Antioxidant activity profile of extract and fraction of kersen (Muntingia calabura L.) fruits prepared by different methods

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Abstract. The public widely uses kersen (Muntingia calabura L) fruit for consumption and treatment because Kersen fruit has various vitamins, minerals, and secondary metabolite compounds that reduce free radicals. The purpose of this study was to see the effectiveness of kersen fruit as an antioxidant agent. In this study, the samples used were ethanol extract (EE), Aqueous ethanol fraction (EF), ethyl acetate fraction (EAF), and n-hexane (HF) fraction from kersen fruit (Muntingia calabura L). The samples were evaluated by observing the antioxidant activity profile using various methods, i.e., nitric oxide, β-carotene bleaching assay, hydroxyl radicals, and iron chelating. The results of this study showed that the IC50 values of EE (31.05 µg/mL), AEF (33.86 µg/mL), FEA (40.48 µg/ml) gave powerful antioxidant activity while HF showed weak results (459 µg/ml). In the nitric oxide method. The hydroxyl radical method with IC50 values of 32.06 µg/mL (EE), 38.73 µg/mL (EF), 22.18 µg/mL (EAF) gave very strong activity, and 53.37 µg /mL (HF) with strong activity. The β-carotene bleaching method gave powerful antioxidant activity results with IC50 values <50 µg/mL in each sample. The Iron Chelation method showed weak antioxidant activity (IC50 > 200 µg/mL) of each sample. Based on the results obtained, it can be concluded that the antioxidant activity of extracts and fractions in kersen fruit has a different activity profile according to the test method used.

Keywords: Antioxidant; Kersen (Muntingia calabura L); Radical Nitrogen Species; Radical Oxygen Species.

1. Introduction
Free radicals are one of the main factors causing the increase in degenerative diseases such as Parkinson's disease, heart failure, atherosclerosis, and several other diseases. Free radicals are unstable and highly reactive molecules because they contain only one or more unpaired electrons. Biochemically, the presence of an endogenous enzyme system can neutralize free radicals in the body, such as the enzymes catalase, glutathione, peroxide, super oxidase dismutase, and glutathione-s-transferase [1,2]. Free radicals have an essential role in tissue damage and pathological processes of living organisms. Excessive levels of free radicals in the body can attack vulnerable compounds such
as lipids, proteins, and DNA which can cause various diseases. Therefore, antioxidant compounds are needed to reduce oxidative stress damage caused by free radicals [3,4].

Antioxidants are compounds that can prevent or delay cell damage in the human body exposed to free radicals. Antioxidants are produced naturally in the body, but the process is ineffective and decreases with age, while free radicals are constantly being formed. Therefore, the body requires an intake of exogenous sources of antioxidant compounds produced from chemical synthesis or natural ingredients, fruits, and vegetables to neutralize the formation of free radicals in the body [5–7].

One of the plants that have the potential as an antioxidant is kersen fruit. Kersen (Muntingia calabura L.) is a plant that has an antioxidant effect [8,9]. Previous research has shown that kersen contains phenols and flavonoids. Based on the results of chemical analysis, the leaves contain alkaloids, proteins, flavonoids, and anthraquinone compounds. In addition, Kersen fruit contains phenolic compounds related to its antioxidant capacity, among others. Galloccatechin, epigalloccatechin, catechin, flavanol, naringenin, quercetin, gallic acid, vanillic acid, chloric acid, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, hydroxycinnamic acid, and myricetin [10,11] and has antimicrobial activity [12,13], anti-aging [8], anti-inflammatory [14] and based on research [8] it has been reported that Kersen fruit has antioxidant activity in reducing DPPH, ABTS, and FRAP radicals from Kersen fruit extract with IC50 values of 20.61 µg/mL, 11.17 µg/mL, and 1.89 µg/mL in the ethyl acetate fraction had a vigorous antioxidant activity with an IC50 value < 50 µg/mL while the hexane fraction had a weak antioxidant activity with an IC50 value > 150 µg/mL in reducing DPPH, ABTS, and FRAP radicals.

Based on this background, the researchers wanted to test the effectiveness of antioxidants from extracts and fractions of kersen fruit with various antioxidant testing methods, such as hydroxyl radicals, nitric oxide, β-carotene bleaching, and iron chelating. This study was conducted to provide additional information and as an initial screening of Kersen fruit as a candidate as an antioxidant.

