Biotechnology Reports 20 (2019) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Cooking with EDTA reduces nutritional value of *Vicia faba* beans

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ARTICLE INFO

Article history:
Received 19 January 2019
Received in revised form 1 March 2019
Accepted 1 March 2019

Keywords:
EDTA
Vicia faba
Nutritional values
Minerals
Amino acids

ABSTRACT

Ethylenediamine terta-acetic acid (EDTA) used to accelerate the cooking process of *Vicia (V. faba)* beans. In this study, the effect of cooking with EDTA on the nutritional value of *V. faba* beans was addressed. Water contents, total proteins, lipids, carbohydrates, minerals and amino acids were determined before and after boiling with EDTA (2 g/L). In both of whole beans and seed coats, the water content was increased after boiling with EDTA. In contrast, the levels of proteins, lipids and carbohydrates were significantly decreased in both the whole beans and seed coats upon boiling with EDTA. Furthermore, the levels of sodium were increased while, the levels of other minerals were decreased. All amino acids were significantly decreased in the whole beans and increased in the seed coats after boiling with EDTA. EDTA addition to *V. faba* beans during the cooking process decreased the nutritional value of the cooked *V. faba* beans.

1. Introduction

Cooked *Vicia faba* beans have been one of the most popular dishes in the Egyptian meals over years [1]. *Faba* beans are rich sources of proteins, vitamins (B1, B2, B3 and B6), minerals (K, Mg, Cu, Zn, P and Fe) and essential fatty acids (linoleic, linolenic). Due to its hard seed coat, prolonged soaking and cooking times are required [2]. The soaking process helps to soften the seed coat and decrease the time required for cooking. In addition, phytic acid which is an antinutrient that interferes with the absorption of minerals is partially eliminated [3]. To accelerate the cooking process, chefs use additives such as citric acid, and sodium bicarbonate [4].

Ethylenediamine tertaacetic acid (EDTA) has been used for metal intoxication chelation therapy including mercury, lead and iron [5]. The chelation properties of EDTA are also employed to prevent blood coagulation [6], preserve ophthalmic preparations [7] and to decrease the complications of certain types of anemia and blood transfusion [8]. The Cr (III) complex with EDTA is used in medical diagnosis to assess the glomerular filtration rate [9]. EDTA was also used as an antimicrobial agent for wound care [10] and intraocular lens implantation [11]. The lowest reported toxic dose of EDTA was found to be 750 mg/kg/day [12]. EDTA addition to food products widely accepted due to the high safety margin renders. Recently, EDTA uses for *faba* beans cooking in Egyptian restaurants. However, using EDTA during the cooking process of beans may affect their nutritional value. To our knowledge until now, this point is not addressed clearly. Therefore, in this study, we aim to assess the direct impact of EDTA addition during the cooking process on the nutritional values of *V. faba* beans regarding to the total proteins, lipids, carbohydrates, some minerals and amino acids.

2. Results

2.1. Cooking of V. faba beans with EDTA decreased the cooking time and increased the color development

The approximate amount of EDTA which might be used by the Egyptian Chiefs to accelerate the beans cooking time is about 1–3 g/L. To assess the exact cooking time of *V. faba* beans in the presence of EDTA, two concentrations of EDTA (0.5 and 2 g/L) were added to certain weight of *V. faba* beans during the cooking process (Fig. 1). The results showed that the color was developed after 15 min of boiling (Fig. 2A–C) and gradually increased with the increase of the boiling time (Fig. 2D–F). As shown in Fig. 1, the color

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https://doi.org/10.1016/j.btre.2019.e00322

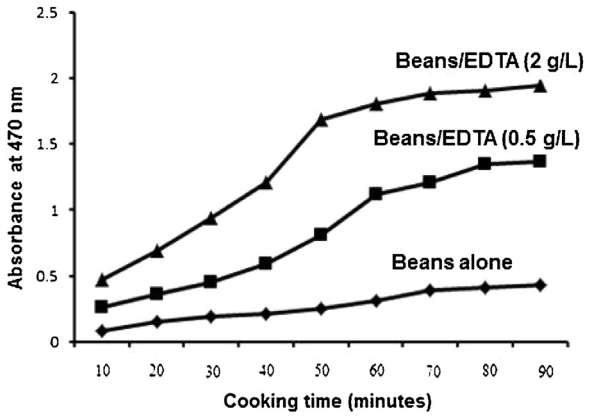

Fig. 1. Spectrophotometric absorbance changes in cooking media after boiling of beans with or without EDTA addition. The absorbance of the developed color was measured every 10 min. in each condition at 470 nm for 90 min.

development was directly proportional with the concentration of EDTA. Ninety minutes of boiling were enough to cook *faba* beans in the presence of 2 g/L EDTA as compared with 6 h in boiling water only (Fig. 2G–I).

