Bioavailability and antioxidant potentials of fresh and pasteurized kiwi juice before and after in vitro gastrointestinal digestion

Ghada Khiralla¹ · Hussein M. Ali²

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Abstract The aim of the present work was to find the changes in the chemical composition, chemical structure and antioxidant activity of bioactive nutrients of kiwi juices upon pasteurization and in vitro gastrointestinal digestion. Results showed that fresh kiwi juice (FKJ) contains total phenols, 162 mg GAE/mL, total flavonoids 1.44 mg QE/mL, ascorbic acid 4.5 mg/mL and fiber 60%; these contents were marginally affected by pasteurization (PKJ) to be 122, 1.02, 2.6 and 47 respectively. On the other hand, gastrointestinal digestion severely lowered total phenols, total flavonoids and ascorbic acid in digested fresh kiwi juice (DFKJ) to 49 mg GAE/mL, 0.16 mg GE/mL and 2.2 mg/mL, and in digested pasteurized kiwi juice (DPKJ) to 42, 0.07 and 1.2 respectively; however fiber contents increased upon digestion as a result of decreasing other nutrients. The antioxidant and reducing power activities followed the same order of decreasing total phenols, total flavonoids and ascorbic acid contents i.e. FKJ > PKJ > DFKJ > DPKJ. The major flavonoid found was apigenin in the form of various glycosides differ in the number (1–3) and position of the sugar units. Pasteurization showed minor effects on the chemical composition of fresh juice while digestion, as a result of gastrointestinal enzymes had more effect but mainly on the number and position of the

sugar units rather than affecting the flavonoid moiety which preserve the main nutritive values.

Keywords Kiwi · Pasteurization · Bioactive components · Antioxidant capacity · Reducing power

Introduction

As part of their concern for public health and food quality, consumers are interested in the consumption of functional foods and beverages that are rich in vitamins, dietary fiber, and other beneficial nutrients. Fruits and vegetables are among the main sources of many important phytochemicals possessing various mechanisms in the body cells e.g. anti-oxidation, enzyme induction and detoxification mechanisms (Schaefer et al. 2006).

In addition to its high nutritional value, fruit juices are easily prepared, preserved and consumed (Rodríguez-Roque et al. 2015). Fruit juice has gained popularity in the world at the turn of the twentieth century, particularly when pasteurization techniques have been developed as a way to increase the safety and shelf-life period of juice consumption; besides, pasteurization allowed, for the first time, the production and storage of juice in factories in commercial scale without spoiling. In the past, the only way to preserve the juice was fermentation, leading to producing alcoholic beverages (Goh et al. 2012).

Studies have shown that the incidence of chronic diseases is mainly due to unbalanced diets that are rich in saturated fats, carbohydrates and poor in bioactive compounds and dietary fiber. The UN General Assembly in 2016 announced a program to improve nutrition around the world in the period between 2016 and 2025 (ONU 2016).

✉ Hussein M. Ali
Hussein_galaleldeen@agr.asu.edu.eg

¹ National Organization for Drug Control and Research (NODCAR), 6-7, AboHazem Street, Pyramids, PO Box 29, Giza, Egypt

² Agricultural Biochemistry Department, Faculty of Agriculture, Ain Shams University, Shubra El-Kheima, Cairo, Egypt

Oxygen is an important and essential element for many human body processes such as energy production through fat and carbohydrate catabolism. However, studies have also shown that oxygen plays a dangerous role in the production of many harmful reactive oxygen species such as peroxides causing many chronic diseases e.g. heart and cancer diseases (Karthiha et al. 2017).

Phenolic and polyphenolic compounds are the most abundant secondary plant metabolites found in the majority of fruits, vegetables, and teas (Lorrain et al. 2013). These bioactive compounds lead to reducing the possibility of many diseases such as arthritis, arteriosclerosis, cancer, inflammation, brain dysfunction and cardiovascular diseases (Attri et al. 2017). Among the fruits, kiwi (*Actinidia arguta*) is an important source of biologically active compounds such as vitamin C, dietary fiber and phenolic and flavonoid compounds which have proven to have many beneficial effects on human health and disease prevention (Lim et al. 2006; Latocha 2017).

