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Extraction and estimation of alpha lipoic acid content in different food samples by reverse phase HPLC: effect of heat treatment

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Abstract

α-Lipoic acid plays vital role in energy metabolism. It is an excellent antioxidant acting inside the cell and at plasma membrane levels. It also acts as therapeutic agents in different diseases including diabetes, neurodegeneration, hypertension, HIV, breast cancer, heavy metal poisoning, radiation injury etc. Considering the nutritional and therapeutic perspectives, this is very important to identify α-lipoic acid-rich food. It is also required to determine whether concentration of α-lipoic acid gets changed with heat treatment. In this investigation, plant and animal foodstuffs were first blended with methanol, and then acidified, after that sonicated and finally centrifuged to extract α-lipoic acid. The concentrations of α-lipoic acid were estimated by the reverse phase high performance liquid chromatography (HPLC). Concentration of α-lipoic acid in the raw form of plant and animal foodstuffs ranged from 1.270671 ng/mg to 22.35785 ng/mg and 2.214241 ng/mg to 13.55982 ng/mg respectively. The level of α-lipoic acid in the boiled form of plant and animal foodstuffs ranged from 0.203982 ng/mg to 3.59858 ng/mg and 1.24481 ng/mg to 7.198254 ng/mg respectively. It was found that raw sample had higher concentration of α-lipoic acid than boiled sample. In our study, loss of α-lipoic acid due to boiling ranged from 10.85% to not detectable level for plant sample and 17.23% to 75.40% for animal sample indicating that α-lipoic acid in animal sample was more stable to boiling than that of plant sample. Further research should be continued to improve the stability of α-lipoic acid in different processed forms of foodstuffs.

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Introduction

Alpha lipoic acid (α-LA) is a disulphide compound found naturally in diverse group of micro-organisms and in a variety of plant and animal tissues (Herbert *et al.,* 1975). It is mainly present in mitochondria and plays a pivotal role in energy metabolism and was first isolated from bovine liver in 1951 (Reed *et al.,* 1951). α-Lipoic acid is present in both free and protein bound form. Lipoate or dihydrolipoate reacts with reactive oxygen species (Biewenga *et al.,* 1997). It is a small molecule that is soluble in both water and lipid. α-Lipoic acid works both inside the cell and at the membrane level providing dual protection (Packer *et al.,* 1995). α-Lipoic acid prevents β-cell destruction, enhances glucose uptake, prevents glycation reactions in some proteins, slows the development of diabetic neuropathy and cataractogenesis (Wagh *et al.,* 1987). α-LA acts as hypotensive agent by increasing tissue GSH levels and preventing deleterious sulfhydryl group modification in Ca^{2+} channels (Vasdev *et al.,* 2000).

It inhibited the replication of HIV-1 in cultured lymphoid T cells (Baur *et al.,* 1991). α-Lipoic acid, protected against radiation injury to haematopoietic tissues in mice (Ramkrishnan *et al.,* 1992). It is a good candidate for the treatment of heavy metal poisoning caused by arsenite, cadmium, and mercury (Grunert, 1960). α-LA prevents ageing process by reducing mitochondrial decay and maintaining normal level of glutathione in cells (Atamna, 2004). It has recently been shown that the application of α-LA to a human breast cancer cell line inhibits cancer metastasis (Lee *et al.,* 2010).

A number of solvents were used to extract free α- LA from health food or dietary supplements. Different approaches such as HPLC/UV (Sun & Chen, 2006), HPLC with coulometric electrode array detection (CEAD) (Durrani *et al.,* 2007) and electrospray ionisation mass spectrometry (ESI-MS) (Durrani *et al.,* 2007), capillary electrophoresis/UV (Sitton *et al.,* 2004), and differential pulse voltammetry at a glassy carbon electrode (Corduneanu *et al.,* 2007) were used to determine α- LA concentration in different food samples. It can be extracted from wheat flours and germs by a mixture of chloroform/methanol/water and determined by thin layer chromatography, where acid hydrolysis yielded lower concentrations of α-LA (Swatiditat & Tsen, 1973). For cleaving covalently protein-bound α-LA from animal samples more drastic conditions were chosen. Eggs and livers of chicken were hydrolysed with 12 M sulphuric acid for 6 hr at 125°C, the hydrolysate was extracted with benzene and α-LA was determined after methylation by GC with a flame ionization detector (Shih & Steinsberger, 1981). Base hydrolysis has also been used to extract α-LA from complex samples. For that purpose, samples were heated at 110° C for 3 hr in 2 M potassium hydroxide solution containing 4% bovine serum albumin. Then, α-LA was converted to its S,S-diethoxycarbonyl methyl ester and quantified by GC with a flame photometric detector. Mild enzymatic hydrolysis of bovine, rat and rabbit tissues using protease released lipoyllysine, which could be determined photometrically by enzymatic NADH oxidation (Akiba *et al.,* 1998), which could also be determined by HPLC and electrochemical detection (Packer, 1997).

