Formulation and Characterization of iron fortified food to treat anaemia in Children

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Abstract

This research aimed to formulate iron-fortified jellies. Raisins were selected as a source of iron as they are easily available in the local markets, cost-effective, and are used as the base material. Iron sulfate was used to enhance the iron content of the raisins. Two different solutions of iron sulfate were prepared (0.1 M and 0.5 M). Raisins were soaked in the solution, covered, and placed in the refrigerator for 7days. The solution was then drained off and iron-rich raisins were then ground to paste and added in gelatin mixture to make jellies. Phytochemical test, atomic absorption spectroscopy, sensory evaluation, proximate composition, and energy calculation were performed on the formulated product. The phytochemical analysis showed the presence of carbohydrates, alkaloids, flavonoids, tannins, terpenoids, and phenolic content while fat (oil), protein, and saponins were absent. The amount of iron content was determined which revealed that 0.5M jellies were better than 0.1M jellies in terms of iron content i.e., 0.5M iron-fortified jellies had more iron content (9.82 ± 1.79) than 0.1M iron-fortified jellies (3.29 ± 0.95). Sensory evaluation results revealed that jellies with less amount of added iron i.e., 0.1 M iron-fortified jellies were appreciated based on overall acceptability, while jellies with 0.5M iron content were bitter and rusty in taste. Proximate analysis was done based on the results of sensory evaluation and overall acceptability on 0.1M iron-fortified jellies which showed that jelly with 0.1 M iron content showed pH 3.82 ± 0.04, acidity $0.10 \pm 0.01\%$, Brix degree $38^0 \pm 0.1\%$, refractive index 1.3947 ± 0.1 nD, moisture content $7.6 \pm 0.1\%$, ash content $4 \pm 0.01\%$, crude fat 0.786 ± 0.001g, crude protein 0 ± 0.001g, crude fiber 87.714 ± 0.001g, and N-free extract 0.004 ± 0.01. This food can also be used as a food cum drug as it can replace traditional medication.

Keywords

Raisins, Iron-fortified jellies, Sensory Evaluation, Phytochemical analysis, Atomic Abs-

orption Spectrophotometer, Proximate Analysis.

1. INTRODUCTION

Iron plays a crucial role in the production of hemoglobin which carries oxygen in the body. Its deficiency can cause major disorders like iron deficiency and iron deficiency anemia (Percy and Mansour, 2017). Not only teenage girls and women mostly are at higher risk of this disorder but children also report this deficiency. The primary causes of iron deficiency are inadequate absorption of iron by the body, low intake of iron in the diet, use of medication (ibuprofen, aspirin), blood loss or heavy menstruation cycle, folic acid, and vitamin B12 deficiency. Different symptoms have been observed in patients (Ahmad and Ahmed, 2019) like pale skin, restless legs, muscle fatigue, brittle nails, dysphagia, altered taste, hair loss, shortness of breath, headache, weakness, feeling low (dizziness), and poor work concentration (Kumar *et al*., 2011). Iron deficiency is the most common nutritional deficiency (Elstrott *et al*., 2020) in the world which is the cause of most cases of anemia (Govindappagari and Burwick, 2019). It can be present with or without anemia and may result in fatigue, poor physical condition, initiate trouble in the learning process, and also can enhance the ratio of illness (Okam *et al*., 2017). Low intake of iron content in diet and its poor bioavailabilty (Aung *et al*., 2013) may cause this type of iron deficiency (Churio *et al*., 2018; Sachdeva *et al*., 2015). It can be diagnosed by performing different tests (V. *et al*., 2013) such as serum iron, ferritin, and transferrin saturation levels, bone marrow aspiration,mean cell volume (MCV), mean cell hemoglobin (MCH) (L. *et al*., 2017; Thomas *et al*., 2013). Upon diagnosis, iron stores should be restocked by using iron-rich diets such as meat, liver, leafy vegetables (spinach, broccoli), legumes, and peas, iron-enriched foods (cereals, grains, rice, and pasta). Most of these foods also have vitamin B, vitamin C, and folic acid (Tuso *et al*., 2013) which also helps to maintain the red blood cells of the body consequently helped to overcome iron deficiency (Saunders *et al*., 2012). Medicinal iron such as oral supplements which include (capsules, syrups, and pills) are available in the market containing different salts (ferrous sulfate, ferrous fumarate, ferrous gluconate). To increase the bioavailability of iron, ascorbic acid or vitamin C should be taken (Froessler *et al*., 2013). Intravenous iron (IV) and blood transfusion (Khalafallah and Dennis, 2012) can also be used in case of chronic disorders (K. and A.J., 2012).

