# ACCEPTABILITY OF NOVEL ANTIOXIDANT ICE CREAM FORTIFIED WITH NUTRITIOUS *Carica papaya* SEED

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**Abstract**

This research aimed to determine the total antioxidant activity and total phenolic content of the papaya seeds. These papaya seeds then were introduced into the prepared ice cream to determine the effect of the papaya seeds to the physicochemical and the acceptability of ice cream by making four different formulations (Control, 1.0%, 2.0%, and 3.0% of papaya seeds). Two methods namely free radical scavenging assay (DPPH) and ferric reducing antioxidant power (FRAP) were used to determine the total antioxidant activity of the papaya seeds, whereas total phenolic content was determined by Folin-Ciocalteu's method (TPC). The papaya seeds showed high total phenolic content in the TPC analysis result. Furthermore, DPPH and FRAP showed high antioxidant activity of the papaya seeds. Acceptability of the ice cream was conducted by sensory evaluation and the results showed that the control (0.0%) papaya seeds ice cream formulation was the most favorable by the panelists followed by 1.0%, 2.0%, and 3.0% papaya seeds formulation. In conclusion, the papaya seeds are proven to contain antioxidants by the results given in DPPH, FRAP, and TPC tests. Surprisingly, the papaya seeds also did not affect the physicochemical of the ice cream and the ice cream was accepted by the panelists.

**Keywords:** Acceptability, Ice Cream, Antioxidant, Papaya Seeds

## Introduction

*Carica papaya* is a giant herbaceous plant in the *Caricaceae* (papaya family) that originates from Central America and is presently developed in tropical areas all around the globe for its large, sweet, melon-like fruits. Papaya (*Carica papaya* Linn.) is well known for its exceptional nutritional and medicinal properties throughout the world. The whole plant including the fruits, leaves, seeds, root, bark, juice and latex obtained from papaya plant used as nutrition, medicine and for various purposes (Kumar and Sreeja, 2017; Egbuonu, 2018). According to Singo and Beswa (2019). today's population increasing the demands and consumes healthy products. People nowadays have acknowledged the awareness of eating and living healthy as they will pay more for healthy food products.

There are many foods products in the market that have been improved according to their nutrition and health qualities such as grain cereal, milk, yogurt, etc. Ice cream is an occasional food that could become functional food as it provides temperature and texture that is suitable to maintain the nutrients. Ice cream has a pleasant taste and appealing texture (Omar and Omar 2018). One of the major problems challenging the food industry throughout the world is how to make full utilization of the waste material. Papaya seeds are usually discarded during the preparation of papaya fruit (Dada et al., 2016). There is plenty of papaya seeds waste produced after the processing and consumption of papaya fruits. This ergonomic waste that usually polluted our habitat could be utilized. The papaya seeds have beneficial value and can be formulated as supplement. Nowadays, the supplement is expensive and in the shape of pills, papaya seed is a cheap source of minerals and fats. Furthermore, the purpose for making ice cream is to provide an alternative way for consumers to take a supplement intake. The aim of this study was to formulate functional food (antioxidant ice cream) by incorporating papaya seeds. Physicochemical characteristics and sensory evaluation were determined for the formulated papaya ice creams.