Kiwi fruits have shown more phenolic contents and higher antioxidant activity than many other common fruits e.g. orange, apple, banana, peach and grapefruit (Iwasawa et al. 2011; Park et al. 2015). In addition kiwi extracts showed strong antioxidant activity (Park et al. 2014; Zhu et al. 2019) and protection against lipid oxidation (Iwasawa et al. 2011), hydrogen peroxide (Iwasawa et al. 2011) and diseases caused or mediated by human pancreatic lipase and/or human fatty acid synthase (Eidenberger 2011). However, the main problem facing kiwi trade is the short shelf-life that leads to the loss of vital compounds found in kiwi (Kim et al. 2018); besides, phenolic compounds can easily undergo oxidation causing fruit browning by polyphenol oxidases (Kroll et al. 2003; Ali et al. 2015, 2016). Consumption as juice, preserved frequently by heat treatment (pasteurization), can solve the problem; nevertheless, heat treatment may affect some biochemical compounds compared to untreated juices (Petruzzzi et al. 2017).

In addition, the digestion stages within the body affect the actual amount absorbed by the body; meanwhile, the in vitro gastrointestinal digestion is a powerful tool for evaluating the concentration of health-related compounds that are released from the food matrix into gastrointestinal organs to be available for absorption (Lucas-González et al. 2018; Quan et al. 2018) yet only few reports have paid attention to changes of individual phenols during gastric digestion (Quan et al. 2018). Therefore, the present study aims to evaluate some of the changes in the phenolic contents and antioxidant activity of kiwi juice upon pasteurization and in vitro gastrointestinal digestion.

Material and methods

Chemicals

All chemicals, standards and gastrointestinal digestion fluids used in the experiments were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chemicals were of analytical grade except for eluting solvents were HPLC grade.

Kiwi juice preparation and pasteurization

Kiwi fruits (*A. arguta*) were procured from the local market of Giza city. Undamaged fruits at the optimal stage of maturity were selected and rinsed with tap then distilled water. For preparing fresh Kiwi juice (FKJ), fruits were peeled, cut and blended with water (1:1, w/v) in electric hand blender (Braun, MQ725). To prepare pasteurized Kiwi juice (PKJ), FKJ was heat-treated by applying a mild-temperature-long-time treatment (MTLT) in water path at 65 °C for 30 min followed by quick cooling in ice bath (Sinhaipant et al. 2013). Samples were kept at – 20 °C until analysis.

In vitro digestion procedure

The in vitro digestion method described by (Cassani et al. 2018) was applied in the present work. The test was divided into two stages; gastric digestion using simulated gastric juice and intestinal digestion using simulated intestinal fluid. FKJ and PKJ (10 mL) were mixed with 10 mL of simulated gastric juice (7.30 g/L NaCl, 0.52 g/L KCl, 3.78 g/L NaHCO₃, 3 g/L pepsin, at a final pH adjusted at 2.5). The mixture was incubated at 37 °C for 1.5 h under continuous shaking at 100 rpm. To stop the gastric digestion the digests were kept in ice-bath for 10 min. Afterwards, 10 mL of simulated intestinal fluid (1.27 g/L NaCl, 0.23 g/L KCl, 0.64 g/L NaHCO₃, 1 g/L pancreatin, 1.5 g/L bovine bile salts) was added and the pH was adjusted at pH 8. The resulting solution was incubated at 37 °C for 3 h under continuous shaking at 100 rpm. To stop intestinal digestion, samples were kept in an ice-bath for 10 min. The test tubes were then centrifuged at 13,500 g for 20 min. The supernatants were collected and filtered using Whatman filter paper. Samples were stored at – 20 °C until analysis. After digestion, the digested FKJ and PKJ were named DFKJ and DPKJ respectively.

Bioactive components in kiwi juice

Total phenolic content

Total phenolic contents of the kiwi juice were determined using the method described by Singleton and Rossi (1965). According to the method, 100 μL of Folin–Ciocalteu reagent, and 300 μL of Na_2CO_3 (20%) were added to 1 ml kiwi juice. Then the volume was completed to 2000 μL with distilled water. After a 30 min incubation period at 45 °C, absorbance was recorded at 765 nm. The results were expressed as mg per mL gallic acid equivalents (GAE).