Raisins belong to the species known as *Vitis vinifera* (Kelebek *et al*., 2013). They are dried grapes included in dried fruits and have valuable nutrients (Effie and Antonia, 2014) including vitamins, iron, potassium, and calcium, also they are fat-free. They have no added sugar and have several health benefits, polyphenols present in the raisins act as antioxidants (Meng *et al*., 2011).They provide an excellent source of dietary fibers and also serve as anti-inflammatory agents in the body (Schuster *et al*., 2017).

Various strategies have been developed to overcome this essential nutrient deficiency like food fortification, bio-fortification, and supple-mentation (Masuda *et al*., 2013). Food fortification involves a process of incorporating micronutrients into processed food sometimes known as enrichment. Staple foods such as cereals, salts, milk, flour, etc. are used to add specific micronutrients or macronutrients. Fortified foods help to reduce micronutrient deficiencies (Lindsay *et al*., 2006). The fortificants used for the iron fortification are, ferrous sulfate, ferrous fumarate, ferrous gluconate, sodium ferrice ethylenediaminetetraacetate (NaFeEDTA), etc. along with ascorbic acid to increase the bioavailability of the fortificants added (Diego Quintaes *et al*., 2017).

This research aimed to develop cost-effective iron-fortified food that is more attractive to children and has a good quantity of iron with high absorption rates. Iron-fortified jellies prepared in this research were cost-effective as everything used in this product was available easily. Many staple foods were fortified with iron recently but iron fortification of jellies has been done for the first time. Jellies have been selected for fortification purposes because children are attracted to them, this product not only provides them a taste but also can help to maintain iron content in their diet. Different techniques were used to characterize plain raisins and fortified jellies qualitatively and quantitatively such as phytochemical test, atomic absorption spectroscopy (AAS), sensory evaluation, proximate analysis, and energy calculation.

2. MATERIALS AND METHODS

Raisins, iron sulfate ($FeSO_4 \cdot 7H_2O$), gelatin, sugar, citric acid, food color, and flavor were used. Raisins were bought from the local market of Lahore and washed thoroughly, then air-dried and kept in an airtight container. Food-grade iron sulfate and gelatin were used for the preparation of iron-fortified jellies. Choosing the most suitable fortificant presented a challenge as products and iron compounds can interact. Out of the three water-soluble compounds listed in WHO and FAO document "Guidelines on Food Fortification with Micronutrients" (Lindsay *et al*., 2006) $FeSO_4 \cdot 7H_2O$ was selected as it is widely used fortificant, easily available, and cost-effective.

For the formulation and characterization of iron-fortified jellies, raisins were soaked in two different concentrations of iron sulfate i.e., 0.1 M and 0.5 M which were covered and placed in the refrigerator for 7 days. Raisins were then taken out from the iron sulfate solution, ground to paste, and boiled in water. This raisin solution was then strained so that pulp could be removed. The filtered solution was then boiled again and a mixture of sugar, gelatin, and citric acid was added. It is boiled until required consistency. Then Food color and flavor were added. Phytochemical analysis of plain raisins and iron-fortified jellies was done qualitatively. To determine iron content in plain raisins, iron-fortified raisins, and iron-fortified jellies AAS analysis was done. Sensory evaluation, proximate analysis, and energy value calculation were also done.

2.1. Phytochemical Analysis

Phytochemical analysis was done to determine the major constituent of the sample qualitatively (Rubab *et al*., 2021; Rubab, Rizwani, HASSAN, *et al*., 2022). 5g of Raisins and jellies were taken in separate conical flasks, then their extract was prepared for each sample using 30ml distilled water and ethanol (Mir *et al*., 2016). The extract was then filtered and used for phytochemical analysis (Lemino Singh and Bag, 2013).

Different tests were conducted on the extract qualitatively by utilizing the established protocols to identify the major chemical compounds found in plants (Rubab *et al*., 2021; Rubab *et al*., 2020; Rubab, Rizwani, Durrani, *et al*., 2022).

