment of nutritional value in selected edible greens based on the chlorophyll content in leaves. *Research in Plant Biology, 3*(5), 45–49.

[6] Isabelle, M., Lee, B. L., Lim, M. T., Koh, W. P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry, 123*, 77–84.

[7] Mavhungu, N. (2011). *Antioxidant properties and cellular protective effects of selected African green leafy vegetables* (PhD thesis). University of Pretoria, South Africa.

[8] Mnkeni, A., Masika, P., & Maphaha, M. (2007). Nutritional quality of vegetable and seed from different accessions of Amaranthus in South Africa. *Water SA, 33*(3), 377–380.

[9] Yadav, A. K., & Singh, S. V. (2014). Osmotic dehydration of fruits and vegetables: A review. *Journal of Food Science and Technology, 51*(9), 1654–1673.

[10] Lanfer-Marquez, U. M., & Sinnecker, P. (2007). Chlorophylls: Properties, biosynthesis, degradation and functions. In C. Socaciu (Ed.), *Food colorants: Chemical and functional properties* (pp. 25–45). CRC Press.

[11] Jiang, H., Zhang, M., & Adhikari, B. (2013). Fruit and vegetable powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders* (pp. 532–552). Woodhead Publishing.

[12] Syamila, M., Gedi, M. A., Briars, R., Ayed, C., & Gray, D. A. (2019). Effect of temperature, oxygen and light on the degradation of β-carotene, lutein and α-tocopherol in spray-dried spinach juice powder during storage. *Food Chemistry, 284*, 188–197.

[13] Kandansamy, K., & Somasundaram, P. D. (2012). Microencapsulation of colors by spray drying – a review. *International Journal of Food Engineering*. https://doi.org/10.1515/1556-3758.2647

[14] Idham, Z., Muhamad, I. I., & Sarmidi, M. R. (2012). Degradation kinetics and color stability of spray-dried encapsulated anthocyanins from Hibiscus sabdariffa L. *Journal of Food Process Engineering, 35*(4), 522–542.

[15] Hsiao, C. J., Lin, J. F., Wen, H. Y., Lin, Y. M., Yang, C. H., Huang, K. S., & Shaw, J. F. (2020). Enhancement of the stability of chlorophyll using chlorophyll-encapsulated polycaprolactone microparticles based on droplet microfluidics. *Food Chemistry, 306*, 125300.

[16] Tontul, I., & Topuz, A. (2013). Mixture design approach in wall material selection and evaluation of ultrasonic emulsification in flaxseed oil microencapsulation. *Drying Technology, 31*(12), 1362–1373.

[17] Piñón-Balderrama, C. I., Leyva-Porras, C., Terán-Figueroa, Y., Espinosa-Solís, V., Álvarez-Salas, C., & Saavedra-Leos, M. Z. (2020). Encapsulation of active ingredients in food industry by spray-drying and nano spray-drying technologies. *Processes, 8*(8), 889.

[18] Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. E., & Saurel, R. M. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40*(9), 1107–1121.

[19] Costa, S. S., Machado, B. A. S., Martin, A. R., Bagnara, F., Ragadalli, S. A., & Alves, A. R. C. (2015). Drying by spray drying in the food industry: Micro-encapsulation, process parameters and main carriers used. *African Journal of Food Science, 9*(9), 462–470.

[20] Bakowska-Barczak, A. M., & Kolodziejczyk, P. P. (2011). Black currant polyphenols: Their storage stability and microencapsulation. *Industrial Crops and Products, 34*(2), 1301–1309.

[21] Xiao, Z., Xia, J., Zhao, Q., Niu, Y., & Zhao, D. (2022). Maltodextrin as wall material for microcapsules: A review. *Carbohydrate Polymers, 298*, 120113.

[22] Hoyos-Leyva, J. D., Bello-Pérez, L. A., Alvarez-Ramirez, J., & Garcia, H. S. (2018). Microencapsulation using starch as wall material: A review. *Food Reviews International, 34*(2), 148–161.