Considering the nutritional and therapeutic perspectives, this is very important to identify α-lipoic acid-rich food. In our country, however, it is yet to be determined the concentration of α-lipoic acid in different food stuffs obtained from animal and plant sources. The aim of this investigation is to extract and estimate α-lipoic acid content in different food samples by HPLC, to identify high α-lipoic acid containing foods, and to study the effect of heat treatment on α-lipoic acid present in food stuffs.

Materials and methods

Chemicals and Reagents

α-lipoic acid (5g) was purchased from Alfa Aesar. Acetonitrile (HPLC grade) was obtained from Merck (Germany). HPLC grade methnol was purchased from Sigma Aldrich. Ammonium acetate (500g) was purchased from Merck (India) while acetic acid (AR Grade) was obtained from SD Fine chemicals (Hyderabad, India). Water (HPLC grade), 0.45 µm

Nylon membrane filters were generously provided by Wazed Miah Science Research Center, Jahangirnagar University.

Instrument

HPLC analysis was performed on Shimadzu LC-20AT Prominence Liquid Chromatograph comprising a LC-20AT pump, Shimadzu SPD- 20AV Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable C18G (250 X 4.6 mm; 5µ). An electronic analytical weighing balance (METTLER TOELDO × P6), digital pH meter (HQ40d HACH pH meter), a sonicator (sonica, model 2200 MH) were used in this study.

Chromatographic conditions

The separation of the components was achieved on a reverse phase C18 column, Enable C18G (250 X4.6 mm; 5µ), mobile phase consisting of a mixture of acetonitrile and ammonium acetate buffer (20 mM, adjusted pH with 30% v/v of glacial acetic acid to 4.6) in the ratio of 50:50, v/v. The mobile phase was set at a flow rate of 0.8 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 215 nm (Rajkumar *et al.,* 2014).

Buffer preparation

The buffer solution is prepared by weighing 1.54g of ammonium acetate and transferring to 1000 ml of HPLC grade water to get 20 mM buffer strength and later pH was adjusted to 4.6 with 30% v/v acetic acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

Mobile phase preparation

The mobile phase was prepared by mixing acetonitrile and buffer in the ratio of 50:50, v/v and later sonicated for 10 minutes for the removal of air bubbles.

Preparation of stock and working sample solution

100 mg of α-lipoic acid were accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of methanol and then sonicated for 5 minutes to dissolve. Later the solution was made up to the mark using the same solvent. This is considered as standard stock solution (1 mg/ml). Then the standard stock solution was diluted to get the concentration 1ng/ml, from which 20 µl was injected into the HPLC system.

Sample collection

Different food samples of animal and plant origin were collected from different supermarkets of Bangladesh.

In this experiment 22 raw and 13 boiled plant foodstuffs as well as 8 raw and 9 boiled animal foodstuffs were used for the estimation of α-lipoic acid. Plant samples includes ginger, cauliflower, banana, teasle gourd, apple, red amaranth, malta, carrot, pointed gourd, bitter gourd, turmeric, cabbage, ladies finger, potato, snake gourd, brinjal, ribbed gourd, tomato, green chilli, plantain, cucumber and grape. Animal sample included chicken, chicken liver, beef, beef liver, mutton, mutton liver, carp fish and halibut fish.

