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Procedia - Social and Behavioral Sciences 121 (2014) 193 – 203

Procedia
Social and Behavioral Sciences

INHAC 2012, Kuala Lumpur

International Halal Conference, Putra World Trade Centre Kuala Lumpur, Malaysia, 4-5 September 2012

The Islamic Perspective Approach On Plant Pigments As Natural Food Colourants

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Abstract

The importance of having halal and *toyyiban* foods has been widely propagated among Muslim all around the world. One of the discussions is the increasing demand for food colourants from natural sources that can serve as substitute to synthetic dyes due to both legislative action and consumer concerns over health issues. Among all pigments, anthocyanin is the target of numerous studies because of its colourant properties and benefits as a potent antioxidants and chemoprotective. This study was conducted to determine the colour changes, anthocyanins stability and antioxidant activity of selected spray dried plant pigment from roselle calyx, and its combination from *senduduk* fruits and purple-flesh sweet potato at the same ratio (50:50). The highest anthocyanin content was obtained in roselle and *senduduk* combination (428.83 ± 5.15 mg/L). It also showed the highest antioxidant activity with FRAP value (52.04 ± 2.67 mg Trolox/g) and DPPH activity (17.94 ± 0.56 %). The phenolic content was 66.97 ± 0.07 mg GAE/g. The colour obtained from the combination of roselle and *senduduk* reported to be the most pinkish with chroma value of 25.43 ± 0.72. It has the greatest potential as a healthy and safe natural colourant to be used as functional ingredient in food products apart from being a natural colouring agent. In short, this concept is in line with maqasid approach of *darruriyat which* signifies five essentials for Muslim community and Islamic legal maxim in promoting the maxim of harm may neither be inflicted nor reciprocated in Islam (*la dharar wa la dhirar*).

Keywords : Plant Pigment; Natural Food Colourants; Maqasid Shariah; Islamic Legal Maxim

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doi:10.1016/j.sbspro.2014.01.1120

1. Introduction

Food colourants have quite a numerous controversial issues throughout the years especially in safety and health issue. Consumers generally associate food colour with safety and quality of fresh foods or of good processing. Some consumers are concerned on the possibility of colourant being used to cover up any misconduct in food preparation by masking deterioration in microbiological, nutritional and flavour of the food. However for food manufacturer, colour is important in terms of marketability and acceptability of food products. It has been shown by studies conducted by many researchers that uses of artificial or synthetic colours in food may increase the risk of getting cancer, allergies and may trigger hyperactivity in children. Due to these, many consumers have shifted their preference towards food products containing natural colourants. Natural pigments from plants sources offer the opportunity to 'colour food with food'. There are various groups of natural colour pigments such as anthocyanins, carotenoid, chlorophyll and betalain. Among these natural pigments, the anthocyanins are the target of numerous studies, due to the colourant properties, as it responsible of the shiny orange, pink, red, violet and blue colours in the flowers and fruits of some plants (Wrolstad, 2000). It is also well known that one of the significant properties of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Konczak and Zhang, 2004).

Even though, anthocyanin is among the natural food colourants permitted by food regulations besides curcumin, chlorophyll, β-carotene, lycopene, and beet red but their applications in food products have not been broadly used. The setbacks are caused by their low stability to processing and storage. Their instability in withstanding harsh conditions and complex reactions during processing affects the final product colour quality, lower their health benefits and reduce acceptance by consumer. Many studies have been conducted to find ways to stabilise anthocyanins. From these studies, several intrinsic and extrinsic factors influencing the pigment stability have been investigated such as species, environmental and agronomic conditions; extraction and processing parameters such as pH, storage temperature, concentration, chemical structure, light, oxygen, proteins, ascorbic acid, sugars, sulfites, enzymes and metallic ions (Cavalcanti et al*.,* 2011; Patras et al., 2010; Rein, 2005). Among all the factors, pH and temperature are the major factors significantly influence the pigment colour variations and stability.

