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Comparison of phenolic content and antioxidant activity of fresh and fried local fruits

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Abstract

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Fried fruits are popularly consumed by many people over the world. Some of the famous local fried fruits are fried cempedak, jackfruit and breadfruit. These fruits are rich in antioxidant, but the antioxidant content after frying is unknown as frying may alter the antioxidant content. This study aimed to compare total phenolic content and antioxidant activity of fresh and fried local fruits. Freeze-dried samples were extracted using 80% methanol and Folin-Ciocalteu assay was used to determine the total phenolic content (TPC) of the samples while FRAP and Beta-carotene assays were used to evaluate their antioxidant activity. Fried jackfruit (76.836 + 0.619 mg GAE/100g) had the highest TPC, followed by fresh jackfruit, fried cempedak, fresh cempedak, fried breadfruit and the lowest TPC was in fresh breadfruit (54.042 + 0.596 mg GAE/100g). Sample with the highest antioxidant activity as measured by FRAP assay was fresh cempedak (3.881 + 0.301 mM Fe^{2+/g}), followed by fresh breadfruit, fresh jackfruit, fried jackfruit, fried breadfruit and the least antioxidant activity was in fried cempedak (0.794 $+$ 0.106 mM Fe²⁺/g). Using Beta-carotene assay, fried cempedak had the highest percentage of antioxidant activity (98.936 + 0.182) followed by fresh jackfruit, fresh cempedak, fried jackfruit, fresh breadfruit and the lowest was observed in fried breadfruit (-76.449 + 8.139). There was no correlation found between TPC and antioxidant activity as measured using both FRAP and beta-carotene assays. In conclusion, frying of fruits resulted in increment of TPC but mixed changes in antioxidant activity of the final product thus suggesting the importance of controlling the frying process in getting the benefits of fruit antioxidants.

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Introduction

People popularly consume fried foods all over the world. Fried foods may contain high amount of fat due to the frying process. General population studies have also shown that diets high in fat, particularly saturated fat, can increase the risk of diseases such as cancer, diabetes and heart disease (Physicians Committee for Responsible Medicine, 2004). The Malaysian Adult Nutrition Survey 2003 which was conducted among 6742 subjects ranked local kuih as the eighth most consumed foods daily among Malaysians (Norimah *et al*. (2008). In Malaysia, some of the famous foods in this category are such as fried banana fritters, karipap, cakoi and peneram in which all are actually fried. Therefore, intake of high fat foods and using fats and oils in food preparation such as frying should be limited in our daily diet. Fruits and vegetables, on the other hand are important food to improve our health. They have high concentrations of vitamins, minerals and phytochemicals especially

antioxidants. On top of that, they are also low in fat, sodium and calories when compared to other foods (Nurul Izzah *et al*., 2012). Dietary antioxidants are defined as substances that protect cells against the effects of free radicals which are molecules produced when the body breaks down food or by environmental exposures like tobacco smoking or radiation (National Institute of Health, 2012). The role of dietary antioxidant in human health and disease is undeniable and sufficient evidence exists to broadly recommend that an increase intake of fruits and vegetables may contribute significantly to good health (Wootton-Beard *et al*., 2011).

 Malaysian fruits such as the cempedak *(Artocarpus interger)*, jackfruit *(Artocarpus heterophyllus)* and breadfruit *(Artocarpus altilis)* are known to be rich in antioxidants (Kolar *et al*., 2011; Almeida *et al*., 2011; Fu *et al*., 2011). In the Malaysian food culture, some fruits are fried before consumed and these three fruits are among the most common fruits being fried. Besides that, fried banana,

fried sweet potatoes and fried tapioca are also famous among Malaysians. The fruits or plants are fried because the high temperature used during the process enables rapid heat transfer resulting in a very short cooking time (Rojas-Gonzalez *et al*., 2006). Frying also produces a desirable flavor and crispy texture of the food being fried. However, when fruits are fried, the increase of heat during frying process may cause changes in the nutrient and antioxidant profile. It is already known that these fruits are rich in antioxidant, but changes in the antioxidant content after frying remains unknown. Therefore, this study was conducted to determine and compare the total phenolic content and antioxidant activity (AA) of fresh and fried samples of jackfruit, breadfruit and cempedak.