2.2. Cooking of V. faba *beans with EDTA showed new peaks by ESI-MS*

To investigate the mechanism by which EDTA decreases the cooking time, ESI-MS analysis of EDTA was assessed before and after cooking with beans. The fragmentation pathway of EDTA was shown in Fig. 3. The results showed that there were three peaks characterized the different forms of EDTA without boiling as a following: a peak at $m/z = 293$ for free EDTA, a peak at $m/z = 315$ for the monosodium salt and a peak at $m/z = 337$ for the disodium salt. The other peaks at $m/z = 132,160,$ 247 are for the hydrolytic fragments of EDTA (Fig. 4A). The effect of temperature on EDTA was studied by boiling with dist. H_2O for 90 min, as shown in Fig. 4B, there were no significant differences observed as compared with ESI-MS analysis of EDTA without boiling. Boiling of EDTA with beans for 90 min showed the appearance of new peaks due to formation of EDTA complexes with leached minerals. The peak of the disodium salt of EDTA disappeared which suggests a displacement reaction between sodium the other mineral that leads to formation metal chelates with EDTA (Fig. 4C).

2.3. Cooking of V. faba *beans with EDTA increased the water contents and decreased proteins, lipids and carbohydrates contents*

We further determined the water contents, total proteins, lipids and carbohydrates in both of the whole beans and the seed coats before and after boiling with EDTA (2 g/L) for 90 min. The data showed that the percentages of water content were increased in the presence of EDTA in both of whole beans and seed coats. The levels of proteins, lipids and carbohydrates significantly decreased in seed coats and slightly decreased in the whole beans after cooking with EDTA (Table 1). We found also that EDTA addition significantly increased proteins, lipids and carbohydrates contents in the cooking media when compared to their control conditions (Data not shown).

2.4. Cooking of V. faba *beans with EDTA changed the* V. faba *minerals contents*

To further study, the mechanism by which EDTA accelerates the cooking process, ICP-MS was used to quantify various mineral including potassium (K), manganese (Mn), iron (Fe), magnesium (Mg), zinc (Zn), sodium (Na) and calcium (Ca) in both the seed coat and the cooking medium after boiling with different concentrations of EDTA for 90 min. As shown in Table 2, the amounts of K, Fe, Mg and Ca significantly decreased in the seed coat after boiling with EDTA and these decreases were dependent on the EDTA concentration. In cooking medium (supernatant) only Mg content showed a marked increase while Cu showed a marked decrease. Interestingly, the level of sodium (Na) significantly increased in the seed coats upon addition of EDTA when compared to their control conditions this increase was concentration dependent (data not shown).

2.5. Cooking of V. faba *beans with EDTA changed the* V. faba *amino acids contents*

The effect of EDTA on the amino acid content of *V. faba* beans was studied after cooking for 90 min. As shown in Tables 3 and 4, addition of EDTA to beans lead to an observable decrease for all the studied amino acids contents. The highest % decrease (relative to the initial amount in the whole beans) was observed with the sulfur-containing amino acids (cysteine and methionine) while the highest absolute decrease was obtained with the acidic amino acids (aspartate and glutamate). Interestingly, adding EDTA to seed coats only and boiled for 90 min showed a significant increase in all amino acids contents (Tables 3 and 4).

Cooking of V. faba beans in the presence of EDTA also led to changes in the levels of vitamins and phytochemical composition (data not shown).

3. Discussion

Vicia faba beans are considered as good source nutrients such as proteins, carbohydrates, lipids, vitamins, fibers, essential minerals such as iron, zinc and calcium [13]. Due to the presence of hard coats of beans, it needs to long time for cooking which increase the coast of the cooking process. Recently, EDTA has been used as an additive to accelerate the cooking process of *V. faba* beans and to reduce the cost effective. Far from several medical applications of EDTA, due to the high safety margin of EDTA, it used for other applications in food industry [14]. United States Standards of Identity and Food Additive Regulations permitted addition up to 165 ppm of disodium EDTA to canned beans [15]. In this study, we addressed the impact of EDTA addition on the nutritional value of *V. faba* beans during the cooking process regarding to their proteins, lipids, carbohydrates, minerals and amino acids contents.