2. Materials and Methods

2.1 Materials

The materials used in this study were aluminum foil, Aquadest (One Med®), Ammonium sulfate, Gallic acid, linoleic acid (Aldrich), CuCl2, 70% ethanol, ethyl acetate (Brand, Germany), 50% H2O2, chloroform (Brand, Germany), n-hexane (Brand, Germany), sodium salicylate, Neocuproine, β-carotene reagent (TCI, Japan), FeSO4 reagent and Tween 20.

2.2 Sample preparation

The Kersen fruit samples that have been obtained from Biringkanaya District, Makassar City was then carried out wet sorting to reduce impurities attached to the Kersen fruit samples, cleaned using running water and cut the Kersen fruit samples to facilitate the drying process, and then put in an oven at a temperature of 40°C to The dried sample of kersen fruit was obtained.

2.3 Extraction and fractionation

The dry Kersen powder was extracted using the maceration method using 70% ethanol solvent in a ratio of 1 kg of dry kersen fruit with 7.5 liters of 70% ethanol solvent for five days and stored in a place protected from light. After the first damping process is complete then filtering is carried out. The filtrate obtained is then collected and evaporated using a rotary evaporator until a thick extract is obtained. The viscous extract obtained was then dissolved in ethanol: water (1:9) in 25 mL and stirred. The mixture was put into a separating funnel and extracted liquid-liquid with 25 mL of n-hexane solvent, then shaken and allowed to stand until completely separated. The n-hexane fraction will be at the top, while the ethanol layer will be at the bottom. Next, the n-hexane and ethanol fractions were separated. The ethanol fraction was extracted again with n-hexane until the solution obtained was clear (colorless). The same is done for the ethyl acetate solvent following the level of polarity ranging from non-polar to polar.
2.4 Hydroxyl radical (OH\(^-\)) method

Testing the antioxidant activity of extracts and fractions of kersen fruit in reducing hydroxyl radicals with a slight modification of [15] based on Fenton reaction by H\(_2\)O\(_2\) with a metal ion to form hydroxyl radicals OH\(^-\). The reaction mixture was made of 1.5 mL of 1.5 mM FeSO\(_4\) dissolved with ten mM Na\(_2\)EDTA, added 1 mL of 10 mM H\(_2\)O\(_2\) and added sodium salicylate then added extract samples and Kersen fruit fractions with varying concentrations (100, 50, 25, 12.5, 6.25, and 3.125 ppm) and the volume was made up to 3 mL with distilled water for incubation at room temperature for 10 minutes. After incubation, the absorbance of the sample was measured using UV-Vis spectrophotometry at a wavelength of 522 nm. The ability of a sample to reduce hydroxyl radicals can be calculated using the following formula [15]:

\[
\% \text{Inhibition} = \frac{\text{Absorbance sample}}{\text{Absorbance control}} \times 100
\]

(1)

2.5 Nitric oxide (NO\(^*\)) method

Testing antioxidant activity using the nitric oxide method is based on the procedure (Nur et al., 2021) with slight modifications. The NO method’s antioxidant activity was carried out by preparing a ten mM sodium nitroprusside reagent solution in 0.5 mM PBS (pH 7.4) and Griess reagent solution (0.33% sulfanilamide in 20% glacial acetic acid and mixed with naphthylenediamine 0.1% w/v ratio 1:1). A series of variations in sample concentration 1-100 g/mL was added with 2 mL of 10 mM sodium nitroprusside and incubated at 27°C for 2.5 hours. After the incubation period, 1 mL of Griess reagent was added, and the volume was filled with H\(_2\)O with a volumetric flask up to 5 mL. Then the absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 541 nm. The formula calculates the percentage inhibition value represented by IC\(_{50}^c\):

\[
\% \text{Inhibition} = \frac{\text{Absorbance sample}}{\text{Absorbance control}} \times 100
\]

(2)