Materials and Methods

Sample preparation

The cempedak *(Artocarpus integer)*, jackfruit *(Artocarpus heterophyllus)* and breadfruit *(Artocarpus altilis)* were purchased from fruits stalls at Pasar Borong Seri Kembangan, Selangor by convenience sampling. Each of the fruits were determined first on their maturity level as only ripe fruits were chosen. One whole cempedak and jackfruit plus two whole breadfruits were bought and prior to being used, all the fruits were stored in an open environment for a day within room temperature of around 21-25 °C. For the study purpose, the fruits were divided into two whereby one half was used for frying and the other half was used as fresh samples. The first half of the jackfruit and cempedak arils were removed from the whole fruit while the breadfruits' skin was removed and then cut into small pieces before being sealed in a container and kept in the freezer at -80°C for a day and freeze-dried. The second halves of the fruits were prepared for frying purpose. Initially, the breadfruit skin was removed and the fruit was cut into a size of 3 cm x 3 cm. Meanwhile, the jackfruit and cempedak arils were used whole one by one but with their seeds removed. The arils of cempedak and jackfruit along with the cut breadfruit were coated with a batter made of wheat flour, rice flour, salt, and water. The amount of wheat flour, rice flour and water was 3:1:1 cups respectively. The fruits were fried immediately after being coated with the batter.

The deep frying method was used whereby food was fully submerged in hot oil and the frying temperature was 175±2°C (Petersen *et al*., 2013). The fruits were fried for approximately 4 minutes until each were golden brown in color. The oil used was palm oil. Variables like temperature for frying,

duration of frying, type of oil used and cut size of the fruits were kept constant to provide proper control of the frying process. Oil was filtered and removed when lifting the fried products from the pan. The fried fruits were left to cool off for a while before sealed in a container to be kept in a -80° C freezer for a day and freeze-dried. After freeze-drying, the samples were grinded and sieved. The powder from each samples were dried until it reached a constant weight in a dessicator at room temperature (24ºC). The powdered samples of both fresh and fried forms of fruits were kept in a -20 \degree C freezer until they were used for analysis.

Extraction of samples

Antioxidant of samples were extracted by adding 2 g of dried sample into 50 ml of 80% methanol. The mixture was kept at room temperature overnight and shaken by an orbital shaker at 50 rpm. The extract was then filtered using the Whatman filter paper no.1 under vacuum and the filtrate was stored at -80ºC until further analysis.

Folin-Ciocalteu assay

A modified version of the Folin-Ciocalteu method by Mohd Fadzelly *et al.* (2009) was used to determine the total phenolic content of samples. About 300 µl of the extract was mixed with 2.25 ml of Folin-Ciocalteu reagent which had been previously diluted 10-fold with distilled water. The mixture was allowed to stand for 5 minutes at room temperature. Later, 2.25 ml of sodium carbonate (60 g/l) solution was added to the mixture. After 90 minutes and at room temperature, the absorbance of the mixture was measured at 725 nm using the spectrophotometer (Fisher Scientific Sdn. Bhd., Shah Alam, Malaysia). The results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

Beta-carotene bleaching assay

A modified version of B-carotene bleaching assay by Amin *et al.* (2002) was used. About 1 ml of B-carotene solution (0.2 mg/ml chloroform) was pipetted into a 50 ml round-bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was evaporated at 40° C for 10 minutes using a rotary evaporator to remove chloroform. The mixture was immediately diluted with 100 ml distilled water after the evaporation. Distilled water was added to the mixture slowly followed by vigorous agitation to form an emulsion.

Approximately 5 ml aliquots of the emulsion were transferred into different test tubes containing 0.2 ml of sample in 80% methanol at 1 mg/ml. The test tubes were mixed gently and then placed in a water bath of 45°C for 2 hours. Absorbance of the samples was measured at 470 nm using a spectrophotometer (Fisher Scientific Sdn. Bhd., Shah Alam, Malaysia) at initial time $(t=0)$ against a blank which consist of an emulsion without B-carotene. Standards at the same concentration as samples were used for comparison. About 0.2 ml of 80% methanol in 5 ml of the above emulsion was used as the control. Measurement was done at 15 minutes intervals.