In this study, we found that the addition of EDTA during the cooking process decreased the cooking time 4 folds. This finding was in agreement with a previous study which showed that addition of some chemical compounds as additives such as citric acid and sodium bicarbonate to *faba* beans accelerated the cooking process by decreasing the cooking time from 120 min to 75 min [16]. According to our findings, the chelation activity of EDTA with some minerals of the seed coat could be the reason to accelerate the cooking time.

During the boiling process, the color of the cooking media of beans alone or beans/EDTA was changed. We have found that an increase in the color development due to EDTA addition and this change was dependent on EDTA concentrations. This finding could be due to the release of some components such as minerals and other elements or due to the chelation activity of EDTA to some metals. The development of the dark color early after adding EDTA to beans could be due to the increase in the degradation rate of the pigments. Our finding was in agreement with previously reported study showed that the presence of Cu and Fe led to change the color of the cooking medium [17].

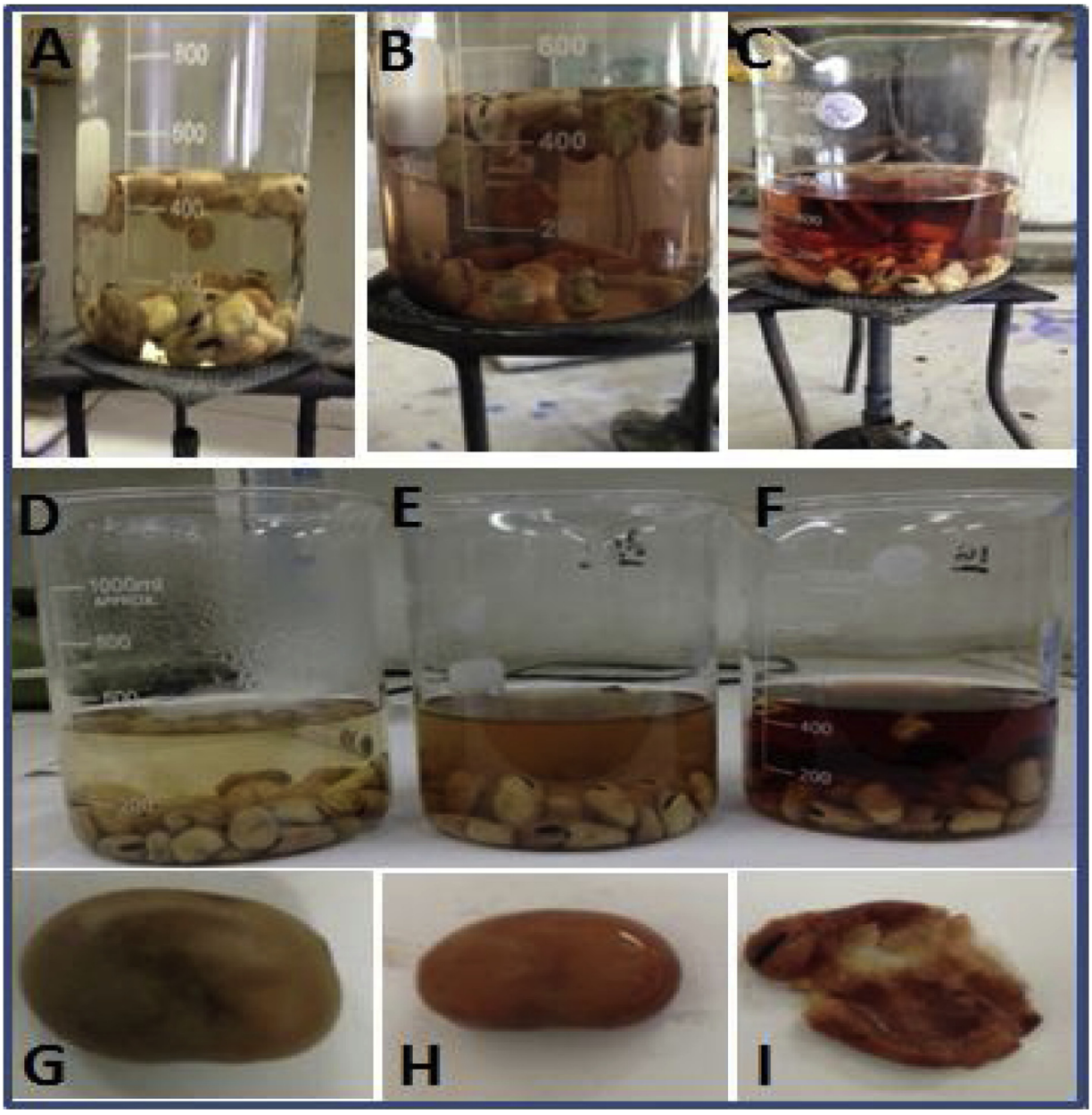

Fig. 2. Effect of cooking of *V. faba* beans with EDTA on the color of the cooking media. The color changes after 15 min. (A) No EDTA, (B) 0.5 g/L EDTA, (C) 2 g/L EDTA. The color changes after 90 min, (D) No EDTA, (E) 0.5 g/L EDTA and (F) 2 g/L EDTA. The degree of ripening after boiling 90 min. (G) No EDTA, (H) 0.5 g/L EDTA and (I) 2 g/L EDTA.

Interestingly, addition of EDTA to the *V. faba* beans during the cooking process increased the level of Na in the seed coats. The ESI-MS analysis showed that the peak of the EDTA disodium was disappeared when EDTA were added to the *V. faba* beans during the cooking process which could be the reason of the increase of the Na level in the seed coats. Several