



REVIEW ARTICLE

Roselle anthocyanin stability profile and its potential role in post-harvest deterioration: A review

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Abstract

The conversion of roselle calyx into a dried extract without decreasing its consistency is a challenge, given the perishability of the calyx and instability of anthocyanin, which can quickly degrade and develop colored or unwanted brown colors because of its high reactivity. The most critical factors influencing anthocyanins' stability are pH, temperature, light and post-harvest-related enzymes. Besides, the calyx suffered wound injury when removing seed from the calyx, causing stress and eventually, microbial degradation. Nonetheless, mature anthocyanins stimulate plants by responding to stress, especially drought, high salinity, excess light and injury; it is also correlated with improved stress resistance as the genes of individual plants are triggered under these conditions modulate anthocyanin biosynthesis. This work investigates the stability and potential role of roselle anthocyanin in post harvest deterioration. Anthocyanin stability can, therefore, be achieved by maintaining low pH and temperature, acylation, glycosylation, copigmentation and encapsulation. In the quest for roselle deterioration biomarkers, the detection of critical enzymes, such as Chalcone synthase CHS and FH3 Flavanone 3 hydroxylase, would offer insight into the genetic modification of anthocyanin.

Keywords

Calyx, Deterioration, Encapsulation, Stress, Stability, Malvaceae

Introduction

The roselle (*Hibiscus sabdariffa* L.) is a member of the Malvaceae family and has been grown in several countries around the globe, including Malaysia (1) (Fig.1). Increasingly, many people believe that medicinal herbs, fruits



Fig. 1. Morphological features of the roselle plant. (a) Roselle farm (b) roselle leaves (c) roselle flower and (d) roselle calyx

and vegetables contribute to a broad range of health advantages (2). Phenolic is well-known for its many health advantages, including “regulating

glucose levels and increasing antioxidant, anti-inflammatory, anti-mutagenic, anticancer and neuroprotective effects," due to its ability to modulate numerous processes and pathways in the human body (3, 4). Many physicians, nutritionists and other health professionals now believe that a daily regimen of herbal remedies, fruits and vegetables helps protect human health (2). Roselle calyces have abundant flavonoids such as anthocyanins and other phenolic compounds. Naturally, flavonoids are distributed as dietary polyphenol. Roselle dried petals can be used for tea, coffee, jam, pudding, ice cream or the petals that can be boiled and used for syrup (1, 5).

Anthocyanidin and their conjugated acylglycosylated or glycosylated derivatives, termed anthocyanins, are both flavonoids and a fascinating family of water-soluble vacuolar pigments (4). They are produced via the flavonoid pathway and are thought to be the primary source of the vibrant red, orange, violet and blue colors found in a variety of edible flowers, vegetables, fruits, some cereals, seeds and plant leaves, as well as their derivatives such as juices, tea and red wines (6). Over 4000 flavonoids have been depicted and classified into many major groups, including phenolic flavonols, anthocyanin, Chalcone, catechin, flavones and isoflavones, according to their phenol structures (7). The active roselle extract has shown to be the anthocyanin in several forms and that delphinidin-3-sambubioside leads in folk medicines and activity in the treatment of many diseases (8). Anthocyanin pigments exist in six separate anthocyanin groups, including "delphinidin, cyanidin, malvidin, pelargonidin, petunidin and peonidin," were found in various highly antioxidant-active vegetables and fruits (9, 10).

Plants activated anthocyanin, due to poor post harvest regulation and in response to stresses like dryness, increased salinity, light and wound degradation, also are associated with increased stress tolerance. Anthocyanins can protect the plant against oxidative stress due to stabilizing unpaired electrons in free radicals. The sensitivity of its antioxidants is higher than that of vitamins C and E (11, 12). Many anthocyanins have demonstrable antiviral, antibacterial and fungicidal activity like other flavonoids (13, 14). Pathogenic microorganism infections can be protected from plants (15, 16). However, the instability of anthocyanin leads to early degradation and develops unwanted brown colors due to its high reactivity. The most critical factors influencing anthocyanins' stability are pH, temperature, light and post-harvest-related enzymes. Therefore, the present study summarizes and critically reviews recent findings concerning the effect of anthocyanin stability and its potential role in roselle post harvest deterioration. Particular emphasis will be placed on the mechanism and stability of anthocyanins' degradation, subject to heat, pH, storage, degradation kinetics and the role of anthocyanin in post harvest deterioration.

Anthocyanin

Anthocyanins constitute a significant class of flavonoids containing many secondary metabolites and natural pigments present in the roselle calyx, fruits and vegetables

(Table. 1). Anthocyanins are polyphenol glycosylated compounds ranging from orange, red and purple to blue in flowers, fruits and vegetative tissue (17). In nature, about

Table 1. Anthocyanin content (mg.100 g-1) of some fruits (128).

Fruits	Anthocyanin	Content (mg.100 g-1)
Roselle (<i>Hibiscus sabdariffa</i>)	Dp 3-xylosylglucoside Cy 3-xylosylglucoside Dp 3-glucoside Cy 3-glucoside	150
Blue berry (<i>Vaccinium corymbosum</i>)	Dp 3-galactoside Dp 3-arabinoside Mv 3-galactoside Mv-arabinoside	25-495
Black berry (<i>Rubus spp.</i> ..Var Cumberland)	Cy 3-glucoside Cy 3-rutinoside Cy 3-sambubioside Cy 3-xylosylrutinoside	428
Strawberry (<i>Fragaria spp.</i>)	Pg 3-glucoside Cy 3-glucoside	450-700
Cranberry (<i>Vaccinium macrocarpon Ait</i>)	Pn 3-galactoside Pn 3-arabinoside Cy 3-galactoside Cy 3-arabinoside	78
Grappe (<i>Vitis vinifera L.</i>)	Mv 3-monoglucoside Pn 3-monoglucoside Dp 3-monoglucoside Mv 3-monoglucoside-p-coumarate Pn 3-monoglucoside-p-coumarate	30-750
Prune (<i>Prunassalicina cv. Sordum</i>).	Cy 3-rhamnoglucoside Cy 3-glucoside	29.5
Sweet cherry (<i>Prunus cerasus L. var. Montmorency</i>)	Pn 3-rutinoside Cy 3-glucoside Cy 3-rutinoside Cy 3-sophoroside Cy 3-2Gglucosylrutinoside	34-8

600 anthocyanins have been discovered (18). Anthocyanins' most prominent plants include "pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin" (17). Anthocyanins protect plants against various biotic and abiotic stresses (19, 20). As seen in (Fig. 2), two benzene

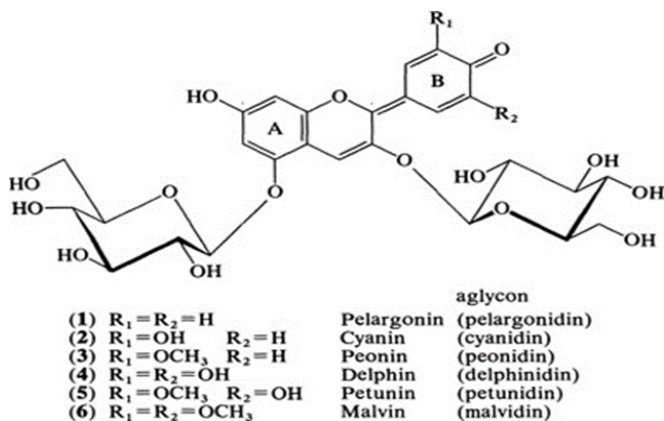


Figure 2. Anthocyanins general structure, highlighting the six most abundant anthocyanidins (25)

rings with a three-carbon linear chain are used for anthocyanins (C2, C3, C4); this means they have a single C6-C3-C6 skeleton (21). Chemically, anthocyanins are anthocya-

nidin fractions obtained from flavylum or 2-phenylbenzopyrylium cation. In polar solvents, such as ethanol, methanol and water, anthocyanins are soluble.

The structures of anthocyanins can be transformed with pH changes, leading to color changes due to the color stability, usually higher than pH 4 (14) (Fig. 5); the acid pH range was suitable for most anthocyanin dyes. The red col-

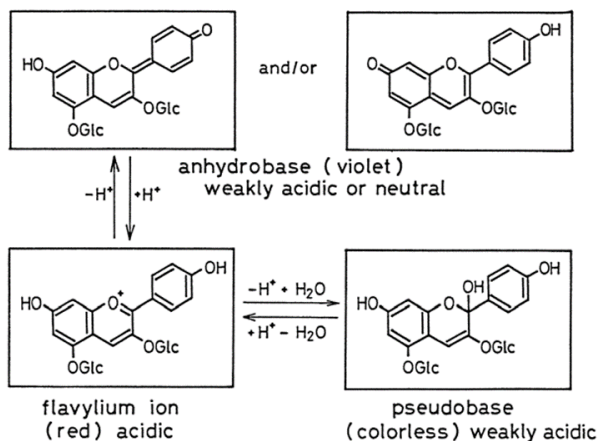


Figure 5. Anthocyanin structure changes with pH in an aqueous solution (131).

or is especially acidic at pH 1-3, with a high pH of about 4, and the majority of colors are blue and purple with pH levels ranging from 6-7 (22). Anthocyanin's various functions include its coloring effects in plants and numerous health effects, including antioxidants, anti-diabetic, antihypertensive, anticancer, cardioprotective and many other medicinal properties. They're considered to be very unstable. pH, temperature, light and storage are the most critical factors influencing stability (22, 23).