2.6 β-Carotene bleaching (BCB) method

The stock solutions prepared are pipetted with varying concentrations (10-1000 μg/mL) and quercetin compared with concentration variations (10-100 μg/mL) into a 5 mL volumetric flask 2 mL of BCB emulsion is added. Then the volume was made up to 5 mL with distilled water in a 5 mL volumetric flask and incubated at 50°C for 20 minutes. After the incubation period, it was measured at a wavelength of 461 nm, and the measurements were monitored for 2 hours with an interval of 30 minutes. Antioxidant activity was calculated based on changes in the degradation of samples and control BCB. Percentage inhibition of β-carotene degradation rate using the following formula:

\[
\% \text{Degradation rate inhibition} = \ln \left[ \frac{(a/b)x(1/t)}{x 100} \right]
\]

(3)

Where a is the absorbance of the control (without sample), b is the absorbance of the sample after 20 minutes of incubation, and t is the absorbance of the sample after 2 hours of incubation. The IC\(_{50}^c\) value is determined by plotting the percent inhibition against each sample concentration [17]

2.7 Iron chelating method

A stock solution of 1000 ppm was made from each extract and a fraction of 10 grams in 10 ml of 70% ethanol. Then made a solution of FeSO\(_4\) as much as 3 mg in 100 ml. The ethanol extract, N-hexane fraction, ethyl acetate fraction, and water-ethanol fraction were pipetted (200; 400; 600; 800 dan 1000 µg/mL). Each was added with 0.1 mL FeSO\(_4\) and added 0.1 mL ferrozine, and then the volume was made up to 2 ml using distilled water so that the final concentration was obtained (100; 200; 300; 400; 500 µg/mL). The mixture was incubated at room temperature for 10 minutes, after which the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 563 nm [18]. The formula calculated the metal ion chelating activity:

\[
\text{Metal chelating activity} = \left[ \frac{(A_0 - A_s)}{A_0} \right] \times 100\%
\]

(4)

Where AO is the control absorbance and As is the extract/standard absorbance.
3. Results

It tested the effectiveness of extracts and fractions from Kersen fruit using several methods with different mechanisms. This research is done to further screen Kersen fruit as an exogenous antioxidant agent derived from natural ingredients. The tests carried out in this study used various antioxidant testing methods, namely hydroxyl radicals, nitric oxide, \( \beta \)-carotene bleaching, and iron chelating agents. According to [8,19], the process of neutralizing free radicals with antioxidants is based on the presence of a hydrogen atom transfer reaction (HAT) and single electron transfer (SET) so that it can neutralize free radicals by two critical mechanisms, namely first, the presence of chain termination by primary antioxidants that can donate electrons to free radicals. Meanwhile, the second mechanism is removing the initiator of the ROS/RNS species by breaking the chain catalyst. The antioxidant capacity of Kersen fruits was determined based on the IC\(_{50}\) values. The IC\(_{50}\) value is determined from the linear regression equation of the sample concentration with the percentage of inhibition from the sample, where the smaller the IC\(_{50}\) value, the greater the activity and vice versa.

3.1 Hydroxyl radical (OH\(^\cdot\)) method

The antioxidant activity of extracts and fractions of Kersen fruit in reducing hydroxyl radicals with the hydroxyl radical method based on the Fenton reaction of Fe\(^{2+}\)-EDTA + \( \text{H}_2\text{O}_2 \) to produce Fe\(^{3+}\) + OH + OH\(^\cdot\) while EDTA is used as a metal chelator. Hydroxyl radicals are one part of Reactive oxygen species (ROS), which are very reactive. The results of testing the antioxidant activity of extracts and fractions of Kersen fruit in reducing hydroxyl radicals can be seen in (Figure 1). The results obtained showed that the ethyl acetate (EAF), ethanol extract (EE), and water-ethanol (EF) fraction had IC\(_{50}\) values of 22.18 \( \mu \text{g/mL} \), 32.06 \( \mu \text{g/mL} \), and 38.73 \( \mu \text{g/mL} \) with a powerful category (< 50 \( \mu \text{g/mL} \)). The same thing was found in the quercetin as a positive control with a powerful category (2.32 \( \mu \text{g/mL} \)). In comparison, the hexane fraction (HF) has an IC\(_{50}\) value of 53.37 \( \mu \text{g/mL} \) with a strong category (50-100 \( \mu \text{g/mL} \)). Based on the results obtained, extracts and fractions from Kersen fruit have antioxidant activity in reducing hydroxyl radicals which are one of the most reactive parts of ROS to prevent Parkinson's disease, Alzheimer's disease, and several other degenerative diseases caused by one of them due to free radicals.

