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# Comparative analysis of some commonly consumed allergic food materials

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## ABSTRACT

**Background:** Food allergy is turning out to be one of the vital causes of death in all countries irrespective of the socio-economic status of the people. The extent of mortality from food allergens has increased several folds in the last decade. This has brought research on food allergy and its causative properties to the front. Human beings have the natural power to fight with an allergen, ranging from viruses to food. But in the case of immune-compromise or some other defects in the immune system, individuals can react against some food allergic component(s). Various studies have indicated that cereal or grain particles are more allergic materials than fruits or vegetables.

**Objective:** This study was carried out with the main objective of understanding the variations in various biochemical parameters of a few foods commonly consumed and known to elicit allergic reactions.

**Methods:** In this study, a few food materials known to elicit allergic reactions in some individuals were selected and comparative analysis (qualitative and quantitative) was performed in an attempt to understand the basis of their differential responses.

**Results:** The studies indicated difference in various biochemical parameters and anti-oxidative properties between equivalent quantities of the food samples.

**Conclusion:** Our study has revealed differential levels of nutrient contents and anti-oxidative properties between equivalent quantities of the samples of allergic food materials. These findings can be used for further research on the underlying mechanisms of their action.

**Keywords**: Food allergy, Biochemical attributes, Non-communicable diseases, Dietary habits, Nutrition

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## INTRODUCTION

The body's immune system keeps the body healthy by fighting off infections and other dangers to good health. A food allergy reaction occurs when the immune system overreacts to a food or a substance in a food, identifying it as a danger and triggering a protective or immune response. The incidence rates of allergies have increased exponentially, affecting more than 60 million people on daily basis. Environmental changes have been identified as one of the prime factors contributing to this increase as they not only affect the intensity and diversity of external exposures, but also alter the normal immune responses [1]. The main causative agent behind the allergies is usually a harmless substance, named allergen, which is basically a type of antigen. This allergen triggers an immune response so that the immune system can fight against any external threat [2]. Allergen can stimulates type-I hypersensitivity reaction in individuals through Immunoglobulin E (IgE) responses [1] and is a Th2-driven immune disorder where food-specific IgE antibodies take necessary actions [3]. Sensitivity against an allergen varies from one person to another. Allergies take place when immunoglobulin E (IgE) binds to food molecules, particularly the protein part of the food and stimulates the release of histamine like inflammatory substances [4, 5].

Food allergy is phenotypically an extremely heterogeneous group of diseases. It can affect many organs at a time, either separately, or in combination, with the severity of reactions ranging from mild to severe and local to full-blown anaphylaxis [3]. Basically all types of foods have the ability to generate an allergic reaction in that person who has become sensitive for that particular antigen. Food allergies, in fact, can cause life-threatening reactions and profoundly influence the quality of life. Presently food allergies have a promising ground in health research [6].

### What is Non-communicable disease and how it is related to food allergy

Recently, the cause of diseases and deaths of non-communicable diseases (NCDs) related with food allergens has increased worldwide [7, 8, 9, 10, 11, 12]. Several factors, such as

abundance and aggressiveness of allergens or allergen carriers due to environment pollutions and novel food engineering technologies, and misuse of antibiotics may be responsible for making a person more susceptible towards allergies. Foods have a high chance to get contaminated with heavy metals such as mercury, lead, chromium and cadmium; especially hybrid foods have the maximum possibility as they get directly affected by the heavy pollution of water resources [13,14]. Some reported studies have tried to understand the interaction between the environment, pathogen and host in context of non-communicable diseases and food allergens [15, 16].

Thus this study was carried out with the objective of understanding the differences in basic characteristics of a few common food materials which are known to generate allergic reactions in some individuals selectively. They were compared with respect to basic biochemical parameters as well as their anti-oxidant capability. The food materials selected included Eggplant (*Solanum melongena*), Oat (*Avena sativa*), Onion (*Allium cepa L.*), Broccoli (*Italica* cultivar group of the species *Brassica oleracea*) and Beetroot (*Beta vulgaris*). Such comparative analyses may help in identifying the molecular mechanisms by which they contribute towards occurrence of various non-communicable diseases.