beaks were also found when EDTA was added to the beans during the cooking process, which could due to the formation of different complexes with EDTA. Due to boiling process, the content of water was increased in the whole beans and their seed coats, and this finding was in agreement with Deshpande & Cheryan, 1986 [18]. Upon boiling of *faba* beans with EDTA for 90 min, the results showed that the water contents significantly increased in these compartments when compared to their control conditions. This could be due to the increase of sodium contents in beans tissues.

It has been reported that, *faba* beans cooking lead to loss of some nutrients including proteins, lipids and carbohydrates [17]. The obtained data showed that total proteins, lipids and carbohydrates were reduced in both of *faba* whole beans and seed coats after boiling with water, Further, along with boiling with EDTA, there was a significant decrease in these nutrients in the whole beans and seed coats were observed compared with the control that boiled in water. EDTA could be facilitate cell wall separation during the cooking process through ion exchange and chelation between monovalent cations (Na^{+}) in solution and divalent cations (Ca^{+2} & Mg^{+2}) in the middle lamella [18]. Online with the previous statements, our results showed that a significant decrease in the Ca and Mg levels and a significant increase in Na levels in seed coats upon boiling with EDTA. This result was in agreement with other studies reported that soaking by using EDTA accelerated the cooking process of *V. faba* beans [12]. Having these facts, the chelation properties of EDTA may affect the mineral contents in beans that in turn decreased their nutritional value.

Our results reported that the addition of EDTA to the whole beans during the cooking process led to a marked decrease in all

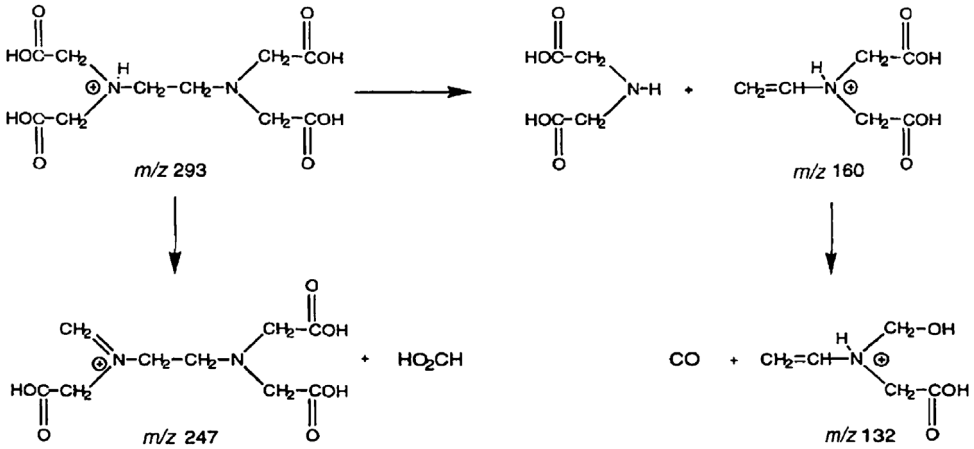

Fig. 3. The pathway of EDTA fragmentation.