![Graph of the test results of antioxidant activity in reducing free radicals of hydroxyl radicals from (EE) ethanol extract, (EF) aqueous-ethanol fraction, (EAF) ethyl acetate fraction, (HF) hexane fraction, and quercetin as positive control determined based on the parameter IC\(_{50}\) value (Inhibition concentration 50 %). The data were observed triplicate (n=3).](image-url)
3.2 Nitrite oxide (NO\(^\cdot\)) method
Testing of antioxidant activity using the nitric oxide NO\(^\cdot\) method is based on the activity of a sample in reducing nitric oxide radicals, which is one of the radicals of RNS (Reactive Nitrogen Species). The results obtained in (Figure 2) show that EE, EF, and EAF have potent antioxidant activity (< 50 µg/mL) compared to HF, which has weak antioxidant activity with IC\(_{50}\) values > 200 µg/mL). Chemical compounds influence the weak antioxidant activity of the hexane fraction with low polarity from the hexane fraction, which can inhibit the action of NO radicals. The results obtained are based on compounds’ content in each extract and fraction in Kersen fruit. Previous research supports this result [8], explaining that the content of phenolic and flavonoid compounds contained in the EE, EF, and EAF of Kersen fruit can neutralize radical by HAT and SET mechanism.

![Graph of antioxidant activity testing using the nitric oxide method from ethanol extract (EE), an aqueous-ethanol fraction (EF), ethyl acetate fraction (EAF), hexane fraction (HF), and quercetin as a positive control, which was determined antioxidant activity based on IC\(_{50}\) values. The data were observed triplicate (n=3).](image)

3.3 β – Carotene bleaching method
They tested the antioxidant activity of extracts and fractions of Kersen fruit using the β-carotene bleaching method based on the inhibition of the degradation rate of β-carotene in the emulsion media. The antioxidant activity of the extract and fraction of Kersen fruit can be seen in Figure 3, which shows that the ethanol extract (EE), an aqueous-ethanol fraction (EF), ethyl acetate fraction (EAF), and hexane fraction (HF) have good activity with powerful category (<50 µg/mL). This result indicates that extracts and fractions of Kersen fruit have activity in reducing lipid peroxide resulting from the oxidation of linoleic acid, which turns into hydroperoxides with an increase in temperature that occurs during the incubation process and then attacks the double bonds of β-carotene in the lipid phase of the emulsion media. Therefore, from our results, the HF (IC\(_{50}\), 6.5 µg/mL) has antioxidant activity with a lower IC\(_{50}\) value compared to EE (IC\(_{50}\), 14.7 µg/mL), EF (IC\(_{50}\), 47.66 µg/mL), and EAF (IC\(_{50}\), 20.51 µg/mL) because compounds with low polarity penetrate the lipid phase in the emulsion media, thereby preventing oxidation of linoleic acid and inhibiting the rate of degradation of β-carotene. However, the results showed the antioxidant activity of each extract and fraction with a potent category.

3.4 Iron chelating method
Testing of antioxidant activity using the iron-chelating method was carried out to determine the potential of a sample to chelate Fe ions from a reagent. This event is based on the ability of antioxidant compounds to prevent complex reactions between Fe ions and ferrozine, which a fading purple color can characterize compared to the blank (no sample). Based on the results obtained from this study (Figure 4) shows that EE, EF, EAF, and HF have antioxidant activity in chelating Fe ions, which is weak with an IC\(_{50}\) value > 200 µg/mL compared to Na\(_2\)EDTA with antioxidant activity in chelating Fe ions with a value of IC\(_{50}\) is 90.59 µg/mL.
Figure 3. Graph of antioxidant activity testing using β–Carotene Bleaching method from ethanol extract (EE), an aqueous-ethanol fraction (EF), ethyl acetate fraction (EAF), hexane fraction (HF), and BHT as control positive which were determined antioxidant activity based on IC₅₀ values. The data were observed triplicate (n=3).

Figure 4. Graph of antioxidant activity testing using iron chelation method from ethanol extract (EE), an aqueous-ethanol fraction (EF), ethyl acetate fraction (EAF), hexane fraction (HF), and Na₂EDTA as a positive control, which was determined antioxidant activity based on IC₅₀ values.