## Literature Review

### Health benefits of papaya seeds

Papaya seeds contain nutrients that have beneficial values on the human body. Papaya seeds contain fatty acids, crude protein, crude fiber, papaya oil, papain, carpaine, caricin, benzyl glucosinate and many more. Medical uses of papaya seeds are carminative, an anti-fertility agent in males, counter-irritant, as a paste in the treatment of ringworm, psoriasis, emmenagogue, vermifuge, liver cirrhosis and abortifacient (Kumar and Sreeja, 2017). Apart from that, isothiocyanate contained in papaya seed, work well for colon, breast, lung, leukemia and prostate cancer. This enzyme also capable of inhibiting both formation and development of a cancer cell. Papain enzyme that high in both unripe papaya and papaya seeds. Papain gives benefit in aiding the protein digestion as it breaks the peptide bonds. Furthermore, Kumar and Sreeja (2017) have stated that papain also effective against cancer as it breaks down the fibrin cancer cell wall and protein into amino acid form. Papaya seeds also contain lycopene which highly reactive towards oxygen and free radicals. In another study, Dwikat and Dini (2010) have reported that papaya seeds have antioxidant that can act as anti-apoptotic effect. According to the National Cancer Institute, apoptotic was defined as a type of cell death in which a series of molecular steps in a cell leads to its death. As claimed by Adesola, and Akande, (2019), papaya seeds contain several phytochemical compounds which have been proven to show antiviral, anti-allergic, anti-inflammatory, anti-tumor and antioxidant activities. Hence, Consumption of papaya seed is a clever way to obtain cheap, natural, harmless, a readily available supplement that perform as a monotherapeutic and could prevent against intestinal parasitosis especially in tropical communities (Okeniyi et al., 2007).

### Antioxidants in the papaya seeds

Generally, papaya has abundant with antioxidants nutrients which is carotenes, vitamin C, and flavonoids like B vitamins and folate; and the minerals, potassium, copper, and magnesium; and fibre. Ali et al. (2011) also has reported that papaya has large amount of antioxidants like beta-carotene, total phenols, tocopherols, and vitamin C. Lichtanhaler (1987) has reported that the carotenes have two classes which are primary group like β- carotene, violaxanthin, and neoxanthior that required on photosynthesis process whereas the secondary group such as α-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, capsanthin, and capsorubin that localized in fruits and flowers.

In a study by Nakamura et al. (2007), the researcher has reported that papaya seeds have rich source of benzyl isothiocyanate (BITC), which is hydrolized from benzyl glucosinolate and it is a bioactive phytochemical with antioxidant activity. Fahey et al. (2001) reported that amino acids precursors are the components of glucosinolates derived which become sulfur containing glycosides with variable side chains. The phytochemicals present in the papaya like beta- cryptoxanthin and benzyl isothiocyanates can confer health benefits on chronic disease like cancer. From previous studies, Kothari and Seshadri (2010) and Norshazila et al. (2010) have reported that the content of the papaya seeds contained different phenolic compounds and vitamin C. Neuzil et al. (1997) has reported that many species which can oxidize vitamin

C that have the potential to be involved in human diseases whereby relevant species are divided into several classes including oxygen related radicals like superoxide, hydroxyl radical, and peroxyl radicals. Besides that, hypochlorous acid, nitrosamines, and other nitrosating compounds, nitrous acid related compounds and ozone are reactive but not radicals. Next is the compound that is formed by reaction with either of the first two classes and then react with vitamin C.

### Nutraceutical ice cream

Gabbi et al (2017) have stated that frozen products such as ice cream, adapted most readily to nutrient fortification and served as good carriers of nutraceuticals because of their low storage temperature, ability to stabilize ingredients and recognition among consumers. Ice cream can be used successfully to dispense unique added nutritional benefits to consumers. There are many factors that have made ice-cream become one of the most favorable desserts. Its texture, taste and its cool serving temperature have made it acceptable by consumers across the ages. Ice cream is commonly appreciated by people of all ages due to its cooling effect and nutritional value (Karaman et al., 2014). Therefore, new varieties of ice cream are being developed targeting the palate and health intention to consumers.

## Methods

### Sample collection and seed extraction

Papaya fruit was obtained from Mydin Mall at Seremban, Negeri Sembilan. The papaya was in the same type, size, color, and weight. The seeds of samples were prepared by isolating them from flesh and cleanly washed with distilled water for impurities removal. The seeds were dried in a hot-air oven at 50 °C for 48 hours. A blender is used to mill the seeds into a fine powder and stored in air-tight bottles at -4 °C.

