Different Methods of Food Preparation affect the Glycation Markers of Commonly Consumed Food Samples and Antioxidant Potential of Erythrocytes

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ABSTRACT

Background: The advanced glycation end products (AGEs) interfere with the normal functioning of the protein, alter the enzyme activity leads to the development of diabetic complications. Food is an exogenous source of AGEs. The long term processes like storing and cooking lead to an elevated level of AGEs content in them. The elevated AGEs are responsible for the generation of oxidative stress and inflammation in a cellular environment. The present study aims to determine the glycation potency of commonly consumed foods samples and evaluate the effect of various food preparation methods on glycation content and its impact on healthy erythrocytes.

Methods: In this investigation from December 2017 to April 2018, Aqueous extracts of 29 food samples were tested for their glycation potency using glycation markers (fructosamine, free thiol groups, β-amyloid content, AOPP). Erythrocytes were treated with food extracts and their antioxidant indices (FRAP, catalase) were determined.

Result: The result shows that protein-rich food had maximum levels of glycation as compared to carbohydrate and fat-rich food. The study indicated that cooking methods like (frying, roasting, baking and boiling) have a different effect on the glycation indices of the food. The food samples cooked by frying method had increased glycation content (p<0.001) and deleterious cellular effect.

Key words: Erythrocytes, Exogenous glycation, Food samples, Liquid extract, Oxidative stress.

INTRODUCTION

Glycation is a reaction leading to the formation of derivatives of glucose-protein and glucose-lipid interactions resulting in the generation of advanced glycation end products (AGEs) (Brownlee, 1995). During industrial processes or home cooking, long-term food storage initiates a Maillard reaction and formed stable amadori products in them (O’Brien and Morrisssey, 1989). The common Maillard products found in foods are Nε-carboxymethyl lysine (CML), methylglyoxal lysine dimmers (MOLD), pentosidine and pyrraline (Delgado-Andrade, 2016). According to the current research background, excessive consumption of these compounds contribute to the many progressive diseases like diabetes, atherosclerosis and Alzheimer’s disease (Goh and Cooper, 2008). Food is an exogenous source of AGEs. The long-term processes like storing and cooking lead to an elevated level of AGEs content and depletion of nutrient content in them (Kamalasundari, Babu and Umamaheswari, 2019). The Maillard reaction in food depends on cooking methods, cooking temperature, cooking time, protein and fat presence (Goldberg et al., 2005; Uribarri et al., 2015; Choudhary et al., 2019). The increasing burden of food-derived AGEs in the body is responsible for causing oxidative stress (OS) and inflammation, which plays a vital role in the causation of chronic diseases, (Uribarri et al., 2010) loss of bone density and muscle mass associated with aging (Odetti et al., 2005). Processed foods along with artificial sweeteners have been reported to cause hyperglycemia (Minekus et al., 2014; O’Brien and Morrisssey, 1989).

Consumption of foods high in sugar and/or foods exposed to high-temperature cooking methods such as deep-frying, broiling, roasting, baking and grilling can increase the total daily AGEs intake by 25% compared to the average adult daily intake (Negrean et al., 2007; Uribarri et al., 2005). Exogenous glycation occurs during foodstuffs preparation at various processes like baking, roasting, frying and grilling (Henle, Schwarzenbolz and Klostermeye, 1997). Food processing, heating, in particular, has a significant accelerating effect in the generation of glyco- and lipoxidation products. The heat helps create tasteful flavors.
and food manufacturers have been using this knowledge to boost the flavor of natural foods by incorporating synthetic AGEs into foods. The amount of AGEs in foods baked or fried in the oven is higher than the amount in steamed or simmered (Ames, 2008). As these activities occur at a high temperature of 120°C, the sugars present in the food undergo browning reaction or Maillard reaction to form brown sugar which results in the formation of AGEs (Henle, Schwarzenbolz, and Klostermeyer, 1997). The Western diets have been investigated for the exogenous glycation modifications. However, little information has been found for diet used in commonly consumed food about the glycation status and its changes during food preparation processes. Overall objectives of the present study are (1) to investigate different exogenous glycation markers in twenty-eight commonly consumed food samples, (2) To determine levels of glycation parameters in processed food samples (solid samples processed by cooking methods and liquid samples by addition of sweeteners) and (3) To evaluate the effect of food modifications on the antioxidant potential of erythrocytes.