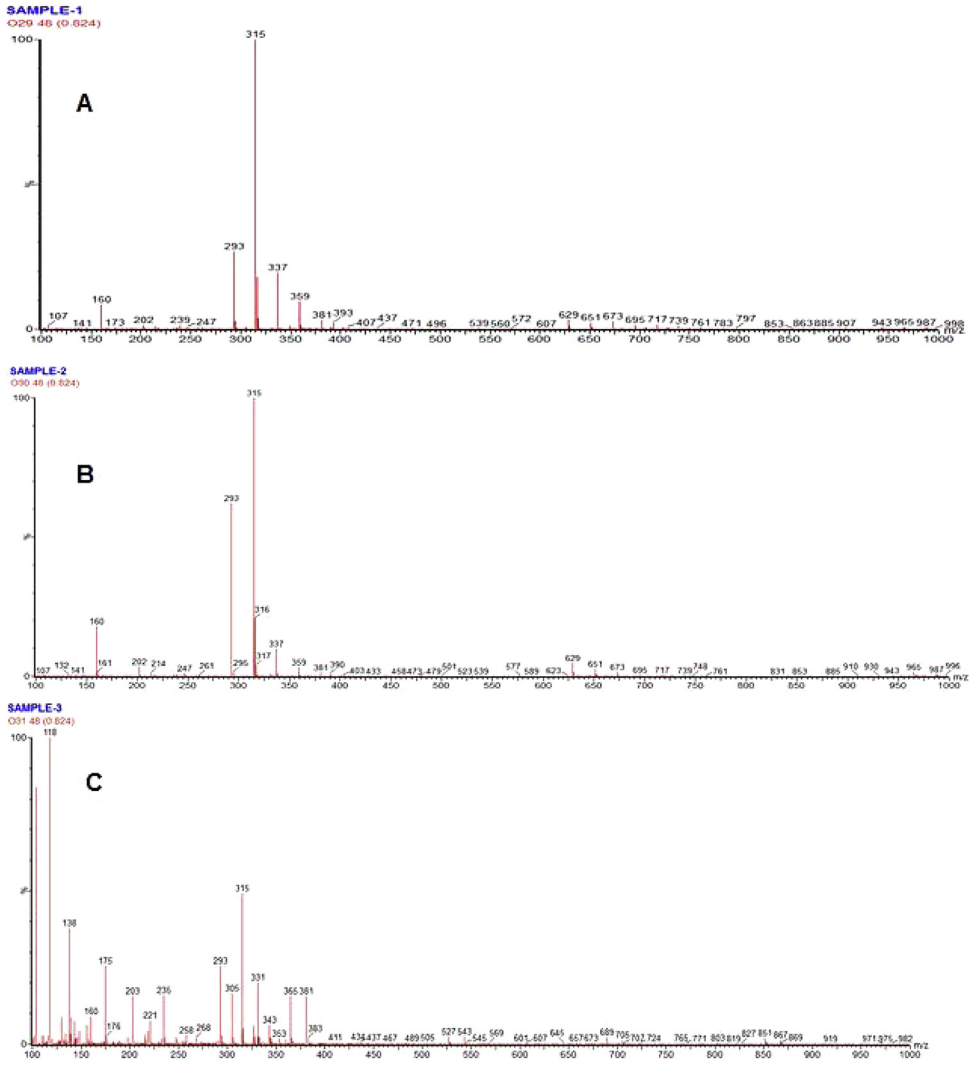

Fig. 4. ESI-MS analysis of EDTA solution, boiled EDTA and boiled EDTA with *V. faba* beans for 90 min. (A) positive ion ESI-MS of EDTA solution (Peak of *m/z* 293 represent the presence of free EDTA; Peaks of *m/z* 315 and 337 represent the presence of EDTA monosodium and disodium; Peaks of *m/z* 132,160, 247 represent a hydrolytic fragment of EDTA). (B) Positive ion ESI-MS of boiled EDTA solution (Peak of *m/z* 293 for free EDTA; Peaks of *m/z* 315 and 337 for EDTA monosodium and disodium; Peaks of *m/z* 132,160, 247 represent a hydrolytic fragment of EDTA). (C) Positive ion ESI-MS of EDTA boiled with *V. faba* beans (Peak of *m/z* 293 represent the presence of free EDTA; Peaks of *m/z* 315 represent the presence of EDTA monosodium).

