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Cooking of Edible Leafy Vegetables Changes their Nutritional Value

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Abstract: ***The current study investigated the effect of various cooking methods (boiling, blanching, sautéing and microwaving) on different nutritional components namely protein, polyphenol, polysaccharide, flavonoid, total antioxidant and free amino acid of four edible leafy vegetables (water spinach, red amaranth, spinach and bottle gourd leaves), widely used in preparation of local diets. The results show that; for protein, polysaccharide, polyphenol, flavonoid and free amino acids the cooking technique of sautéing had maximum retention for all four edible leaves. The total antioxidants of the edible leaves; varied greatly across the different cooking techniques used. The antioxidant activity increased in red amaranth leaves when blanched, in spinach leaves when microwaved and in bottle gourd leaves for all cooking techniques. On the contrary, the antioxidant activity of water spinach leaves decreased for all cooking methods.***
Keywords: Cooking, Edible Leafy Vegetables, Nutrition

I.INTRODUCTION

Human nutrition can be broken down into two classes: macronutrients and micronutrients. Macronutrients mainly include fats, proteins and carbohydrates. These are required in considerable quantities by humans and are primarily responsible for enabling most, if not all of the energetic and assimilative activities of the body. Micronutrients include numerous vitamins and dietary minerals which serve a diverse array of non-assimilatory or energy producing roles including enzyme cofactors, bio-chemical ligands etc (Al-Whaibi, 2011).

Humans are heterotrophs. Therefore, humans acquire nutrients from the surrounding environment for growth, sustenance and energy production in the form of what we generalize as “food”. It should also be noted that apart from macronutrients and micronutrients a diverse variety of organic compounds having documented influences on human biochemical systems are also present in “food”. Examples of that can include flavonoids and carotenoids, to name a few. Although human food can refer to an extremely diverse and elaborate collection of edible substances of plant, animal and even microbial origin, they have some common attributes.

Firstly, human catabolic enzymes can break down the complex (generally polymeric) substances comprising the food materials into much simpler monomeric products and the body can assimilate them and/or convert them to energy. Secondly, the chemicals/chemical groups/ bioactive compounds present in the food do not have any known negative effects on the human body. Thirdly, macronutrients and micronutrients comprising food are present in different physical and chemical states and forms. This is essential as the exact state of a particular substance in food can affect how much of it can actually be utilized by the body. Finally, combinations of different food ingredients/items found naturally or artificially constructed can significantly affect the net nutritive value of the food. The above statements summarize the considerations associated with the consumption of raw, unprepared food. Since most food items consumed by humans are subjected to a multitude of preparatory methods, how the food is cooked becomes the biggest consideration as far as food and nutritive value correlation are concerned. An overgeneralization of cooking would be the application of varying degrees of thermal energy on food ingredients for varying amounts of time. However, a high degree of variation is possible as far as varying cooking time and heating intensity for different cooking methods are concerned (Wang et al., 2004, López-Berenguer et al., 2007). Moreover, several cooking methods are commonly associated with the addition of different types of edible lipids making the downstream effects of cooking on food nutrients even more difficult to predict. The recent decades have also seen a steady increase in the application of microwave radiation to cook food which may or may not have differing effects on nutrients as compared to traditional fire heated food (El-Adawy, 2002). To complicate matters even further, different nutrients have wildly different sensitivities to heat and radiation which is often subject to variation when interacting with other nutrients. The samples were subjected to each of the below-mentioned cooking techniques._Sautéing is cooking food in a small quantity of fat or oil. In sautéing, a very small amount of oil is placed in a shallow pan, and when it is sufficiently hot, the food is put into it. Foods that are to be sautéed are usually sliced thin or cut into small pieces, and they are sometimes turned frequently during the process of cooking. Blanching is a cooking technique involving boiling food (usually vegetables and fruits) in water for a very short time.