The preparation of a methanolic extract of the seeds is based on the method of Irondi et al (2013), 0.5 g sample was added to 25 ml of methanol in a covered 50 ml centrifuge tube, shaken continuously for one hour at 25 °C. By using centrifuge, the mixture was centrifuged at 3000 rpm for 10 minutes, and then the supernatant (subsequently referred to as methanolic extract) was collected and stored in 50 ml amber bottle at -4 °C until analysis. All the chemicals used for analysis are analytical grade.

### Folin-Ciocalteu Assay

The Folin-Ciocalteu method to determine the total phenolic content of samples methanolic extract is according to Irondi et al. (2013). 300 µl of extract was dispensed into test tube (in triplicates). Next, 1.5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) was added to the test tube and followed by adding 1.2 ml of sodium carbonate solution (7.5 % w/v). The reaction mixture was shaken and allowed to stand for 30 minutes at room temperature. Absorbance was measured at 760 nm against a blank by using UV-spectrophotometer (Varian Cary 50). Blank was prepared by dispensing 300 µl of distilled water instead of sample extract. Total phenol content was expressed as gallic acid equivalent (GAE) in mg/g material.

### Free radical scavenging assay

The free radical scavenging ability of methanolic extracts against 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical was evaluated as described by Irondi et al. (2013) with modification. 2 ml of extract was mixed with 2 ml of 0.1 mM methanolic solution of DPPH radicals. The mixture was left in the dark for 30 minutes before the absorbance at 517 nm was taken. Blank was prepared by adding 2 ml of DPPH reagent with 2 ml of distilled water. Percentage inhibition (%Inh) was used to calculate the decrease in absorbance of DPPH on the addition of test samples in relation to the control:

### Ferric reducing antioxidant power (FRAP)

The reducing property of the methanolic extracts was determined by assessing the ability of the extract to reduce ferric chloride solution as described by Irondi et al. (2013). A 2.5 ml of aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes and then 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 minutes by using centrifuge. Then 5 ml of supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. Absorbance was measured at 593 nm by using UV-spectrophotometer (Varian Cary 50).

### Ice cream production

The ice cream was produced according to Carolina et al. (2018) with some modifications. The ice cream formulation contains 40.0% papaya puree, 70.0% milk, 20.3 % stabilizer, 100.0% whipped cream, 0.08% vanilla extract, 0.2% salt and 42.4% sugar (w/w) (Carolina et al., 2018). Four experimental were prepared as follow: ice cream with seed powder (2.5 %, 5.0 % and 10.0 % w/w) and ice cream without seed powder (0.0 %) as a control. The batches were prepared by mixing all the ingredients and pour into the ice cream machine at -18 °C for 30 minutes. Then stored in plastic containers (500 ml) under refrigeration (-18 °C).

### Sensory analysis

The sensory characteristics of the ice-creams were evaluated according to Pedredo and Pangborn (1989). Samples were tested by 60 untrained consumers using 9-points hedonic scale. 1 to 9 (1: poor, 9: excellent) points for six attributes including color, appearance, texture, flavor, and overall acceptability (refer appendix I). An amount of 15 g of the sample was served in plastic containers, coded with a random 3-digit number.

## Results and Discussion

### Folin Ciocalteu's assay

The data obtained from the absorbance reading of UV-visible spectrophotometer at 760 nm were shown in Table 1 as the total phenolic content was expressed as gallic acid equivalent (GAE) in mg/g material. The TPC value of the papaya seeds were significantly high according to Maisarah et al. (2013) as the research had lower value (30.32) compare to batch 1 and batch 2 papaya seeds which had the value of 71.868 and 64.356 respectively. Besides that, the difference in reading for both batches may due to the factors affecting the nutrients uptake of the papaya plant which were soil solution, root systems, rate of flow of water to the root and light intensity (Brewster, 1971). Table 1 showed the absorbance of standard gallic acid and Figure 1 showed the calibration curve of standard gallic acid.

Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny, 2001). Flavonoids are phenolic compounds, which are very effective antioxidants (Yanishlieva-Maslarova, 2001). Ciocalteu method is a rapid and widely used assay, to investigate the total phenolic content but it is known that different phenolic compounds have different responses in the Folin Ciocalteu method (Kähkonen et al., 2001). Therefore, in this work, the total phenolic content was calculated in units of mg gallic acid equivalent of phenolic compound as shown in Table 1.