[23] Ghosh, S., Sarkar, T., Das, A., & Chakraborty, R. (2022). Natural colorants from plant pigments and their encapsulation: An emerging window for the food industry. *Lebensmittel-Wissenschaft + Technologie, 153*, 112527. https://doi.org/10.1016/j.lwt.2021.112527

[24] Ngo, T., & Zhao, Y. (2007). Formation of zinc-chlorophyll-derivative complexes in thermally processed green pears (Pyrus communis L.). *Journal of Food Science, 72*(7), C397–C404.

[25] Guzmán, G. R., Dorantes, A. L., Hernández, U. H., Hernández, S. H., Ortiz, A., & Mora, E. R. (2002). Effect of zinc and copper chloride on the color of avocado puree heated with microwaves. *Innovative Food Science & Emerging Technologies, 3*(1), 47–53.

[26] Porrarud, S., & Pranee, A. (2010). Microencapsulation of Zn-chlorophyll pigment from Pandan leaf by spray drying and its characteristic. *International Food Research Journal, 17*(4), 1031–1042.

[27] Chaiwanichsiri, S., Dharmsuriya, N., Sonthornvit, N., & Janjarasskul, T. (2000). Process improvement to preserve the color of instant pennywort Centella asiatica (Linn.) Urban. *Journal of Scientific Research, Chulalongkorn University, 25*(2), 233–243.

[28] Cao, H. K. N., & Dong, T. A. D. (2023). Study on the effect of metal salts on chlorophyll pigment extraction from fresh Sauropus androgynus leaves. *Journal of Agriculture and Food Research, 14*, 100699.

[29] Zheng, Y., Shi, J., Pan, Z., Cheng, Y., Zhang, Y., & Li, N. (2014). Effect of heat treatment, pH, sugar concentration, and metal ion addition on green color retention in homogenised puree of Thompson seedless grape. *LWT - Food Science and Technology, 55*(2), 595–603.

[30] Selan, O. T., Hawu, H. N., Poy, L. D., Cunha, T. D., & Darmakusuma, D. (2021). The substitution of Mg2+ with Mn2+/4+ metal ions in chlorophyll structure isolated from Gliricidia sepium leaves. *Journal of Physics: Conference Series, 2017*(1), 012002.

[31] Code of Federal Regulations. (2020). *Title 21 - Food and Drugs. Subchapter B: Food for Human Consumption. Part 184 and 182: Direct food substances affirmed as Generally Recognised As Safe. Section 184.1261 - Copper sulfate and Section 182.8985- Zinc chloride*. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1261

[32] Ganje, M., Jafari, S. M., Dusti, A., Dehnad, D., Amanjani, M., & Ghanbari, V. (2016). Modeling quality changes in tomato paste containing microencapsulated olive leaf extract by accelerated shelf life testing. *Food and Bioproducts Processing, 97*, 12–19.

[33] Zhang, X., Yi, W., Liu, G., Kang, N., Ma, L., & Yang, G. (2021). Colour and chlorophyll level modelling in