1.1. Issues in food colourant

In Malaysia, food colouring is under Food Additive Regulation in The Food Act 1983 and the Food Regulations 1985. Some synthetic dyes are allowed to be used as colouring substances in food and must comply with the permitted level determined by the authority. The usages of synthetic dyes are found mostly in any food application because of the easy application, availability and its stability in food during processing. Synthethic food colourant can be divided into dyes and lakes. Dyes are certified, water soluble synthetic food colourants. They are manufactured as powders, granules or liquids. There are several types of synthetic dyes based on the compound and structure. In the 1936-1960 periods, several studies on the safety of the synthetic colourants were carried out and it was found that some were considered unsafe for consumption. Consumers who are concerned with the safety of synthetic colourant are encouraging food manufacturers to replace the synthetic colourant to natural colouring ingredients. Recently, The European Union (EU) had required all food manufacturers to put a warning label on any products that contained 'southampton six colourant'. These colourant are tartrazine, quinoline yellow, sunset yellow, carmoisine, ponceau red and allura red. There are studies that link the hyperactivity in children with these food colourant. A study reported on the significant increased of ADHD (attention deficit hyperactivity disorder) in children after consumption of drinks with azo dyes and benzoic acid

(Buchweitz et al., 2012). While in animal study of cochineal red A/ red 2G, it was found that the kidney of the mice was enlarged and deposition of iron has increased. The mice also experienced an accelerated erythropoiesis and rats showed necrosis of elastica. Furthermore, the erythrosine, another original colours permitted by the United States have been accepted and used globally. The toxicological study showed a neurotransmission inhibition however no deleterious effect was reported (Delgado-Vargas and Paredes-Lopez, 2003).

1.2. Studies on natural colourant

A replacement for synthetic red dyes such as amaranth and carmosine from natural sources are the top priorities in the food industry currently. There are quite a number of studies done on new viable sources of anthocyanin as natural food colouring. Recently, Buchweitz et al. (2012) reported on the suitability of sugar beet pectin in the stabilisation of natural blue anthocyanin-based soluble food colourant without adding aluminium and iron. While Osorio et al., (2011) studied on the chemical characterisation of anthocyanin in tamarillo (*Solanum betaceum* Cav.) and Andes berry (*Rubus glaucus* Benth.) fruits. Giusti and Wrolstad (2003) reviewed on the acylated anthocyanins from edible sources and it application in food systems. They reported that the acylated anthocyanin with increased stability may impart desirable colour and able to withstand various kind of processing used in commercial food application. Radish and red potatoes are among the most potential sources as alternative for red synthetic colourant. Another potential colourant sources from purple clover (*Oxalis triangulis*) was studied by Pazmino-Duran et al., (2001a). They reported that the main anthocyanin found were malvidin-3-rutinoside-5-glucoside and other acylated derivatives containing one and two molecules of malonic acid esterified to the main structure. Its attractive hue, high anthocyanin content and edible characteristic of *O.triangulis* make it a potential desirable source of natural colourant.

Research by Obon et al. (2009) reported on the *Opuntia stricta* fruit juice as a source of natural red purple colourant. The betacyanin-containing juice was dried using co-current spray dryer with glucose syrup (29 DE) as a carrier. The resultant powder showed good colour strength and stable at room temperature storage for a month. The incorporation in yogurt and soft drinks exhibited a vivid red-purple hue that was very attractive to consumers and the colour was maintained and stable for a month under refrigeration temperature. Hager et al. (2008) studied the processing and storage stability of anthocyanin from processed black raspberry on monomeric anthocyanin, percent polymeric colour and its antioxidant capacity for 6 months. Their findings showed that thermal processing caused significant loss of total anthocyanin in clarified and unclarified black raspberry juice. Low antioxidant capacity value was also observed in the fruit juice, however the individually quick-frozen berries retained its anthocyanin content and antioxidant capacity during 6 months storage at -20^{o}C.

Study on comparison between natural pigments and synthetic dyes had also been conducted by Cevallos-Casals and Cisneros-Zevallos (2004). The stability of anthocyanin in Andean red sweet potato and purple corn extracts at different pH, temperature and light conditions was compared to commercial colourants (purple carrot, red grape, red 40, and red 3). They concluded that red sweet potato and purple carrot colourants showed higher stability than purple corn and red grape colourants due to its acylated anthocyanins. After storage at 20^{o}C for 138 days, red sweet potato was found the most stable followed by purple carrot, purple corn and red grape. Many researches on the natural colourants have been conducted whereby large numbers of high potential plant sources and natural pigments have been recommended for food use at some stage. A replacement for red, yellow and orange dyes was achieved through anthocyanin, betalains and carotenoid despite their inferior stability (Buchweitz et al., 2012).

1.3. Development of new colourant

The development of new colourant was conducted using three plant sample which were found abundantly in Malaysia which were roselle calyx, *senduduk* fruits and purple-fleshed sweet potato (PFSP).