Total flavonoid content

The total flavonoid content in FKJ and PKJ was determined before and after digestion as described by Viacava and Roura (2015). Water extracts (0.2 mL) were mixed with 1.28 mL of deionized water and 0.06 mL of NaNO_2 (50 g/L). After 5 min at 20 °C, 60 μL of AlCl_3 (100 g/L) was added; 6 min later, 0.4 mL of NaOH (40 g/L) was incorporated in the same conditions. The mixtures were stirred in vortex, and the absorbance was read at 510 nm (A_{510}). The total flavonoid content was expressed as mg of quercetin equivalent/mL (QE/mL) of juice. The standard curve of quercetin was made up in the range of 0.05–1.2 g/L.

Ascorbic acid content

Ascorbic acid content of prepared Kiwi juices was determined according to titration method described by Ballentine (1941) with some modifications. Briefly, 1 ml of kiwi juice is added to 25 ml of 2 N sulfuric acid and 75 ml distilled water then titrated with 0.5 N iodine solution using starch solution (1% w/v) as indicator.

Total dietary fiber

Total Dietary Fiber was determined by filter bag technique (AOAC 1990) using ANKOM 200 fiber analyzer. Air dried juice samples were placed in filter bags and sealed. Digestion was performed first by sulfuric acid (0.255 N) with heat and agitation for 40 min. followed by rinsing with water and agitation for 5 min. Second, bags were treated with NaOH (0.313 N) with heat and agitation for 40 min. then rinsed with water for 5 min. After digestion, bags were soaked in acetone for 5 min. then dried in oven at 102 °C for 2 h. Total Dietary Fiber was expressed as percentage in dried samples.

Recovery index of bioactive components

Bioavailability of bioactive component (BAC) after in vitro digestion was determined by calculating the recovery index as described by Ortega et al. (2011). For each BAC (total phenols, total flavonoids, ascorbic acid and total dietary fiber) the recovery index was calculated as following:

$$\text{Recovery index of BAC \%} = \left[(\text{BAC}_{\text{after digestion}} / \text{BAC}_{\text{before digestion}}) \right] \times 100$$

where $\text{BAC}_{\text{before digestion}}$ and $\text{BAC}_{\text{after digestion}}$ are the content of each bioactive component before and after digestion respectively.

Antioxidant potential

DPPH radical-scavenging activity

DPPH radical-scavenging activity was evaluated according to method described by Celep et al. (2013). Briefly, 0.1 mL kiwi juice (or 0.1 mL distilled water in control) was added to 5 mL of 0.1 mM DPPH (2,2 diphenyl-1-picrylhydrazyl) solution freshly prepared in methanol. After being vortexed and incubated in the dark at room temperature 25 ± 3 °C, the absorbance of the mixture was measured at 517 nm (A_{517}). The percent of antiradical activity (ARA) against DPPH was calculated according to the following equation:

$$\text{ARA\%} = ((A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}) \times 100.$$

Reducing power (RP)

Mixture of 0.1 mL kiwi juice, 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1% w/v) was prepared and incubated at 50 °C for 30 min. Afterwards, (2.5 ml) trichloroacetic acid (10% w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. Supernatant (2.5 mL) was diluted with distilled water (2.5 mL) and shaken with 0.5 mL freshly prepared ferric chloride (0.1% w/v). Absorbance was measured at 700 nm (A_{700}). Butylated hydroxyl toluene (BHT) was used as positive control (Jayaprakasha et al. 2001).

Total antioxidant capacity

Total antioxidant capacity (TAOC) of kiwi juice was performed by using phosphomolybdenum method (Prieto et al. 1999). Three hundred microliters of samples were mixed with 3 mL of reagent solution consisting of 28 mM sodium phosphatemonobasic, 4 mM ammonium molybdate and 0.6 M sulfuric acid. After an incubation period of 90 min

at 95 °C, the absorbance was read at 695 nm. The total antioxidant capacities were expressed as mg ascorbic acid equivalent (AAE) per 1 mL juice.