Anthocyanin Biosynthesis

A general flavonoid pathway synthesizes anthocyanins. Three malonyl-CoA is condensed by CHS and 4-coumaroyl-CoA molecules generated from tyrosine phenylalanine (shikimate or phenylpropanoid pathway) into naringenin, which is furthermore converted to naringenin via CHI (chalcone isomerase). F3H and flavonoid 3'5'-hydroxylase (F3'5'H) enzymes modified naringenin, resulting in different DFR (dihydroflavonols-4 reductase). These molecules were reduced to make leucoanthocyanidins (leucocyanidin, leucopelargonidin and leucodelphinidin) via DFR (26). Anthocyanidin synthase (ANS) oxidation of leucoanthocyanidins produces unstable flavylium cation anthocyanidin that is further bound to C3 monosaccharide residue in ring C or other flavonoid glucosyltransferases (UFGT)-catalyzed glycosylation and methyltransferase (TM) positions, resulting in the formation of complicated aglycones and anthocyanin, creating stable anthocyanin molecules (25) Fig. 3. Glucose is the most abundant sugar; natural anthocyanins have galactose and xylose (26). Additional modifications are also possible, including hydroxyl ring B methylation and acylation (24). Such intrinsic improvements are intended to improve the anthocyanin's stability or diversify anthocyanin colors (19).

Economic and Medicinal Benefit of Anthocyanin

Anthocyanins are generally used in the medical, food man-

ufacturing and cosmetics industries (26). Anthocyanins are more prominent than the food industry dyes and can provide alternatives to synthetic dyes. The return of red or blue colorants to anthocyanin sources dramatically increases anthocyanin intake (27). The demand for food color has risen steadily in recent years and is expected to rise from 10 % to 15% per annum. The market for natural food coloring is expected to expand at a reasonably fast pace to over \$7.7 billion in 2019 compared to synthetic hue (28). Similarly, it was argued that the overall production of food colors keeps on expanding globally (29). Global markets are expected to expand by sales at an average growth rate of 6.22% in 2015-2019. Anthocyanins, carotenoids and chlorophyll are also used for coloring food. Roselle is a unique food in terms of nutritional properties because of its high concentration of vitamin C and anthocyanins. Roselle calyces, according to nutritionists are a good source of calcium, potassium, magnesium, sodium, niacin, riboflavin and iron (29).

Consumers are becoming more mindful of what they are consuming owing to the variety of illnesses currently impacting the globe (30). The production of healthcare in human life has proved to be facilitated by anthocyanins and other nutritious bioactive. Daily colorful fruit and vegetable consumption is essential for a balanced way of life to protect against chronic diseases (31). Low fruit and vegetable intake are estimated to be one of the causes of 1.7 million deaths worldwide (32). Recent experimental studies have shown that colored food, fruit and vegetable compounds may inhibit baked-food mutation. In preventing diseases linked to lifestyle, the use of roselle anthocyanins may have an essential role including, hyperglycemia, neurological and cancer disorders, antioxidant, anti-hypertension among others (33).

Anticancer

Anthocyanins from *H. sabdariffa* induce apoptosis in HL-60 cancer cells by activating p38 MAP kinase, which phosphorylates the target protein C-Jun, initiating apoptotic protein cascades that include Fas-mediated signaling and culminate in the release of cytochrome C from mitochondria and caspase-3 cleavage (34). Delphinidin 3-sambubioside from *H. sabdariffa* causes dose-dependent apoptosis in human promyelocytic leukemia (HL-60) cells via a mitochondrial malfunction pathway mediated by reactive oxygen species (ROS) (35). It was shown for the first time that anthocyanins from *H. sabdariffa* changed mitochondrial activity and accelerated cell death in MCF-7 cells via autophagy and necrosis rather than programmed cell death (36). The previous research establishes that anthocyanins produced from roselle have anticancer activity.

Antioxidant Activity

Although free radicals are produced naturally during metabolism, their accumulation can be harmful to cells, causing oxidation of cellular components (such as nucleic acids, proteins and fatty acids) and lipid peroxidation, which accelerates aging and contributes to the development of a variety of chronic diseases such as cancer, atherosclerosis and ulcerative colitis (37). Antioxidants made from synthetic materials have been linked to various health problems,

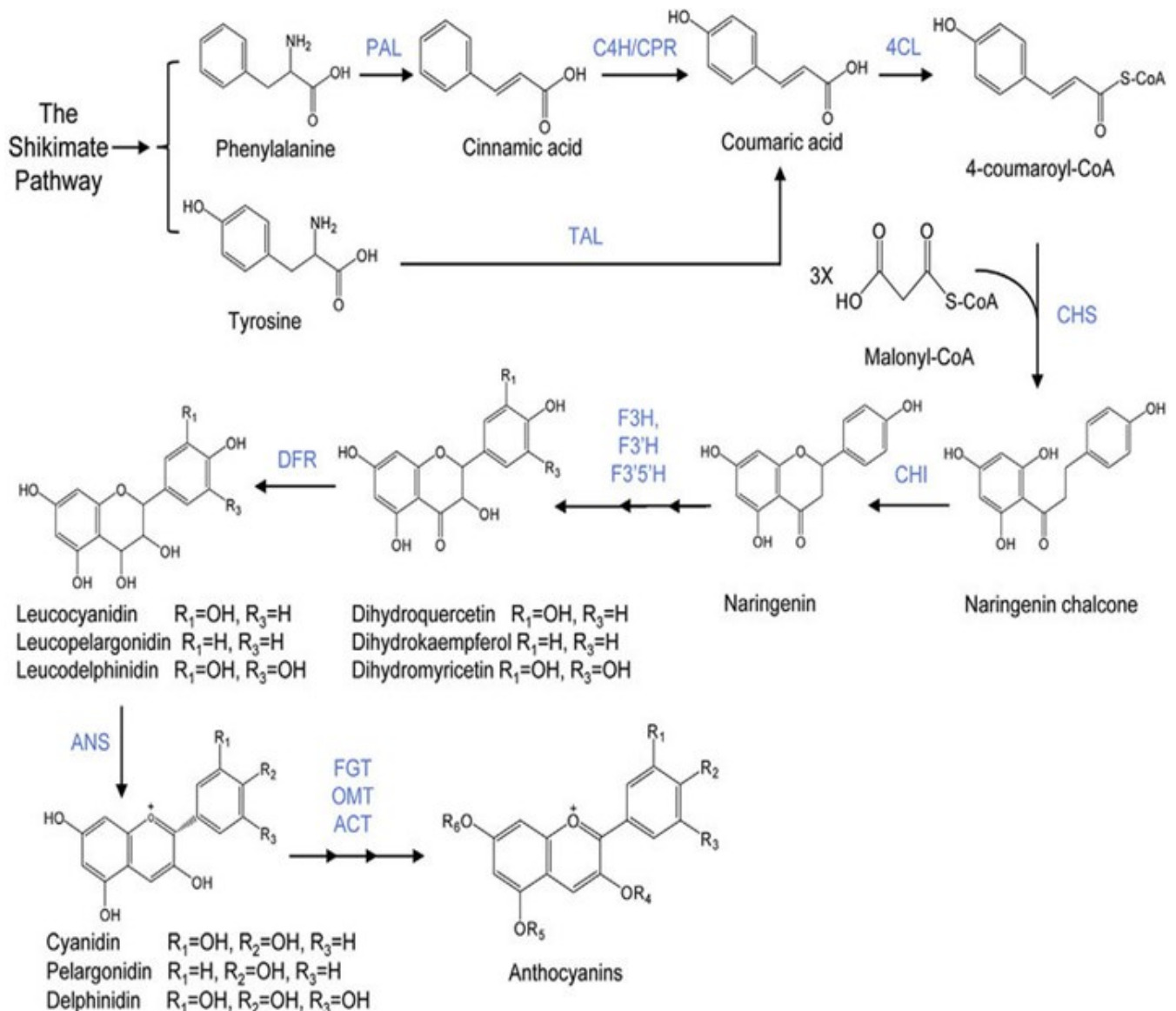


Figure 3. Anthocyanin biosynthesis pathway (129)

which is why more and more people are turning to natural sources. The antioxidant properties of anthocyanins are widely documented. Antioxidant activity of roselle extracts has been shown; in actuality, this plant has only recently emerged due to the growing interest in natural antioxidants. According to Hai-Yao Wu, the roselle extract is rich in anthocyanins and has significant antioxidative properties (38). Researchers used *H. sabdariffa* extract to reduce the severity of the drug-induced sperm abnormalities and enhance sperm motility by increasing antioxidant capacity and the activity of testicular antioxidant enzymes.

Antihypertensive

According to the studies, *H. sabdariffa* is a safe and effective therapy for mild to moderate essential hypertension and that it is on par with current pharmaceutical antihypertensive medicines. Roselle may be the first line of defense against increasing blood pressure (39). For the first time in humans, it was showed the antihypertensive effects of *Hibiscus sabdariffa* extracts on angiotensin-converting enzyme (ACE), giving scientific legitimacy to the use of roselle extract in traditional medicine to regulate blood pressure (40). As the anthocyanin content increases, enzyme activity

decreases because the anthocyanins compete with the active site for electron flow. The enzyme ACE turns angiotensin I into angiotensin II.

Antimicrobial Activity

To combat the emergence of antimicrobial resistance in bacteria over time, natural products have received considerable attention due to their abundance of metabolites with antimicrobial, antifungal and antiparasitic properties. Indeed, these processes serve as a defensive mechanism for plants against diseases and illnesses throughout their development and growth. Among these phytochemicals, anthocyanins have previously been shown to be capable of inhibiting the replication and development of a variety of Gram-negative and Gram-positive bacteria and parasites (4, 41). According to the studies, flavonoids in roselle extract have antibacterial properties because they form complexes with bacterial cell walls and enhance the extract's permeability to the surface of the cells. Increasing the permeability of the plasma membrane, which in turn inhibits electron transport protein translocation and other enzyme-dependent functions, may result in an ion leakage from bacterial cells as one potential mode of action (42, 43). Pro-

anthocyanidins found in Roselle combine or disrupt the structural entity of bacterial cells' P-fimbriae, limiting their ability to adhere and produce biofilms *in vitro*.