## MATERIALS AND METHODS

***Sample preparation:*** For sample preparation, 1 gm of each food sample was added either in 5 ml of water, PBS or Etanol to make stock solution. The dissolved samples were centrifuged at 10,000 rpm for 5 minutes. The supernatant was collected for performing the tests.

***Qualitative Analysis of the Samples***

***(i) Determination of presence of Sugar in Samples***

Benedict's test was used to test for simple carbohydrates. The Benedict's test identifies reducing sugars (mono-saccharide's and some disaccharides), which have free ketone or aldehyde functional groups. For performing this test, approximately 1 ml of sample was placed into a clean test tube. 2 ml (10 drops) of Benedict's reagent ($CuSO_4$) was added to it. The solution was then heated in a boiling water bath for 5 minutes and color change was observed in the solution of test tubes. The observed data were collected and tabulated.

***(ii) Determination of presence of Vitamin C in Samples***

In this test, a blue substance called 2, 6-dichlorophenolindo phenol (or DCPIP for short) acts as an indicator. Its colour changes from blue to red with acids but is lost in the presence of certain chemicals, one of which is ascorbic acid (vitamin C). Thus, DCPIP solution can be used to test for the presence of vitamin C in foods. For performing this test, a small amount of the sample was put into a test tube to a depth of about 2cm. An equivalent similar amount of distilled water was added to it and the mixture was stirred with a glass rod. Next, it was allowed to stand for a few minutes. Subsequently, a small amount of the clear liquid was transferred into to a test tube and DCPIP solution was added to it dropwise. If the extract is acid, the colour will change from blue to red. More DCPIP solution was added to check whether the colour disappeared altogether. Decolourisation of DCPIP showed that a vitamin C is probably present. Other chemicals can do this in food and drink, but vitamin C is the main one.

***(iii) Determination of presence of Starch in Samples***

Iodine solution is used to test for the presence of starch. If starch is present in a food item, it turns an intense blue-black colour upon addition of aqueous solutions of tri iodide anion, due to the formation of an intermolecular charge-transfer complex. In the absence of starch the brown colour of the aqueous solution remain same. This interaction between starch and tri iodide is also the basis for iodometry. For performing this test, the test tubes containing samples were placed in a water filled beaker and the beaker was placed over a hot plate. The mixture was heated for 5 minutes while continuously stirring the water in beaker with a glass rod. The solutions were filtered from one test tube to another using filter papers. 5 clean test tubes were taken and some of the filtrate was poured into it. A few drops of iodine solution was taken using a dropper and added to the filtrates and color change was observed.

***(iv) Determination of presence of Lipid in Samples***

2 ml of ethanol was added to the food sample and the mixture was shaken well. It was allowed to settle in a test tube rack for 2 minutes for the food to dissolve in ethanol. Any clear liquid was emptied into a test tube containing 2 ml of distilled $H_2O$. Appearance of a milky-white emulsion indicated a positive result (presence of lipid). If the mixture remained clear, it indicated that no fats are present in the sample.

***Quantitative Analysis of the Samples***

***(i) Determination of pH of Samples***

The pH electrode was immersed in pH 7 buffer and then in pH 4 buffer to calibrate the pH meter. pH electrode was then immersed into the sample and pH value was noted down for each sample.

***(ii) Determination of presence of Iron in Samples***

For this assay, 2 gm each samples were weighed. The samples were heated strongly in an evaporating dish or crucible until a gray ash remained. After cooling 5 ml of distilled water was added to the ash and stirred well. The filtrates were filtered and transferred to another set of test tubes of the same size as the standards. Subsequently, 5 ml of 0.1 M KSCN solution (previously prepared) was added and the color was compared to the standards to understand how much Iron was present in 2 gm of each sample. O.D. was taken at 450 nm to test the absorbance of the standards and unknowns using Spectrophotometer. Using O.D. standard curve was prepared and exact concentration of iron was found in each sample.

