COMPARATIVE STUDIES ON THE NUTRITIONAL QUALITY OF AULIFLOWER GROWN IN KOLKATA MUNICIPAL SOLID WASTE DUMPING SITE (DHAPA LAND-FILLED GROUND) WITH NORMAL GROUND

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Abstract: The largest open waste dumping ground, where the Municipal solid wastes (MSW) of the city of Kolkata are dumped is popularly known as Dhapa. A vast area of Dhapa, which was land filled previously by MSW is presently used for the cultivation of vegetables adjoining the current core MSW dump site. Cauliflower is one of the most abundant vegetable cultivated in Dhapa Landfilled Ground (DLG). The main objective of this study is to evaluate and compare the nutritional quality of cauliflower, which include various nutrients, anti-nutrients, and antioxidant activity, grown on a municipal waste dumping site (i.e. DLG) and Normal Ground (NG). The cauliflower samples were collected throughout the winter season from five selected fields of DLG & NG and analysed. The results stated that there are no significant differences exist ($p>0.05$) in nutrients, anti-nutrients, antioxidants content and total antioxidant activity of cauliflower between Normal Ground and Dhapa Landfilled Ground, except ash, pH, soluble and total protein, tannin and flavonoid contents. Although differences ($p < 0.05$) present within the different fields of DLG & NG in terms of nutritional quality except for moisture, pH, carbohydrate, total protein and ascorbic acid. From the study it has been concluded that the MSW did not affect the overall nutritional quality of Cauliflower grown in Dhapa Landfilled Ground in comparison the Cauliflower grown in Normal Ground.

Keywords: Cauliflower, Dhapa Land-Filled Ground (DLG), Normal Ground (NG), Nutrients, Anti-Nutrients, Antioxidant Activity

Introduction: Municipal Solid Waste (MSW) constitutes that portion of solid waste stream originating from households, institutions, commercial and service establishments, offices and public facilities and construction and demolition sites.(Ghaly et al., 2010). It not only contains 'valuable' and often re-usable materials (such as glass, paper, plastic and food remains) but also contains increasing amount of hazardous substances .Typical of the latter is mercury from batteries, cadmium from fluorescent tubes, pesticides and bleaches as well as a wide range of toxic chemicals such as solvents, paints, disinfectants and wood preservations (Biwas, 1989). The long-term environmental impact of municipal solid waste (MSW) landfilling is still under investigation due to the lack of detailed characterization studies. A MSW landfill site, popularly known as Dhapa, in the eastern fringe of the city of Kolkata,West Bengal, India, is the subject of present study, where MSW of the city of Kolkata has been dumped since the middle of the 19th century. Dumping of MSW is still continuing in certain parts of Dhapa and the rest of the vast stretch of land (approx. 2000 acres) is used by the farmers to grow vegetables . The Dhapa soil is found to be polluted with heavy metals such as Cu, Zn, and Pb (highly elevated) and Ti, Cr, Mn, Fe, Ni, and Sr (moderately elevated), compared to the natural countryside soil. (Gupta et al., 2007).

Thus it is expected that the vegetables grown there may also contain elements in higher concentrations than those in uncontaminated soil. But the most notable feature discovered by Gupta et al., 2011 was the similar elemental concentrations in the edible flower part of all cauliflower samples irrespective of the type of soil , although the elemental concentrations in the root soils and leaves of the samples vary from field to field, whereby the concentrations of Cupper, Zinc and lead in root soils of MSW contaminated fields were higher by almost an order of magnitude compared to uncontaminated fields. In this regard,Cauliflower, one of the most widely cultivated vegetable in Dhapa Landfilled Ground (DLG) was selected as a sample of study and the overall nutritional quality are assessed.