Extraction of α-lipoic acid from foodstuffs in raw form and boiled form

50 gm food sample in raw form or boiled (50 gm sample was boiled for 25 min in 500 ml water) form was cut and weighed. Then it was blended with methanol and after blending, final volume was measured. The volume that contained (50-150) mg foodstuffs was taken on eppendroff tube. Then 1 ml of acidified methanol was added to this eppendroff tube. The sample was then sonicated for 1hr at room temperature followed by centrifugation for 10 min at 10000 rpm. 500 µl supernatant following centrifugation will be collected and preserved at 4◦C. From this supernatant, 20 µl was injected into the HPLC system (Durrani, 2008).

Data analysis

All the data were analyzed by Microsoft Excel.

Results and discussion

This study was conducted on plant and animal foodstuffs in the raw and boiled form to estimate the

concentration of α-lipoic acid by reverse phase HPLC method. Previously, different approaches were made to determine α-lipoic acid from health food, dietary supplements, animal food stuff, vegetables and in biological samples (Kataoka *et al.,* 1993). In our investigation, acidified methanol-treated blending followed by ultrasonication was used for the extraction of α-lipoic acid from plant and animal foodstuffs.

Table 1. Loss of α-lipoic acid content due to boiling for 25 min in plant sample (only detectable values are given).

Plant sample	Loss in %
Bitter gourd	10.85
Plantain	31.24
Brinjal	56.06
Pointed gourd	77.20
Ladies finger	79.5
Potato	84.03
Cucumber	85.67
Tomato	85.88
Teasle gourd	88.42
Snake gourd	89.49
Carrot	91.19
Ribbed gourd	92.15

Table 2. Loss of α-lipoic acid content due to boiling for 25 min in animal sample.

Animal sample	Loss in %
Halibut fish	17.23
Carp fish	21.82
Beef liver	65.11
Chicken	65.82
Beef	68.47
Mutton liver	71.34
Chicken liver	75.40

After analyzing obtained data, plant and animal foodstuffs contained higher and lower concentration α-lipoic acid in raw and boiled form were determined. Chromatogram of standard α-lipoic acid has been shown in fig. 1.

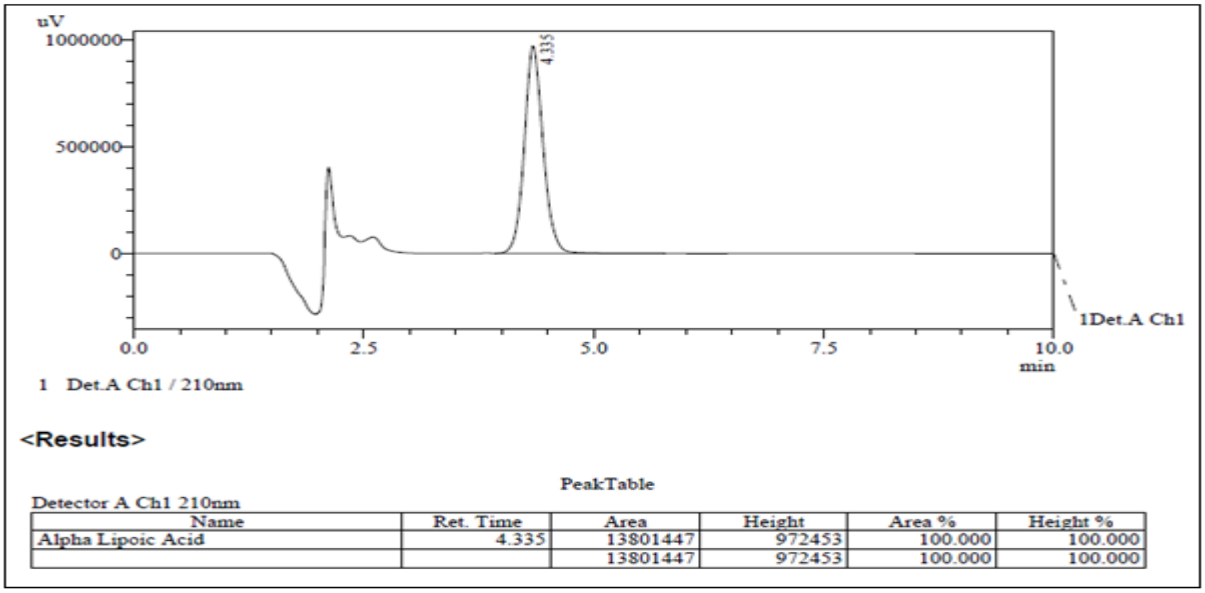

Fig. 1. Chromatogram of standard α-lipoic acid.