**Table 1 Total phenolic content (TPC) of papaya seeds**

| Papaya seeds | Total phenolic content (mg GAE/g) |
|---|---|
| Batch 1 | 71.868 |
| Batch 2 | 64.356 |

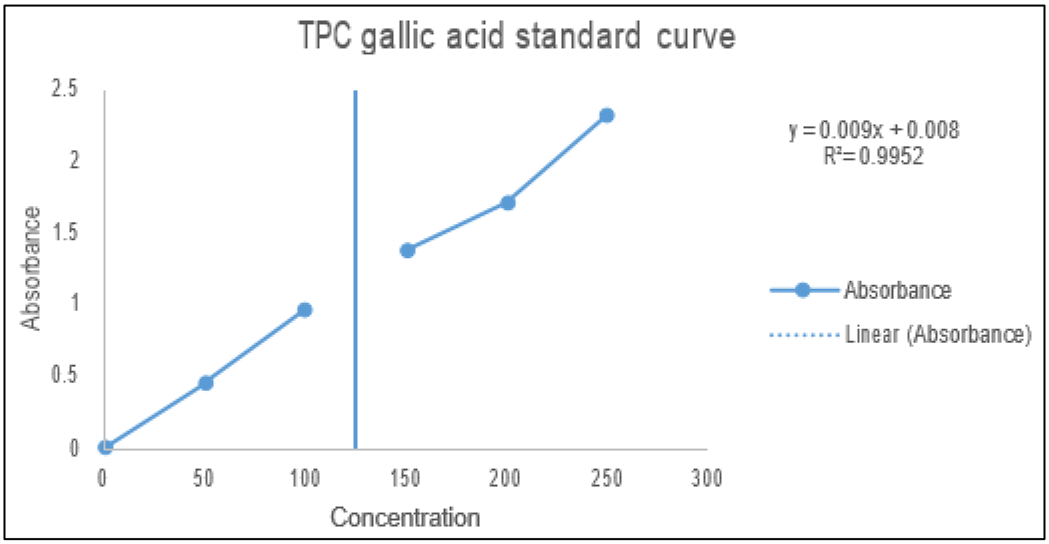


**Fig 1 TPC calibration curve of standard gallic acid at 760nm**

## Free radical scavenging assay

The free radical scavenging activity of the papaya seeds were tested through DPPH method and the results was presented in the Table 2. The free radical scavenging activity in the examined papaya seeds using the DPPH method was expressed in terms of percentage of inhibition. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades when antioxidant molecules quench DPPH free radicals (i.e by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on DPPH molecule) and convert them into a colorless or bleached product (i.e 2,2-diphenyl-1-hydrazine or substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz et al., 2003). The antioxidant activity of the papaya seeds is expressed in terms of percentage of inhibition (%). The lower the absorbance, higher the free radical scavenging activity (Murali, Ashok and Madhavan, 2011).

**Table 2 Percentage of inhibition (%)**

| Papaya seeds | Percentage inhibition |
|---|---|
| Batch 1 | 39.36883 |
| Batch 2 | 38.72703 |

## Ferric reducing antioxidant power (FRAP)

The data obtained from the absorbance reading of UV-visible spectrophotometer at 593 nm were shown in Table 3 as the FRAP value was expressed as gallic acid equivalent (GAE) in mg/g material. Whereas, Table 4 showed the absorbance of standard gallic acid and Figure 4.3 showed the calibration curve of standard gallic acid. The results for the FRAP value of papaya seeds were equivalent to some research. According to Irondi et al. (2013), the research had the FRAP value of 6.132 which was equivalent to the value of batch 2 (5.718) and lower from batch 1 value (11.758).