Anti-inflammatory

There were investigations on the effect of roselle extract on streptozotocin-induced diabetic rats (44). TNF- was found to be reduced at doses of 72 mg/day/200 g body weight and 288 mg/day/200 g body weight, indicating that Roselle possesses anti-inflammatory properties.

Government policy recommendations like the US dietary guidelines and bodies as the National Fruit and Vegetable Alliance Guidelines note that the contribution made by bioactive nutritional compounds such as anthocyanin should be taken into account (27). Today China has established a specific proposed anthocyanin daily intake level of 50 mg/day (31). Similarly, "NHANES recorded anthocyanin's dietary intake in 2007-2008 at; 11.6 ± 1.1 mg / d per person aged ≥ 20 y. Females had more anthocyanin consumption a day (12.6 6.5 mg / d) than males (10.5 6.8 mg / d). The median ingestion of anthocyanins in different racial/ethnic groups has also been shown to differ considerably, with whites having more mean daily intakes (12.5 , 6.3 mg / d) than Hispanics (10.1 , 6.2 mg / d and non-Hispanic black)" in the United States (45). Since anthocyanin is poorly bio-available, the risk of food supply toxicity is low. The Joint FAO/WHO Committee on Food Additives has concluded that anthocyanins have an unquestionable daily intake of 2.5 mg/kg (46). Anthocyanin protection and toxicology tests suggest that animal acute toxicity is extremely poor, and there are no findings that anthocyanin usage in humans is adverse with a regular dietary intake (47). Similarly, it was

reported that anthocyanins are considered safe and are recommended, along with physical activity, to reduce stress-related diseases and no adverse effects of anthocyanin consumption have been documented (27).

Mechanism of anthocyanin degradation

The anthocyanin color change was described (48). Anthocyanins are commonly used in aqueous solutions as four pH species: QB (quinoid base), FC (flavylium cation), carbinol, or PB (pseudo base) and Chalcone. Anthocyanins are used in acidic conditions ($\text{pH} < 2$) in deep-red cation (FC). Increased pH values cause a rapid loss of proton blue or violet quinoid (QB) form. "Flavylium cation (FC) hydration occurs at the same time as carbinol, or pseudo base (PB) is produced, eventually entering the colorless Chalcone (CH) balance. The pH of FC, QB, PB and CH relatively varied (48); this means that ionic anthocyanins modify the molecular structure according to the prevailing pH, leading to varying colors and shades at respective pH values". The pH modification can achieve the average stabilization period of anthocyanine; experience can greatly aid food producers. The degradation index (DI) comprises three degradation components: first, the increase in absorption due to browning; The other is absorbance from colorless carbinol bases and the impact of bathochromic changes due to less stable anthocyanin structures (49). At high temperatures, anthocyanin degrades more quickly than at low temperatures (50). During two stages, heating damage can occur. First and foremost, hydrolysis takes place in glycosidic anthocyanin bonds so that aglycons are unstable and therefore released into carbinol and Chalcone (Fig. 4) (51).

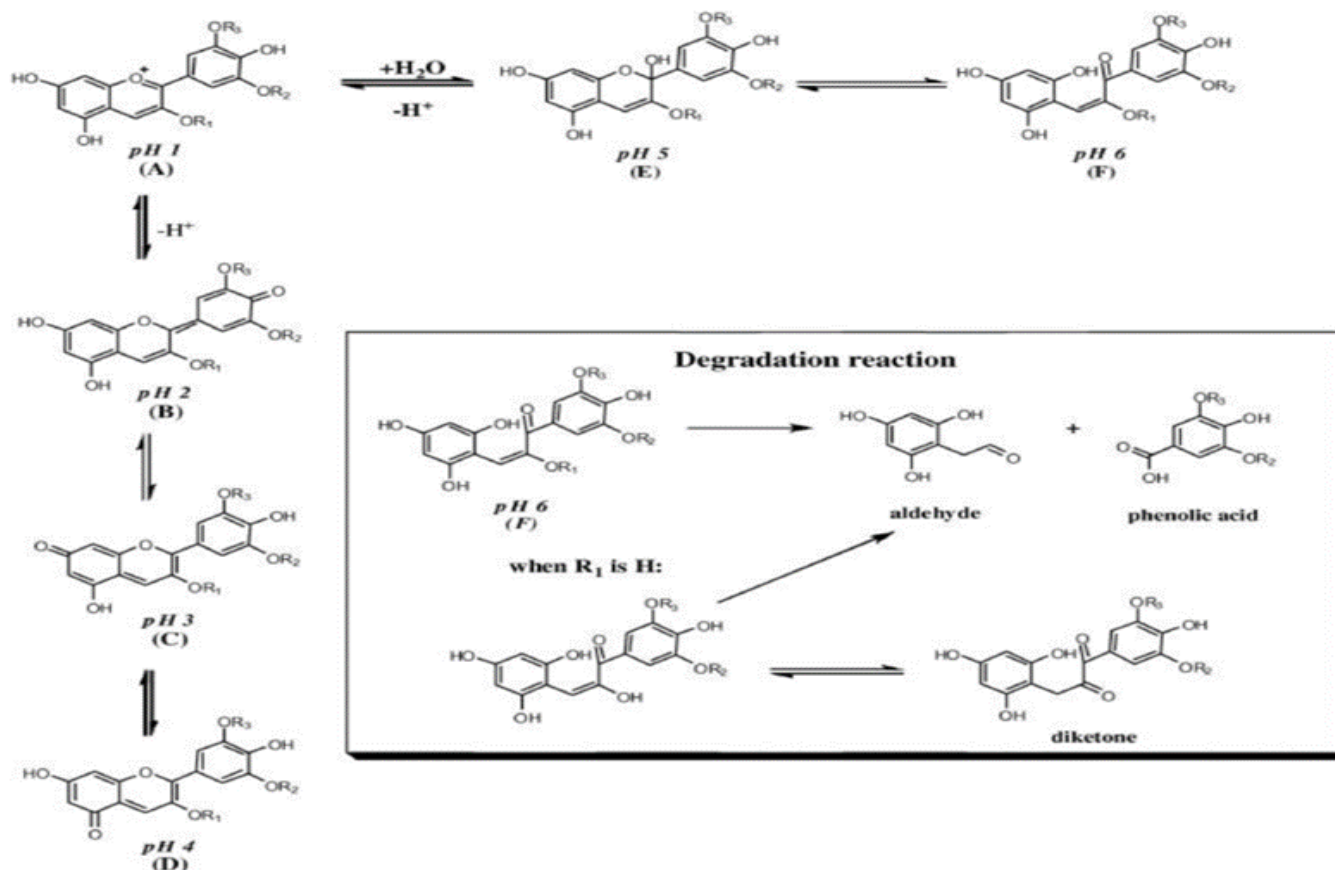


Figure 4. Degradation mechanism of anthocyanin compounds (130)

Anthocyanin decoloration may be caused by active enzyme-driven breakdown processes (52, 53). (52), suggesting that three families are involved in the degradation of anthocyanin: Peroxidase, polyphenol oxidase and β -glucosidase. There are two known mechanisms for anthocyanin degradation. Another method is to oxidize Peroxidase directly. The second step involves deglycosylation of glucosides and oxidation of polyphenol or Peroxidase. There are non-enzymatic factors that affect the color and stability of anthocyanin, which may improve its tolerance to anthocyanin degradation enzymes (53, 54). "Degradation of anthocyanins through isothermal heating is expressed in obeying first-order kinetics for juice and concentrate of sour cherry" (48), strawberries (55) and blackberries (56). Kinetic models are also used to test food health scientifically, quickly and economically. The critical quality parameters can also be predicted using kinetic modelings such as constant rate, reaction order and energy activation to predict food losses during storage and thermal processing. Nutrient deficiency is a significant factor in food production.

Anthocyanin stability

Anthocyanins are natural food coloring agents, but consistency issues limit their use (57). Anthocyanin is relatively unstable and can rapidly degrade and form colorless or undesired brown compounds during extraction and storage due to its high reactivity (58). The most significant factors affecting stability are pH, temperature, light, storage, oxygen, enzymes and metal ions (22). Besides affecting food products directly, the deterioration of anthocyanin can result in benzene ring aldehydes production that affects human health (38).

pH stability

Shivon Sipahli reported that anthocyanin HCl-acidified ethanol extracts provide excellent stability when exposed to low pH, low heat and dark light. Anthocyanins' kinetic degradation has indicated that it could be heated to up to 70 °C at gradually decreasing antioxidant content (57). It was also clearly shown (59) that increasing pH causes more destruction of anthocyanin. It also indicates at lower pH (< 5.0), anthocyanin is stable while unstable at alkaline (29), decreased pH to 2.8 in anthocyanin solutions, flavylium cation structure changes provide more extended stability (38). It was also reported that roselle anthocyanin is stable at an acidic pH (1-4) (60).