Antioxidant activity (AA) was measured in terms of successful bleaching of B-carotene by using the formula below.

$$
\mathrm{AA} = \left. \begin{array}{cc} 1 - \left[\left(\frac{\left(A_0 - A_t\right)}{\left(A^\circ_{\ 0} - A^\circ_{\ t}\right)}\right)\right] \times 100 \end{array} \right.
$$

In this method, since the absorbance is measured at 120 minutes, then A_t and A_t° were at 120 minutes. A° and A_0° were the absorbance values measured at the initial time of incubation for the samples and controls respectively while A_t and A_t° were the absorbance values measured in the samples or standards and control at t=120 minutes.

Ferric reducing/antioxidant power (FRAP) assay

A modified version of FRAP assay by Fu *et al.* (2011) was used. The FRAP assay was prepared from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1 respectively. The FRAP reagent was freshly prepared upon usage and was warmed in a water bath of 37°C before used. About 100 µl of the diluted sample was added to 3 ml of the FRAP reagent and the absorbance of the mixture was measured at 593 nm using a spectrophotometer (Fisher Scientific Sdn. Bhd., Shah Alam, Malaysia). The standard curve was constructed using FeSO_4 solution and the results were expressed as µmol Fe (II)/g wet weight of the fruit.

Statistical analysis

All of the experiments were performed in triplicates. The results were expressed as mean \pm standard deviation. Analysis of variance (ANOVA) was conducted to identify the significant difference between each samples (p<0.05). Fisher's Least Significant Difference (LSD) test was used for post-hoc analysis. Independent sample t-test was conducted to compare the means between fresh and fried samples for all three fruits. Meanwhile, the Pearson correlation test was run to determine the correlations between TPC and AA.

Results and Discussions

Total phenolic content (TPC)

The values for total phenolic content for each sample are shown in Table 1. The TPC did not vary much ranging from 54.042 ± 0.596 mg GAE/100g fresh weight (FW) to 76.836 ± 0.619 mg GAE/100g FW. Fried jackfruit had the highest TPC, followed by fresh jackfruit, fried cempedak, fresh cempedak, and fried breadfruit. The lowest TPC was in fresh breadfruit.

A one-way ANOVA was used to compare the TPC between each fruit for both fresh and fried samples. There was a statistically significant difference (p<0.05) in TPC between fresh jackfruit and fresh breadfruit, between fresh jackfruit and fresh cempedak and between fresh breadfruit and fresh cempedak. For fried samples, there were statistically significant differences (p <0.05) in TPC between fried jackfruit and fried breadfruit, between fried jackfruit and fried cempedak and between fried breadfruit and fried cempedak. Besides, an independent sample t-test to compare the means of TPC between fresh and fried samples of each fruits showed statistically significant differences in TPC between all fresh and fried fruits (jackfruit, breadfruit and cempedak).

Generally, the TPC for the fried samples could not be compared with previous findings as there were no studies reported on this matter to date. However, a number of studies that determined the TPC of fresh fruits were found. The TPC for fresh jackfruit was almost similar to the TPC reported by Fu *et al.* (2011) which was 60.960 ± 3.690 mg GAE/100g FW. However, the TPC for fresh breadfruit differed when compared to a study in India by Kolar *et al.* (2011). The study stated that their breadfruit sample contained approximately 172.300 mg GAE/100g FW of TPC which was very high compared to findings of this study $(54.042 \pm 0.596 \text{ mg } \text{GAE}/100 \text{g } \text{FW})$. A study by Lee *et al.* (2013) found approximately half the amount of TPC for fresh cempedak compared to the current study which was 38.446 mg GAE/100g FW. The difference between the findings could be due to the different place of origin of the fruit samples. Other variables such as fruit ripeness, cultivar specificities, cultural practices, geographic origin season and postharvest storage conditions are also the factors that cause differences (Balamurugan, 2014).