**Table 3 FRAP value of papaya seeds**

| Papaya seeds | FRAP (mg GAE/g) |
|---|---|
| Batch 1 | 11.758 |
| Batch 2 | 5.718 |

**Table 4 Absorbance of gallic acid in FRAP reagent**

| Concentration | Absorbance |
|---|---|
| 0 | 0.2514 |
| 20 | 0.3072 |
| 40 | 0.6809 |
| 60 | 1.0151 |
| 80 | 1.3660 |
| 100 | 1.6323 |

The ferric reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals (Ogunmoyole et al., 2009). This reducing power activity, which may serve as a significant reflection of antioxidant activity was determined using a modified Fe (III) to Fe (II) reduction assay; the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of the samples. The presence of antioxidants in the samples caused the reduction of the $Fe^{2+}$/Ferricyanide complex to the ferrous form. Therefore, $Fe^{3+}$ can be monitored by measuring the formation of Perls Prussian blue at 593 nm (Ferreira et al., 2007).

**Sensory evaluation**

Four different papaya ice creams with different percentage amount of papaya seeds, 0.0 % (control), 1.0 %, 2.0 %, and 3.0 % of papaya seeds added were subjected to the consumers. Hedonic test was used to investigate the panelist's degree of liking towards the ice cream. Based on the aim of the experiment, researcher would like to examine the acceptability and the best formulation of the ice cream based of the panelist's view which consisted of appearance, aroma, texture, flavor, and overall acceptance. The data on the scores attributes was shown on Table 5. Hence to test the probability, the following hypotheses were used:

$H_o = \mu 1 = \mu 2 = \mu 3$
$H_a$ = *At least one mean differs*

The test statistic for the appearance, F=19.50 had p-value of 0.000. The p-value was the probability of obtaining a test statistics F=19.50. Since the p-value was less than α=0.05 at 95% confidence interval, the null hypothesis was rejected, and the alternative hypothesis was accepted. Thus, it can be concluded that there was at least one mean differ. The mean data for 0.0%, 1.0%, 2.0%, and 3.0% were 7.617, 6.800, 5.950, and 5.850 respectively. Based on the data in Table 4.6, it can be concluded that mostly consumers like the ice cream with 0.0% of papaya seeds. It might due to the color preference of ice cream in the panelist prefer which is brighter color rather than darker ice cream. The darker color is caused by the papaya seeds. Hence, the null hypothesis of the appearance attributes was rejected and accepted the alternative hypothesis of the research.

The aroma attributes were analyzed based on the data presented in Table 5, it showed that the panelists preferred sample with 0.0 % papaya seeds, 6.917 compared to other samples, followed by samples with 1.0 %, 2.0 %, and 3.0 % papaya seeds that are 6.433, 6.033, and 5.733. Since the p-value was less than α=0.05 at 95 % confidence interval, the null hypothesis was rejected, and the alternative hypothesis was accepted. Thus, it can be concluded that there were significant differences between ice cream with different percentage of papaya seeds with 0.0 %, 1.0 %, 2.0 %, and 3.0 %.

Next, attribute that had been analyzed was the texture of the four samples. The test statistic for the texture attribute, F=0.37 with p-value of 0.771. Since the p-value was higher than α=0.05 at 95 % confidence interval, the null hypothesis was accepted that all mean was considered the same. Thus, it can be concluded that there was no significant difference between the four samples.

Move on to another attribute which is flavor, panelists like to have their choices on the control ice cream with 0.0 % of papaya seeds followed by ice cream with 1.0 %, 2.0 %, and 3.0 % papaya seeds. The preference of the panelist on the control sample over other samples was might due to the pure sweetness of the papaya puree and the other samples with the papaya seeds had been less favorable because of the papaya seeds had affected the sweetness of the ice cream by its bitter taste. The p-value obtained was less than α value, thus an assumption can be made that there was significantly different between all the samples.