Table 1
Water content, proteins, lipids and carbohydrates in the whole beans and beans seed coats before and after boiling with water or with EDTA.

Parameters*	Whole beans		
	Before boiling	Boiling/H_2O	Boiling/EDTA (2 g/L)
Water contents	3.4 %[c]	24.07 % [b]	39.43 % [a]
Total proteins	14.2 ± 0.61[a]	13.9 ± 0.78[a,b]	11.8 ± 0.87[b]
Total lipids	15.2 ± 0.09 [a]	13.4 ± 0.07 [b]	12.5 ± 0.06 [c]
Total carbohydrates	18.9 ± 0.14 [a]	8.9 ± 0.12 [b]	4.1 ± 0.15 [c]
	Seed coats		
Water contents	8.23 %	17.03 % [b]	54.28 % [a]
Total proteins	14.0 ± 0.85 [a]	9.5 ± 0.68 [b]	6.1 ± 0.76 [c]
Total lipids	14.7 ± 0.71[b]	16.5 ± 0.61 [a]	10.2 ± 0.51 [c]
Total carbohydrates	15.5 ± 0.11 [a]	7.8 ± 0.13 [b]	2.6 ± 0.16 [c]

* Total proteins, lipids and carbohydrates were expressed as mg/100 mg d. wt.
T-test was used to compare the values of the means from two samples, significant differences were indicated when $p < 0.05$.

Table 2
Electrolytes concentration levels in the whole beans and seed coats before and after boiling with water or with EDTA.

Parameters*	Whole beans		
	Before boiling	Boiling/H_2O	Boiling/EDTA (2 g/L)
Potassium (K)	80 ± 0.1[a]	73 ± 0.6 [b]	70 ± 0.09 [c]
Manganese (Mn)	0.057 ± 0.04 [b]	0.076 ± 0.04 [a]	0.067 ± 0.005 [a, b]
Iron (Fe)	1.2 ± 0.05 [c]	1.8 ± 0.05 [a]	1.5 ± 0.09 [b]
Magnesium (Mg)	11.8 ± 0.2 [b]	12.4 ± 0.2 [a]	10.1 ± 0.05 [b]
Zinc (Zn)	0.196 ± 0.04 [a]	0.198 ± 0.04 [a]	0.260 ± 0.07[a]
Sodium (Na)	14.3 ± 0.3 [b]	12.1 ± 0.3 [c]	43 ± 0.09 [a]
Calcium (Ca)	19.6 ± 0.1[b]	20.4 ± 0.1[a]	15.3 ± 0.1[c]
	Seed coats		
Potassium (K)	72 ± 0.12 [a]	61 ± 0.6 [b]	48 ± 0.7 [c]
Manganese (Mn)	0.088 ± 0.04[b]	0.112 ± 0.04 [a]	0.023 ± 0.008[b]
Iron (Fe)	1.1 ± 0.05 [a]	0.7 ± 0.05 [b]	0.5 ± 0.03 [c]
Magnesium (Mg)	21.7 ± 0.2 [a]	17.3 ± 0.2 [b]	7 ± 0.4 [c]
Zinc (Zn)	0.052 ± 0.04 [c]	0.066 ± 0.04 [b]	0.1 ± 0.07 [a]
Sodium (Na)	24.8 ± 0.3 [b]	13.4 ± 0.3 [c]	56 ± 0.08 [a]
Calcium (Ca)	40.7 ± 0.1 [a]	41.6 ± 0.1 [b]	17.4 ± 0.1 [c]

T-test was used to compare the values of the means from two samples, significant differences were indicated when $p < 0.05$.

Table 3
Free amino acids constitute in the whole beans seeds after boiling with water or with EDTA.