***(iii) Determination of presence of Protein in Samples***

1 ml of stock solution was added in 1 ml of Bradford reagent. After that the whole mixture was half diluted. For blank 1 ml of water was added in 1 ml of Bradford reagent. The mixture was incubated at room temperature for 30 minutes in dark. O.D. was taken at 595 nm to test the absorbance of the standards and unknowns using Spectrophotometer. Using O.D. standard curve was prepared and exact concentration of protein was found in each sample.

**Calculation:** Regression curve was prepared by plotting optical density on the 'y' axis against standard protein, i.e. BSA. The protein in the sample was calculated from the standards BSA graph.

***(iv) Estimation of Total Phenolic contents (TPC) (Follin-Ciocalteau Assay):***

Total phenolic contents of the samples were estimated by Folin-ciocalteau method (with some modifications). For this assay, each sample was diluted 15 times and 300 µl of the diluted sample was mixed with 1500 µl of 1:10 dilued Floin-ciocalteau reagent. Subsequently, each of these mixtures was

incubated with 1200 µl of 7% sodium bicarbonate solution for 2 hours. Finally, absorbance was measured at 765 nm in spectrophotometer. The observations were interpreted on the basis of standard curve prepared by using a solution of Gallic acid [17].

***(v) Estimation of Total Flavanoid contents (TFC) (Aluminium chloride Assay):***

Total flavonoid contents of the samples were estimated using aluminium chloride colorimetric method, with some modifications. For this assay, each sample was diluted 15 times. 300 µl of each diluted sample was incubated with 90 µl of 5% $NaNO_2$ for 5 minutes followed by incubation with 90 µl of 10% $AlCl_3$ for 6 minutes. In the last step, the mixture was incubated with 600 µl of 1M NaOH and the absorbance was measured at 510 nm in spectrophotometer. A solution of Catechin as standard for interpreting the absorbance values of the samples [18].

***(vi) Determination of DPPH radical scavenging activity:***

Antioxidant activity of the samples was estimated with the help of DPPH radical scavenging assay, with some modifications [19]. The absorbance was measured at 540 nm in spectrophotometer. The absorbance values of the samples were compared with that of the control. Percentage scavenging activity was calculated using the formulae: % Scavenging = [(Control absorbance- sample absorbance)/Control absorbance] * 100

## RESULTS AND DISCUSSION

***The Results of Qualitative Analysis of the Samples:***

***1. Determination of Solubility:***

All the samples were suspended in various solvents to get an estimate of their solubiliy. Figure 1 summarizes the findings.

**Result Of Solubility Test Of The Samples**

| Solvent | Eggplant | Oats | Onion | Broccoli | Beetroot |
|---|---|---|---|---|---|
| **Water** | **Fully** | **Partially** | **Fully** | **Fully** | **Fully** |
| **PBS** | **Fully** | **Partially** | **Fully** | **Partially** | **Partially** |
| **Ethanol** | **Fully** | **Fully** | **Fully** | **Fully** | **Fully** |

Figure 1: Summary of solubility of samples in various solvents

***2. Determination of presence of sugar, vitamin C, starch and lipids:***

All the samples were tested for presence of sugar, vitamin C, starch and lipids in their contents. Oats were found to be rich source of starch, whereas sugar and vitamin C were found to be abundant in eggplant, broccoli, onion and beetroot. Lipids were not detected in any of these samples. Figure 2 summarizes the findings.

**Result Of Qualitative Analysis Of The Samples**

| Sample | Sugar Test | Vit C | Starch | Lipid Test |
|---|---|---|---|---|
| **Eggplant** | ✓ | ✓ | ✗ | ✗ |
| **Oats** | ✗ | ✗ | ✓ | ✗ |
| **Onion** | ✓ | ✓ | ✗ | ✗ |
| **Broccoli** | ✓ | ✓ | ✗ | ✗ |
| **Beetroot** | ✓ | ✓ | ✗ | ✗ |