Retained activity

Retained activity (%RA) of scavenging activity, reducing power and total antioxidant activity after treatment (pasteurization and/or gastrointestinal digestion) was calculated as follows:

$$\% \text{ Retained activity} = (\text{Activity after treatment} / \text{Activity of FKJ}) 100$$

LC–MS–ESI analysis of phenolic compounds

Samples were dissolved in methanol (80%) and filtered with a 0.45 µm micropore membrane prior to injection. Phenolic compounds were analyzed using Agilent 1200 LC–MS–ESI instrument equipped with a diode array detector (DAD) and an Agilent Zorbax Eclipse Plus C18 column (Agilent Technologies, 100 mm × 2.1 mm, 1.8 µm Palo Alto, CA, USA). The elution gradient consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile). The gradient program was: 0–10 min 5–20%B, 10–30 min 20–80%B then 30–35 min 5%B. The flow rate was set at 0.8 mL/min and injection volume was 5 µL. Electrospray ionization (ESI) was operated at positive ion mode in full scan analysis range 100–900 m/z using nitrogen as collision gas.

Statistical analysis

For each treatment, data from three independent measurements were collected and the mean values ± standard deviations (SD) were determined and analysed by a one-way ANOVA using ‘Proc Mixed’ (SAS 8.2, Cary, NC). Differences between treatments were determined by Duncan’s method. In all cases, the level of statistical significance was of $P < 0.05$.

Result and discussion

Bioactive components of kiwi juices

Fresh kiwi juice (FKJ) and pasteurized kiwi juice (PKJ) were prepared in the present study. The effect of in vitro simulated gastrointestinal digestion of both juices on their bioactive components and antioxidant potential were investigated. Table 1 presented important bioactive components in kiwi juices before (FKJ and PKJ) and after

(DFKJ and DPKJ) digestion. The results showed that FKJ had high contents of total phenols, flavonoids, dietary fiber and ascorbic acid (162 mg/mL, 1.44 mg/mL, 60% and 4.5 mg/mL respectively). This result indicated that the FKJ could be considered as a good source of bioactive compounds. In agreement with this finding, Liang et al. (2017) stated that kiwi can be defined as a distinct source of antioxidants and other bioactive compounds. From the data of PKJ presented in Table 1, it can be noticed that pasteurization had some effects on reducing total phenols and flavonoids (122 and 1.02 mg/mL respectively). On the other hand, dietary fiber and ascorbic acid were strongly reduced to 47% and 2.6 mg/mL respectively.

In contrary to pasteurization, gastrointestinal digestion severely decreased total phenols, flavonoids and ascorbic acid in both fresh and pasteurized juice. These bioactive contents, in fresh juice (FKJ), were dramatically reduced from 162, 1.44 and 4.5 to 49, 0.16 and 2.2 mg/mL respectively while in pasteurized juice (PKJ) they were decreased from 122, 1.02 and 2.06 to 42, 0.07 and 1.2 mg/mL respectively after gastrointestinal digestion. (Table 1). This reduction could be due to changes in molecular structure of the studied bioactive components as a result of the enzymatic action (pepsin and pancreatin) and consequently in its availability and solubility (Kroll et al. 2003). Several studies have confirmed a significant reduction in bioactive compounds after gastrointestinal digestion (Ortega et al. 2011; Rodríguez-Roque et al. 2015; Lucas-Gonzalez et al. 2016). Lucas-González et al. (2018) mentioned that the loss of biologically active compounds after gastrointestinal digestion may be due to oxidation reactions, polymerization, interaction with other nutrients, and phenol-derivatives formation.

On the other hand, increase in the dietary fiber percentages were observed in digested samples (DFKJ and DPKJ) after in vitro gastrointestinal digestion (76 ± 6 and $78 \pm 5\%$) compared to those recorded before digestion (60 ± 5 and $47 \pm 3\%$) in FKJ and PKJ, respectively (Table 1) which could be attributed to the observed degradation of the other bioactive compounds.

Recovery index of total phenols, total flavonoids, dietary fibers and ascorbic acid of fresh and pasteurized kiwi juices after in vitro gastrointestinal digestion was illustrated in Fig. 1. The most affected bioactive component was the total flavonoids content, where the recovery index of digested fresh and pasteurized kiwi juices was only 11 and 9.5%, respectively. The highest recovery index was recorded for total fiber content of both digested fresh and pasteurized juices because of the increase in its concentration in juice samples after digestion.