Amino acid	Boiling/H_2O	Boiling/EDTA (2 g/L)	*t- Value*	*p- Value*
Aspartic acid	247.24 ± 2.1	221.42 ± 1.02	19.156	0.000
Threonine	84.33 ± 1.5	73.98 ± 1.00	9.944	0.001
Serine	83.79 ± 0.7	77.37 ± 1.25	7.762	0.001
Glutamic acid	342.57 ± 1.4	316.43 ± 0.73	28.676	0.000
Proline	199.52 ± 0.9	185.46 ± 0.86	19.563	0.000
Glycine	90.43 ± 1.1	79.35 ± 0.93	13.323	0.000
Alanine	98.71 ± 1.03	98.08 ± 1.1	0.724	0.509
Cysteine	79.98 ± 1.03	62.40 ± 0.73	24.119	0.000
Valine	77.02 ± 0.6	69.10 ± 0.28	20.718	0.000
Metheonine	15.93 ± 1.13	13.15 ± 1.38	2.700	0.054
Isoleucine	60.03 ± 1.09	52.21 ± 0.97	9.283	0.001
Leucine	142.20 ± 0.7	129.24 ± 0.94	19.153	0.000
Tyrosine	52.13 ± 1.5	49.14 ± 0.98	2.890	0.045
Phenyl alanine	71.08 ± 1.8	65.05 ± 1.11	4.939	0.008
Histidine	101.81 ± 1.2	95.01 ± 1.03	7.448	0.002
Lysine	143.21 ± 1.4	128.79 ± 0.98	14.615	0.000
Arginine	158.94 ± 1.9	149.83 ± 1.36	6.753	0.003

T-test was used to compare the values of the means from two samples, significant differences were indicated when $p < 0.05$.

amino acids composition, particularly in the relative percentage of cysteine and methionine that could be an indication of the effect of EDTA on protein denaturation might be mainly through breaking the disulfide linkage. In contrast, there was significant increase in

Table 4
Free amino acids constitute in beans seed coats after boiling with water or with EDTA.

Amino acid	Boiling/H_2O	Boiling/EDTA(2 g/L)	*t- Value*	*p- Value*
Aspartic acid	12.29 ± 1.05	27.30 ± 1.04	17.592	0.000
Threonine	3.71 ± 1.00	9.61 ± 1.02	7.154	0.002
Serine	3.98 ± 1.05	11.08 ± 0.94	8.726	0.001
Glutamic acid	12.45 ± 1.12	26.31 ± 0.73	17.957	0.000
Proline	3.92 ± 0.96	13.15 ± 1.10	10.950	0.000
Glycine	8.22 ± 1.17	34.35 ± 0.94	30.156	0.000
Alanine	5.41 ± 1.14	19.79 ± 1.06	16.000	0.000
Cysteine	5.70 ± 0.88	17.69 ± 1.15	14.341	0.000
Valine	3.17 ± 0.81	12.14 ± 0.92	12.675	0.000
Metheonine	0.21 ± 0.11	1.85 ± 0.55	5.064	0.007
Isoleucine	2.57 ± 0.85	8.50 ± 1.28	6.685	0.003
Leucine	5.46 ± 0.90	17.48 ± 1.05	15.054	0.000
Tyrosine	2.56 ± 0.74	12.57 ± 0.79	16.017	0.000
Phenyl alanine	2.47 ± 0.73	9.81 ± 1.23	8.888	0.001
Histidine	5.76 ± 1.12	17.68 ± 0.93	14.182	0.000
Lysine	8.80 ± 0.95	19.20 ± 0.89	13.838	0.000
Arginine	7.34 ± 0.85	11.41 ± 1.16	4.902	0.008

T-test was used to compare the values of the means from two samples, significant differences were indicated when $p < 0.05$.

all amino acids contents of the bean's seed coats upon boiling with EDTA when compared to seed coats without boiling or boiled in water. This finding could be due to the capability of EDTA to chelate different minerals from the seed coats and/or to degrade proteins constituents in the seed coat membranes percolating free amino into the boiling water of cooking media. It has been shown that high ionic strength anions in EDTA are thought to denature protein and then shortening the cooking times [19]. This decrease in the amino acid content diminishes the nutritional value of *V. faba* beans and emphasis the importance of finding alternative ways for a healthy and efficient cooking process.

4. Conclusion

Addition of EDTA decreased the nutritional values of *Vicia faba* beans including proteins, lipids, carbohydrates, minerals and amino acids. Therefore, adding EDTA to the *V. faba* beans during the cooking process to reduce the cooking time is not recommended due to the loss of essential nutrients.

5. Materials and methods

5.1. Materials

Vicia faba beans were purchased from the local market in Egypt. Ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma (Sigma-Aldrich, CO. LLC, USA).

5.2. Estimation of the cooking time and color development

Fifty grams (50 g/L) of *V. faba* beans alone, 50 g/L of *V. faba* beans/EDTA (0.5 g/L) and 50 g/L of *V. faba* beans/EDTA (2 g/L) were prepared, where the ratio of beans to EDTA was 25:1. These mixtures were boiled by using the laboratory benzene flame and beans were examined every 10 min to test the cooking time required for ripening. To estimate the change of the cooking media color in the above mentioned conditions, 1 ml of the cooking media was used to detect the development of the color by reading the absorbance of colors developed every 10 min at 470 nm.