Temperature and light stability

The stability of anthocyanins was greater at the lowest storage temperature (4 °C) (61, 62), which indicated that acylated anthocyanins were much more stable than nonacylated anthocyanins at all storage temperatures. Both extracts, however, were stable when stored at 0 °C. Therefore, the consequence of low temperature (≤ 50 °C) on the stability of anthocyanin is imperative. Because the heat treatment at 55 °C had no discernible effect on the color, it may be assumed that the roselle extract's red hue stabilized at that temperature, Anthocyanins' thermal degradation temperature of 80 °C (63, 64) also reported a decrease in the absorbance of anthocyanins in higher temperatures (100 °C). At

marginally increased pH 5.0 were discovered New anthocyanin and gum arabic acid solutions (65). A solution of "0.51 mg/ml was heated to 80 °C and 126 °C for 80 min (66). A similar report indicated that adding coumaric, cinnamic and ferulic acids as co-pigments to roselle anthocyanin extracts resulted in significant anthocyanin concentration and color stability over 60 days storage at 10 °C (67).

Investigation was on the effect of temperature on anthocyanin at different temperatures (37, 50 and 100 °C) (under light) (7). Higher temperatures (50 to 100 °C) destroy anthocyanin faster, implying that it should be avoided for anthocyanin processing, storage and usage. On the other hand, the anthocyanin is stable between 4 °C and 37 °C and may therefore be utilized for storage (68).

Investigated anthocyanin degradation under light and discovered that it is temperature-dependent and that exposure to light may destroy anthocyanin molecules (48). According to anthocyanin studies, the chalcone type of anthocyanin has little effect in the visual range but loses substantially in absorbance as temperature increases. Cooling the copigments solution produces changes in the copigments complex, responsible for quantitative color recovery (48). Encapsulation was used to obtain light stability of anthocyanin (69).

Color stability

As consumers increasingly reject synthetic pigments, there has been increasing interest in food colorants from natural or naturally derived sources (70). The worry is increasing since it has been revealed that synthetic pigments or anti-oxidants may affect cardiovascular disease (70). Anthocyanin is a natural colorant that is extensively utilized in the food industry. Spray-dried encapsulated anthocyanins Color stability, encapsulated with polysaccharides accompanied by sufficient processing to improve anthocyanin stability for practical usage in food systems, noticed that maltodextrin and Arabic gum combinations had the highest encapsulation performance. C3 is more efficient in stabilizing diglycosides as compared to mono glycosides. Glycosylation of C5 reduces the pigment density. Acylation improves anthocyanin stability, and the increase in acyl moieties tends to alter the colors from red to blue (71). For instance, metal ions such as iron and magnesium enhance the stability of anthocyanins by creating complexes (29).

Storage stability

In a research, there examined the durability of pigment-copigments complexes generated during a 6 month storage period (72). Throughout the storage period, the addition of ferulic and caffeic acids significantly increased the color intensity of pelargonidin 3-glucoside. According to a study, no deterioration was seen during the refrigerated storage of anthocyanin at (4 °C) (73). It was indicated that anthocyanin stability and color concentration was achieved for over 60 days storage at 10 °C through addition of coumaric, cinnamic and ferulic acids as co-pigments (67).

Enzymatic stability

The stability of anthocyanins is due to their modification. Following the production of anthocyanidin aglycones, cyto-

solic modification processes include glycosylation, methylation and acylation (Fig. 6). Knowledge of these modifications of their instability and degradation during processing and storage. Encapsulation is one of the best approaches

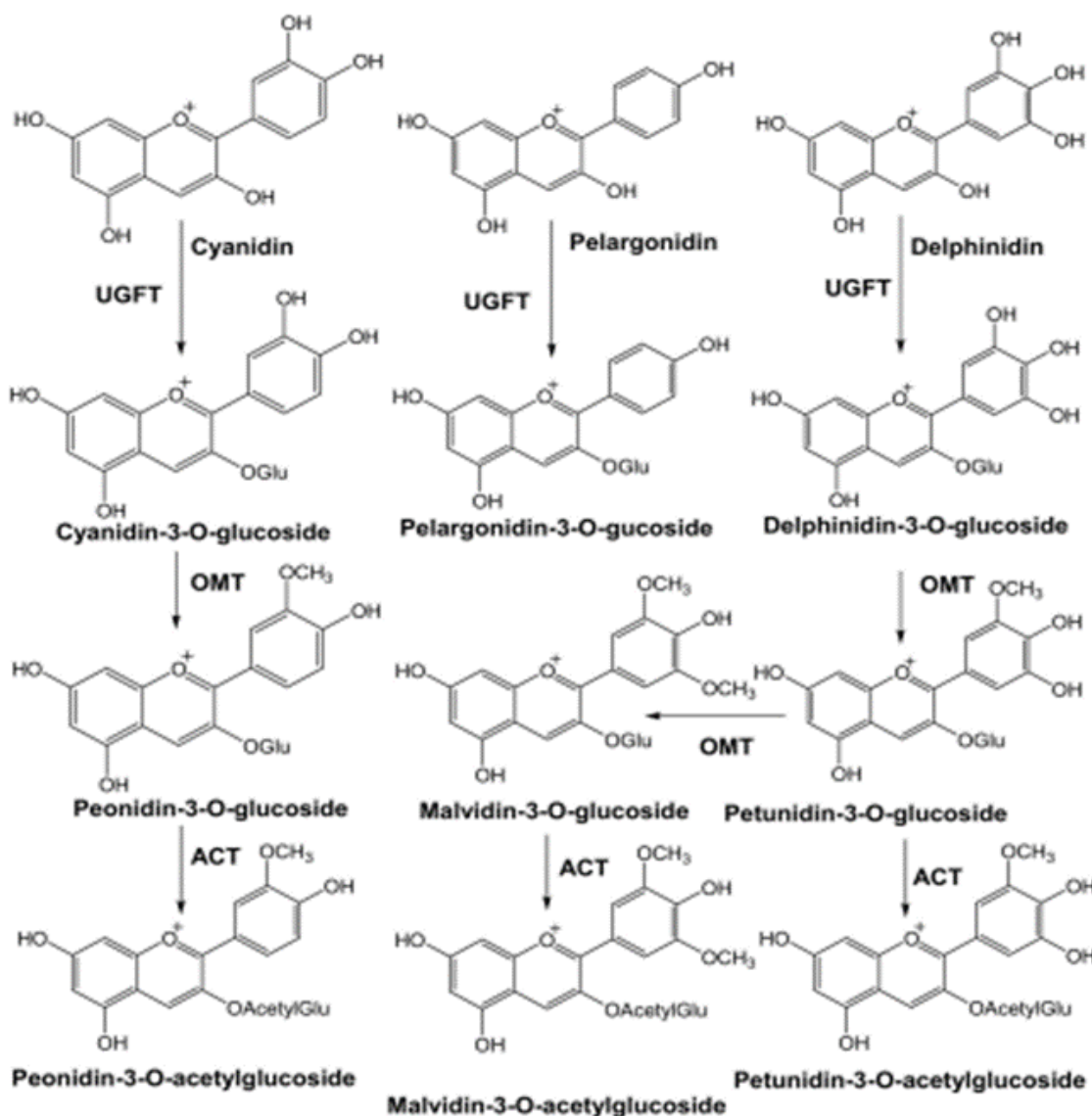


Figure 6. Anthocyanidins modulated via acylation, glycosylation and, methylation (80)

tion processes' biochemistry and molecular biology, as well as the enzymes involved, has exploded in the past decade attributable to the molecular cloning of the enzyme genes from various plant species (74). Reports are on anthocyanins' decolorization to the activity of a β -glycosidase (75). Several additional researchers, notably (76) shown that crude vegetable extracts may substantially degrade anthocyanins only when phenols are present. Polyphenol oxidase, they claim, is the enzyme responsible for this activity. In this case, the anthocyanin would be destroyed as a consequence of its interaction with the quinone generated by the oxidation of an appropriate phenol substrate. According to research, acylation increases the stability of anthocyanins, whereas structural changes increase their bioactivity (77).

Anthocyanin encapsulation

Different delivery methods have been developed to address the fact that the integration of phenolic compounds into foods and pharmaceutical products is a challenge because

among them all (4). Encapsulation is an effective method for avoiding colorant degradation and premature color development. When anthocyanins are encapsulated, their stability improves because of an effective barrier between them and external environmental variables like light and temperature. They are also protected against enzymes and reactive substances by encapsulation. Spray drying (78), freeze-drying (79, 80), emulsification (36, 81, 82), liposomal encapsulation (83), gelation (84, 85) and complexation have all been described (86). Due to the anthocyanin instability and sensitivity to deterioration, during processing and storage, different delivery methods have been devised to protect these phenolic compounds and ensure their use in foods and pharmaceuticals (87).

Natural colors that have been encapsulated are more resistant to changes in temperature, light and pH. Products with color encapsulation have a longer shelf life, are more stable over a broader pH range and do not develop color during storage. As a consequence, the chosen hue

is instantly accessible following manufacturing. At the moment, food researchers are very interested in microwave encapsulation owing to its many potential benefits, including reduced drying time, cheap cost, better product quality, and the ability to produce a range of dried products (88). For example, (88) reported that Margarine with roselle encapsulated anthocyanin was more stable than non-encapsulated anthocyanin. Also, previously published reviews addressed encapsulating agents and methods for stabilizing and delivering anthocyanins (82, 89, 90).