As seen in Table 1, the values for TPC in fried samples of the three fruits were higher compared to the fresh samples. This could be mainly due to the cooking process used which was deep frying. Temperature can easily affect phenolic content

Values in the same column with different letters are significantly different at $p<0.05$ (ANOVA). Values in the same row with different symbols are significantly different at $p<0.05$ (T-test).

of foodstuff and also the polyphenol compounds present in the oil used for frying (Ruiz-Rodrigez *et al.*, 2008). A study by Sultana *et al*. (2008) also found a pattern of increase in TPC in vegetables fried under the temperature of 170° C in samples of white turnip and carrot. The study concluded that the increase was due to the cooking treatments which may have attributed to the extractability and bioavailability of antioxidants from the vegetables. The TPC may have increased because of the softening or disruption of plant cell walls and the destruction of complex phenolics (Sultana *et al*., 2008).

Another study by Miglio *et al.* (2008) also found an increase in certain phenolics (caffeic acid and p-coumaric acid) in samples of carrots and broccoli. The increase was explained to be due to hydrolysis of certain phenolics (chlorogenic acid) into other phenolic compounds (caffeic acid and p-coumaric acid) (Miglio *et al.*, 2008). This could also be explained due to the contribution of phenolics from the cooking oil used to fry the fruits. For this study, palm oil was used to fry the jackfruit, breadfruit and cempedak. A study by Sambanthamurthi *et al.* (2011) discovered major phenolic components in oil palm phenolics such as protocatechuic acid, p-Hydroxybenzoic acid, caffeoylshikimic acid and some gallic acid equivalents (Sambanthamurthi *et al.*, 2011).

Besides, the flour used for making the batter could have also contributed to the increase in phenolic content in fried fruits. The phenolic antioxidant in wheat flour may contribute to the total phenolic present. A study by Lv *et al.* (2012) identified the presence of TPC as high as 2.00 mg of GAE/g within 10 samples of wheat flour (Lv *et al*., 2012). Another study by Wang *et al.* (2013) found the presence of various phenolic acids in different wheat cultivars.

Table 2. Antioxidant activity of fresh and fried fruits measured using FRAP assay.

Values in the same column with different letters are significantly different at $p<0.05$ (ANOVA). Values in the same row with different symbols are significantly different at p<0.05 (T-test)

Among the phenolic acids found were ferulic acid, syringic acid, p-coumaric acid, caffeic acid, chlorogenic acid and gentisic acid.

FRAP (ferric reducing antioxidant power)

The AA of the six samples measured using FRAP assay are shown in Table 2. The AA between the six samples measured using FRAP assay did not vary much, with the highest value observed being 3.881 ± 0.301 mM Fe²⁺/g FW and the lowest value was 0.794 ± 0.106 mM Fe²⁺/g FW. The sample with the highest AA per 1g dry weight was observed in fresh cempedak followed by fresh breadfruit, fresh jackfruit, fried jackfruit and fried breadfruit. The least AA was in fried cempedak.

A one-way ANOVA was used to compare the AA of the FRAP values between fruits in both fresh and fried forms. For fresh samples, there was a statistically significant difference (p <0.05) in AA as measured using FRAP assay between fresh jackfruit and fresh breadfruit, between fresh jackfruit and fresh cempedak and between fresh breadfruit and fresh cempedak. For fried samples, there were statistically significant differences (p<0.05) in the AA measured using FRAP assay between fried jackfruit and fried breadfruit and between fried jackfruit and fried cempedak but not between fried breadfruit and fried cempedak. Moreover, using the independent sample t-test, there were statistically significant differences in the AA between the fresh and fried forms of all three fruits.

Stangeland *et al.* (2011) reported a FRAP value of 0.15 ± 0.07 mmol Fe²⁺/100g FW for fresh jackfruit pulp. Another study by Fu *et al.* (2011) reported a FRAP value of 2.57 µmol Fe^{2+}/g FW for jackfruit. Kolar *et al.* (2011) reported a FRAP value of 16280.62 ascorbic acid equivalent (AAE)/g FW for breadfruit.

Values in the same column with different letters are significantly different at p<0.05 (ANOVA). Values in the same row with different symbols are significantly different at p<0.05 (T-test)

However, there was no study which determined the antioxidant activity using FRAP assay for fresh cempedak. In addition, no studies that determined antioxidant activity using FRAP assay for fried fruits were found. Generally, the trend for AA as measured using FRAP assay showed reduction activities after the fruits were fried for all three fruits.