Blanching is often followed by plunging the food into ice water to stop the cooking process. Boiling refers to cooking food in boiling hot water. In most populated parts of the world, plain water boils at temperatures from about 95°C to 100°C. As the water heats in the process of boiling, tiny bubbles appear on the bottom of the vessel in which it is contained and rise to the surface. Then, gradually, the bubbles increase in size until large ones form, rise rapidly, and break, thus producing constant agitation of the water. Microwaving refers to cooking food using electromagnetic waves in a microwave powered by electricity. Microwaves activate the water molecules or particles of food, causing heat by friction which cooks the food. Thus, studies on the retention of nutritional value of food with respect to different cooking techniques would help to determine not only the nutritionally superior version of cooked food but also to better our understanding of food and nutrition biochemistry (Wu et al., 2003, Reda 2011). In our work, we aimed to study the effect of cooking on the nutritional content of water spinach, red amaranth, spinach and bottle gourd leaves by quantitatively measuring the content of protein, polysaccharide, flavonoids, polyphenols, free amino acids and total antioxidants using UV- Vis spectrophotometry.

II. MATERIALS AND METHODS

A. Reagents and Chemicals

Aluminium chloride, disodium phosphate, monosodium phosphate, anhydrous sodium carbonate, dextrose, Folin's reagent, sodium nitrate and sodium hydroxide were purchased from Merck. Other chemicals such as ethanol from Rankem – RFCL Limited, New Delhi, Bradford reagent from G-quant, bovine serum albumin from Himedia, phenol from Sigma- Aldrich, concentrated sulphuric acid from Finar, gallic acid, glycine and ascorbic acid from SD - Fine Chemical Ltd, methanol and quercetin-1, 1-diphenyl-2-picrylhydrazyl from Sisco Research Laboratories Pvt. Ltd., Mumbai, ninhydrin from Research – Lab Fine Chem industries, Mumbai and distilled water from Biobharati Lifesciences was purchased and utilized for different experimental purposes.

B. Sample Collection and Preparation

Fresh leaves of water spinach (kalami saag), bottle gourd (lau saag), red amaranth (lal saag) and spinach (palong saag) were collected from the local markets of Kolkata, West Bengal. Raw leaves were used as a control. The leaves were washed under running tap water and subjected to the processing techniques of blanching, boiling, microwaving and sautéing in oil. During boiling the sample was subjected to boiling in 1L of tap water until cooked. For blanching, the leaves were immersed in hot water until cooked and then immediately sieved out and transferred into an ice bath to immediately stop the cooking process. Microwaving was carried out in a conventional microwave oven with a splash of water until cooked. For sautéing, the leaf samples were sautéed in vegetable oil on the stove top on low to medium flame until cooked. The cooked leaves were then grinded using a mortar and pestle kept over ice in 10% weight/volume solution of phosphate buffer. The obtained solution was centrifuged at 10,000 rpm for 10 minutes after which the supernatant was collected and kept frozen at -20ºC until further use.

C. Determination of the protein content

Initially, 100µl of the extract was mixed with 1ml of Bradford reagent. The solution was then incubated in dark for 30 minutes. The absorbance was measured at 595nm. The calibration curve was prepared by using BSA as a standard.

D. Determination of the polyphenol content

In 500µl of extract, 500µl of phosphate buffer, 500µl of folin (1:10 dilution), 5 ml of distilled water and 6 ml of sodium carbonate solution (7.5%) was added and mixed well. The absorbance was measured at 760 nm after 30 minutes of incubation in dark. The calibration curve was prepared by using gallic acid as a standard (MoDNicki and Balcerek, 2009).

E. Determination of the polysaccharide content

In 30µl of extract, 470µl of phosphate buffer was added followed by addition of 500µl of 5% phenol and 2.5ml of sulphuric acid. The solution was then incubated for 30 minutes, after which the absorbance was measured at 488nm. The calibration curve was prepared by using dextrose as a standard (Daniel and Krishnakumari, 2015).