Anthocyanin copigmentation

Copigmentation is a "process through which anthocyanins form complexes with copigments such as phenolic, metal ions and biopolymer (48). In addition to increasing color intensity, this process also protects colored flavylum cations from water molecules' nucleophilic assault and improves antioxidant capabilities. Copigmentation ideas from theory and experiment have recently been presented in a complete description of modulating anthocyanin stability and color via copigmentation (91). The kind of copigments used is critical for successful copigmentation. For instance, phenolic substances such as hydrolyzable tannins (92), flavonoids (93) and phenolic acids (94, 95) all copigments with anthocyanins through - stacking and hydrogen" bonding. It was demonstrated that adding phenolic acids (ferulic, cinnamic and coumaric) to roselle anthocyanin extracts increased anthocyanin levels and improved color stability during storage and also showed noticeable antioxidant and antimicrobial activities (66). Similarly, in their Copigmentation experiments of *H. sabdariffa* anthocyanin extract with ferulic acid (96) found that ferulic acid significantly enhanced the heat stability of anthocyanins. It was showed that β -carotene bleaching assay and higher color stability during storage at 25 °C, 40 °C and 60 °C than original blueberry" anthocyanins (97).

Effect of stability on the medicinal value of anthocyanin

Rich anthocyanin foods can enhance overall health by providing nutrients. Its poor environmental stability complicates the incorporation of anthocyanin into food and medicinal items (71). Some studies have shown that anthocyanins, a healthy food ingredient used throughout the globe, may pose significant risks to public health (cardiovascular disease, inflammation, obesity and diabetes) from food additive chemical synthesis (90, 98). Furthermore, anthocyanin degradation can also result in benzene ring aldehyde, posing health issues (99). Anthocyanins' pharmacological and other functions are directly proportional to their antioxidant activity, which can also be lost due to stability issues. Anthocyanin isolates and Flavonoids -rich mixtures can protect against DNA cleavage, estrogen activity, inhibition of the enzyme, enhanced cytokines synthesis (i.e., regulatory immune), anti-inflammation, lipid Peroxidation, reducing the capillary permeability and membrane improvements (100, 101).

Post-harvest deterioration mechanism

Post harvest degradation refers to several unwanted physical and biochemical modifications that reduce the shelf life and durability of the products and make them inappropri-

ate for consumption (102). Post-harvest is the last step in crop production that includes harvesting, processing, decorating, washing, pre-cooling, grading at packaging, storage, transportation and post harvest treatments. When a crop is harvested from the parent plant, it starts to deteriorate (103). Both the plants, after harvesting, are still living entities; hence they are deprived of a supply of hormones, nutrients and water. Therefore they should be treated carefully and these perishables are very vulnerable to injury.

There are two differentiated mechanisms for deterioration: physiological or primary and secondary or microbiological (104). The fundamental cause of the decline of calyx and widespread brown color, leading to shelf life and decreased inconsistency, is physiological degradation (105–107). Primary degradation includes an increase in the function of phenolic oxidative enzymes, including catechin and anthocyanidin, that eventually polymerize into condensed tannins (108–110). Mechanical damage, an inevitable consequence of harvesting, causes physiological post harvest degradation. Calyx was typically injured during decorating, i.e., removing roselle calyx from the seeds, resulting in stress production, anthocyanin instability and ultimately, pathogenic decay, fermentation, or calyx softening (111, 112). Secondary post harvest deterioration sometimes happens when a calyx has moderate to substantial injury from a wide variety of pathogens, typically 5 to 7 days later (113, 114). The gene coding for Catalase, ascorbate peroxidases and secretive peroxidases catalyzes the reduction of H₂O₂ by utilizing ascorbate or a variety of organic or inorganic substrates as an electron donor is expressed during post harvest physiological deterioration (PPD) in cassava (115). (112, 113), Also, Hydroxyprolinerich glycoprotein HRGP1 (111), and two Cytochrome P450 and CYP79D2 (AAF27289 and AAF27290;(115) previously expressed during post harvest storage. Quantitative and qualitative losses for crops between harvest and consumption are unavoidable natural processes. Human involvement is required to mitigate this loss (116). For a global population of about 9.8 billion predicted to hit 2050 (117), food shortages, waste or both would further intensify food security issues globally. The average losses recorded in Malaysia for fruit and vegetables are still about 20% (103). Sufficient efforts should be made that would revamp existing procedures to reduce food waste and losses.

Role of anthocyanin in post-harvest deterioration

Anthocyanins can also play a significant role in enhancing post harvest processes and protective effects by preserving membrane integrity to decrease cell senescence and inhibiting lipid peroxidation (118). Anthocyanins improve fruit's antioxidant capacity, eliminating reactive oxygen species (ROS) activity and signaling mechanism. Therefore, it may delay over-breaking, limiting cell death induction and fungal spread (53). Due to its extraordinary antioxidant power, anthocyanins were more widely used during the past 20 years (112, 119, 120) and anthocyanins are widely considered to contribute to *Hibiscus sabdariffa* preventive impact significantly. A correlation between roselle antioxidant activity and anthocyanin content has also been identified, indicating that these compounds may contribute to ro-

selle's antioxidant impact (120). In addition to protecting plants from oxidative stress, anthocyanins may donate hydrogen atoms to free radicals, balancing the unpaired electrons. Furthermore, pathways by which anthocyanins help plants in the control of abiotic stress are becoming increasingly understandable. Many functions, including ROS scavengers, were forecast for anthocyanins generated during abiotic stress, photoprotectants, stress signals (121–123), xenohormesis (e.g., therapeutic stress). As a result of the growing interest in mechanisms by which abiotic stress-tolerating anthocyanins help plants cope with abiotic stress (124–127) it has been reported that inductive anthocyanin synthesis is the result of gene activation, which enhances the plant's response to antioxidants so that tissues that are directly or indirectly affected by stress maintain their physiological status. According to a study, the genes of particular plants are also activated under these circumstances and regulate the production of flavonoids, such as anthocyanins, resulting in a rise in phytochemicals under stress exposure (11).

Conclusion and future developments

Roselle anthocyanin has multiple roles due to its antioxidant activity, including food coloring, free radical scavenging, pharmacological and medicinal properties. They are considered very unstable due to pH, temperature, light, enzymes and storage. From this review, anthocyanin is more stable in cold storage and low pH. A low pH and temperature are required to keep anthocyanins stable. Other methods of stabilizing anthocyanins include glycosylating, copigmenting, acylating and encapsulating the pigments. Roselle calyx is protected from post harvest deterioration by scavenging free radicals by anthocyanin. It may also indicate degradation throughout the detection of high levels of anthocyanin in the harvested calyx. To this date, there is no study available in the literature on roselle post harvest deterioration biomarkers. Still, in other plants like cassava, they reported that PPD might be a peroxidase-mediated process. So, the identification of significant anthocyanin biosynthesis genes such as CHS and F3H may serve as a potential biomarker of post harvest deterioration.

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Authors contributions

AAL wrote the manuscript. NSM brought the research idea and supervised the writing of the manuscript MMK reviewed the manuscript and made corrections while MAD proofread the manuscript and participated in the manuscript report.

Compliance with ethical standards

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References

- Ahmed, Abdelatif. Roselle (*Hibiscus sabdariffa* L.) in Sudan, cultivation and their uses. *Bull Environ Pharmacol Life Sci.* 2012;1(6):48–54.
- Mannino G, Gentile C, Ertani A, Serio G, Berteza CM. Anthocyanins: Biosynthesis, distribution, ecological role and use of biostimulants to increase their content in plant foods — A Review. 2021; <https://doi.org/10.1016/j.foodchem.2019.125515>
- Newman DJ., Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod.* 2020;83:770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
- Gonçalves AC, Nunes AR, Falcão A, Alves G, Silva LR. Dietary effects of anthocyanins in human health: A comprehensive review. *Pharmaceuticals.* 2021;14(7):1–34. <https://doi.org/10.3390/ph14070690>
- Kouakou TH, Konkon NG, Ayolie K, Obouayeba AP, Abeda ZH, and Kone M. Anthocyanin production in calyx and callus of roselle (*Hibiscus sabdariffa* L.) and its impact on antioxidant activity. *J Pharmacogn Phytochem.* 2015;4(3):9–15.
- Pervaiz T, Songtao J, Faghihi F, Haider MS, Fang J. Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. *J Plant Biochem Physiol.* 2017;05(02).
- Labib S., Akraus M, Wikert T, Richling E. The Pig Caecum Model; A suitable tool to study the intestinal metabolism of flavanoids. *Mol Nutr Food Res.* 2004;48(4):326–32. <https://doi.org/10.1002/mnfr.200400022>
- Cahlíková L, Ali BH, Havlíková L, Locárek M, Siatka T, Opletal L et al. Anthocyanins of *Hibiscus sabdiffera* calyces from Sudan. *Nat Prod Commun.* 2015;10(1):77–9. <https://doi.org/10.1177/1934578X1501000120>
- Aurelio, López D, Edgardo, Gabriel R, Navarro-Galindo, Salvador. Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv. 'Criollo') infusion. *Int J Food Sci Technol.* 2008;43(2):322–25. <https://doi.org/10.1111/j.1365-2621.2006.01439.x>
- Kähkönen MP, Heinonen M. Antioxidant Activity of Anthocyanins and Their Aglycons. *Journal of Agricultural and Food Chemistry.* 2003;51(3):628–33. <https://doi.org/10.1021/jf025551i>
- Hernández I, Alegre L, van Breusegem F, Munné-Bosch S. How relevant are flavonoids as antioxidants in plants?. *Trends Plant Sci.* 2009;14(3). <https://doi.org/10.1016/j.tplants.2008.12.003>
- Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MN V. Heavy metal-induced oxidative damage, defense reactions and detoxification mechanisms in plants. *Acta Physiol Plant.* 2013;35(4):985–99. <https://doi.org/10.1007/s11738-012-1169-6>.
- Konczak I, and Zhang W. Anthocyanins – more than nature's colours. *J Biomed Biotechnol.* 2004;239–40.
- Wrolstad, E. R. Anthocyanin Pigments — Bioactivity and coloring properties. *J Food Sci.* 2004;69(5):419–21. <https://doi.org/10.1111/j.1365-2621.2004.tb10709.x>
- Padmavati M, Sakthivel N, Thara KV, Reddy AR. Differential sensitivity of rice pathogens to growth inhibition by flavonoids. *Phytochemistry.* 1997;46:499–502. [https://doi.org/10.1016/S0031-9422\(97\)00325-7](https://doi.org/10.1016/S0031-9422(97)00325-7)
- Werlein H-D, Kutemeyer C, Schatton G, Hubbermann EM, Schwarz K. Influence of elderberry and blackcurrant concen-