vacuum-precooled green beans during storage. *Journal of Food Engineering, 301*.
[34] Weemaes, C. A., Ooms, V., Loey, A. M. V., & Hendrickx, M. E. (1999). Kinetics of chlorophyll degradation and colour loss in heated broccoli juice. *Journal of Agricultural and Food Chemistry, 47*, 2404–2409.
[35] Gaur, S., Shivhare, U. S., Sarkar, B. C., & Ahmed, J. (2007). Thermal chlorophyll degradation kinetics of mint leaves puree. *International Journal of Food Properties, 10*(4), 853–865.
[36] Ryan-Stoneham, T.A., & Tong, C.H. (2000). Degradation kinetics of chlorophyll in peas as a function of pH. *Journal of Food Science*, 65, 1296–1302.
[37] Rudra, S. G., Sarkar, B., & Shivhare, U. (2008). Thermal degradation kinetics of chlorophyll in pureed coriander leaves. *Food and Bioprocess Technology*, 1(1), 91–99.
[38] Lau, M. H., Tang, J., & Swanson, B. G. (2000). Kinetics of textural and color changes in green asparagus during thermal treatments. *Journal of Food Engineering*, 45(4), 231–236.
[39] Kang, Y.-R., Lee, Y.-K., Kim, Y. J., & Chang, Y. H. (2019). Characterisation and storage stability of chlorophylls microencapsulated in different combinations of gum Arabic and maltodextrin. *Food Chemistry*, 272, 337–346.
[40] Comunian, T. A., Monterrey-Quintero, E. S., Thomazini, M., Balieiro, J. C., Piccone, P., Pittia, P., & *Favaro-Trindade*, C. S. (2011). Assessment of production efficiency, physicochemical properties and storage stability of spray-dried chlorophyllide, a natural food colourant, using gum Arabic, maltodextrin and soy protein isolate-based carrier systems. *International Journal of Food Science & Technology*, 46(6), 1259–1265.
[41] Mohd Amin, S.F., Karim, R., Yusof, Y.A., & Muhammad, K. (2023). Effects of metal concentration, pH, and temperature on the chlorophyll derivative content, green colour, and antioxidant activity of amaranth (Amaranthus viridis) purees. *Applied Sciences*, 13(3), 1344. https://doi.org/10.3390/app13031344
[42] Amin, S.F.M., Karim, R., Yusof, Y. A., & Muhammad, K. (2021). Effects of enzymatic liquefaction, drying techniques, and wall materials on the physicochemical properties, bioactivities, and morphologies of zinc-amaranth (Amaranthus viridis L.) powders. *International Journal of Food Science*, Oct 21.
[43] Dere, S., Günes, T., & Sivaci, R. (1998). Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany*, 22, 13–17.
[44] Costache, M. A., Campeanu, G., & Neata, G. (2012). Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. *Romanian Biotechnological Letters*, 17(5), 7703–7708.
[45] Ee, S., Jamilah, B., Muhammad, K., Hashim, D., & Adzahan, N. (2014). Physico-chemical properties of spray-dried red pitaya (Hylocereus polyrhizus) peel powder during storage. *International Food Research Journal*, 21(1)
[46] Tonon, R. V., Brabet, C., & Hubinger, M. D. (2010). Anthocyanin stability and antioxidant activity of spray-dried açai (Euterpe oleracea Mart.) juice produced with different carrier agents. *Food Research International*, 43(3), 907–914.
[47] Larrauri García, J.A., & Saura Calixto, F. (2000). Evaluation of CIE-lab colour parameters during the clarification of a sugar syrup from Mesquite pods (Prosopis Pallida L.). *International Journal of Food Science & Technology*, 35, 385–389.
[48] Selim, K. A. K., Abdel-Bary, M. S., & Abdel-Azeim, N. A. (2008). Extraction, encapsulation and utilisation of red pigments from roselle (Hibiscus sabdariffa L.) as natural food colourants. *Alexandria Journal of Food Science and Technology, Special Volume Conference*, 7–20.
[49] Cai, Y., Sun, M., Wu, H., Huang, R., & Corke, H. (1998). Characterisation and quantification of betacyanin pigments from diverse amaranthus species. *Journal of Agricultural and Food Chemistry*, 46(6), 2063–2070.
[50] Shaaruddin, S., Ghazali, H. M., Hamed Mirhosseini, S., & Muhammad, K. (2017). Stability of betanin in pitaya powder and confection as affected by resistant maltodextrin. *LWT - Food Science and Technology*, 84, 129–134.
[51] Cai, Y. Z., & Corke, H. (2000). Production and properties of spray-dried Amaranthus betacyanin pigments. *Journal of Food Science*, 65(7), 1248–1252.
[52] Ramakrishnan, Y., Adzahan, N. M., Yusof, Y. A., & Muhammad, K. (2018). Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation. *Powder Technology*, 328, 406–414.
[53] Marzena Anna, P., Arkadiusz, S., Edyta, S., Małgorzata, G., & Elżbieta, D. (2018). α- and β-Carotene stability during storage of microspheres obtained from spray-dried microencapsulation technology. *Polish Journal of Food and Nutrition Sciences*, 68(1), 45–55.
[54] Koca, N., Karadeniz, F., & Burdurlu, H. S. (2007). Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry*, 100(2), 609–615.
[55] Vongsawasdi, P., Nopharatana, M., Sasaeng, K., Tantek, P., & Wongphaisitpisan, S. (2010). Kinetics of chlorophyll degradation in pandanus juice during pasteurisation. *Asian Journal of Food and Agro-Industry*, 3(1), 44–51.
[56] Bilek, S. E., & Özkan, G. (2018). Encapsulation of zinc-chlorophyll derivatives in whey protein matrix by emulsion/cold-set gelation. *The Journal of Food*, 43(1), 174–183.
[57] Reyes, L. F., & Cisneros-Zevallos, L. (2007). Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (Solanum tuberosum L.). *Food Chemistry*, 100(3), 885–894.
[58] Schwartz, J., & Lorenzo, V. (1990). Chlorophyll in food. *Food Science and Nutrition*, 29(1), 1–12.
[59] Bustos-Garza, C., Yáñez-Fernández, J., & Barragán-Huerta, B. E. (2013). Thermal and pH stability of spray-dried encapsulated astaxanthin oleoresin from *Haematococcus pluvialis* using several encapsulation wall materials. *Food Research International*, 54(1), 641–649.
[60] Gunawan, M. I., & Barringer, S. A. (2000). Green colour degradation of blanched broccoli (Brassica oleracea) due to acid and microbial growth. *Journal of Food Processing and Preservation*, 24, 253–263.
[61] Reshmi, S., Aravindhan, K., & Devi, P. S. (2012). The effect of light, temperature, pH on stability of betacyanin pigments in basella alba fruit. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4), 107–110.
[62] Cai, Y., Sun, M., Schliemann, W., & Corke, H. (2001). Chemical stability and colourant properties of betaxanthin pigments from *Celosia argentea*. *Journal of Agricultural and Food Chemistry*, 49(9), 4429–4435.