F. Determination of the flavonoid content

In 1.2ml of the extract, 90µl of $NaNO_2$ (5%), 90 µl of $AlCl_3$ (10%), 600µl of NaOH (1M) and 1020µl of water was added and mixed well. The solution was incubated for an interval of 5 minutes, 6 minutes, 2 minutes and 10 minutes respectively after addition of the reagents. Then the absorbance was measured at 510 nm. The calibration curve was prepared by using quercetin as a standard (Kalita et al., 2013).

G. Determination of the total antioxidant content

The absorbance of 0.2mM concentration of DPPH solution was measured at 517nm. The absorbance was subsequently adjusted to 1.05 after 30 minutes of incubation using methanol. 100µl of the extract was mixed 2.9ml of the DPPH solution. The absorbance was measured at 517nm after 30 minutes of incubation. The calibration curve was prepared using ascorbic acid as a standard (Vithya et al., 2012).

H. Determination of the free amino acid content

In 100µl of extract, 100µl of ninhydrin and 100µl of 50% ethanol was added and mixed well. The samples were then kept in a water bath (80ºC) for 15 minutes. The samples were taken out of the water bath and 2ml of distilled water was added to each of the tubes. Then the absorbance was measured at 570 nm. The calibration curve was prepared by using glycine as a standard.

I. Instrumentation

A systronics UV-Vis spectrophotometer was used to measure the absorbances of the different assay reactions.

III. RESULTS

A. Effect of cooking on protein concentration

The protein content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 1**. As expected, the raw edible leaves have the highest protein content excepting water spinach (kalami saag) where the variation in the protein content of the raw and cooked in oil samples are within the margin of error.

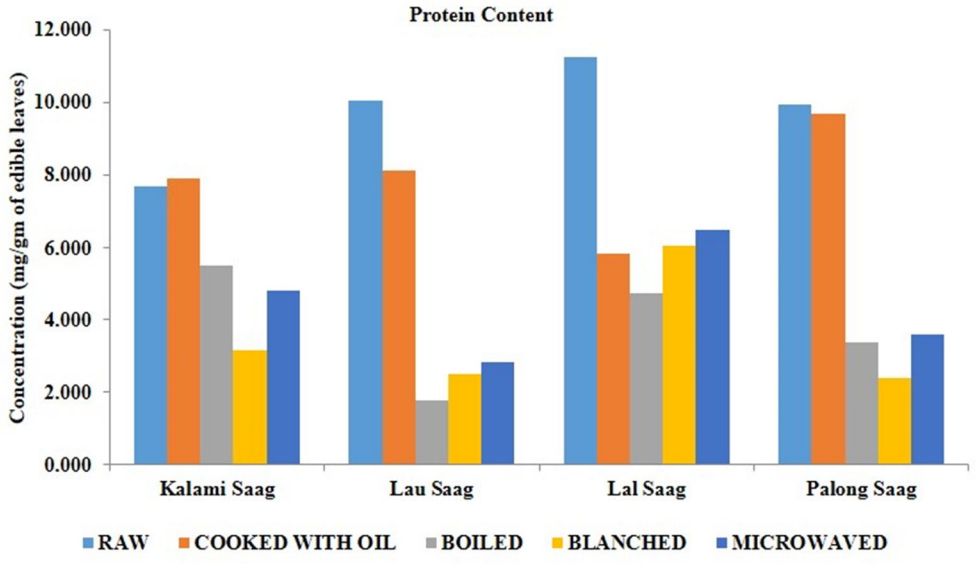

Figure 1. Protein content in different edible leaves before and after cooking using various cooking techniques.

The protein retention was observed to be highest in water spinach leaves (kalami saag) and lowest in bottle gourd leaves (lau saag) after boiling. However, when cooked with oil the protein retention was highest in spinach leaves (palong saag) and lowest in red amaranth leaves (lal saag). After blanching the protein retention was observed to be highest in red amaranth leaves (lal saag) and lowest in spinach leaves (palong saag). Also, the protein retention was highest in red amaranth leaves (lal saag) and lowest in bottle gourd leaves (lau saag) when microwaved.