Cooking factors including method, temperature, cooking time and portion size strongly affect the antioxidant activity of cooked food. Thermal treatment especially can affect the AA of food samples (Hwang *et al.*, 2012). Similar to the results of this study, Hwang *et al.* (2012) showed decreased antioxidant capacities after thermal treatment of frying and other cooking methods in samples of carrots, onions, white cabbage, colored peppers and other cabbage studied which is in contrast to a study by Ferracane *et al.* (2008). Ferracane *et al.* (2008) reported that, when certain antioxidants like phenolics, carotenoids and flavonoids increase, there should also be an increase in AA accompanying it as these antioxidant compounds contribute to the activity of fruit or vegetables (Ferracane *et al.*, 2008).

Miglio *et al*. (2008) also showed similar findings whereby carrots, courgettes and broccoli cooked using three different methods (frying, boiling and steaming) all showed an increase in AA when measured using three assays, FRAP, TRAP and TEAC assay. Besides that, the increment of total antioxidant capacity may have been contributed by the formation of new molecules such as the Maillard reaction products with high antioxidant capacities (Miglio *et al*., 2008). Matrix softening and increased extractability upon cooking were accompanied by the conversion of polyphenol into very active chemical species, which may lead to high antioxidant capacity (Miglio *et al*., 2008). The overall increase of total antioxidant capacity values observed in the study is in partial disagreement with the concept that processed vegetables have lower nutritional quality

compared to raw ones thus; suggesting that for each sample, there is a certain preferential cooking method that could preserve or improve its nutritional and physicochemical qualities.

Beta-carotene bleaching assay

The AA of the six samples measured using betacarotene bleaching assay are shown in Table 3. A wide range of activities was observed between the highest and lowest values for the AA $(%)$ whereby the lowest value was (-76.449 ± 8.139) for fried cempedak while the highest value was (98.936 ± 0.182) for fried breadfruit. Fried cempedak had the highest percentage of AA (%) followed by fresh jackfruit, fresh cempedak, fried jackfruit, and fresh breadfruit. The lowest was observed in fried breadfruit.

One-way ANOVA that compared the percentage of AA measured using beta-carotene bleaching assay between fruits in both fresh and fried forms showed statistically significant differences ($p<0.05$) in activity between samples (between fresh jackfruit and breadfruit; between fresh breadfruit and cempedak). While for fried samples, statistically significant differences $(p<0.05)$ in the percentage of AA between fried jackfruit and breadfruit, fried jackfruit and cempedak and fried breadfruit and jackfruit were observed. The independent sample t-test that compared the means of percentage of AA between fresh and fried samples showed statistically significant differences in the percentage of AA between fresh and fried jackfruit, fresh and fried breadfruit and fresh and fried cempedak.

In this study, a mixed pattern in the percentage

of AA can be observed. Based on Table 3, it can be seen that for jackfruit and breadfruit, there was a decrease in percentage of AA after the fruits were fried. However, for cempedak, the percentage of AA showed an increment when the fruit was fried. Not many of the previous studies have used the betacarotene bleaching assay to evaluate AA in these fruits. Therefore, it was difficult to make comparison for the values of AA (%) for these samples. Literature search on this assay found other studies mostly used different parts of fruit such as hull, seed and leaves.

The negative value of AA observed in fried breadfruit sample indicates that the sample might contain pro-oxidant. Pro-oxidant is a species that causes or promotes oxidation (Winterbourn, 2009). Previously, Papetti *et al*. (2006) investigated the effects of thermal treatment on antioxidant and prooxidant activity of certain vegetables. The study found negative AA values in samples of vegetable juice, indicating that the samples contains pro-oxidant. It was explained that after the samples had undergone thermal treatment of boiling at 102° C, the percentage of protective activity of the vegetables decreased and their pro-oxidant activity increased (Papetti *et al*., 2006). In the study, red vegetables were found to have higher pro-oxidant activity compared to green vegetables (Papetti *et al.*, 2006).