Table 1 Bioactive components* of kiwi juices before and after in vitro gastrointestinal digestion

Kiwi juice	T. Phenols (mg GAE/mL)	T. Flavonoids (mg QE/mL)	Dietary fiber (%)	Ascorbic acid (mg/mL)
FKJ	162 ^a ± 9	1.44 ^a ± 0.02	60 ^a ± 5	4.5 ^a ± 0.2
DFKJ	49 ^b ± 2	0.16 ^b ± 0.01	76 ^b ± 6	2.2 ^b ± 0.1
PKJ	122 ^c ± 6g	1.02 ^c ± 0.01	47 ^c ± 3	2.6 ^b ± 0.2
DPKJ	42 ^b ± 3	0.07 ^b ± 0.01	78 ^b ± 5	1.2 ^c ± 0.1

FKJ fresh kiwi juice, PKJ pasteurized kiwi juice, DFKJ digested fresh kiwi juice, DPKJ digested pasteurized kiwi juice

*Means ($n = 3$) ± standard deviation (SD) with the same letter in the same column has no significant differed ($p > 0.05$)

Fig. 1 Recovery index (%) of bioactive components (BAC) before and after simulated gastrointestinal digestion of Kiwi juices

Antioxidant potential of kiwi juices

The antioxidant potential of FKJ, PKJ, DFKJ and DPKJ samples were evaluated using different antioxidant models including scavenging activity, reducing power and total antioxidant capacity (Table 2). The FKJ was characterized by its high antioxidant properties; scavenging activity

(75%), reducing power (0.146) and total antioxidant capacity (350 mg AAE/mL). Again, pasteurization showed less effect where the retained activities were 69.3%, 89.0 and 91.4% respectively while digestion had more effect on retained activities i.e. 66.7%, 82.2 and 51.4% in DFKJ and 56.0%, 75.3 and 47.1% in DPKJ respectively. The decrease in the antioxidant activities is clearly explained by the above mentioned loss in the bioactive contents where the descending order of the antioxidant activities matches the order of decreasing total phenols, flavonoids and ascorbic acid i.e. FKJ > PKJ > DFKJ > DPKJ.

Identifying changes in the chemical structure of nutrients

To identify the nature of structural changes upon pasteurization and digestion, the four juice samples (FKJ, PKJ, DFKJ and DPKJ) were subjected to LC–MS–ESI analysis. Fresh juice sample (FKJ) gave main fractions at Rt 1.725, 1.860 and 25.484 min (Fig. 2). The three fractions indicated different apigenin (Ap) hexose (Hex) derivatives which explains their solubility in the aqueous medium. Their structures depend on the number and site of the binding sugar unit. Spectrum of fraction at Rt 1.725 min (Fig. 3) showed peaks at m/z 269 and 433 for (Ap-1) and (Ap + Hex + 1) respectively in addition to peaks at m/z 105 and 187 for fragments (104 + H) and (189-2H) respectively as illustrated in Fig. 4. Fraction at 1.860 min

Table 2 Antioxidant potential* before and after in vitro gastrointestinal digestion of kiwi juices

Kiwi juice**	Scavenging capacity %ARA (%RA) ^b	Reducing power A_{700} (%RA)	TAOC mg AAE/mL (%RA)
FKJ	75 ^a ± 5	0.146 ^a ± 0.008	350 ^a ± 9
PKJ	52 ^b ± 3 (69.3)	0.130 ^b ± 0.003 (89.0)	320 ^b ± 5 (91.4)
DFKJ	50 ^b ± 3 (66.7)	0.120 ^c ± 0.006 (82.2)	180 ^c ± 7 (51.4)
DPKJ	42 ^c ± 3 (56.0)	0.110 ^c ± 0.005 (75.3)	165 ^c ± 5 (47.1)

FKJ fresh kiwi juice, PKJ pasteurized kiwi juice, DFKJ digested fresh kiwi juice, DPKJ digested pasteurized kiwi juice, TAOC total anti-oxidant capacity

*Means ($n = 3$) ± standard deviation (SD) with the same letter in the same column has no significant differed ($p > 0.05$)

^bRetained activity

Fig. 2 Chromatograms of FKJ, PKJ, DFKJ and DPKJ samples

gave the same 269, 433 and 105 peaks as well as a base peak at 203 m/z for ($^{13}B + \text{Hex-CHOHCHOH-OH}$) (Fig. 4). Fraction at Rt 25.484 min gave in addition to Ap + H ion peak (m/z 271) a peak at m/z 308 for (Ap + Hex-(CHOH)₃-2OH) ion. In recent literature (Park et al. 2014; Zhu et al. 2019), more phenolic compounds were identified in alcoholic extract of kiwi fruits; however, the present work deals with water extract, to imitate natural juice, which more likely contains only highly polar glycosidic phenols; besides, not all minor fractions were identified..