B. Effect of cooking on polyphenol concentration

The polyphenol content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 2.** The polyphenol content of the leaves increases especially when cooked with oil and varies greatly for other cooking techniques used. The polyphenol retention was observed to be highest in water spinach leaves (kalami saag) and lowest in red amaranth leaves (lal saag) after boiling. Similarly, when cooked with oil the polyphenol retention was highest in spinach leaves (palong saag) and lowest in red amaranth leaves (lal saag).

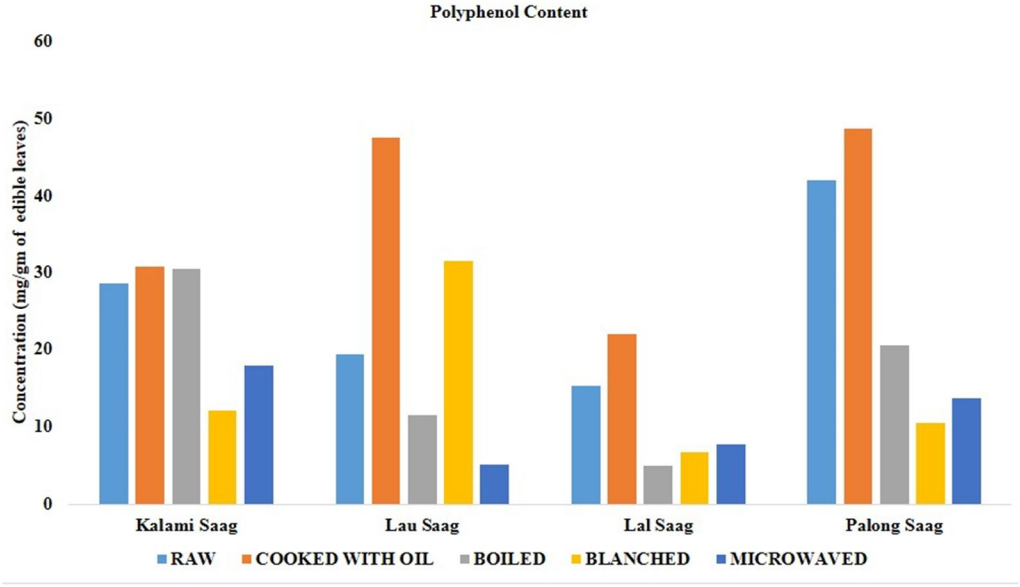

Figure 2. Polyphenol content in different edible leaves before and after cooking using various cooking techniques.

After blanching the protein retention was observed to be highest in bottle gourd leaves (lal saag) and lowest in red amaranth leaves (lal saag). Also, the polyphenol retention was highest in water spinach leaves (kalami saag) and lowest in bottle gourd leaves (lau saag) when microwaved.

C. Effect of cooking on polysaccharide concentration

The polysaccharide content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 3.** The polysaccharide content of the samples increases especially when cooked with oil and varies greatly for other cooking techniques used.