[63] Akbas, E., Kilercioglu, M., Onder, O. N., Koker, A., Soyler, B., & Oztop, M. H. (2017). Wheatgrass juice to wheatgrass powder: Encapsulation, physical and chemical characterisation. *Journal of Functional Foods*, 28, 19–27.
[64] Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology*, 26(7), 816–835.
[65] Min, D. B., & Boff, J. M. (2002). Chemistry and reaction of singlet oxygen in foods. *Comprehensive Reviews in Food Science and Food Safety*, 1(2), 58–72.
[66] Boris, D. B., Svetlana, V. R., Andrey, P. M., & Mikhail, B. B. (2004). Chemical transformations of chlorophyll and its application in the design of a new generation of environmentally safe dyes. *Russian Chemical Reviews*, 73(2), 185.
[67] Martins, T., Barros, A. N., Rosa, E., & Antunes, L. (2023). Enhancing health benefits through chlorophylls and chlorophyll-rich agro-food: A comprehensive review. *Molecules*, 28(14), 5344.
[68] Lee, E., Ahn, H., & Choe, E. (2014). Effects of light and lipids on chlorophyll degradation. *Food Science Biotechnology*, 23, 1061–1065.
[69] Acosta Murillo, R. (2021). Analysis of light exposure time and its effect on photosynthetic pigment chlorophyll α and β degradation. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 7(6), 17.
[70] Petrović, S., Zvezdanović, J., & Marković, D. (2017). Chlorophyll degradation in aqueous mediums induced by light and UV-B irradiation: An UHPLC-ESI-MS study. *Radiation Physics and Chemistry*, 141, 8–16.