1.4. Process flow for obtaining new food colourants from plants

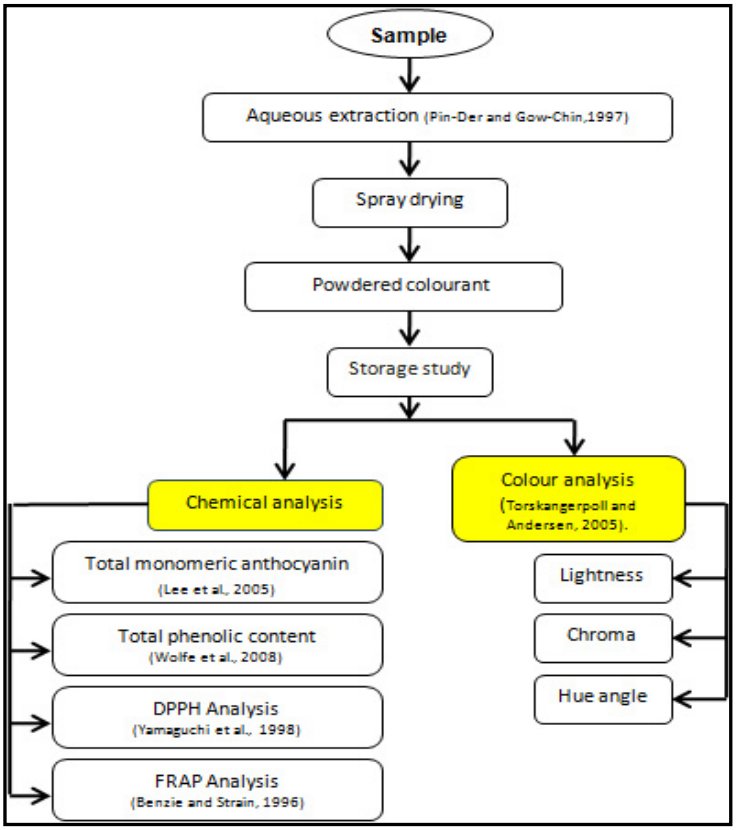

Figure 1: Process flow for obtaining natural food colourant from plant sample

1.5. Sample preparation

All samples were washed and clean prior to further treatments. For *senduduk* fruits and PSP, the samples were peeled to remove the skins and unwanted residues. Roselle calyx was washed and the seeds were removed. The combination of the roselle calyx with *senduduk* fruit and roselle calyx with purple fleshed sweet potato at 50: 50 ratio were carried out. This combination is termed as copigmentation, a condition in which the pigments and other colourless organic compounds or metallic ions to form molecular or complex associations, generating a change or an increment in the colour intensity (Boulton, 2001). This interaction is considered important because colour is one of the main quality factors in a product acceptance (Eiro and Heinonen, 2002). Some studies had proposed that the copigmentation of

anthocyanins with other compounds (copigments) is the main mechanism for stabilisation of colour in plants as well (Castaneda-Ovando et al., 2009).

The combination samples were aqueous extracted according to the method of Pin-Der and Gow-Chin (1997) with some modification of water to sample ratio. 200 ml of water was added to 100 g of dry sample and heated to 60 ^{o}C for 10 minutes. According to Patras et al., (2010) heating step such as blanching to approximately 50 ^{o}C have a positive effect on the anthocyanin retention due to enzymes polyphenol oxidase deactivation. The soften sample was then ground and filtered with Whatman No. 4 filter paper in a Buchner funnel under vacuum. The sample obtained was kept at 4 °C in an amber bottle until spray drying process.

1.6. Spray drying process

All samples that undergone aqueous extraction was added with food grade citric acid powder to achieve pH 2.3. Initial total soluble solid of the aqueous extract was determined using a handheld refractometer (N2, Atago, Japan). Maltodextrin, the carrier agent was added to obtained 25^{o} Brix. Each preparation were homogenised using a homogeniser (T25, IKA, Germany) at 10000 rpm for 20 minutes. Then, all combinations were spray dried using a mini spray drier (B-191, Buchi, Switzerland). The inlet and outlet temperature was controlled at 160 ± 5 ^{o}C. Powdered samples were kept in an amber bottle at room temperature until further analysis.