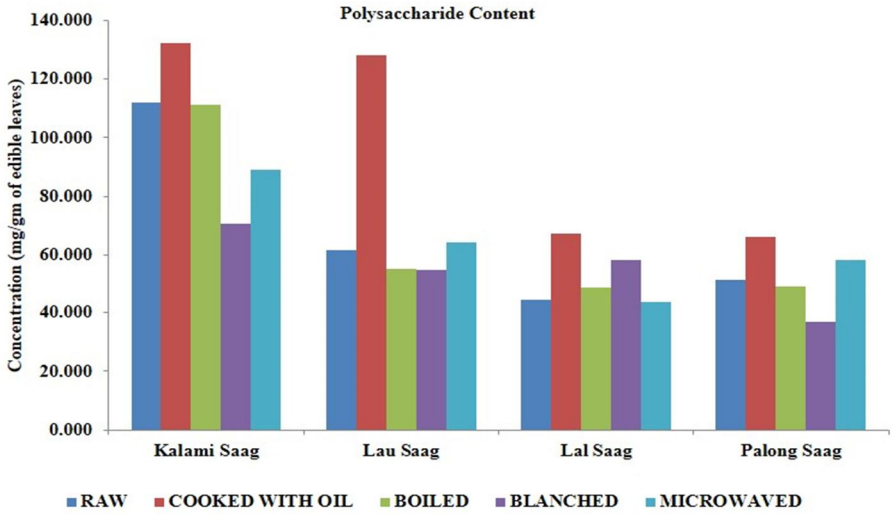

Figure 3. Polysaccharide content in different edible leaves before and after cooking using various cooking techniques.

The polysaccharide retention was observed to be highest in water spinach leaves (kalami saag) and lowest in red amaranth leaves (lal saag) and spinach leaves (palong saag) after boiling. Similarly, when cooked with oil the polysaccharide retention was highest in water spinach leaves (kalami saag) and lowest in spinach leaves (palong saag). After blanching the polysaccharide retention was observed to be highest in water spinach leaves (kalami saag) and lowest in spinach leaves (palong saag). Also, the polysaccharide retention was highest in water spinach leaves (kalami saag) and lowest in red amaranth leaves (lal saag) when microwaved.

D. Effect of cooking on flavonoid concentration

The flavonoid content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 4.** The flavonoid content of the leaves is considerably increased when cooked with oil.

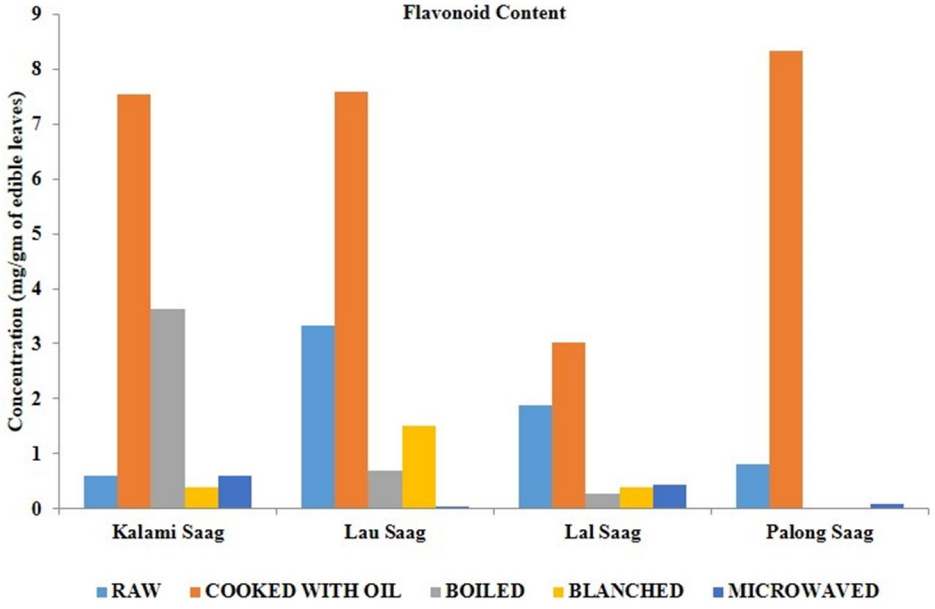

Figure 4. Flavonoid content in different edible leaves before and after cooking using various cooking techniques.

The flavonoid retention was observed to be highest in water spinach leaves (kalami saag) and lowest in spinach leaves (palong saag) after boiling. Similarly, when cooked with oil the flavonoid retention was highest in spinach leaves (palong saag) and lowest in red amaranth leaves (lal saag). After blanching the flavonoid retention was observed to be highest in bottle gourd leaves (lal saag) and lowest in spinach leaves (palong saag). Also, the flavonoid retention was highest in water spinach leaves (kalami saag) and lowest in bottle gourd leaves (lau saag) when microwaved.