The pro-oxidant effect could also be due to the presence of available free fatty acids in the samples, which in this study are the fruits. Golden and Williams (2001) reported in their study that breadfruit actually contains a lot of free fatty acids. The highest fatty acid observed in a ripe breadfruit was myristic acid (11.18mg/100g fresh weight) and oleic acid (10.34mg/100g fresh weight). Other fatty acids are caproic, capric, lauric, palmitic, linolenic, linoleic, stearic, arachidic and behenic acid (Golden and Williams, 2001). Another source of free fatty acid is from frying oil. A study by Frega *et al.* (1999) investigated the pro-oxidant effect in filtered oils and confirmed the pro-oxidant effect of the free fatty acids when a methyl ester was added to the oil sample and the intensity of this effect was related to free fatty acid concentration (Frega *et al*., 1999).

A study by Chowdhury *et al*. (1997), reported that the edible part of jackfruit contains palmitic acid (57.0 mg/100g of whole fruit) and oleic acid (48.3 mg/100g of whole fruit). However, when compared to other parts of the jackfruit such as the inner nonedible part, inner perianth, inner stick and outer bark, the edible part of jackfruit had the lowest content of free fatty acid. No studies were found to report the free fatty acid content in jackfruit. Therefore, since breadfruit contains a variety of free fatty acids, accompanied with the free fatty acids from the cooking oil used for frying, this could have led to the negative value of antioxidant activity measured using the beta-carotene assay.

Correlation between TPC and antioxidant activity of samples (FRAP and beta carotene bleaching assay)

Table 4 shows the relationship between TPC of samples and their AA measured using FRAP and beta-carotene assays. No significant correlation exists between TPC and AA using both assays for fresh and fried samples of jackfruit, breadfruit and cempedak. Therefore, other compounds such as flavonoids, anthocyanins and tannins could have contributed to the AA of the studied samples (Hosu *et al.*, 2014). Carotenoids are also known to have protective effect against oxidation which can contribute to AA (Mezadri *et al*., 2008). Therefore, it can be said that the TPC in samples is most probably not the main component responsible for the AA in the fruit samples.

Maisarah *et al.* (2013) showed similar findings whereby there was no correlation for AA analyzed using beta-carotene bleaching assay with TPC in papaya. Scalzo *et al.* (2005) also reported a negative correlation between TPC and AA in strawberries which was measured using TEAC assay. The study explained that the antioxidant capacity might not always correlate with the amount of phenolics. In fact, the negative correlation could be due to the very high content of ascorbic acid of strawberries, which was 20% higher compared to oranges and this accounted for most of the TEAC value. No studies on jackfruit, breadfruit and cempedak were found to support the negative correlation between TPC and AA measured using FRAP assay.

Interestingly, these findings are in contrast with the findings from a study by Fu *et al.* (2011) which reported that there is a highly positive correlation between TPC and FRAP value of the fruits studied. They suggested that phenolic compounds may be responsible for the reducing ability of the fruits (Fu *et al*., 2011). Kolar *et al*. (2011) also reported similar findings whereby their study found good correlation between TPC and FRAP values for breadfruit samples. Another study done by Thaipong *et al.* (2006) also showed positive correlation between AA by FRAP assay and TPC in their sample of guava fruit extracts. However, not many studies were found which investigated the correlation between total phenolic content and AA measured using betacarotene assay and also between TPC and AA for fried fruits.

Conclusion

As a conclusion, frying of fruits resulted in increment of TPC and mixed changes in antioxidant activity of the fried product. Certain antioxidants are enhanced by the frying process such as phenolics. Results showed that fried jackfruit, breadfruit and cempedak contained higher amounts of phenolics compared to the fresh samples of the fruits. Analysis of antioxidant activity using FRAP assay showed that fresh fruits had higher antioxidant activity compared to fried fruits. However, when beta-carotene bleaching assay was used to measure antioxidant activity, the results did not show similar pattern. Higher percentage of antioxidant activity in fresh fruits compared to fried fruits was only observed in jackfruit and breadfruit, but not for cempedak. Besides, no correlation was observed between total phenolic content and antioxidant activity measured using FRAP and beta-carotene assays. Generally, frying process of fruits must be controlled and optimized in getting the benefits of fruit antioxidant besides enjoying the good flavor, sweet taste and crunchy texture of fried fruits.

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