1.7. Chemical analysis

1.7.1. Total monomeric anthocyanin content

This is a simple method to determine the amount of anthocyanin pigment in a sample that present a wide range of colours from red, blue to purple. Basically, the monomeric anthocyanin pigments are able to change its colour reversibly with a change in pH. At pH 1, the majority of coloured oxonium form exist while the colourless hemiketal form predominat at pH 4.5. The differences between the absorbance of the pigment at certain wavelength is proportional to the pigment concentration. The result will be expressed as cyanidin-3-glucoside basis. The degraded anthocyanin, in the polymeric form are resistant to any colour change and was not included in the measurement. Total anthocyanin analysis was performed using a spectrophotometric differential pH method, with few modifications (Lee et al*.,* 2005). The UV-Visible spectrophotometer (Heλios α, Thermo Scientific, England) at 524 nm, and 700 nm was used in the analysis. Each sample was analysed in triplicate and the results were expressed as the averages of the three measurements.

1.7.2. Antioxidant analysis

One of the significant properties of anthocyanins is their antioxidant activity, which helps in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Konczak and Zhang, 2004). Antioxidant is a compound capable of slowing or preventing the oxidation of other molecules. Anthocyanins in plants are capable of dealing with oxidative stresses from physiological stress or environmental adverse effects such as air, light and temperature (Ramamoorthy and Bono, 2007). It is also react towards free radicals in plants that been produced during photosynthesis process. For the antioxidant analysis, there are various methods to compare and monitor the antioxidant activity in a sample. For this study, the total phenolic content (TPC) was measured and two antioxidant analyses were conducted. A quick and easy method to measure the antioxidant activity was using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). It was use to test the ability of a compound to act as free radical

scavenger. This method can be used for any types of sample not specific to a particular antioxidant component. It also indicates the overall antioxidant capacity of the sample. Another method was Ferric reducing antioxidant power method (FRAP). This method principally measures the ability of antioxidant to reduce ferric iron to the ferrous form at low pH. The reduction was measured by the change in absorption at 593 nm, using a diode array spectrophometer. A measure of total antioxidant capacity helps understand the functional properties of foods.

1.7.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the crude extracts was determined using Folin-Ciocalteu reagent following the method of Wolfe et al., (2008) using gallic acid as a standard. The volume of 0.1 ml diluted extract (1 mg of extract/1 ml of distilled water) solution was added in a 10 ml volumetric flask. Then, 0.5 ml of Folin-Ciocalteu reagent was added and the content of the flask was thoroughly mixed. After 1 to 8 minutes, 1.5 ml of Na_2CO_3 (20%) was added and the volume was made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis Spectrophotometer (Lambda 35, Perkin Elmer, USA). Total phenolic content was determined as mg of gallic acid equivalent (GAE) per milligram of crude extract.

1.7.4. Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was performed according to Benzie and Strain (1996) but with some modification. FRAP reagent (10:1:1), 10 volumes of 300 mm acetate buffer, pH 3.6 was added with 1 volume of 10 mm 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mm HCl and 1 volume of 20 mM Fe_2Cl_3. The solvent mixture was incubated at 37 ^oC for 10 minutes. 0.1 ml sample then was added with 2.9 ml of the solvent mixture and incubate again for another one hour. The absorbance was measured at 593 nm using UV-Visible spectrophotometer. Five different concentrations of Trolox (100, 250, 500, 750, 1000 µmol/l) were used to construct a standard curve and the results for the samples were expressed as µmol/g.

1.7.5. DPPH radical scavenging activity

The effect of plant extracts on 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical is estimated according to the method of Yamaguchi et al. (1998) with some modifications. 600 µl of the extracts (0.2 mg /1 ml distilled water = 200 ppm) was added to 4.5 ml of DPPH solution (1 mM in ethanolic solution). The mixture was vortex and left to stand at room temperature for 20 minutes in a dark place. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The radical scavenging activity was measured and calculated by the following formula:

$$\text{Scavenging affect (\%)} = 1 - \left(\frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}} \right) \times 100$$

1.8. Physical analysis

1.8.1. Determination of colour

The changes in colour were determined using chromameter (CR400, Minolta, Japan). The colour indices was measured using CIE L*C*H^o colour space (The International Commission on Illumination, Vienna, Austria) with illuminant of D65 and 2^o observer. L* is a measure of lightness ranging from 0 (black) to 100 (white) and colour coordinates, *a** which takes positive values for redness colour and

negative values for greenness and *b** positive for yellowness colour and negative for blueness. From these coordinates, other colour parameters are calculated, chroma (C*) is the quantitative attribute of colour intensity or saturation. The higher chroma value indicated a more saturated colour was observed. Chroma values were calculated as following equation.