E. Effect of cooking on the total antioxidant concentration

The total antioxidant content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 5.** The total antioxidant content of the samples varied greatly across the different cooking techniques used.

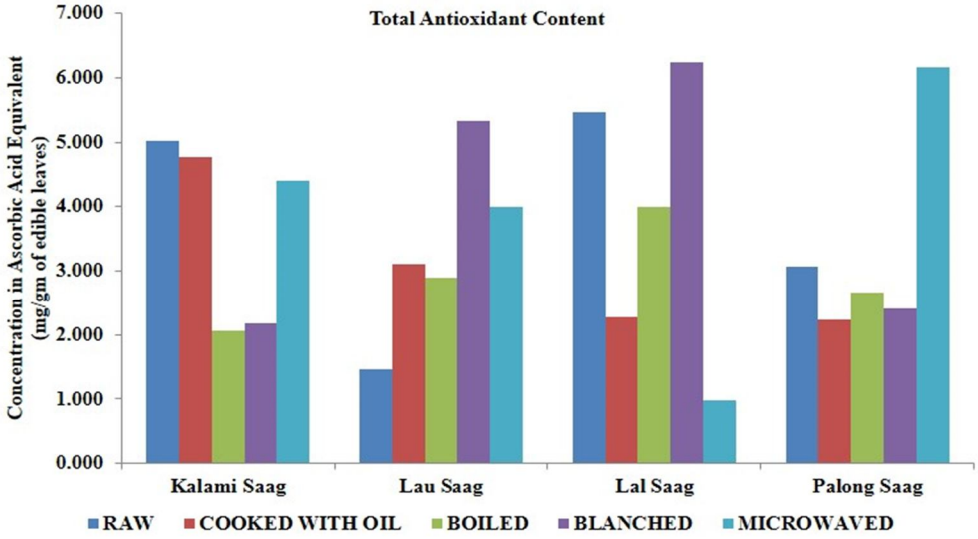

Figure 5. Total antioxidant content in different edible leaves before and after cooking using various cooking techniques.

The total antioxidant retention was observed to be highest in red amaranth leaves (lal saag) and lowest in water spinach leaves (kalami saag) after boiling. Similarly, when cooked with oil the total antioxidant retention was highest in water spinach leaves (kalami saag) and lowest in spinach leaves (palong saag). After blanching the total antioxidant retention was observed to be highest in red amaranth leaves (lal saag) and lowest in water spinach leaves (kalami saag). Also, the total antioxidant retention was highest in spinach leaves (palong saag) and lowest in red amaranth leaves (lal saag) when microwaved.

H. Effect Of Cooking On The Free Amino Acid Concentration

The free amino acid content of the edible leaves raw (untreated) and cooked under different conditions are presented in **Figure 6.** The free amino acid retention was observed only in water spinach leaves (kalami saag) after boiling.