Materials & Methods:

Sample collection: The sample was collected throughout the year from five selected fields of Dhapa land-filled ground (designated as DP_1,DP_2, DP_3, DP_4, & DP_5) and also from selected Normal ground (designated as NG_1, NG_2, NG_3, NG_4, & NG_5). All the time sample was collected in sterile zip lock plastic bag and preserved in ice bag during transportation from land to the laboratory. For analysis flower with small portion of adjoining stem were used. It may be further mentioned that for identification, whole plants of selected samples were collected and herbarium sheets were prepared after sun drying. These plants were identified and classified by a plant taxonomist of Botanical Survey Of India, Shibpur, Howrah. The details of each plant species are elaborated in Table 1.

Table 1: Vegetables collected for the study and pats used for proximate analysis.

English name	Species name	Family name	Local name	Parts used	Status
Cauliflower	Brassica oleracea L.	Brassicaceae	Phulcopi	Flower	Cultivated

Dry matter determination: For determination of the dry matter content, appropriate amount of raw sample (as triplicate) was dried in a convection oven at 70°C for at least 3 days until reaching constant weight

Preparation of extract:

For antinutrients assay: Prior to extraction and analysis the vegetable (cauliflower) was washed and dried on paper towel and then subjected to size reduction using a knife. They were oven dried and finally grounded by using pestle and motor.

- **For Oxalate (Baker, 1952):** Appropriate amount of grounded plant samples (dry weight basis) were extracted in 2N HCl with mechanical shaking for about 2 hours. It was then centrifuged and filtered with whatmaan 40 filter paper and the filtrate was taken for oxalate estimation.
- **For Phytate (Thompson & erdman, 1982):** Appropriate amount of grounded plant samples (dry weight basis) were extracted in 3% TCA with mechanical shaking for about 30 minutes. It was then centrifuged and supernatant was taken for phytate estimation.
- **For Tannin (Schanderi, 1970):** Appropriate amount of grounded plant samples (dry weight basis) were boiled with distilled water for 30 minutes and cooled. It was then centrifuged and filtered with whatmaan 1 filter paper and the filtrate was taken for tannin estimation.

For antioxidant assay:- Prior to extraction and analysis all vegetables were washed and dried on paper towel and then subjected to size reduction using a knife and blended with a kitchen mixer to get a thick paste. Appropriate amount of plant paste sample was extracted with---------

- 6 % metaphosphoric acid for vitamin C estimation and
- 80% methanol and left it overnight. These were then centrifuged at 10,000 rpm for 15 min and the supernatants were decanted into polypropylene tubes and filtered through Whatman No.1 filter paper. The clear extracts were analyzed both for determination of phenolic contents and antioxidant activity.**(Prior et al., 2005).**

Analysis :

(i) **Determination of moisture content :** Moisture content was determined by drying 10 g of samples at 105°C in a drying oven to a constant weight **(AOAC, 1990).**

(ii) **Determination of ash content :** Ash content was determined by weight difference method by using muffle furnace **(Raghuramulu et al., 2003)**

(iii)Determination of pH : The pH of the sample was determined using pH meter **(khosa et al., 2011).**

(iv)Determination of total carbohydrate content: The fresh sample was blended and used for the estimation of total carbohydrate content by using Anthrone Method **(Mc.Cready et al., 1950).** To the 0.1gm blended sample, 5ml 2.5(N) HCl was added and the mixture was hydrolyzed for 3 hours in a water bath. After cooling, the hydrolyzed mixture was neutralized with Na2CO3 and the volume was made up to 100 ml by distilled water and centrifuged at 3000 RPM for 15 minutes to remove any residues. To 1.0 ml of supernatant, 4ml of Anthrone reagent (0.2% in conc. H2SO4) was added and mixed thoroughly. The mixture was allowed to heat for 8 minutes in water bath and cooled. This was followed by recording absorbance in spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) at 630 nm wavelengths against a blank. The blank was prepared by taking 1.0 ml distilled water instead of sample extract.