Up to now, there are few quantitative data on α-lipoic acid contents in biological and food samples and, due to the different method of analysis employed, very contrasting data are reported in the literature. α-Lipoic acid contents have been found to be high (ranging from 0.55 to 2.36 ppm) in animal foods derived from tissues with a high metabolic activity (Kataoka *et al.,* 1993), but very low (0.09 ppm) or not detectable in plant foods.

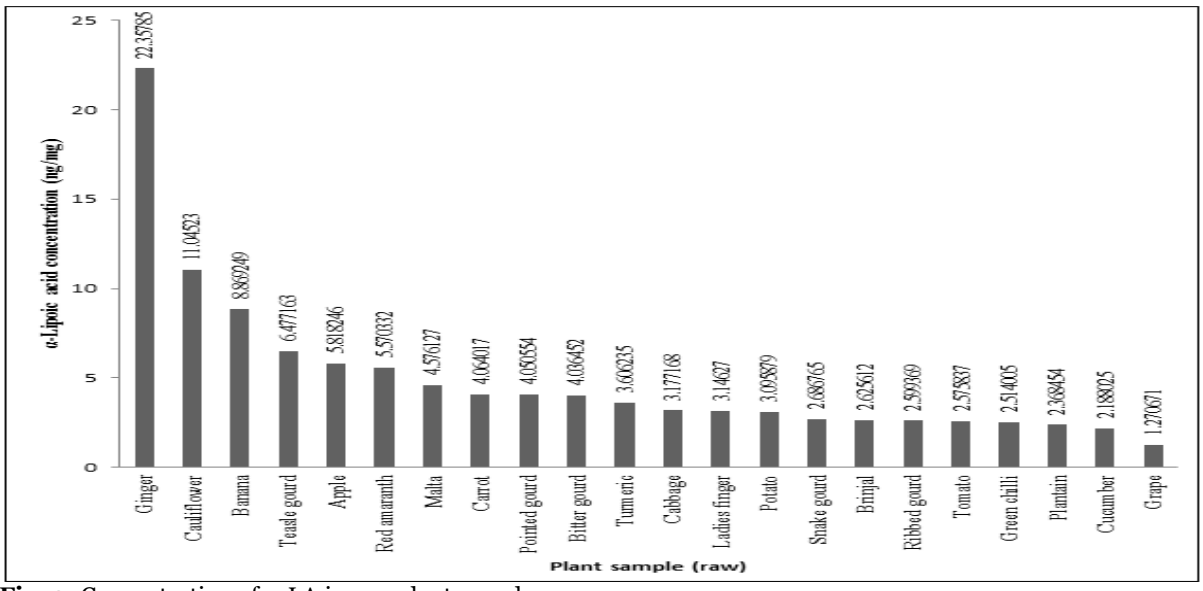

Fig. 2. Concentration of α-LA in raw plant sample.