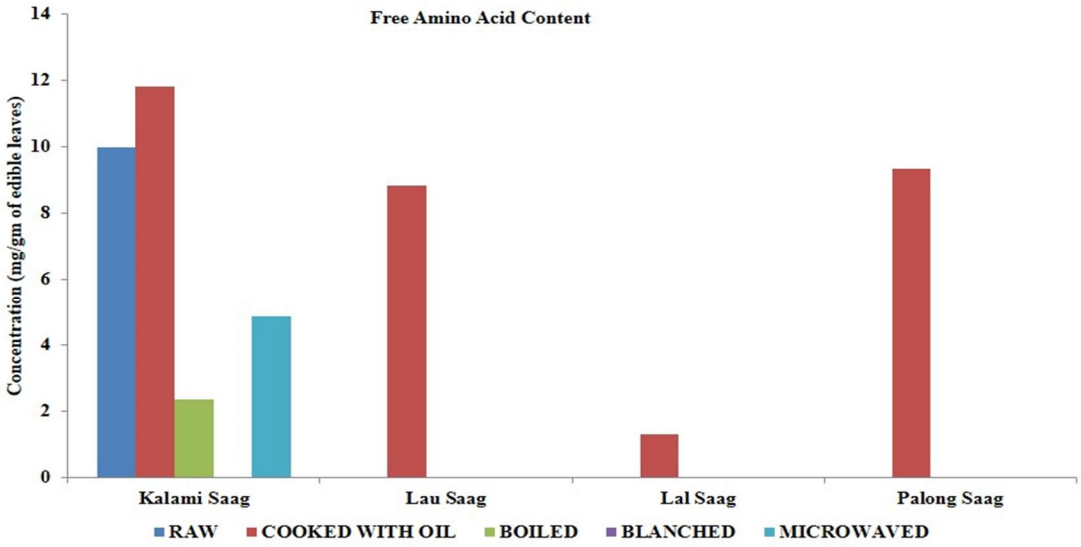

Figure 6. Free amino acid content in different edible leaves before and after cooking using various cooking techniques.

Similarly, when cooked with oil the free amino acid retention was highest in water spinach leaves (kalami saag) and lowest in red amaranth leaves (lal saag). After blanching, no free amino acid was retained in any of the samples. Also, the free amino acid retention was observed only in water spinach leaves (kalami saag) when microwaved.

IV.CONCLUSION

For protein and polysaccharide, the cooking technique of sautéing showed maximum retention (Figure 1 and Figure 3). For the macromolecules, this can be due to the presence of oil that forms a layer, which reduces the degradation in the leaf samples and/or due to the loss of water during the process of cooking that concentrates the nutrient content (Ayala et al., 2005). Also, this can be due to a less uniform thermal conductivity compared to other cooking techniques (boiling, blanching and microwaving) that reduce macromolecular degradation.

Polyphenol and flavonoid retention followed a similar pattern with the different cooking techniques (Figure 2 and Figure 4). For polyphenol, sautéing could have inactivated the polyphenolic oxidases preventing polyphenol decomposition and/or it could have promoted dietary fibre-binding type polyphenols decomposition into free phenolic compounds that increased the value (Stewart et al., 2000, Yamaguchi et al., 2003). For flavonoid, the heating process also makes available most of the phenolic compounds trapped in fibre of green leafy vegetables as explained by Adefegha and Oboh (Adefegha and Oboh, 2011). The oil used for cooking could also contain certain phenolic compounds.

For total antioxidants, each cooking technique had different effects on different leaf samples (Figure 5). The DPPH free radical scavenging ability increased in red amaranth leaves when blanched, in spinach leaves when microwaved and in bottle gourd leaves for all cooking techniques. The increase in antioxidant activity in vegetables can have different possible reasons as suggested by Morales and Babbel (Morales and Babbel, 2002). For water spinach leaves the antioxidant activity decreased for all cooking techniques. Several studies have reported reduced antioxidant activity after thermal treatments (Ismail et al., 2004, Roy et al., 2007, Chuah et al., 2008, Falher and Fialho, 2009)

The maximum retention of free amino acids was observed in the leaf samples sautéed in oil (Figure 6). Under the heat treatment, some proteins and short peptides may have degraded into free amino acids (Cho et al., 2007). While in other cooking techniques, the free amino acid concentration is low (or negligible). The proteins might have denatured to form polypeptides but did not degrade completely to form free amino acids. Also, the cooking time is usually more when sautéing the leaf samples in oil compared to boiling, blanching or microwaving and should be a point of consideration. No perceptible patterns were observed between protein degradation and free amino acid concentration. Other factors might have a contributing role in this aspect. More elaborate studies are required to assess the pattern of protein degradation and free amino acid concentration.

Fried leaf samples were observed to demonstrate data slightly inconsistent with the other cooking methods. This may be due to additional theoretical, practical and assay considerations when dealing with lipid-rich samples.