**MATERIALS AND METHODS**

The study was conducted from December 2017 to April 2018 at Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth (Deemed to be University), Pune. All food samples such as white bread, brown bread, biscuit, toast, cake, potato, corn, soya chunks, raisins, apple, sweet lime, pineapple, pomegranate, chickoo, lemon, orange, carrots, watermelon, muskmelon, beetroot, bottle gourd, spinach, coriander, nuts, cashew, eggs, moong, wheat flour were obtained from the local market. These food samples were divided into solid and liquid samples.

**Modifications of solid food samples**

Selected solid samples were further processed by different food preparations such as boiling (selected food samples were modified by boiling them in water) baking/roasting (selected food samples were modified by using flame), frying (selected food samples were modified by frying them in oil). The modified samples were potato (boiled, baked, fried), corn (boiled, baked, fried), wheat (chapatti, paratha, puri), moong (boiled, roasted, fried), eggs (boiled, scrambled, omelet), nuts (roasted, fried), cashews (roasted, fried). The liqui, sweet lime, orange, lemon, watermelon, muskmelon and beetroot were used in juice. Selected juice samples such as apple, pineapple, sweet lime and watermelon juice were modified by the addition of various sweeteners such as white sugar, brown sugar, sugar-free, honey. Aqueous extracts of solid food samples (50 mg/mL; w/v) and liquid samples (50 µL/mL; v/v) were prepared in double-distilled water as per the method of Saraswat et al., 2009 with some modification. All the extracts were then kept on a shaker at 150 rpm for 3 hours, followed by centrifugation (3,000 rpm, 10 min) (SuperspinR- V/FM, Plasto craft, India). The clear supernatant was used for further studies. The extracts were stored at 4°C in plastic vials, until further use. All the analyses were performed in triplicates.

**Measurement of glycation in food samples**

**Estimation of fructosamine**

Nitroblue Tetrizolium (NBT) assay was used to determine the fructosamine level (Barker et al., 1985). Fructosamine content was calculated by using standard 1-deoxy-1-morpholino-D-fructose and results were expressed as mM of fructosamine/mL.

**β amyloid content**

Congo red binding to the amyloid cross-β structure was estimated by measuring absorbance at 530 nm (Klunk, Jacob, and Mason, 1999). Absorbance was recorded at 530 nm of the food samples. Results were expressed as OD 540 nm/mg protein.

**Protein thiol estimation**

Thiol groups of food samples were measured according to Ellman's assay using DTNB (Ellman, 1959). Free thiol content was calculated by using the molar extinction coefficient (ε 410 nm = 13.6 mM-1 cm-1) and results were expressed as nM free thiols/mg of protein.

**AOPP**

Advanced oxidative protein products (AOPP) were measured according to the method given by (Anderstam et al., 2008). The absorption is measured at 340nm.

**Measurement of toxicity in erythrocytes by modified food samples**

**In vitro treatment of erythrocytes with selected modified food samples**

The healthy volunteer (23 years; female) was recruited at Bharati Hospital, Pune, India. The study’s ethical approval was obtained from the Human Institutional Ethical Committee of Bharati Medical College of BVDU (Ref No. BVDU/MC/E1, Dt 24.02.2017). The 4% solution of fructosamine was prepared in PBS. 2.5mL of diluted erythrocytes were added to 2.5mL of the sample and incubated at 37°C for 3 hours. After incubation, the solution was centrifuged at 3000rpm for 10 minutes and hemolysate was prepared in lysis buffer.

**Catalase assay**

Catalase activity was assayed by the method of Aebi, 1984. The absorbance was measured at 0 and 60 sec at 240 nm. The results were expressed as Katal unit (KU)/gm hemoglobin. The catalase activity is determined by using the following formula:

\[
\text{Activity/mg protein} = \frac{\text{specific activity/ mg protein}}{\epsilon \times C} 
\]

**Ferrous reducing antioxidant property (FRAP) assay**

The erythrocytes’ intracellular antioxidant power was determined by FRAP assay (Benzie and Strain, 1999). The absorbance of the sample was recorded at 593 nm against a reagent blank.
Glycation potential of food samples

An elevated level of fructosamine indicates high glycation content. The processed samples such as white bread, brown bread, biscuits, toast and cakes showed the highest fructosamine (Fig 1a) content compared with other raw and unprocessed samples. In fruit samples, raisins and apple showed the highest amount of fructosamine compared to all the solid and liquid samples. Amongst liquid samples, apple, pineapple, sweet lime, and watermelon juices have nearly the same amount of fructosamine levels that are marginally higher than orange and muskmelon. Fat-rich samples like groundnuts and cashews also have low fructosamine levels. The amyloid level increases if there is an increase in the glycation process. The amyloid formation in white bread and brown bread was less than that of other processed food samples like biscuit, toast and cakes (Fig 1b). The glycation level was lower in starch and protein-rich samples like potato, corn, and soya chunks, whereas glycation level was elevated in fruits and vegetables. Amongst juices, in lemon juice, the negligible formation of amyloid content was observed while beetroot, muskmelon and sweet lime had high glycation.