(v) Determination of protein content:

(a) **Estimation of soluble Protein:** The quantity of soluble protein can be determined by colorimetric method introduced by Lowry et al., **(Lowry et al., 1951).** 1000 mg the fresh sample was crushed with 10 ml cold phosphate buffer (pH 7.5, 0.1M) using a chilled mortar and pestle. The homogenate was kept overnight for complete extraction of protein and centrifuged at 5000 rpm for 30 min in a cold centrifuge (4°C). The supernatant was then taken for protein analysis according to the method of Sawhney and Randhir (Sawhney and Randhir, 2006). Briefly 5.0 ml of alkaline copper sulfate reagent [prepared by mixing 1.0 ml of copper sulfate reagent (0.5 % $CuSO_4.5H_2O$ in 1.0% sodium potassium tartarate) with 50 ml of alkaline sodium carbonate reagent (2% Na_2CO_3 in 0.1 (N) NaOH solutions)] was added in 1.0 ml of supernatant and mixed thoroughly. The mixture was then allowed to stand for ten minutes. Now 0.5 ml of Folin

ciocalteu reagent (1:1v/v in Distilled water) was added into it and incubate for 30 minutes in room temperature. The absorbance of the blue colored solution obtained was measured at 750 nm by spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) against a blank. The blank is prepared by taking 1.0mL of 0.1 M phosphate buffer in place of sample. Standard Bovine serum albumin protein at various concentrations was used to draw a standard curve and the amount of proteins in different samples was estimated from the standard curve.

(b) **Estimation of Total Protein:** The total Protein content was determined using BIS method (**Bureau of Indian Standard, IS No. 7219:1973)** based on Kjeldahl method. Once the nitrogen content has been determined, it is converted to a protein content using the appropriate conversion factor (N factor i.e. 6.25).

(vi) Determination of oxalate content: Briefly, the determination was as previously described by (**Baker, 1952)** with some modifications. The sample extract was weighed after taking in a beaker and boiled for 15 mins. This was then adjusted to previous volume with distilled water and volume was made upto 100ml with 2N HCl. The mixture was shaken well and filtered (whatman 40). To the 25 ml of filterate , 5 ml of phosphoric tungstate reagentwas added & mixed well & once or twkept overnight . Next day the mixture was centrifuged for 10 minutes at 3000 rpm and filtered (whatman 40) . 20 ml of clear solution was then taken to a 50 ml centrifuge tube and 2-3 drops of methyl red was added & neutralized with ammonia. Then 5 ml of Calcium chloride reagentwas added to it and stirred with a fine glass rod and kept tube overnight in a refrigerator at 5 - 7 ^{o} C. Next day the mixture was again centrifuged for 10 minutes at 3000 rpm and filtered (whatman 40) .The ppt was then dissolved in distilled water followed by 5 ml of 2N sulphuric acid. Then the mixture was placed in a water bath over 80 ^{o} C for 2 minutes and titrated the oxalic acid with N/100 potassium permanganate solution to a faint pink colour which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titre value. The overall redox reaction is: $2MnO^{-} + 5C_2O_4^{2} + 16H^{+} \rightarrow 2Mn^{2} + 8H_2O + 10CO_2$

(vii) Determination of phytate content: Total phytate content of the extract was determined according to the supernatant difference method of **Thompson & Erdman (1982).**Sample extract (10 ml) was mixed with 4 ml $FeCl_3$ solution and heated in a boiling water bath for 45 mins. If the supernatant is no clear after 30 mins, add one or two drops of 3% Sodium sulphate in 3% TCA and continue heating.It was then Centrifuged for 10 – 15 mins and carefully decant the clear supernatant. After that precipitate was washed twice by dispensing well in 20 – 25ml of 3% TCA and heated in boiling water bath for 5 – 10 mins and centrifuged. The process of washing with water repeated. The precipitate was then dispersed in 27ml of water and 3 ml of 1.5N NAOH with mixing and the volume was made upto approximately 30ml with water and allowed to heat in boiling water bath for 30mins. It was then filtered hot through a moderately retentive paper Whatman No.2. The precipitate was washed with 6 – 7oml hot water and the filtrate was discarded. Then the precipitate from the paper was dissolved with 40ml 3.2N HNO_3 into a 100ml volumetric flask and the volume was made upto the mark. 5ml aliquot was transferred to another 100ml volumetric flask and diluted to approximately 70ml. 20ml of 1.5M KSCN was added to it and diluted to the volume. Finally read at 480nm of wavelength. Standard curve was prepared by using $Fe(NO)_3$. The (µg) iron present in the standard curve was found out and phytate P was calculated as per the equation.