5.3. Electrospray ionization-mass spectrometry (ESI-MS) analysis

To prepare samples for ESI-MS analysis, 2 g/L of EDTA was used at ambient temperature added to 50 g beans. After boiling for

90 min, the supernatants were filtered and then stored at −20 °C for ESI-MS analysis. ESI-MS positive and negative ion acquisition modes were carried out on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA, USA).

5.4. Determination of water content

Water contents in the whole beans and seed coats under different treatments were determined as follows: A specific weight of samples were placed in clean dry and pre-weighed crucible and then placed in an oven at 100 °C for 2 h, cool then weight. Further placements in the oven will be carried out until approximately a constant weight will be obtained. Moisture content (%) = (W2 – W1) – (W3 – W1) / (W2 – W1) X 100. Where: W1 = weight of empty crucible, W2 = weight of crucible with the sample, W3 = weight after drying.

5.5. Determination of total proteins, lipids and carbohydrates

Whole beans and seed coats without any treatments were used as controls, beans and their seed coats were boiled in the presence and absence of EDTA, tissues under different conditions were collected, dried, homogenized and kept for analysis.

Lowry et al. method was followed to estimate the total protein. Briefly, proteins were precipitated by 10% trichloroacetic acid, collected by centrifugation and digested in NaOH (0.1 N), then centrifuged. To 1 mL of supernatant, 5 mL of alkaline copper reagent and 0.5 mL of Folin-Ciocalteu Phenol reagent (1 N) were added and read at 700 nm after 30 min, using bovine serum albumin as standard [20].

Total lipids were determined according to Knight et al. (1972). Briefly, homogenized samples in chloroform/methanol mixture (v/v) were heated at 100 °C for 10 min, centrifuged then 0.25 mL of supernatant and 0.1 mL of concentrated sulfuric acid were heated again at 100 °C for 10 min, cooled, 2.4 mL of phosphor-vanillin reagent was added. The pink colors were read at 490 nm after 5 min, using oleic acid as standard [21].

Carbohydrates were extracted with 80% ethanol at 80 °C (three times) and were assessed by the phenol–sulfuric method of Dubois et al. (1956) [22].

5.6. Determination of some minerals using inductively coupled plasma (ICP) analysis

In the presence of 50 g of the whole bean and seed coats, 2 g/L of EDTA was added. By using benzene flame, these mixtures were boiled for 90 min. The cooked whole beans and seed coats in the presence and absence of EDTA were dried and kept in clean place for ICP analysis to determine minerals level. Whole beans and seed coats without any treatments were used as controls. Ten mL of conc. HNO_3 were added to 0.5 g of dry tissues, then samples were digested by using microwave digestion system (Milestone, Ethos Easy model: ACT36). Serial dilutions were then prepared from a stock solution (1 g/L) of K, Mn, Fe, Mg, Zn, Na and Ca to prepare concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 mg/L) using deionized water. Different electrolytes standards were measured at ICP (Perkin Elmer Model: Optima 700 DV) to get the standard curves. Using the WINLAB 32 software, samples concentrations were assessed as mg/dl.

5.7. Free amino acids analysis

To determine amino acids contents in the whole beans and in seed coats, in the presence or absence of EDTA, 50 g of whole beans or bean's seed coats were boiled with or without 2 g/L of EDTA. After 90 min, the whole beans and their seed coats were collected and dried in oven, then processed for amino acids assessment by using amino acid analyzer (Sykam S433 model). Briefly, one gram of each sample was de-fated by diethyl ether, dried then 5 mL of 6 N HCl was added to each sample and placed in oven at 110 °C for 24 h, cooled, placed on filter paper and washed by adding 5 mL dist. H_2O then incubated at water bath for 5 h for complete digestion. Excess H_2O and HCl were then eliminated; the samples were dissolved in 2 mL buffer, filtered by 0.22 μm syringes, diluted and then injected into amino acid analyzer system.

5.8. Statistical analysis

One–way analysis of variance (ANOVA) was used to assess the significant differences among groups. All data are presented as mean ± SD. The accepted level of statistical significance was $p < 0.05$.

Conflict of interest

All authors declare that there was no conflict of interest.

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