**(A)**

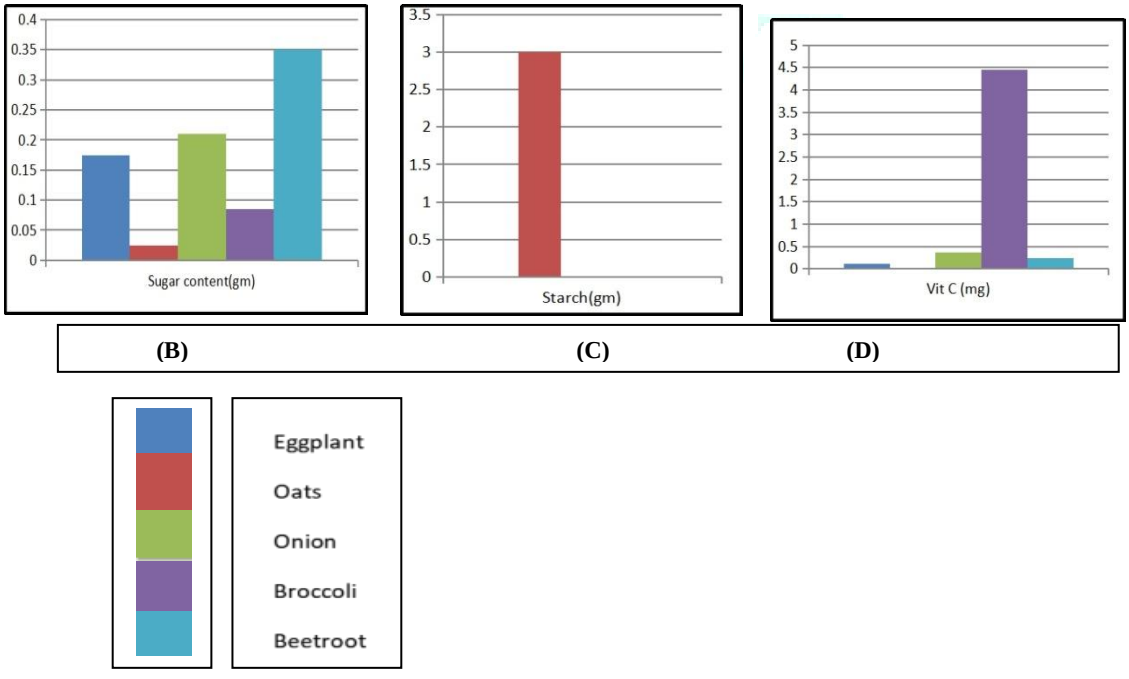
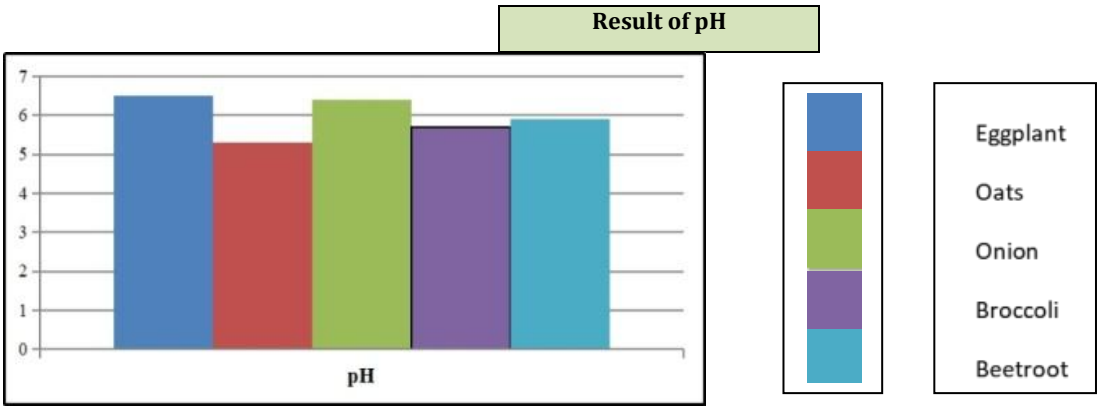



Figure 2: Summary of qualitative analysis of the samples for detection of sugars, vitamin C, starch and lipids. (A) summary of presence/absence, (B) graphical representation - sugar, (C) graphical representation - vitamin C, (D) graphical representation - starch

### 3. *Determination of pH:*

All the samples were tested for their pH. Figure 3 summarizes the details of the findings.