Phytate P present in the sample = µg of Fe x 15 / Weight of the sample (g)

(vii) Determination of tannin content: Tannin content in the plant extract (extracted in boiled water) was determined as described by (**Schanderi, 1970**) , using tannic acid as the standard. The extract solution (1 ml) was mixed with the Folin denis reagent (5ml) and super saturated solution of Na2CO3(10 ml) and volume made upto 100ml by distilled water. After 30 minutes of incubation at room temperature, the absorbance of the reaction compound at 700 nm was measured spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) The overall tannin content was expressed as mg of tannic acid equivalents / gm dry weight .

(ix) Determination of antioxidant content:

(a) Determination of total phenols: Total phenol content in the plant extract (extracted in 80% methanol, kept overnight) was determined as described by **Singlaton and Rossi (1965)** , using gallic acid as the standard. The extract solution in 80% methanol (1 ml, 50 mg ml-1) was mixed with the FC reagent (10%, 1 ml) and an aqueous solution of Na2CO3(7.5% , 0.8 ml) and volume made upto 10ml by distilled water. After 30 minutes of incubation at room temperature, the absorbance of the reaction compound at 765 nm was measured spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) The overall phenol content was expressed as mg of gallic acid equivalents (GAE)/ gm dry weight .

(b) Estimation of total flavonoids: Total flavonoid content of the extract was determined according to a

modified colorimetric method **of Bao , Cay et al. (2005).** Sample extract (1 ml, 50 mg ml-1) was mixed with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes , 0.15ml of 10% $AlCl_3.H_2O$ solution was added. After 6 minutes, 2 ml of 4% Sodium hydroxide was added and volume made upto 10ml by distilled water. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer(Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as micrograms of quercetin equivalents (QE) per gram dry weight of the sample.

(c) Estimation of ascorbic acids: Ascorbic acid content in the plant extract (extracted in 6% metaphosphoric acid) was determined as described in **Interscience publishers (1947) and J. Biol. Chem. (1944)** using ascorbic acid as the standard (1mg/ml). The extract solution (2 ml) was mixed with equal amount of acetate buffer (ph 4.0) and dye (sodium salt of 2,6 dichlorophenol indophenol)solution in the separating funnel. The content was mixed well and 10ml xylene was added . This was then mixed well and allowed to stand for 6 seconds for separating the layers and then the water layer was removed and the colour in xylene was measured in a spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) at 500 nm. The ascorbic acid content was calculated as..

$$= \frac{0.1 \times \text{Blank- Sample}}{\text{Blank- Standard}} \times 100$$

Amount of the sample taken (mg/100gm fresh weight)

(x) Determination of total antioxidant activity of the extract :

(a) ***Determination of ferric reducing/antioxidant power assay (FRAP):*** FRAP assay was carried out according to the method of **Benzie and Strain (1999)**. FRAP reagent was prepared from acetate buffer (1.6 g sodium acetate and 8 ml acetic acid make up to 500 ml) (pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in oven prior to use. A total of 50 µl samples extract were added to 1.5 ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using using microplate reader spectrophotometers (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer) after 4 mins. Samples were measured in three replicates.