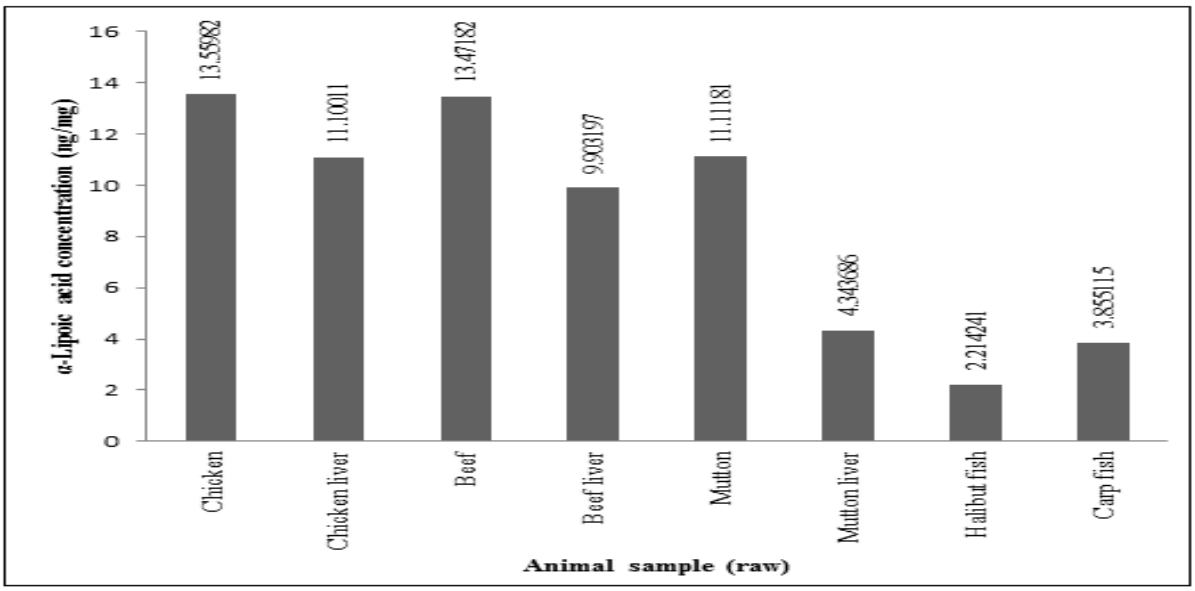

Fig. 3. Concentration of α-LA in raw animal sample.

In our study, the concentration of α-lipoic acid in plant food stuffs in raw form ranged from 1.270671 ng/mg to 22.35785 ng/mg (Fig. 2). The highest concentration of α-lipoic acid in raw plant foodstuff was appeared in ginger (22.35785 ng/mg) while the lowest concentration was appeared in grape (1.270671 ng/mg). Packer L. (1997) found the highest concentration of α-LA in "green" tissues such as spinach and broccoli correlating with the metabolic activities of tissues. In this investigation, the level of α-lipoic acid in potato was 3.095879 ng/mg. Lachman *et al.,* (2000) reported α-LA as one of the potential

antioxidants of potato. The early reported values for α-LA contents in flours, derived from endosperm of wheat after hydrolysis, ranged from 1 to 1000 ppm (Sullivan *et al.,* 1961; Swatdid and Tsen, 1973; Vianey-Liaud, 1994), which is much higher than the range we found. Kataoka *et al.* (1997) reported a contents of α-LA of 0.09 ppm in asparagus and not detectable amounts in garlic, soybean and welsh onion.