The pasteurized sample (PKJ) gave similar chromatogram (Fig. 2) and spectra to those of the fresh juice sample (FKJ) indicating similar chemical composition of fresh and pasteurized samples which is also indicated by the little differences in the total phenolic and flavonoid contents in both samples and explains the high retained

antioxidant activity after pasteurization discussed above. On the other hand, gastrointestinal digested sample (DFKJ) gave different chromatogram where the fractions at Rt 1.725, 1.860 and 25.484 min vanished while appeared other major peaks in the chromatogram (Fig. 2) with different spectra (Fig. 3) at Rt 2.631, 3.117, 3.402 and 3.620 min. Spectrum of fraction at Rt 2.631 min gave ions at m/z 242 (Ap-CO), 316 ($^{13}A + \text{Hex} + 2$) and 298 (Ap + Hex-134) depicted in Fig. 4). The fraction at Rt 3.117 min gave the peaks at 269, 104 and 203 peaks, previously interpreted, in addition to peaks at m/e 219 (fragment with m/z 203 + O), 231 (fragment with m/z 203 + CO) and 175 (fragment 176–1 or 174 + 1, Fig. 4). Fraction at Rt 3.402 min gave also the previously mentioned peaks at m/z 219, 231, 269 and 308. Fragment at Rt 3.620 min gave fragment (m/z 203) and $^{13}B + \text{Hexose-H}_2\text{O-1}$ ion (m/z 261). Sample DPKJ gave similar chromatogram to that of DFKJ but showed new fraction at 2.748 min which gave spectrum (Fig. 3) with peaks at m/z 203 and 219.

Note that peaks at m/z 187, 203, 219, 231 and 261 indicate that the sugar unit is bonded to B-ring while peaks at m/z 316 and 381 point to bonding sugar to A-ring; therefore, it could be concluded that in FKJ sample, both fractions at Rt 1.725 min, that contains peaks at m/z 187, 269 and 433, and fraction at 1.860 min with peaks at m/z 203, 269 and 433 are for Ap with sugar unit bonded to B-ring but can differ in the number of sugar units bonded consequently to the B-ring, especially spectrum of fraction 1.725 min shows ions with m/z higher than 432 for Ap + Hexose while fraction at 25.484 min shows only peaks of Ap nucleus (m/z 271 and 308).

In DFKJ sample, the fraction at 2.631 min gave fragment at m/z 316 characteristic for hexose bonded to A-ring while fractions at 3.117, 3.402 and 3.620 showed fragments at m/z (203, 219, 231), (219, 231) and (203, 261) respectively which are all characteristic of sugar bonded to B-ring, in addition to peaks related to Ap. Fraction at 2.748 min of DPKJ sample gave spectrum (Fig. 3) with peaks at m/z 203 and 219 for sugar bonded to B-ring in addition to new fragment at m/z 381 which indicates bonding two sugar units on line to A-ring, as presented in Fig. 4, indicating that this fraction is for glycoside with at least three sugar units.

These results indicate that one of the main gastrointestinal digestion effects is changing the number and position of the sugar unit of the flavonoid glycosides which could be rationalized since the gastrointestinal digestion fluids contains mainly hydrolase enzymes; in addition; the number and position of the glycosylation sites affect the number of available phenolic groups and thus the antioxidant activity of polyphenols (Heim et al. 2002; Verma and Pratap 2010; Ali and Ali 2017, 2019).

Fig. 3 Spectra of major fractions in FKJ, DFKJ and DPKJ samples

Fig. 4 MS fragmentations of Ap-hexoses. Facing arrows with similar colors indicates fragments. The bond number of ring C (0–4) and notations ^{1,3}A, ^{1,3}B and ^{0,4}B of flavone fragments were assigned in accordance with Cuyckens and Claeys (2004)

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