$$\text{Chroma} = (a^{*2} + b^{*2})^{½}$$

Hue angle (H^o) is the qualitative attribute of the colour expressed as (0º/360º) red, (90º) yellow, (180º) green and (270º) blue and calculated using the following equation

$$\text{Hue angle} = (\tan^{-1} a^*/b^*)$$

1.9. Storage study

A storage stability study of ready-to-drink pink guava juice (PGJ) incorporated with spray-dried anthocyanin colourant of roselle and *senduduk* at 50:50 ratios was carried out for 12 weeks. The spray dried combination of roselle and *senduduk* was selected to be incorporated in pink guava juice as it have the highest value (428.83±5.15 mg/L) in monomeric anthocyanin content and antioxidant activity, DPPH assay (17.94±0.56 %) and FRAP assay (52.04± 2.67 mg Trolox/g) as compared to combination of roselle with purple fleshed sweet potato. Pink guava juices were stored in glass bottle and wrap with aluminium foil to eliminate light exposure and to mimic the commercial aseptic carton packaging. Two storage conditions were evaluated, at refrigerated temperature, 4 ^oC and room temperature, 25 ^oC. Analyses of total monomeric anthocyanin content, total phenolic content, antioxidant activites (FRAP assay and DPPH radical scavenging activity) and colour were conducted for every week for a period of 12 weeks.

2.0. Statistical analysis

All presented numeric values are means of three or more measurements as stated ± standard deviation (SD). The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor, R^2. One-way ANOVA performed in Statistical Analysis System SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used to determine whether the differences between measurements are significant. Differences were considered significant at a confidence level superior to 95% ($p < 0.05$).

2.1. Results

From the results obtained, the combination of roselle and *senduduk* gave a significant highest anthocyanins content (428.83 ± 5.15 mg/L) among all combinations while powdered roselle alone (250.54±6.77 mg/L) reported having the lowest value of anthocyanins. The effort combination of different samples-containing anthocyanin was the main colour-stabilizing mechanism in plants (Bakowska et al., 2003). For the antioxidant determination, the total phenolic content was the highest in roselle and PFSP (66.97±0.07 mg GAE/g), the highest inhibition percentage (DPPH) was in roselle and *senduduk* (17.94±0.56 %) and the highest FRAP assay value was roselle and *senduduk* was 52.04 ± 2.67 mg Trolox/g. The colour obtained from the combination of roselle and *senduduk* also reported to be the darker (40.09 ± 0.83) red pinkish in appearance with chroma value of 25.43 ± 0.72. These results showed that the combination of roselle and *senduduk* has the greatest potential as natural colourant to be used as functional ingredient in food products application apart from being a natural colouring agent.

In storage stability study, PGJ stored at 4 ^oC had a higher total monomeric anthocyanin content compared to PGJ stored at 25 ^oC. The total monomeric anthocyanin at 4 ^oC range between the lowest 34.98 mg/L to the highest of 74.48 mg/L. The high temperature led to a faster anthocyanin degradation,

which was expected, since these pigments are highly thermo sensitive (Tonon et al., 2010). This negative influence of temperature on anthocyanin stability has been observed by Pacheco-Palencia et al. (2007) who verified the anthocyanin stability in the whole, semi-clarified and clarified acai pulp, had a degradation rate 3.5 times higher when samples were stored at 20 ^{o}C compared to 4 ^{o}C.

As for the chromaticity parameter, the lightness values fluctuate at 4 ^{o}C significantly throughout 3 months of storage in which the highest value reached up to 37.77 ± 0.19 and the lowest value was 35.18 ± 0.07. At 25 ^{o}C storage, there was also a fluctuation from the first to sixth week and the value was almost constant from week 7 to the end of the storage period. The slight degradation of the lightness could due to the polymerisation of anthocyanins at high temperature during juice pasteurisation indicating a loss of red colour in pink guava juice. A fluctuation of chroma was observed at both temperatures during storage period. The highest value (12.19 ± 0.09) on first week and the lowest (9.97 ± 0.45) during sixth week was observed at 4 ^{o}C storage.