FRAP value of Sample (µM) = (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of **standard**from 0 to 4 minute) X FRAP value of standard {Ascorbic Acid (M.W. 176.13) 1000 µ} **Note: FRAP value of Ascobic acid is 2**

(b) Determination of free radical scavenging using DPPH method: Antioxidant activity was determined by the 2,2,-di- phenyl-2- picryl-hydrazyl (DPPH) method of **Zhang and Hamauzu (2004)** with some modifications. The concentration of the methanol (80%) extracts of fresh vegetable was adjusted to 10 mg/ml (on dry basis), which was chosen as an appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. An aliquot of 2 ml of 0.1 mM DPPH radical in methanol was added to a test tube with 0.1 ml of vegetable extract, at 10 mg/ml volume made upto 4ml by methanol (80%). Instead of methanolic extract of vegetables, pure methanol was used as control. The reaction mixture was vortex mixed and let to stand at room temperature in the dark for 30 min before the decrease in absorbance at 517 nm was measured at spectrophotometer (Perkin Elmer, Lambda 25, UV/VIS Spectrophotometer). Samples were measured in three replicates. Percentage of DPPH scavenging activity was calculated as % inhibition of DPPH = [Abs control – Abs sample / Abs control] x 100.

Table 2 : NUTRIENTS - Moisture, Ash, pH, Total Carbohydrate, Soluble Protein, and Total Protein contents (gm % fresh weight) of Cauliflower grown in selected five fields of Dhapa land-filled ground (DLG) and Normal Ground (NG)

SAMPLES	MOISTURE CONTENT (gm/100gm FW) ## N = 15	ASH CONTENT (gm/100gm FW) # N = 15	PH # N = 15	CARBOHYDRATE (gm/100gm FW) ## N = 15	SOLUBLE PROTEIN CONTENT (gm/100gm FW) # N = 15	TOTAL PROTEIN CONTENT (gm/100gm FW) # N = 15
CAU (DLG)	90.374 ± 0.411**	1.152 ± 0.140*	5.794 ± 0.015**	4.480 ± 0.098**	2.188 ± 0.090*	2.444 ± 0.011**
CAU (NG)	90.820 ±	0.964 ±	5.807 ± 0.009**	4.384 ± 0.036**	2.222 ± 0.020*	2.360 ±** 0.014

	0.391**	0.082*				

Table 3 : ANTI NUTRIENTS - Tannin, Oxalate and Phytate contents (per gm dry weight) of Cauliflower grown in selected five fields of Dhapa land-filled ground (DLG) and Normal Ground (NG)

SAMPLES	TANNIN CONTENT (mg/gm DW) # N = 15	OXALATE CONTENT (mg/gm DW) ## N = 15	PHYTATE CONTENT (µg/gm DW) ## N = 15
CAU (DLG)	11.250 ± 0.469*	2.827 ± 0.282*	19.911 ± 0.323*
CAU (NG)	10.742 ± 0.805*	3.040 ± 0.116*	19.775 ± 0.349*

SAMPLES	TOTAL PHENOL CONTENT (mg/gm DW)## N = 15	FLAVONOID CONTENT (mg/gm DW)# N = 15	ASCORBIC ACID CONTENT (mg/100gm FW)## N = 15	FRAP VALUE (mM/100gm DW)## N = 15	DPPH ACTIVITY## at 1000µg /ml
CAU (DLG)	94.680 ± 8.020*	21.600 ± 4.711*	56.595 ± 0.233**	4.401 ± 0.292*	17.938 ± 1.628*
CAU (NG)	95.353 ± 4.222*	20.0667 ± 2.154*	56.513 ± 0.274**	4.469 ± 0.277*	17.82 ± 1.445*

Table 4 : ANTIOXIDANTS – Total phenols, Flavonoids, Ascorbic acids content and FRAP Assay and DPPH Radical Scavenging Activity (dry weight basis) of Cauliflower grown in selected five fields of Dhapa land-filled ground (DLG) and Normal Ground (NG)
All results are expressed in MEAN ± SD
*determines significant variation exits within the fields of DLG & NG;
** determines no significant variation exits within the fields of DLG & NG;
# determines significant variation exits between the fields of DLG & NG ;
determines no significant variation exits between the fields of DLG & NG.