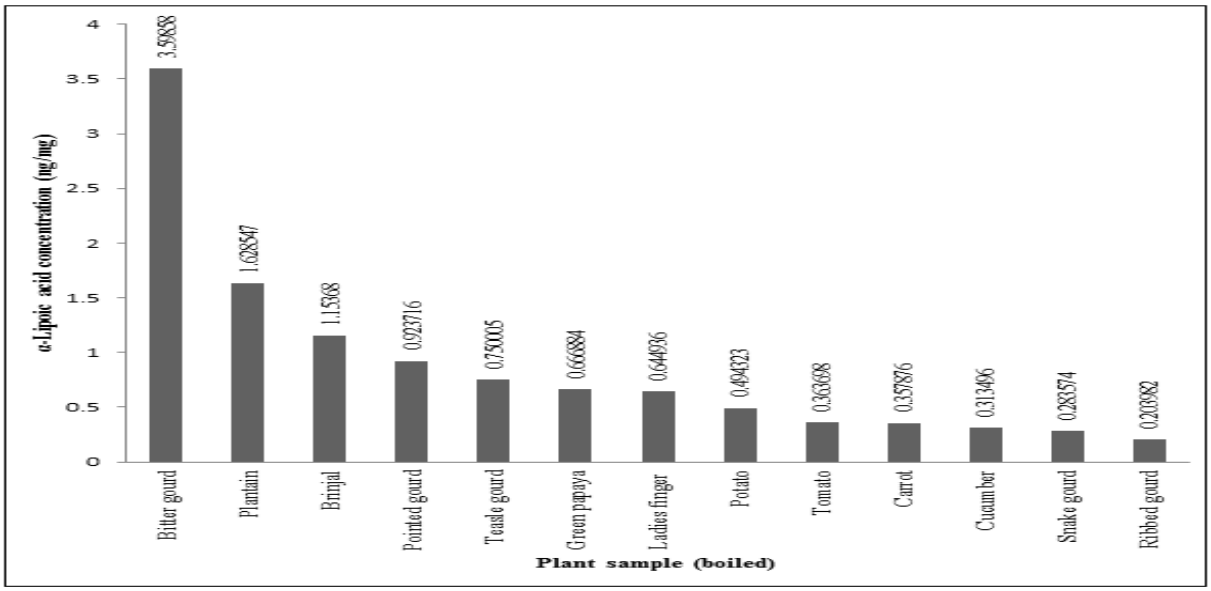

Fig. 4. Concentration of α-LA in boiled plant sample.

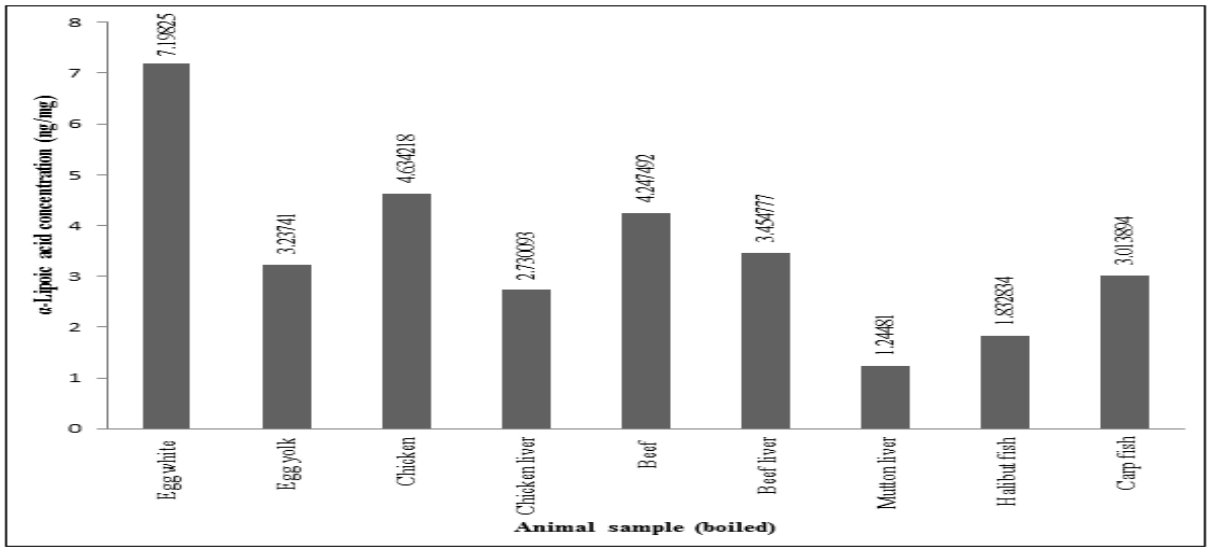

Fig. 5. Concentration of α-LA in boiled animal sample.

In this investigation, the concentration of α-lipoic acid in animal food stuffs in raw form ranged from 2.214241 ng/mg to 13.55982 ng/mg (Fig. 3). The highest concentration of α-lipoic acid in raw animal food stuff was appeared in chicken (13.55982 ng/mg) while the lowest concentration was appeared in halibut fish (2.214241 ng/mg). A group of scientists found α-lipoic acid contents egg powder, mayonnaise, fine peas and potatoes ranged from 0.1 to 4.2 µ/g (Durrani *et al.,* 2010) which are much higher than the range we found. Our study showed that in case of chicken, beef and mutton; the level of α-lipoic acid was higher in muscle than that of liver (Fig. 3). Packer L. (1997) estimated lipolylysine of bovine tissues and found the highest concentration of lipolylysine in the kidney, heart and liver contrasting our results.

We also determined α-lipoic acid concentration in boiled form of plant and animal food stuffs. The concentration of α-lipoic acid in plant food stuffs in boiled form ranged from 0.203982 ng/mg to 3.59858 ng/mg (fig. 4). However, the presence of alpha lipoic acid in many boiled sample was almost below the limit of detection. Among the boiled plant food stuffs the highest concentration was observed in bitter gourd (3.59858 ng/mg) and the lower concentration of that was found in ribbed gourd (0.203982 ng/mg).