The overall acceptance score for sample with 3.0 % papaya seeds was only 5.533 compared to the control sample with 0.0 % papaya seeds 7.650 which was higher. The decreasing in taste of papaya puree has cause the decreasing in acceptability of the samples to the panelists. The overall acceptance of the ice cream was ranked in the following order: 3.0 % < 2.0 % < 1.0 % < 0.0 %. The overall acceptance data showed that the ice cream without the papaya seeds was still acceptable by the panelists. But, the samples with 1.0 %, 2.0 %, and 3.0 % papaya seeds were significantly different with the control sample in their attributes. Referring to the data in Table 5, a spider web was developed to see overall degree of liking of consumers towards the products and can be seen in Figure 2.

**Table 5 The attribute scores evaluated by 60 panelists**

| Attributes | 0.0% seeds (control) | 1.0% seeds | 2.0% seeds | 3.0% seeds |
|---|---|---|---|---|
| Appearance | 7.617±1.223[a] | 6.800±1.338[b] | 5.950±1.523[c] | 5.850±1.676[c] |
| Aroma | 6.917±1.381[a] | 6.433±1.555[ab] | 6.033±1.402[b] | 5.733±1.755[b] |
| Texture | 5.733±2.449[a] | 5.750±2.039[a] | 5.417±1.994[a] | 5.500±1.944[a] |
| Flavour | 7.567±1.332[a] | 6.567±1.489[b] | 6.050±2.020[bc] | 5.400±2.286[c] |
| Overall acceptance | 7.650±1.162[a] | 6.733±1.506[b] | 6.050±1.721[bc] | 5.533±2.095[c] |

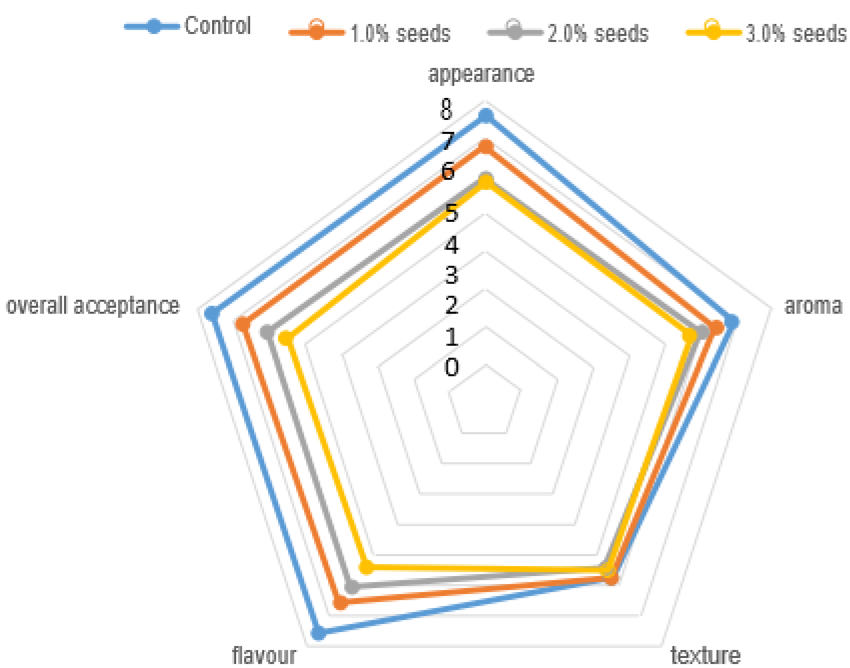


**Figure 2 Spider web attributes represents panelist's degree of liking**

## Conclusion

As a conclusion, there are two methods that have been used to test the total antioxidant activity of the papaya seeds namely free radical scavenging assay and ferric reducing antioxidant power (FRAP) whereas total phenolic content was determined by Folin-Ciocalteu's method. The papaya seeds showed high value of activity for all three methods used. Next, the ice cream was highly accepted by the panelists even though the control ice cream without the papaya seeds had the highest scores between the other ice creams. Further studies should be conducted on the proximate analysis and the nutritional composition of the papaya seeds products. The papaya seeds could be of domestic and industrial use in the supplementation or fortifications of other food products. For the sake of knowledge, further studies should be conducted using animals' test to determine its acceptability to human health if consumed.

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