- trates on the growth of microorganisms. *Food Control* 2005;16:729–33. <https://doi.org/10.1016/j.foodcont.2004.06.011>
17. Tanaka YNSAO. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Plant J*. 2008;54:733–49. <https://doi.org/10.1111/j.1365-313X.2008.03447.x>
 18. Smeriglio, Smeriglio A, Barreca D, Bellocchio E, Trombetta D. Chemistry, pharmacology and health benefits of anthocyanins Research Phyther. 2016;30(8):1265–86. <https://doi.org/10.1002/ptr.5642>
 19. Chalker-Scott L. Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol*. 1999;70(1):1–9. <https://doi.org/10.1111/j.1751-1097.1999.tb01944.x>
 20. Ahmed NU, Park JI, Jung HJ, Yang TJ, Hur Y, Nou IS. Characterization of dihydroflavonol 4-reductase (DFR) genes and their association with cold and freezing stress in *Brassica rapa*. *Gene*. 2014;550:46–55. <https://doi.org/10.1016/j.gene.2014.08.013>
 21. Wilska JJ. Food colorants. In Z. E. Sikorski (Ed.), *Chemical and functional properties of food components*. Boca Rat CRC Press. 2007;245–74. <https://doi.org/10.1201/9781420009613>
 22. Amr A, Al-Tamimi E. Stability of the crude extracts of *Ranunculus asiaticus* anthocyanins and their use as food colourants. *Int J Food Sci Technol*. 2007;42(8):1365–2621. <https://doi.org/10.1111/j.1365-2621.2006.01334.x>
 23. Bagchi D, Sen CK, Bagchi M, & Atalay M. Anti-angiogenic, antioxidant and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. *Biochem*. 2004;69(1):75–80.
 24. Zhao Y, Chen P, Lin L, Harnly JM, Yu L, Li Z. Tentative identification, quantitation and principal component analysis of green pu-erh, green and white teas using UPLC/DAD/MS. *Food Chem*. 2011;126(3):1269–77. <https://doi.org/10.1016/j.foodchem.2010.11.055>
 25. Jaakola L. New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends Plant Sci*. 2013;18(9):477–83. <http://dx.doi.org/10.1016/j.tplants.2013.06.003>
 26. Tsuda T. Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Mol Nutr Food Res*. 2012;159–70. <https://doi.org/10.1002/mnfr.201100526>.
 27. Wallace TC, Guisti MM. Anthocyanins. *Adv Nutr*. 2015;7. <https://doi.org/10.3945/an.115.009233>
 28. Future Market Insight. *Natural Food Colours Market: Significant Demand for Clean Label and Naturally Sourced Ingredients in Food Products Spurring Revenue Growth: Global Industry Analysis (2013 - 2017) and Opportunity Assessment (2018 - 2028)*. 2018).
 29. Cortez R, Luna-Vital DA, Margulis D, Gonzalez de Mejia E. Natural pigments: Stabilization methods of anthocyanins for food applications. *Compr Rev Food Sci Food Saf*. 2017;16(1):180–98. <https://doi.org/10.1111/1541-4337.12244>
 30. Sipahli S. Identification, characterization and application of a natural food colourant from *Hibiscus sabdariffa* [Internet]. 2016. Available from: <http://openscholar.dut.ac.za/handle/10321/2620>
 31. Chinese Nutrition Society. *Chinese DRIs handbook*. Beijing (China): Standards Press of China; 2013. <https://doi.org/10.3390/nu9030221>
 32. WHO. *Global strategy on diet, physical activity, and health: promoting fruit and vegetable consumption around the world*. 2004.
 33. Shaik A, Naidu KK, Panda J. A Review on Anthocyanins: a Promising Role on Phytochemistry and Pharmacology. *Int Res J Pharm*. 2018;9(1):1–9.
 34. Chang YC, Huang HP, Hsu JD, Yang SF, Wang CJ. Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. *Toxicol Appl Pharmacol*. 2005;205(3):201–12. <https://doi.org/10.1016/j.taap.2004.10.014>
 35. Hou DX, Tong X, Terahara N, Luo D, Fujii M. Delphinidin 3-sambubioside, a Hibiscus anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. *Arch Biochem Biophys*. 2005;440(1):101–19. <https://doi.org/10.1016/j.abb.2005.06.002>
 36. Wu CH, Huang CC, Hung, CH, Yao FY, Wang CJ, Chang YC. Delphinidin-rich extracts of *Hibiscus sabdariffa* L. trigger mitochondria-derived autophagy and necrosis through reactive oxygen species in human breast cancer cells. *J Funct Foods*. 2016;25:279–90. <https://doi.org/10.1016/j.jff.2016.05.018>
 37. Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski*. 2020;48(284):124–47.
 38. Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules*. 2018;23(6).
 39. Walton RJ, Whitten DL, Hawrelak JA. The efficacy of *Hibiscus sabdariffa* (rosella) in essential hypertension: A systematic review of clinical trials. *Aust J Herb Med*. 28(2):48–51.
 40. Ojeda D, Jiménez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, Alvarez L. Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. *J Ethnopharmacol*. 2010;127(1):7–10. <https://doi.org/10.1016/j.jep.2009.09.059>
 41. Ma Y, Ding S, Fei Y, Liu G, Jang H, Fang J. Antimicrobial activity of anthocyanins and catechins against foodborne pathogens *Escherichia coli* and *Salmonella*. *Food Control* [Internet]. 2019;106 (June):106712. Available from: <https://doi.org/10.1016/j.foodcont.2019.106712>
 42. Al-Hashimi AG. Antioxidant and Antibacterial Activities of *Hibiscus sabdariffa* L. extracts. *African J Food Sci*. 2012;6(21):506–11. <https://doi.org/10.5897/AJFS12.099>
 43. Fullerton M, Khatiwada J, Johnson JU, Davis S, Williams LL. Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 isolated from food, veterinary and clinical samples. *J Med Food*. 2011;14(9):950–6. <https://doi.org/10.1089/jmf.2010.0200>
 44. Mardiah, Zakaria FR, Prangdimurti E, Damanik R. Anti-inflammatory of purple roselle extract in diabetic rats induced by *Streptozotocin*. *Procedia Food Sci* [Internet]. 2015;3:182–9. Available from: <http://dx.doi.org/10.1016/j.profoo.2015.01.020>
 45. Sebastian RS, CW E, JD G, CL M, Steinfeldt, LC *et al*. New database facilitates characterization of flavonoid intake, sources and positive associations with diet quality among U.S. adults. *J Nutr*. 2015;145:1239–48. <https://doi.org/10.3945/jn.115.213025>
 46. European Food Safety Authority. Scientific opinion on the reevaluation of anthocyanins (E 163) as a food additive. *EFSA J*. 2013;11:1–51. <https://doi.org/10.2903/j.efsa.2013.3145>
 47. Burton-Freeman B, Sandhu A, Edirisinghe I. Anthocyanins. *Nutraceuticals effic saf toxic*. 2016;489–500.
 48. Cavalcanti RN, Diego TS, Maria AAM. Non-thermal stabilization mechanisms of anthocyanins in model and food systems - An overview. *Food Res Int*. 2011;44:499–509. <https://doi.org/10.1016/j.foodres.2010.12.007>
 49. Patras A, Brunton NP, O'Donnell C, Tiwari BK. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci Technol*. 2010;21(1):3–11. Available from: <http://dx.doi.org/10.1016/j.tifs.2009.07.004>
 50. Moldavan B, Daud L, Chisbora CCC. Degradation kinetics of anthocyanin from European Canberry bush (*Viburnum opulus* L.) fruit extracts effect of temprature, pH and storage solvent molecules. 2012;11655–66.
 51. Hayati EK, Budi US, R. H. Konsentrasi. Total senyawa antosianin ekstrak kelopak bunga rosella (*Hibiscus sabdariffa* L.): Pengaruh Temperatur dan pH. *J Kim*. 2012;6(2):138–47.
 52. Oren-Shamir M. Does anthocyanin degradation play a significant