V. ACKNOWLEDGEMENT

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VI. DECLARATION OF INTEREST

The authors declare no conflict of interest for the current study.

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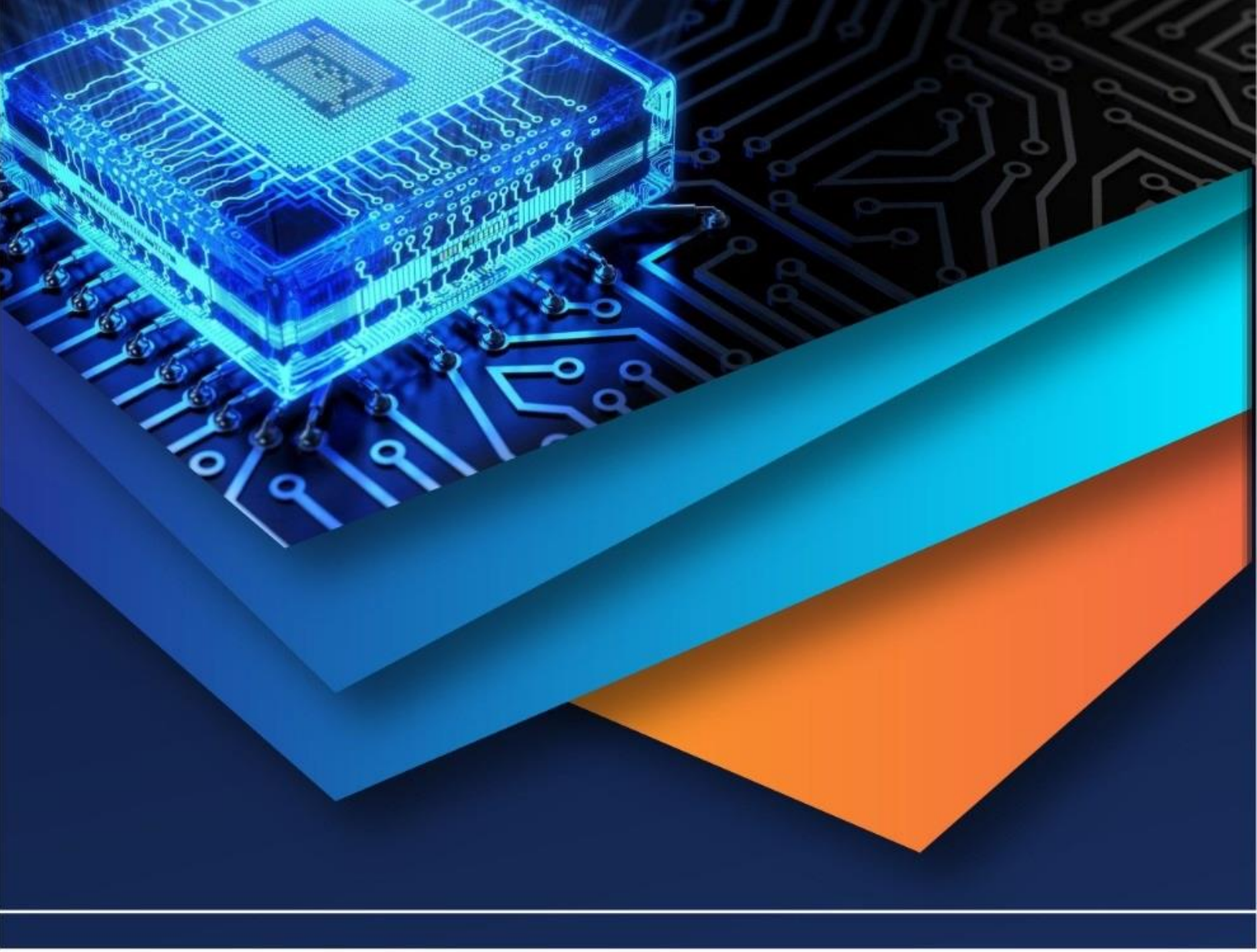

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