Juices such as apple, pineapple and watermelon had moderate amyloid formation when compared with all the other juices. The Maillard reaction in food depends on several factors - composition, availability of pro and antioxidants, reaction time, processing temperature, the concentration of reductants, availability of water and pH (Chuyen, 2006; Blanc et al., 2011) Processed samples were baked at high temperature with minimum time and as reported by Goldberg et al. (2005) exposure of food to high temperature elevates the level of glycation. Raw food samples - potato, corn, soya chunks, raisins, apple, pomegranate, chickoo, lemon, orange, carrots, nuts and cashew are a rich source of antioxidants which can inhibit the glycation reaction hence the minimum level of fructosamine content were observed in them.

In food samples, glycation processes lead to oxidation of free thiol groups. As observed in Fig 1c Spinach and coriander show a maximum number of thiol groups. In processed food samples, biscuits have a high level of thiol groups as compared to white bread, brown bread, toast and cake. Samples such as potato, corn, soya chunks, raisins, apple, orange, beetroot and bottle gourd have similar levels of free thiol groups considerably lower than spinach and coriander. Amongst fat-rich food samples, nuts are found to be less glycated than that of cashews. All juices show no or minimal levels of free thiol groups that indicate high glycation compared to all solid samples. More amounts of oxidative protein products were observed in spinach, coriander, and bottle gourd amongst solid food samples. Starch and protein-rich samples like potato, corn and soya chunks have increased levels of AOPP (Fig 1d).

All other food samples have similar AOPP content results but are lesser than spinach, coriander and bottle gourd. Processed foods like biscuit and toasts have more formation of AOPP than bread samples. Amongst all juice’s pineapple, muskmelon and beetroot have no or minimal amount of AOPP. Juices have the least amount of AOPP formation as compared to all the food samples. An increase in glycation product hampers the antioxidant property of samples by the generation of oxidative products. The dry heat was found to be a promoter for a generation of AGEs. This modern cooking method is capable of altering native defense and distribution of the antioxidant status of food. To bake biscuits and toast dry heat is used which leads to the reduction in antioxidant status of the food. Hence the rate of formation of oxidative products in these foods was high as compared to the raw sample. Whereas fruits and vegetables contain high amounts of antioxidant substances hence can control the formation of the oxidative product. Glycation markers in processed solid food sample

Foods are a complex mixture of many different constituents, and various cooking methods initiate modification. High fructosamine levels (p<0.001) were observed in fried potatoes as compared to the baked and boiled sample. In corn, fructosamine levels were high in fried (p<0.001), baked (p<0.01) samples in comparison to the boiled sample. Amongst protein-rich samples moong has low glycation as compared to eggs (Fig 2a). Fried moong has more fructosamine (p<0.001) levels than roasted and boiled moong. The overall trend of glycation content value observed was frying > roasting > boiling. Fat rich samples like nuts and cashews have no significant difference in glycation levels as per their cooking modifications. Different types of thermal processing (boiling, roasting and frying) lead to the alteration of protein structure (Blanc et al., 2011). High temperature causes major chemical changes, thermal degradation, dehydration and recombination of fragments all of which promote glycoxidation (Goldberg et al., 2005). As observed in the results, processes like boiling and frying lead to an elevated level of fructosamine content. These processes might cause amadori rearrangement of sugars and protein within samples.

Food high in lipid and protein content are more prone to glycation. The amyloid content in protein-rich food like moong and eggs is highest amongst carbohydrate and fat-rich samples (Fig 2b). Amino acid oxidation in protein could induce sequential glycation and oxidative reactions.
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Comparative analysis of sample extracts (50 mg/ml, 50 µl/ml) in terms of (a) Fructosamine, (b) Amyloid, (c) Free thiol groups, (d) AOPP levels. All parameters were performed in triplicates in aseptic condition. Values are means (n = 3), with standard deviations represented by vertical bars.