**(A)**

| Samples | pH |
|---|---|
| Eggplant | **6.5** |
| Oats | **5.3** |
| Onion | **6.4** |
| Broccoli | **5.7** |
| Beetroot | **5.9** |

**(B)**

Figure 3: Summary of pH of samples in various solvents; (A) graphical representation, (B) values

### 4. *Determination of protein concentration:*

All the samples were analysed for their protein content. Figure 4 summarizes the findings.

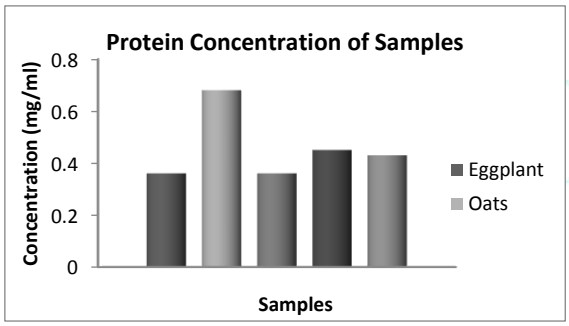



Figure 4: Protein concentrations of various samples

### 5. *Determination of iron concentration:*

All the samples were analyzed for their iron content. Figure 5 summarizes the findings.

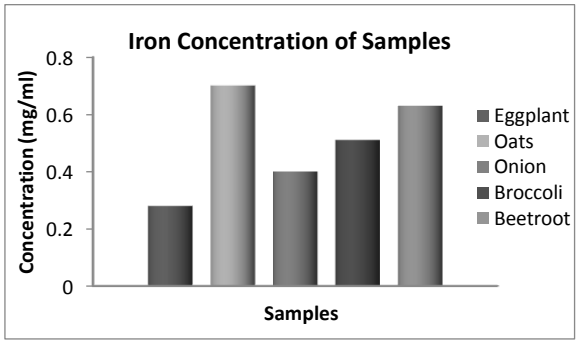



Figure 5: Iron concentrations of various samples

### 6. *Estimation of Total Phenolic contents (TPC):*

All the samples were analyzed for their TPCs. The findings of this assay are summarized in Figure 6.

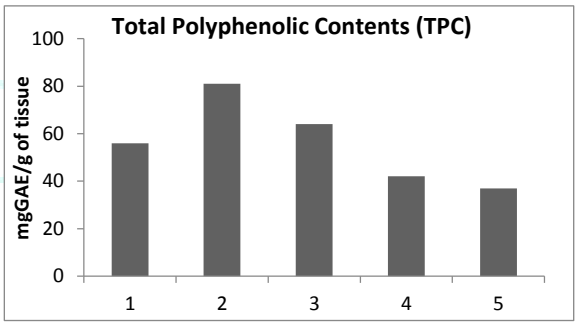



Figure 6: Total Polyphenolic Contents (TPC) of various samples; 1-Eggplant, 2-Oats, 3-Onion, 4-Broccoli, 5-Beetroot

### 7. *Estimation of Total Flavanoid contents (TFC):*

All the samples were also analyzed for their TFCs. The findings of this assay are summarized in Figure 7.

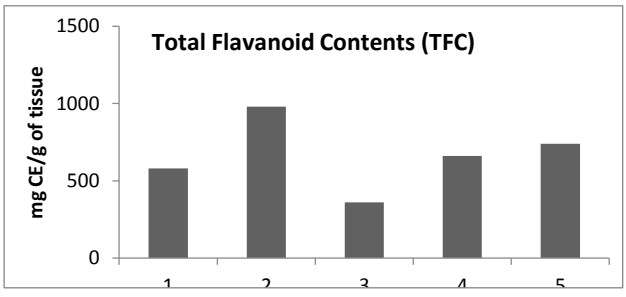



Figure 7: Total Flavanoid Contents (TFC) of various samples; 1-Eggplant, 2-Oats, 3-Onion, 4-Broccoli, 5-Beetroot

### *8. Determination of DPPH scavenging activity:*

All the samples were analyzed for their ability to scavenge DPPH. The findings of this assay are summarized in Figure 8.