2.1.1 Test for Carbohydrates

1ml of the filtered extract was placed in the test tube. Few drops of Benedict's reagent was added to the test tube and warmed gently. Orange-red precipitate would indicate reducing sugars.

2.1.2. Test for Oil

A little amount of the extract was pressed between the two filter papers. Oil stain on the filter papers would suggest the presence of oil.

2.1.3. Test for Proteins

Xanthoproteic test was performed to check the presence of proteins. A small quantity of concentrated nitric acid were incorporatedinto the filtered extract which was taken in a test tube. The emergence of yellow color would show the presence of proteins.

2.1.4. Test for Alkaloids

1ml of the filtered extract was processed with few drops of picric acid, yellow-colored precipitates would show the presence of alkaloids.

2.1.5. Test for Flavonoids

To the extract, a few drops of alkaline reagent i.e., sodium hydroxide solution were introduced. The appearance of the yellow color would show the presence of flavonoids.

2.1.6. Test for Tannins

Few drops of ferric chloride solution were added to the extract, intense green, purple, blue, or black color would show the presence of tannins.

2.1.7. Test for Terpenoids

Salkowski's test was conducted to detect the presence of terpenoids in which 1ml of the extract was taken in the test tube, few drops of chloroform were added to the extract proceeded by the addition of a few drops of concentrated sulfuric acid, and reddish-brown color would indicate the presence of terpenoids.

2.1.8. Test for Saponins

2-3 ml of distilled water was taken and added to the extract, shaken strongly. This test is also known as the Froth test. Foam formation would show the presence of saponins.

2.1.9. Test for Phenolic compounds

In the lead acetate test few drops of lead acetate solution were added to the extract, white precipitates would indicate the presence of tannins.

2.2. Sensory Evaluation of Iron Fortified Jellies

Sensory evaluation of the iron-fortified jellies was done by a jury of 5 semi-trained members. Two samples of jelly with iron salt (0.1M and 0.5M) were evaluated. Jelly made from a premade mixture available in the market was used as a control. The jury was allowed to taste the products one by one and the Performa was filled by them. Assessment of sensory characteristic was done using a basic five points hedonic scale, where 5: extremely like, 3: not like nor a dislike, and 1: extremely disliked (Basu *et al*., 2011; Younas *et al*., 2021). The assessment was grounded on five characteristics: aroma, color, taste, appearance, and overall acceptability (Garrido *et al*., 2015).

2.3. Iron Content Determination

AAS analysis was done to assess the iron content in plain raisins, iron-fortified raisins, and iron-fortified jellies. For the AAS analysis, the sample was digested using 25ml aqua regia (HCl: HNO_3; 3:1) and deionized water. Deionized water was also used to reduce the concentration of the solution. The solution underwent the process of filtration using Whatman filter paper 0.42µ and stored in vials for further analysis (Demirel *et al*., 2008). The iron content was assessed using atomic absorption spectrophotometer (Varian 240-AAS).

2.4. pH, Acidity, Proximate Analysis & Energy Value Calculation

pH, acidity, refractive index, Brix value, moisture, ash content, crude fat, crude protein (Rubab *et al*., 2020), crude fiber, nitrogen-free extract, and energy calculations were determined. A pH meter was utilized to establish the pH of the sample.

For this objective, 0.3g of jelly sample was dissolved in 30mL of distilled water to make 0.1% solution. The pH meter was dipped in the sample solution and pH was noted. The acidity of the sample was determined using the acid-base titration method.

To determine the refractive index and Brix value of the sample refractive index was used. Readings must be noted at 25^0C for accuracy. Moisture, ash content, crude fat, crude protein, crude fiber, nitrogen-free extract, and energy calculations were determined by using standard analytical methods. All these factors were established based on the AOAC method (Ayanda *et al*., 2018) and readings were taken in triplicate.

The percentage calories in the iron-fortified jellies were calculated (Gul and Safdar, 2009) by multiplying the percentage of crude fat with 9 and crude protein and crude fiber with 4.