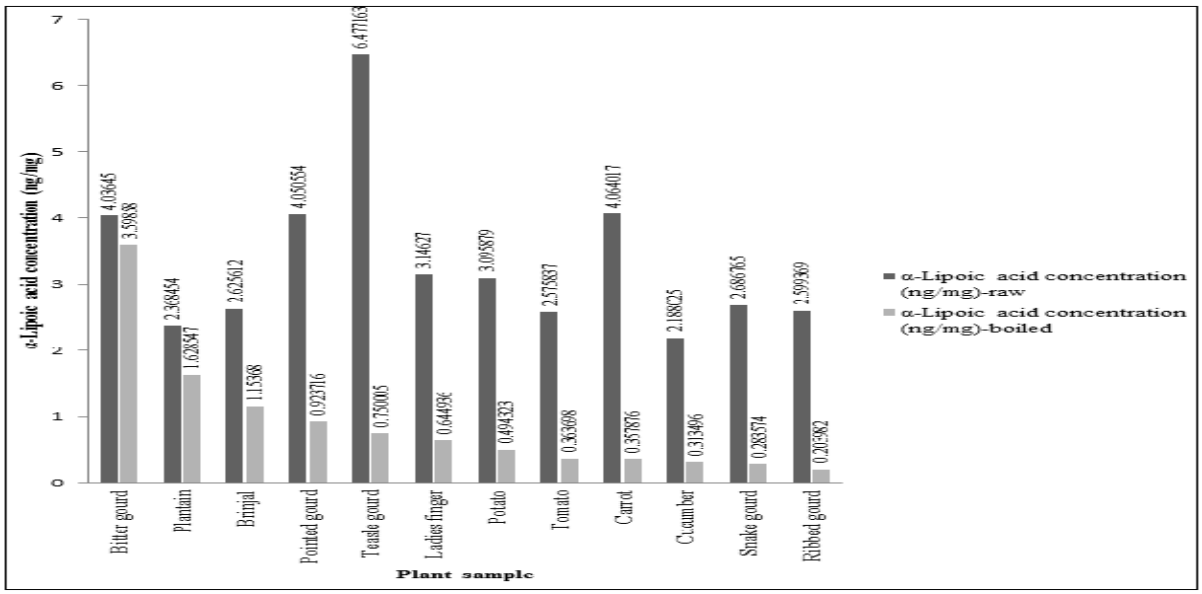

Fig. 6. Comparison of α-LA concentration in raw and boiled form of plant sample.

The concentration of α-lipoic acid in animal food stuffs in boiled form ranged from1.24481 ng/mg to 7.198254 ng/mg (fig. 5). Among the boiled animal food stuffs, the highest concentration was observed in egg white (7.198254 ng/mg) and that contained lower concentration after boiling was mutton liver (1.23481 ng/mg).

Each data of this investigation was analyzed for making comparison of α-lipoic acid concentration between raw and boiled plant and animal sample. It was interesting to note by comparing data between raw and boiled (25 min) form of both plant and animal sample that, raw sample had higher concentration of lipoic acid than boiled form (fig. 6 and 7).

The loss of % of α-lipoic acid content due to boiling for 25 min was calculated from α-lipoic acid contents in raw and boiled form (Table 1 & Table 2). Matsugo *et al.* (1996) reported that photoirradiation of α-LA caused the homolytic rupture of S-S bond of the 1,2-dithiolane ring in lipoic acid producing dihydrolipoic acid. Park *et al.* (2009) also reported that the bond in alpha-lipoic acid can be homolytically cleaved by near UV light and heat where the dithiolane ring structure forms two thiyl radicals. The lower concentration of α-lipoic acid in the boiled form of plant and animal sample might be due to this type of homolytic rupture to heat treatment. The lower concentration of α-lipoic acid in the boiled form of plant and animal sample might be due to the possibility of its instability to heat treatment. It may also be due to the possibility that under heat treatment structural rearrangement of α-lipoic acid takes place. In our study loss of α-lipoic acid due to boiling for 25 min ranged from 10.85% to not detectable level in case of plant sample and 17.23% to 75.40% in case of animal sample (Table 1).