Statistical Analysis: Each experiment was repeated three times for l the sample collected from selected fields of DLG (i.e. DP1, DP2, DP3, DP4, & DP5) and NG (i.e. NG1, NG2, NG3, NG4, & NG5). The results are presented with their means, and standard deviation using Microsoft Office Excel 2010. The statistical analysis was done using repeated measures ANOVA with the help of the software SPSS 16.0. P<0.05 indicates significant variation exists at 95% confidence level.

Results: The result of analysis of nutrients ,anti• nutrients and antioxidant activity in Dhapa land filled Ground (DLG) as well as Normal Ground (NG) are given in Table 2 , Table 3 , and Table 4 respectively.

Discussions:

- **Table 2 : Nutrients:** The result of analysis (Table2) showed variation in proportions of nutrients (Moisture, Ash, pH, Total Carbohydrate, Soluble Protein and Total Protein Content) in• Dhapa land filled Ground (DLG) as well as Normal Ground (NG). It is found that , there are no significant differences in moisture contents , pH and total protein contents of cauliflower grown within the five selected fields of DLG and NG (P>0.05). Except moisture & carbohydrate contents, there are significant differences present between the fields of DLG & NG in terms of ash, pH, soluble protein & total protein contents (P < 0.05). Accordingly these are found to be high for cauliflower samples grown in DLG as compared to NG, except soluble protein which is found to be slight high in the cauliflower samples collected from different fields of NG.

Table 3 : Anti-Nutrients: Table 3 reveals that there are significant differences exist in Tannin , oxalate, & phytate contents of cauliflower grown within the five selected fields of DLG and NG (P < 0.05) . Moreover tannin contents of samples differ significantly (P < 0.05) between the fields of DLG & NG, but there are no significant differences found in oxalate and phytate contents between the fields of DLG & NG (P>0.05).

Table 4 : Antioxidants: The results of table 4 showed both the antioxidant contents (total phenols, flavonoids, & ascorbic acids) and total antioxidant activity (FRAP Assay & DPPH Radical Scavenging Activity at 1000µg /ml, DW) of the cauliflower

samples collected from Dhapa land filled Ground (DLG) as well as Normal Ground (NG). All the results are expressed in dry weight basis. Data reveals that the total phenols & flavonoids content, FRAP value and & DPPH Radical Scavenging Activity (1000µg /ml) of cauliflowers differ significantly within the five selected fields of DLG and NG ($P < 0.05$). Moreover analysis of ascorbic acids content of cauliflower samples showed that no significant differences exist within the five selected fields of DLG & NG ($P > 0.05$). Accordingly, there are also no significant differences present between the fields of DLG & NG ($P>0.05$) in terms of total phenols , ascorbic acids, FRAP Assay & DPPH Radical Scavenging Activity (1000µg /ml),except the flavonoids content ($P < 0.05$) in cauliflower samples.

Conclusion: The observations of the present study suggest that there were no significant differences exist in the Nutrients, Anti-Nutrients and Antioxidant content of cauliflower, except ash, pH, soluble and total protein, tannin and flavonoid contents between Normal Ground (NG) and Dhapa Landfilled Ground (DLG). Although some variations have been found in their distributions within different fields of DLG and NG, except for moisture, pH, carbohydrate, total protein and ascorbic acid content. Collected samples of cauliflower also show no significant difference in their total antioxidant activity for both DLG and NG sites. It was further noted that ash, total Proteins and flavonoids content of the samples of DLG are higher than those of NG. From this study it has been concluded that the nutritional quality of cauliflower are almost similar in both places viz. Normal Ground and Dhapa Land filled Ground (except ash, pH, soluble and total protein, tannin and flavonoid contents).

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