3. RESULTS AND DISCUSSION

Iron-fortified jellies were prepared and ready to use as a supplement for children of growing ages as they are attracted to jellies and candies. Jellies were prepared by soaking the raisins in two different concentrations of standard solutions i.e., 0.5 M and 0.1 M $FeSO_4$ solutions.The quantity of the raisins and the solutions were the same for each formulation. Then the mixture of gelatin containing citric acid and sugar was added and cooked under the same conditions and parameters. The jellies were then characterized qualitatively (phytochemical analysis, sensory evaluation) and quantitatively (AAS, proximate analysis, and energy calculation).

3.1. Phytochemical Analysis

Phytochemical analysis was done by following standard procedures (Sadiqa *et al*., 2021) on plain raisins and fortified jellies using water and ethanol as extracting material to reveal the presence of carbohydrates, oil, proteins, alkaloids, flavonoids, tannins, terpenoids, saponins, and phenols.

Phytochemical analysis of the plain raisins and iron-fortified jellies was done under the same conditions and results show the presence of carbohydrates, alkaloids, flavonoids, tannins, terpenoids, and phenols. The presence of these components in plants was reported by (Rani and Vijayanchali, 2021).

Phytochemical analysis of the fortified jellies having different quantities of salts showed the presence of carbohydrates, alkaloids, terpenoids, and phenols. Phytochemical analysis showed the presence of phenolic content in the raisins and iron-fortified jellies which can act as antioxidants in the body (Ghrairi *et al*., 2013).

The results obtained by the phytochemical analysis of plain raisins and fortified jellies were shown in Tables 1 and 2 respectively.

Table 1. Comparative analysis of phytochemical constituents using different solvents in a plain sample of raisins

Phytochemical Constituents	Chemical Tests	Ethanol Extract of Plain Raisins			Water Extract of Plain Raisins		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Test for Carbohydrates	Benedict's Test	+ve	+ve	+ve	+ve	+ve	+ve
Test for Oil		-ve	-ve	-ve	-ve	-ve	-ve
Test for Proteins	Xanthoproteic Test	-ve	-ve	-ve	-ve	-ve	-ve
Test for Alkaloids	Picric Acid Test	+ve	+ve	+ve	+ve	+ve	+ve
Test for flavonoids	Alkaline Reagent Test	+ve	+ve	+ve	+ve	+ve	+ve
Test for Tannins	Ferric chloride Test	+ve	+ve	+ve	+ve	+ve	+ve
Test for Terpenoids	Salkowoski's Test	+ve	+ve	+ve	+ve	+ve	+e
Test for Saponins	Froth Test	-ve	-ve	-ve	-ve	-ve	-ve
Test for Phenolic Compound	Lead acetate Test	+ve	+ve	+ve	+ve	+ve	+ve

Keys used: +ve = present; -ve = absent

Table 2. Comparative analysis of phytochemical constituents using different solvents in 0.1M, 0.5M formulated jellies

Phytochemical Constituents	Chemical Tests	Ethanol Extract of 0.1M Jellies	Water Extract of 0.1M Jellies	Ethanol Extract of 0.5M Jellies	Water Extract of 0.5M Jellies
Test for Carbohydrate	Benedict's Test	+ve	+ve	+ve	+ve
Test for Oil		-ve	-ve	-ve	-ve
Test for Proteins	Xanthoproteic Test	-ve	-ve	-ve	-ve
Test for Alkaloids	Picric Acid Test	+ve	+ve	+ve	+ve
Test for flavonoids	Alkaline Reagent Test	-ve	-ve	-ve	-ve
Test for Tannins	Ferric chloride Test	-ve	-ve	-ve	-ve
Test for Terpenoids	Salkowoski's Test	+ve	+ve	+ve	+ve
Test for Saponins	Froth Test	-ve	-ve	-ve	-ve
Test for Phenolic Compound	Lead acetate Test	+ve	+ve	+ve	+ve

Keys used : +ve = present; -ve = absent

3.2. Sensory Evaluation

Sensory evaluation of the iron-fortified jellies was done by a jury of 5 semi-trained members. The results of the sensorial evaluation are presented in Figs. 1 and 2 for 0.5 M and 0.1 M iron-fortified jellies respectively. The findings indicated that there was no notable distinction in the color and aroma of all the fortified samples (Podder *et al.*, 2018). The rating for the appearance indicated that there was a substantial variance between the appearance of 0.5M and 0.1M iron-fortified jellies, while 0.5M iron-fortified jellies were off tone.