4. Discussion
The hydroxyl radical method is one of the antioxidant activity tests to see the activity of a sample in reducing hydroxyl radicals. Hydroxyl radicals are the most reactive products of Reactive Oxygen Species (ROS) produced because of the reduction of one electron in cellular metabolism and can induce severe damage to adjacent biomolecules. Hydroxyl radicals are produced by a Fenton reaction between hydrogen peroxide (H₂O₂) and metal ions (Fe²⁺ or Cu⁺) to form hydroxyl radicals, which undergo oxidative damage by abstracting hydrogen from the amino acid carrier carbon be protein radicals centered on carbon and fatty acids. Unsaturated will affect the formation of lipid radicals and induce the breaking of DNA strands, thus triggering the emergence of degenerative diseases such as atherosclerosis, neurological disease, and several other degenerative diseases [20–22]. However, hydrogen peroxide is not a reactive free radical product. Hydrogen peroxide is a reducing agent in the Fenton reaction and will then react with metal ions or superoxide to form by-products, namely hydroxyl radicals (OH•). Under stress conditions, excess levels of O₂•⁻ superoxide will release free iron from ferritin, and the released free iron will react in a Fenton reaction to form OH• and OH• radicals can also occur by the presence of a Haber-Weiss reaction between superoxide radicals and H₂O₂ to form OH• radicals [20,22].
Fe$^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}^- \text{(Fenton Reaction)}$

The nitric oxide method is one of the evaluations of the antioxidant activity of a sample based on NO radical reduction. The principle of this method is based on the reduction of NO radicals produced by the formation of a purplish red color. NO radicals formed will be stabilized by the presence of antioxidant compounds. NO radical attenuation by the presence of antioxidant compounds can be characterized by color fading [16]. In the body, NO is produced by breaking arginine into citrulline by an enzyme in NADPH called nitric oxide synthase (eNOS). NO produced can be inhibited by cytochrome c oxidase, and some enzymatic reactions can neutralize NO. However, the increase in NO in the body triggers ROS and RNS in mitochondria, changing various processes such as mitochondrial biogenesis, respiration, and oxidative stress [23].

β-carotene bleaching is one of the methods used to evaluate a sample in inhibiting lipid peroxidase in linoleic acid emulsion media. The principle of the β-carotene bleaching method is based on inhibiting the rate of degradation of β-carotene during the oxidation process that occurs when linoleic acid turns into hydroperoxides that attack the double bonds of β-carotene so that β-carotene oxidation occurs, which causes loss of chromophore groups which gives color fading from β-carotene. The lipid peroxide radical species produced from linoleic acid hydroperoxide in the β-carotene emulsion system can be inhibited by non-polar antioxidant compounds to prevent the occurrence of linoleic acid hydroperoxides in the lipid phase. Meanwhile, antioxidant compounds with high polarity will be in the aqueous phase so that they are less effective in reducing linoleic acid hydroperoxides [17,24]. Corresponding results were obtained from this study which showed that the aqueous-ethanol fraction (EF) had a high IC$_{50}$ value compared to other samples. This result shows that the compounds contained in the EF are predominantly polar compared to the ethyl acetate and hexane fractions which have low IC$_{50}$ values. The low IC$_{50}$ value indicates the minor concentration capable of inhibiting the action of radicals by up to 50%.

The chelating iron method tests antioxidant activity by looking at the activity of a sample in chelating iron ions to prevent iron ion complexes from forming with ferrozine reagents, which will form complex bonds. The principle of the iron-chelating method is based on inhibition by the presence of a compound from the sample that can prevent the complex bonding that occurs in iron ions with a ligand, namely ferrozine, which can be characterized by the activity of a sample with a purple color change [18]. The tests show that the extract and fraction of Kersen fruit provide complex inhibition between Fe ions and Ferrozine ligands of > 200 µg/mL. These results showed weak activity when compared to the Na$_2$EDTA positive control. However, chemical components from extracts or fractions of Kersen fruit are still influenced by its bioactivity. Antioxidant activity testing with the Iron chelating inhibition method provides an overview of the effects of Fe Ions in the body. Iron is one of the essential elements in the body, and the body has tightly regulated the distribution of iron needs. However, excess iron in the body can cause genetic and non-genetic disorders. It can trigger free radicals in the body by an enzymatic reaction that can form free radicals that can cause several degenerative diseases [25,26]. According to [26], 71% of deaths in heart disease patients are due to iron accumulation in the myocardium, which causes significant complications from iron overload. This case can be avoided by suppressing LPI (Labile Plasma Iron) and eliminating excess iron. In the case of beta-thalassemia significant and hereditary hemochromatosis, the bleeding process is avoided because the patient is anemia, and one of the best treatments, in this case, is iron chelation therapy. Iron chelators (chelators) can bind metal ions for the drastic removal of excess iron.