Among the plant and animal samples α-lipoic acid was stable in bitter gourd and halibut fish, respectively. It may be noted that α-lipoic acid in animal sample was more stable to boiling than that of plant sample. Studies showed that complexation of α-

LA with cyclodextrins (CDs) increased its stability to humidity and high temperature or acidic pH conditions (Ikuta *et al.*, 2013; Takahashi *et al.*, 2011). In plant and animal samples α-LA can exist as free or form complex with protein (Bradford *et al.*, 1987). There may have variation in the number of free and protein bound α-LA between plant and animal samples. There may also have variation in the nature or intensity of this complex formation. Compared with this experiment, we noted that alpha lipoic acid concentration is changeable in different form like raw, boil, short term storage or long term storage.

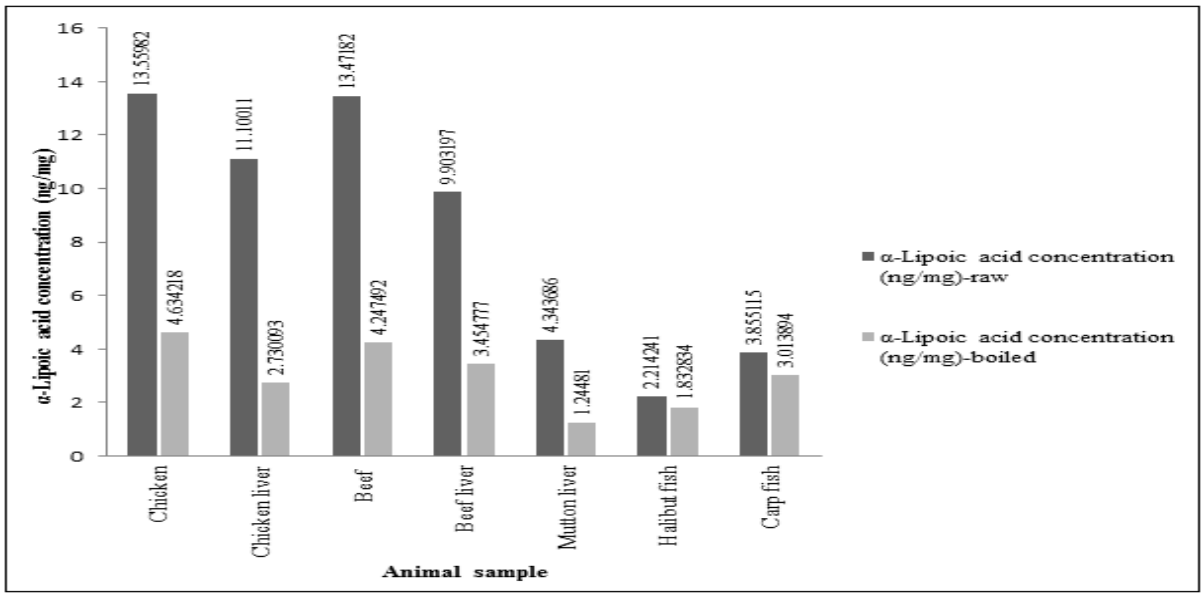

Fig. 7. Comparison of α-LA concentration in raw and boiled form of animal sample.

Conclusion

α-Lipoic acid is an ideal powerful antioxidant acting as a therapeutic agent for diverse conditions, including diabetes, atherosclerosis, insulin resistance, neuropathy, neurodegenerative diseases and ischemia reperfusion injury as described previously. In our study, higher α-lipoic acid concentration was observed in some plant foodstuffs like ginger, cauliflower, banana, teasle gourd, apple, red amaranth and also in some animal foodstuffs like chicken, beef, chicken liver and beef liver. Our study observes that the concentration of α-LA decreases due to heat treatment. This decrease is higher in plant food stuffs compared to animal food stuffs indicating that α-lipoic acid in animal sample may be more stable to boiling than that of plant sample. Thus, how stability of α-LA can be increased in different processed forms of plant and animal foodstuffs might be a considerable issue to address in further study.

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