Fig 1: Comparative analysis of glycation marker in sample extract.
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**Fig 2:** Comparative analysis of glycation marker in different sample modification.

Comparative analysis of sample modifications in terms of (a) Fructosamine, (b) Amyloid, (c) Free thiol groups, (d) AOPP levels. All parameters were performed in triplicates in aseptic conditions. Values are means (n = 3), with standard deviations represented by vertical bars. Data are represented as the mean ± SD. The level of significance is indicated as *** = p<0.001, ** = p<0.01, * = p<0.05 by comparing fried food samples against respective boiled, baked or roasted food modifications.
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the most rapid and effective glycating agents when compared to other sugars and indicates that high dietary simple sugars consumption can represent a substantial source of endogenous AGEs (Cannizzaro et al., 2017). Some population-based studies evidenced that a high intake of fructose-containing drinks and foods in the general population is associated with the induction of lipogenesis with hypertriglyceridemia and insulin resistance, paralleled by oxidative stress, which a relevant factor is contributing to the glycation process (Stanhope et al., 2009; Crescenzo et al., 2013; Ishimoto et al., 2013). From the overall result, we can assume that long-time use of white sugar as a sweetener can lead to an extended glycation level.

Effect of processed food sample on erythrocytes

In cellular microenvironment, hyperglycemia directly increases ROS generation at the mitochondrial level since glucose undergoes autoxidation to generate OH radicals (Aroni et al., 2019). As observed in Fig 4a, all fried food samples show low catalase activity except for potato and moong amongst carbohydrate and protein-rich samples, indicating high glycation in fried samples compared to baked and boiled ones. Food samples such as fried corn, puri and eggs show high glycation as compared to their cooking modifications. Roasted nuts and cashews have low glycation as compared to their respective fried samples. Whereas Anti-oxidant activity was lowest in fried modified food samples as compared to roasted/baked and boiled carbohydrate-rich, protein-rich and fat-rich samples, suggesting high glycation activity in all fried food samples (Fig 4b). Carbohydrate-rich samples show similar antioxidant activity patterns, i.e. boiled samples have low glycation than fried and baked ones. Protein-rich samples such as moong and eggs show the lowest glycation in boiled moong and scrambled eggs respectively. Amongst fat-rich food samples, roasted nuts show low glycation than fried nuts whereas, roasted cashew show high glycation than fried cashew. Cooking methods such as frying decrease catalase and antioxidant activity, which depicts the increased glycation level that is toxic for the cells. Also, dietary-induced glycation could play a role in initiating liver inflammation (Nowotnya, Schrötera, Schreinerb and Grunea, 2018).

When considering the effects of cooking on the formation of glycation in Indian food samples, the traditional cooking methods like boiling have lower adverse effects on the food than modern/western cooking methods like frying. Furthermore, research needs to be cited for the effects of glycation on commonly consumed Indian food samples. This report provides the rationale and the initiation point for a database to be used in clinical studies aiming to evaluate this newly identified dietary factor as a risk for diabetes and other chronic disorders. These findings also support the reevaluation of contemporary meal patterns in the context of major health epidemics. Ongoing studies are needed to further expand the database involving details of exogenous glycation in Indian food samples and investigate additional methods for reducing the formerly mentioned process during home cooking and food processing.

![Figure 4](image-url)

**Fig 4:** Effect of liquid extract of different food products on erythrocytes toxicity.

The effect of various cooking procedures of food extracts (50 mg/ml) on erythrocytes toxicity in terms of (a) catalase, (b) FRAP levels. Results of the measured parameter are expressed as % inhibition with respect to the positive control. Data are represented as the mean ± SD.
CONCLUSION
The study shows that food-derived glyco- and lipoxidation products are potent promters of chemicals and intracellular oxidative stress. This study reveals that protein-rich food had maximum levels of glycation as compared to carbohydrate and fat-rich food. Food samples that are fried indicated more glycation levels than boiled and baked ones. Sweet lime and watermelon juices have more glycation than an apple, pineapple juices. Further, the addition of white sugar in juices induces more glycation than sugar-free and honey. Cellular catalase and antioxidant activity were less in fried food samples as compared to boiled and baked samples. Collectively, frying increases glycation levels in food samples among different cooking methods, which can be toxic for the cells. Therefore, the inclusion of foods with fewer glycation modifications in diets could minimize their intake and associated chronic diseases.

Conflict of interest
The author declares that they have no conflict of interest.

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