- role in determining pigment concentration in plants? *Plant Sci*. 2009;177(4):310–16. <https://doi.org/10.1016/j.plantsci.2009.06.015>
53. Ying Liu, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: A review. *Front Chem*. 2018;6(MAR).
 54. Rivas-Gonzalo J, M S. Analysis of polyphenols. In: *Methods in Polyphenols Analysis*; Santos-Buelga, C., Williamson, G., (Eds); Royal Society of Chemistry. Vol. 95. (Athenaeum Press, Ltd.); Cambridge, U.K.; 2003. 338-358. p.
 55. Kirca A, Cemeroglu B. Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chem*. 2003;81(4):583–87. [https://doi.org/10.1016/S0308-8146\(02\)00500-9](https://doi.org/10.1016/S0308-8146(02)00500-9)
 56. Garzón GA, Wrolstad RE. Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate. 67; *Journal of Food Science*. 2002. 1288–99. <https://doi.org/10.1111/j.1365-2621.2002.tb10277.x>
 57. Siphali S, Mohanlall V, Mellem, Jason J. Stability and degradation kinetics of crude anthocyanin extracts from *H. sabdariffa*. *Food Sci Technol*. 2017;37(2):209–15. <https://doi.org/10.1590/1678-457X.14216>
 58. Durst RW., Wrolstad RE. Separation and characterization of anthocyanins by HPLC. In *Current Protocols in Food Analytical Chemistry*; 2001. Wrolstad, R. E., Ed.; John Wiley & Sons: New York,.
 59. Rakkimuthu, Palmurugan, Shanmugapriya. Effect of temperature, light, pH on the stability of anthocyanin pigments in *Cocculus hirsutus* fruits. *Int J Multidiscip Res Mod Educ*. 2016;2(2):91–6.
 60. Arroyo-Maya IJ, Campos-Terán J, Hernández-Arana A, McClements DJ. Characterization of flavonoid-protein interactions using fluorescence spectroscopy: Binding of pelargonidin to dairy proteins. *Food Chem [Internet]*. 2016;213:431–9. Available from: <http://dx.doi.org/10.1016/j.foodchem.2016.06.105>
 61. Martinsen, Karoline B, Aaby, Kjersti, Skrede, Grete. Effect of temperature on stability of anthocyanins, ascorbic acid and color in strawberry and raspberry jams. *Food Chem [Internet]*. 2020;126297. Available from: <https://doi.org/10.1016/j.foodchem.2020.126297>
 62. Nuzhet T, A S, EHI B. Effect of storage temperature on the stability of anthocyanins of a fermented black carrot (*Daucus carota* var. L) Beverage: Shalgam. *Chem J Agric Food*. 2004;52:3807–13. <https://doi.org/10.1021/jf049863s>
 63. Ingrid HM, Jaka, Santoso H. Natural red dyes extraction on roselle petals. *IOP Conf Ser Mater Sci Eng*. 2016;162(1).
 64. Jenshi J, Saravanakumar M, Aravindhan KM, Suganya P. The effect of light, temperature, pH on stability of anthocyanin pigments in *Musa acuminata* bract. *Res plant Biol [Internet]*. 2011;1(5):5–12. Available from: www.resplantbiol.com
 65. Guan Y, Zhong Q. The improved thermal stability of anthocyanins at pH 5.0 by gum arabic. *LWT - Food Sci Technol [Internet]*. 2015;64(2):706–12. Available from: <http://dx.doi.org/10.1016/j.lwt.2015.06.018>
 66. Sharara S, Magda. Copigmentation Effect of Some Phenolic Acids on Stabilization of Roselle (*Hibiscus sabdariffa*) Anthocyanin extract. *Am J Food Sci Technol [Internet]*. 2017;5(2):45–52. Available from: <http://pubs.sciepub.com/ajfst/5/2/3>
 67. Idham Z, Muhamad II, Mohd Setapar SH, Sarmidi MR. Effect of thermal processes on roselle anthocyanins encapsulated in different polymer matrices. *J Food Process Preserv*. 2012;36(2):176–84. <https://doi.org/10.1111/j.1745-4549.2011.00572.x>
 68. Idham Z, Muhamad II, Sarmidi MR. Degradation kinetics and color stability of spray-dried encapsulated anthocyanins from *Hibiscus sabdariffa* L. *J Food Process Eng*. 2012;35(4):522–42. <https://doi.org/10.1111/j.1745-4530.2010.00605.x>
 69. Joana Gomes., Carmo Serrano., Conceição Oliveira 3, Ana Dias and MM. Thermal and light stability of anthocyanins from strawberry by-products non-encapsulated and encapsulated with inulin. *Acta Sci Pol Technol Aliment*. 2021;20(1):79–92.
 70. Stintzing FC, Carle R. Functional properties of anthocyanins and betalains in plants, food and in human nutrition. *Trends food Sci Technol*. 15(1):19–38. <https://doi.org/10.1016/j.tifs.2003.07.004>
 71. Lachman J, Hamouz K, Šulc M, Orsák M, Pivec V, Hejtmánková A *et al*. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. *Food Chem*. 2009;114(3):836–43. <https://doi.org/10.1016/j.foodchem.2008.10.029>
 72. Eiro MJ, Heinonen M. Anthocyanin color behavior and stability during storage: Effect of intermolecular copigmentation. *J Agric Food Chem*. 50(25):7461–66.
 73. Zozio S, Pallet D, Dornier M. Évaluation de la stabilité des anthocyanes au cours du stockage d'une boisson colorée par des extraits de mures andines (*Rubus glaucus* Benth.), d'açaï (*Euterpe oleracea* Mart.) et de carottes noires (*Daucus carota* L.). *Fruits*. 2011;66(3):203–15.
 74. Sakakibara H, Ogawa T, Koyanagi A, Kobayashi S, Goda T, Kumazawa S, Shimoi K. Distribution and excretion of bilberry anthocyanins in mice. *J Agric Food Chem*. 2009;57(17):7681–86. <https://doi.org/10.1021/jf901341b>
 75. Sakamura S, Watanabe S, Obata Y. Anthocyanase and anthocyanin occurring in eggplant (*Solanum melangena* L.). *Agric Biol Chem*. 1965;29(3):181–90. <https://doi.org/10.1080/00021369.1965.10858372>
 76. Color of anthocyanin solutions expressed in lightness and chromaticity terms. Effect of pH and Type of Anthocyanin. *Journal Food Sci*. 1974;325. <https://doi.org/10.1111/j.1365-2621.1974.tb02886.x>
 77. Alappat B, Alappat J. Anthocyanin pigments: Beyond aesthetics. *Molecules*. 2020;25(23). <https://doi.org/10.3390/molecules25235500>
 78. Mahdavi SA, Jafari SM, Ghorbani M, Assadpoor E. Spray-drying microencapsulation of anthocyanins by natural biopolymers: A Review. *Dry Technol*. 2014;32(5):509–18. <https://doi.org/10.1080/07373937.2013.839562>
 79. He K, Li X, Chen X, Ye X, Huang J, Jin Y, *et al*. Evaluation of antidiabetic potential of selected traditional Chinese medicines in STZ-induced diabetic mice. *J Ethnopharmacol [Internet]*. 2011;137(3):1135–42. Available from: <http://dx.doi.org/10.1016/j.jep.2011.07.033>
 80. Xue J, Wu T, Dai Y, Xia Y. Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chem Rev*. 2019;119(8):5298–415. <https://doi.org/10.1021/acs.chemrev.8b00593>
 81. Huang Y, Zhou W. Microencapsulation of anthocyanins through two-step emulsification and release characteristics during *in vitro* digestion. *Food Chem [Internet]*. 2019;278:357–63. Available from: <https://doi.org/10.1016/j.foodchem.2018.11.073>
 82. Liu J, Tan Y, Zhou H, Muriel Mundo JL, McClements DJ. Protection of anthocyanin-rich extract from pH-induced color changes using water-in-oil-in-water emulsions. *J Food Eng*. 2019;254 (January):1–9. <https://doi.org/10.1016/j.jfoodeng.2019.02.021>
 83. Guldiken B, Gibis M, Boyacioglu D, Capanoglu E, Weiss J. Physical and chemical stability of anthocyanin-rich black carrot extract-loaded liposomes during storage. *Food Res Int [Internet]*. 2018;108:491–97. Available from: <https://doi.org/10.1016/j.foodres.2018.03.071>
 84. Celli GB, Brooks MSL, Ghanem A. Development and evaluation of a novel alginate-based in situ gelling system to modulate the release of anthocyanins. *Food Hydrocoll [Internet]*. 2016;60:500–58. Available from: <http://dx.doi.org/10.1016/j.foodhyd.2016.04.022>