The highest amount of phenolic content was detected during the early weeks of storage at both 4 and 25 ^{o}C storage and decreased over 12 weeks of storage. At 4 ^{o}C, the highest TPC was during the first week with 28.96 ± 0.74 mg GAE/g and the lowest content was on 12th week, with 53 % decreased from the first week. This was in accordance with Ibrahim et al. (2011), who suggested that all treatments, time and temperature of storage significantly affected phenolic degradation caused by non-enzymatic reaction. In antioxidant activity, the radical scavenging activity of DPPH assay in PGJ at 4 ^{o}C demonstrated the strongest activity of radical scavenging activity (95.99 ± 0.21%) on week 12. Both temperatures exhibited a similar increased in DPPH radical scavenging capacity with time. In this case, the efficiency of scavenging capacity could be contributed by several compounds that were normally added into the juice for example the carotenoid from pink guava puree, ascorbic acid and clouding agents. For FRAP assay, the highest value was observed in the fresh PGJ on the first week of refrigerated and room temperature storage with value of 452.38 ± 4.34 and 481.72 ± 2.00 mg Trolox/g and decreased throughout the three months storage.

2.2. Maqasid Shariah and Islamic Legal Maxim

2.2.1. Darruriyat

Darruriyat signifies essentials and it is one of the categories under *Maqasid Shari'ah*. *Shari'ah* Law is established to preserve five essentials for Muslim community, namely, religion, human life, progeny, material wealth and the human faculty of reason. These are seen as absolute requirements to the survival and spiritual well being of individuals. When those essentials are not preserved, it would introduce chaos and demise normal order of the society.

Overall, *Shari'ah* aims to protect and promote these essentials values and validates all measures necessary for their preservation and advancement. For example, *jihad* has been validated in protecting religion and *qisas* in protecting life. Hence, it is an essential substance to protect human life. Islam encourages people to develop the new technology and beneficial innovation especially in food technology in oder to enable the individual to be in a better condition of lifestyle. It also prescribes measures to ensure that innovation and technology is in line with Shariah guideline. In addition, Islam promotes healthy and safety life, therefore the new innovation of food colourants from natural sources is very important especially concerning the consumers' health issues.

2.2.2. Legal Maxims

In literal meaning, Qawa'id Fiqhiyyah or known as Islamic legal maxim means by a set of principles related to any issues in computing the law. In other words, Legal Maxims are statements of principles that are derived from the detailed reading of the rules of *fiqh* on various themes (Kamali). For technical meaning, it is a general principle in legal text form which has an act institution or policy where Great Muslim jurist derived from *Qur'an* and *Sunnah*. As mentioned earlier, the concept of Maqasid Shari'ah entails understanding the Islamic principle of removing hardship and preventing harm.

As regard to this issue of natural food colourant, the suitable legal maxim which is derived form Qur'an and Sunnah is "*La darara wa la dirara fil Islam*" or harm may neither be inflicted nor reciprocated in Islam. In order to protect consumers from any harm and kind of deception, the maxim "*Ad-dararu yuzal*" or harm must be eliminated is designed. In relation with that, Islam is very serious in ensuring that human life always being preserved and protected from any harmful and dangerous element. The using of natural sources in food colourant as been mentioned in this study is very essential and in line with this concept of Islamic legal maxim. As a nutshell, *Qawa'id Fiqhiyyah* is very useful in protecting the *Maslaha*h and preventing harmful and dangerous element among the society.

2.3. Conclusion

Based on the results, the spray dried roselle and *senduduk* gave the most consistent and good results in its anthocyanin content and antioxidant activity. Eventhough its phenolic content was the second highest after combination of roselle and PFSP, the antioxidant activities were the highest and able to act on free radical. Pink guava juice incorporated with 50:50 ratio of roselle and *senduduk* in spray dried form showed good stability compared to storage at 25 ^{o}C. Chill temperature was the best condition to maintain anthocyanin properties. Hence, the amount of phenolic content detected during the early weeks of storage at both 4 ^{o}C and 25 ^{o}C was maintained over 12 weeks of storage. However the radical scavenging and reducing capacity showed a significant decrease with the increase of storage time. Therefore, it has the most commercial potential value to be commercialized as natural food colourant and functional ingredient replacing the synthetic one. Furthermore, the production of food colourant from anthocyanin pigment was assured to support the concept of Islamic legal maxim"*Ad-dararu yuzal*" in order to supply safe and sound product to the consumer.

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