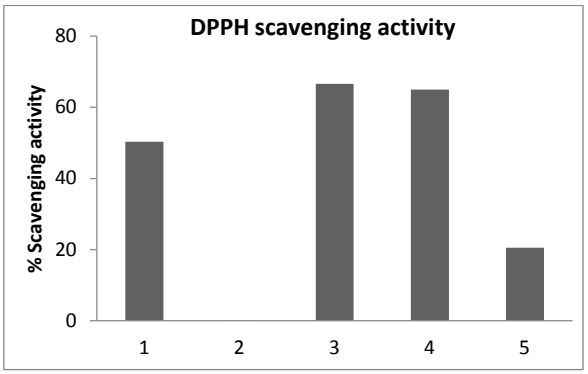



Figure 8: DPPH scavenging activity of various samples; 1-Eggplant, 2-Oats, 3-Onion, 4-Broccoli, 5-Beetroot

The present study clearly indicates that out of the five allergic foods that have been analyse, there is distinct variation both in terms of the biochemical properties as well as anti-oxidant levels. Such variations may be the causative factors of differential allergic reactions in selective individuals. Further detailed study on the molecular mechanisms of their action on healthy versus diseased individuals/model systems will help to get a better understanding of the effects of such differences in their characteristics.

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## REFERENCES

1. Lamson RW, Inman V. *Cal West Med,* 1932; 36(1):24.
2. Bousquet J, Lockey R, Malling HJ. *J Allergy Clin Immunol,* 1998; 102(4 Pt 1):558.
3. van Ree R, Poulsen LK, Wong GW, Ballmer-Weber BK, Gao Z, Jia X. *Zhonghua Yu Fang Yi Xue Za Zhi,* [Chinese] 2015; 49(1):87.
4. Devdas JM, Mckie C, Fox AT, Ratageri VH. *Indian J Pediatr*, 2017. doi: 10.1007/s12098-017-2535.
5. Sicherer SH, Sampson HA. *J Allergy Clin Immunol,* 2014; 133(2):291.
6. D' Auria E, Mameli C, Piras C, Cococcioni L, Urbani A, Zuccotti GV, Roncada P. *J Proteomics*, pii: 2018; S1874-3919(18)30049-6. doi: 10.1016/j.jprot.2018.01.018. [Epub ahead of print] PubMed PMID: 29408543.
7. Miranda JJ, Kinra S, Casas JP, Davey Smith G, Ebrahim S. *Trop Med Int Health*, 2008; 13(10):1225.
8. Upadhyay RP. *Iran J Public Health,* 2012; 41(3):1.
9. Dye C. *Philos Trans R Soc Lond B Biol Sci,* 2014; 369(1645):20130426.
10. Li D. *J Sci Food Agric*, 2014; 94(2):169.
11. Gilles S, Traidl-Hoffmann C. *J Dtsch Dermatol Ges,* 2014; 12(5):395.
12. Siegel KR, Patel SA, Ali MK. *Br Med Bull,* 2014; 111(1):31.
13. Boyce JA, Assa'a A, Burks AW, Jones SM, Sampson HA, Wood RA et al. *Nutrition*, 2011; 27(2):253.
14. Valenta R, Hochwallner H, Linhart B, Pahr S. *Gastroenterology*, 2015; 148(6):1120.
15. Popescu FD. *World J Methodol,* 2015; 5(2):31.
16. Garg A, Anand T, Sharma U, Kishore J, Chakraborty M, Ray PC, Ingle GK. *J Family Med Prim Care,* 2014; 3(2):112.
17. Singleton V.L., Rossi J.A. *American Journal of Enology and Viticulture*, 1965; 16:144.
18. Jia Z.S., Tang M.C. *Food Chemistry*, 1999; 64:555.
19. Blois MS, *Nature,* 1958; 181:1199.