5. Conclusion
The research results on antioxidant bioactivity from extracts and fractions from kersen fruit (Muntingia calabura L) gave different activities based on the testing method. In addition, the differences in each sample indicate differences in antioxidant power. The study results showed that the ethyl acetate fraction and ethanol extract of kersen fruit gave extreme activity.
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References
[1] Nur S, Mubarak F, Jannah C, Winarni D A, Rahman D A, Hamdayani L A and Sami F J 2019 Food Res. 7
[2] Pratama A N and Busman H 2020 J. Ilm. Kesehat. Sandi Husada 11 497–504
[3] Petersen R C 2017 Free-radicals and advanced chemistries involved in cell membrane organization influence oxygen diffusion and pathology treatment 54
[4] Xu D-P, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang J-J and Li H-B 2017 Int J Mol Sci 32
[5] Bagchi D, Bagchi M, Stohs S J, Das D K, Ray S D, Kuszynski C A, Joshi S S and Pruess H G 2000 Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention 12
[6] Moo-Huchin V M, Gonzalez-Aguilar G A, Moo-Huchin M, Ortiz-Vazquez E, Cuevas-Glory L, Sauri-Duch E and Betancur-Ancona D 2017 Chiang Mai J Sci 44 605–16
[7] Oroian M and Escriche I 2015 Food Res. Int. 27
[8] Nur S, Angelina A A, Aswad M, Yulianty R, Burhan A and Nursamsiar 2021 J Pharm. Pharmacogn. Res. 9 409–21
[9] Preethi K, Vijayalakshmi N, Shamna R and Sasikumar J M 2010 Pharmacogn. J. 2 8
[10] Rosalina Y K and Zakarias A M 2018 J Appl. Chem Sci 5 488–90
[11] Rotta E M, Haminiuk C W I and Maldaner L 2017 Int. J. Food Sci. Technol. 52 10
[12] Arum Y, Supartono and Sudarmin 2012 J. MIPA UNESA 35 165–74
[13] Mogollón O F C, Gonzalez-Cuello R E and López J S G 2018 Contemp. Eng. Sci. 11 881–90
[14] Preethi K, Premasudha P and Keerthana K 2012 Pharmacogn. J. 4 6
[15] Kutlu T, Kasim T, Bircan eken and Murat K 2014 J. Med. Plants Res. 8 715–26
[16] Nur S, Aisyah A N, Lukitaningsih E, Rumiyati, Juhardi R I, Rezkiawati Andirah and Hajar A S 2021 J. Appl. Pharm. Sci.
[17] Nur S, Rumiyati R and Lukitaningsih E 2017 Maj. Obat Tradis. 22 63
[18] Berker K I, Güçlü K, Demirata B and Apak R 2010 Anal. Methods 2 1770
[19] Dhaliwal J S and Singh H 2015 Free Radicals and Anti-oxidants in Health and Disease 2 3
[20] Lipinski B 2011 Oxid. Med. Cell. Longev. 9
[21] Phaniendra A, Jestadi D B and Periyasamy L 2015 Indian J. Clin. Biochem. 30 11–26
[22] Treml J and Šmejkal K 2016 Compr. Rev. Food Sci. Food Saf. 15 720–38
[23] Ozcan A and Ogun M 2015 Biochemistry of Reactive Oxygen and Nitrogen Species Basic Principles and Clinical Significance of Oxidative Stress ed S J T Gowder (InTech)
[24] Bogacz-Radomska L and Harasym J 2018 Food Qual. Saf. 2 69–74
[25] Kontoghiorghes G and Kontoghiorghe C 2020 Cells 9 1456
[26] Mobarra N, Shanaki M, Ehteram H, Nasiri H, Sahmani M, Saecidi M, Goudarzi M, Pourkarim H and Azad M 2016 A Review on Iron Chelators in Treatment of Iron Overload Syndromes. Volume 10 9