The ratings for the taste and overall acceptability showed a slight difference in all fortified samples. The panelists gave preference to the jellies fortified with the least amount of salt i.e., 0.1M iron-fortified jellies. Based on the taste and overall acceptability it was concluded by the panelists that the flavor of the product became rusty and rancid with the increasing amount of salt.

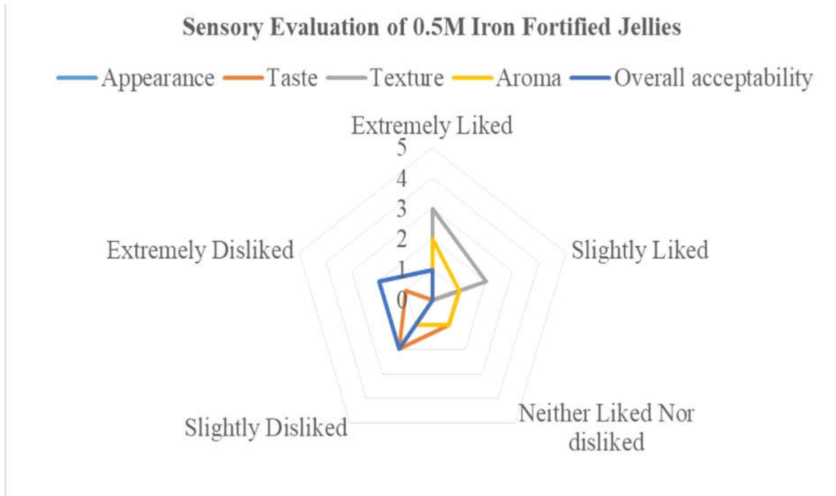

Fig. 1: Sensory evaluation of 0.5M iron fortified jellies

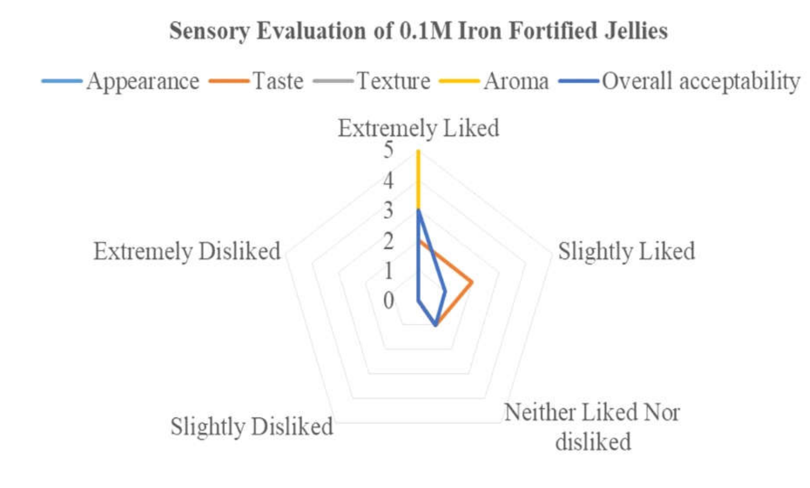

Fig. 2: Sensory evaluation of 0.1M iron fortified jellies

3.3. Determination of Iron Content

Atomic absorption spectrophotometer was employed to determine the iron content in plain raisins and iron-fortified jellies quantitatively. Table 3 showed the iron content in raisins (plain samples, soaked samples), and iron-fortified jellies.

A comparative analysis was done to check the quantitative values of iron in plain raisins, soaked raisins, and fortified jellies. The values showed a significant difference in iron content. The concentration of iron content in iron-fortified jellies elevated with a rise in iron content in the salt solution (Podder *et al.*, 2017). While the concentration of iron in iron-fortified jellies decreased with the decreased amount of iron in the salt solution. In all these samples, jellies fortified with 0.1M and 0.5M iron sulfate solution have 3.29 ± 0.95ppm and 9.82 ± 1.79ppm of iron content respectively.