85. Tan C, Dadmohammadi Y, Lee MC, Abbaspourrad A. Combination of copigmentation and encapsulation strategies for the synergistic stabilization of anthocyanins. *Compr Rev Food Sci Food Saf*. 2021;20(4):3164–91. <https://doi.org/10.1111/1541-4337.12772>
86. Ge J, Yue P, Chi J, Liang J, Gao X. Formation and stability of anthocyanins-loaded nanocomplexes prepared with chitosan hydrochloride and carboxymethyl chitosan. *Food Hydrocoll* [Internet]. 2018;74:23–31. Available from: <http://dx.doi.org/10.1016/j.foodhyd.2017.07.029>
87. Martín J, Kuskoski EM, Navas MJ, Asuero AG. Antioxidant capacity of anthocyanin pigments. flavonoids - from biosynth to hum heal. 2017;
88. Zaidel DNA, Sahat NS, Jusoh YMM, Muhamad II. Encapsulation of anthocyanin from roselle and red cabbage for stabilization of water-in-oil emulsion. *Agric agric sci procedia* [Internet]. 2014;2:82–89. Available from: <http://dx.doi.org/10.1016/j.aaspro.2014.11.012>
89. Sharif N, Khoshnoudi-Nia S, Jafari SM. Nano/microencapsulation of anthocyanins; a systematic review and meta-analysis. *Food Res Int* [Internet]. 2020;132(January):109077. Available from: <https://doi.org/10.1016/j.foodres.2020.109077>
90. Yousuf B, Gul K, Wani AA, Singh P. Health benefits of anthocyanins and their encapsulation for potential use in food systems: A Review. *Crit Rev Food Sci Nutr*. 2016;56(13):2223–30. <https://doi.org/10.1080/10408398.2013.805316>
91. Trouillas P, Sancho-García JC, De Freitas V, Gierschner J, Otyepka M, Dangles O. Stabilizing and modulating color by copigmentation: Insights from theory and experiment. *Chem Rev*. 2016;116(9):4937–82. <https://doi.org/10.1021/acs.chemrev.5b00507>
92. Tuominen A, Sinkkonen J, Karonen M, Salminen JP. Sylvatiins, acetylglucosylated hydrolysable tannins from the petals of *Geranium sylvaticum* show co-pigment effect. *Phytochemistry* [Internet]. 2015;115(1):239–51. Available from: <http://dx.doi.org/10.1016/j.phytochem.2015.01.005>
93. Chatham LA, Howard JE, Juvik JA. A natural colorant system from corn: Flavone-anthocyanin copigmentation for altered hues and improved shelf life. *Food Chem* [Internet]. 2020;310:125734. Available from: <https://doi.org/10.1016/j.foodchem.2019.125734>
94. Bimpilas A, Panagopoulou, M., Tsimogiannis D, Oreopoulou V. Anthocyanin copigmentation and color of wine: The effect of naturally obtained hydroxycinnamic acids as cofactors. *Food Chem*. 2016;197:39–46. <https://doi.org/10.1016/j.foodchem.2015.10.095>
95. Qian BJ, Liu JH, Zhao SJ, Cai JX, Jing P. The effects of gallic/ferulic/caffeic acids on colour intensification and anthocyanin stability. *Food Chem* [Internet]. 2017;228:526–32. Available from: <http://dx.doi.org/10.1016/j.foodchem.2017.01.120>
96. Reshma V. Jadhav SSB. Effect of copigmentation on thermal stability of *Hibiscus sabdariffa* anthocyanins. *Res J Pharm Tech*. 2019; <https://doi.org/10.5958/0974-360X.2019.00496.7>
97. Liu S, Li S, Lin G, Markkinen N, Yang H, Zhu B *et al*. Anthocyanin copigmentation and color attributes of bog bilberry syrup wine during bottle aging: Effect of tannic acid and gallic acid extracted from Chinese gallnut. *J Food Process Preserv*. 2019;43(8):1–13. <https://doi.org/10.1111/jfpp.14041>
98. He, Fei, Mu, Lin, Yan, Liang G *et al*. Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules*. 2010;15(12):9057–91. <https://doi.org/10.3390/molecules15129057>
99. Peng B., Li H., Deng Z. Degradation of anthocyanins in foods during heating process and its mechanism. *J Food Safe Qual*. 2016;7:3851–58.
100. Acquaviva R, Russo A, Galvano F, Galvano G, Barcellona ML, Li Volti G. Cyanidin and cyanidin 3-O-β-D-glucoside as DNA cleavage protectors and antioxidants. *Cell Biol Toxicol*. 2003;19(4):243–52.
101. Lazzé MC, Pizzala R, Savio M, Stivala LA, Prosperi E, Bianchi L. Anthocyanins protect against DNA damage induced by tert-butylhydroperoxide in rat smooth muscle and hepatoma cells. *Mutat Res - Genet Toxicol Environ Mutagen*. 2003;535(1):103–15. [http://dx.doi.org/10.1016/S1383-5718\(02\)00285-1](http://dx.doi.org/10.1016/S1383-5718(02)00285-1)
102. Mayani JM, Desai CS, Desai SC, Vagadia S. Post harvest management of horticultural crops. Post harvest management of horticultural crops. Jaya publishing house Delhi-110095 (India); 2016.
103. Mahmud Tengku MM. post harvest: An Unsung Solution for post harvest. 2017.
104. Acedo JZ, Acedo AL. Controlling post harvest physiological deterioration and surface browning in cassava (*Manihot esculenta Crantz*) roots with hot water treatment. *Acta Hort*. 2013;989:357–62.
105. Naziri D, WQ, BSSW, Viet Phu TBB. The diversity of post harvest losses in cassava value chains in selected developing countries. *J Agric Rural Dev Trop Subtrop*. 2014;115:111–23.
106. Salcedo A, ADVBSVOAOPM. Comparative evaluation of physiological post-harvest root deterioration of 25 cassava (*Manihot esculenta*) accessions: visual vs. hydroxycoumarins fluorescent accumulation analysis. *African J Agric Res*. 2010;5(3):138–44.
107. Sayre R, JR B, EB C, CECFJF. The BioCassava plus program: bio-fortification of cassava for sub-Saharan Africa. *Annu Rev Plant Biol*. 2011;62:251–72.
108. García JA, TSHCLA. Non-destructive sampling procedure for biochemical or gene expression studies on post harvest physiological deterioration of cassava roots. *Post Harvest Biol Technol*. 2013;86:529–35.
109. Zidenga T, Leyva-Guerrero E, Moon H, Siritunga D, Sayre R. Extending cassava root shelf life via reduction of reactive oxygen species production. *Plant Physiol*. 2012;159(4):1396–1407. <https://doi.org/10.3389/pls.2017.00220>
110. Buschmann H, Rodriguez MX, J T, R. BJ. Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of cassava (*Manihot esculenta Crantz*). *Ann Bot*. 2000;86(1):153–60. <https://doi.org/10.1006/anbo.2000.1285>
111. Han, Yuanhuai, Gómez-Vásquez, Rocío, Reilly, Kim. Hydroxyproline-rich glycoproteins expressed during stress responses in cassava. *Euphytica*. 2001;120(1):59–70.
112. Njoku DN, Amadi CO, Mbe J, Amanze N. Strategies to overcome post-harvest physiological deterioration in Cassava (*Manihot esculenta*) root: A Review. *Niger Agric J* [Internet]. 2014;45:51–62. Available from: <http://www.mendeley.com/research/geology-volcanic-history-eruptive-style-yakedake-volcano-group-central-japan/%0Ahttps://doi.org/10.1016/j.actatropica.2019.02.002%0Ahttps://doi.org/10.1016/j.actatropica.2018.07.028%0Ahttp://dx.doi.org/10.1016/j.ijppaw.201>
113. Uritani I, Hirose S, Data ES, Villegas RJ, Flores P. Relationship between secondary metabolism changes in cassava root tissue and physiological deterioration. *Agric Biol Chem*. 1983;47(7):1591–98.
114. Reilly K, D B, F CD, R G-V, J T, R. BJ. Towards identifying the full set of genes expressed during cassava post harvest physiological deterioration. *Plant Mol Biol*. 2007;64:187–203.
115. Andersen MD, Busk PK, Svendsen I, Møller BL. Cytochromes P-450 from cassava (*Manihot esculenta Crantz*) catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin. Cloning, functional expression in *Pichia pastoris* and substrate specificity of the isolated. *J Biol Chem*.g 2000;275(3):1966–75.
116. Kader AA. Increasing Food Availability by Reducing Post harvest Losses of Fresh. 2005.
117. UN. World Population to hit 9.8 Billion by 2050. 2017.
118. Yuzhi Jiao. Studies on antioxidant capacity of anthocyanin ex-

- tract from purple sweet potato (*Ipomoea batatas* L. African J Biotechnol. 2012;11(27):7046–54. <http://dx.doi.org/10.5897/AJB11.3859>
119. HuangHP, ChangYC, CH W, HungCN, CJ W. Anthocyanin-rich Mulberry extract inhibit the gastric cancer cell growth *in vitro* and xenograft mice by inducing signals of p38/p53 and c-jun. Food Chem. 2012;129:1703–09.
 120. Tsai, Jen P, McIntosh, John, Pearce, Philip. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. Food Res Int. 2002;35(4):351–56. [https://doi.org/10.1016/S0963-9969\(01\)00129-6](https://doi.org/10.1016/S0963-9969(01)00129-6)
 121. Kovich N, Kayanja G, Chanoca A, Otegui MS, Grotewold E. Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signal Behav. 2015;10(7).
 122. Marko D, N P, Z T, S J, G P. The substitution pattern of anthocyanidins affects different cellular signaling cascades regulating cell proliferation. Mol Nutr Food Res. 2004;48:318–25. <https://doi.org/10.1002/mnfr.200400034>
 123. Pourcel L, NG I, AJ K, A B-R, GA H, A GE. chemical complementation approach reveals genes and interactions of flavonoids with other pathways. Plant J 2013; 2013;74:383-97; <https://doi.org/10.1111/tpj.12129>
 124. Christie PJ, Alfenito MR, Walbot V. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta. 1994;194.
 125. Garriga M, J R, S R, P C, GA. L. Chlorophyll, anthocyanin and gas exchange changes assessed by spectroradiometry in *Fragaria Chiloensis* under salt stress. J Integr Plant Biol. 2014;56:505–15.
 126. Olsen KM, Lea US, Slimestad R, Verheul M, Lillo C. Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J Plant Physiol. 2008;165(14):1491–99.
 127. Zhang, Swarts, Yin. Antioxidant properties of quercetin. Adv Exp Med Biol. 2011;701:283–89. https://doi.org/10.1007/978-1-4419-7756-4_38
 128. Mazza, G., and Miniati E. Anthocyanin in fruits, vegetables and grains. Boca Raton, FL, USA.: CRC Press, Boca Raton, FL, USA.; 2000. <https://doi.org/10.1201/9781351069700>
 129. Zha J, Koffas MAG. Production of anthocyanins in metabolically engineered microorganisms: Current status and perspectives. Synth Syst Biotechnol [Internet]. 2017;2(4):259–66. Available from: <https://doi.org/10.1016/j.synbio.2017.10.005>
 130. Singh S, Gaikwad KK, Lee YS. Anthocyanin – A natural dye for smart food packaging systems. Korean J Packag Sci Technol. 2018;24(3):167–80. <http://dx.doi.org/10.20909/kopast.2018.24.3.167>
 131. Goto T. Structure, stability and color variation of natural anthocyanins. 1987;113–58. https://doi.org/10.1007/978-3-7091-8906-1_3

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