Table 3. Iron content in plain raisins, soaked raisins and iron fortified jellies using AAS

Iron Content (ppm) in plain raisins, soaked raisins and iron fortified jellies		
	Samples	**Iron (ppm)**
Plain Samples	Sample A	3.03 ± 0.89
Soaked Samples 0.1M $FeSO_4$	Sample B	143.8 ± 12.1
Soaked Samples 0.5M $FeSO_4$	Sample C	114.0 ± 1.38
Formulated Jellies 0.1M $FeSO_4$	Sample D	3.29 ± 0.95
Formulated Jellies 0.5M $FeSO_4$	Sample E	9.82 ± 1.79

3.4. pH, Acidity, Proximate Analysis & Energy Value Calculation

pH, acidity, and proximate analysis were done based on AAS results and sensory evaluation which showed the least content of iron in the jellies in 0.1 M iron-fortified jellies because they were given the best results for sensory acceptability.

The analysis included pH, acidity, Brix degree, refractive index, moisture, and ash content. Table 4 showed the outcomes of the pH, acidity, and proximate analysis.

According to the results pH of the jellies was 3.82 ± 0.04 which is near to the authorized ratio of 2.8-3.4 (Silva and Lidon, 2016), while the acidity of the sample was 10 ± 0.01%. Brix degree value for jellies was allowed to be 40^0 while the Brix degree of the jellies was 38^0 ± 0.1%which was near to the allowed values and the refractive index 1.3947 ± 0.1 nD was corresponding to the Brix degree (Shams Najafabadi et al., 2021). The ash content of the jellies was 4 ± 0.01% while the moisture content of the jellies was 7.6 ± 0.1%.

Table 4. Results of pH, acidity and proximate analysis in 0.1 M iron-fortified jellies

Proximate tests	**0.1 M Iron fortified jellies**
pH	3.82± 0.04
Acidity	0.10± 0.01%
Brix degree	38^0± 0.1%
Refractive index	1.3947± 0.1 nD
Moisture content	7.6± 0.1%
Ash content	4 ± 0.01%

Energy value calculation (Jannaty *et al.*, 2020) was done by following the standard procedures which include the determination of moisture content 7.6 ± 0.1%, ash content 4 ± 0.01%, crude fat 0.786 ± 0.001g, crude protein 0 ±0.001g, crude fiber 87.714 ±0.001g, and N-free extract 0.004±0.01. Table 5 showed the result of the energy value calculation of the 0.1M iron-fortified jellies was calculated as 358 Kcal/100 g.

Table 5. **Energy value calculation of 0.1M iron fortified jelly**

Nutrient	**0.1 M Iron Fortified Jelly**
Moisture content	7.6± 0.1%
Ash content	4 ± 0.01%
Crude fat	0.786 ± 0.001g
Crude protein	0 ±0.001g
Crude fiber	87.714 ±0.001g

N-free extract	0.004±0.01
Energy value	358 Kcal/100 g

Comparative analysis of 0.5 M and 0.1 M iron-fortified jellies showed that one jelly is better than the other in different aspects. 0.5 M jelly has more iron content than 0.1 M jelly while 0.1 M jelly was good in taste, appearance, aroma, and overall acceptability.

In this research raisins and iron salts were used as a base material and the iron content of theraisins was increased by soaking them in two different concentrations of iron. The AAS analysis of iron-fortified raisins and iron-fortified jellies showed an increase in iron content. However, the flavor and appearance of the product can be improved by the use of micro-encapsulated iron salts. Furthermore, taxonomic studies are recommended (Bahadur *et al*., 2020).

4. CONCLUSION

Iron deficiency is a widespread problem in developing countries in the world. It affects men, women, and children but mostly it affects the women and growing kids. Severe iron deficiency may result in iron deficiency anemia ultimately death. This research led to the preparation of iron-fortified jellies for consumption by children to overcome their iron deficiencies as children are attracted to jellies and sweets. The analysis of the phytochemicals revealed the presence of phenolic content in the jellies which are good antioxidant agents. The sensory evaluation revealed that jellies that contain the least salt ($FeSO_4$) were good in overall attributes, while jellies with higher salt had some rusty taste and were not appreciated by the panel. AAS values showed an increased iron content in the jellies than in plain raisins.

Proximate analysis was also done to check the pH, the acidity of the fortified jellies, and results showed the allowed range of those attributes. The energy value calculation showed that it contains 0.786 g of fats, 0 g of proteins, 87.714 g of crude fiber, and the energy value of the 0.1 M iron-fortified jellies was 358 Kcal/100 g.

Conflict of Interest

The authors declare no conflict of interest.

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