

EFFECT OF ONION (*Allium cepa* L.) EXTRACT ON MAILLARD REACTION UNDER *in vitro* CONDITIONS

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Plant extracts have their own importance and now being studied extensively due to having little or no side effects. Protein glycation takes place when elevated levels of reduced sugars react with amino groups in proteins, reaction known as Maillard reaction. If this process continues, it will lead to the formation of complex, often unstable, irreversible and reactive compounds "AGEs", a process that may take weeks or even months to accomplish. In present study onion was selected and used to check the Maillard reaction inhibitory activity. Different combinations of glucose, protein and onion extracts were made under *in vitro* conditions and their activity was monitored with Trichloro acetic acid treatment method at 350 nm. Maillard reaction products/ AGEs were more with high glucose and high protein concentration and these were decreased by highest concentration of onion extract i.e. 25 mg/mL or 250 μ L. Lower concentrations of plant extract produced either no or least response against Maillard reaction.

Keywords: Maillard reaction, AGEs, Onion extract, TCA treatment

INTRODUCTION

In 1912, Louis Camille Maillard described the browning of proteins in food and called it as Maillard reaction. This is also known as non-enzymatic glycation of proteins, or a process which links chronic hyperglycemia to a series of physiopathological alterations considered important in the development of chronic complications of different diseases like diabetes (Takeuchi *et al.*, 2004). These glycated proteins further rearrange and give rise to a stable Amadori product that degrades into a variety of compounds which, more reactive than the sugars from which they are derived (Wautier and Schmidt, 2004). These propagators again form yellow-brown, often fluorescent (some are non fluorescent), irreversible compounds, usually called Advanced Glycation End-Products (AGEs) or Maillard products. Candidate active AGE compounds include *N*-(carboxymethyl)-L-lysine (CML) pyrraline, pentosidine and their cross-links (Kaysen, 2001).

Plants have been the major source of drugs in the world and in sub-continent system of medicinal therapy. Information on such plants in sub-continent has been systematically organized (Satyavati *et al.*, 1987). It is known that medicinal plants have little or no side effects. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world (Eshrat and Hussain, 2002). Metformin is the only ethical drug approved for the treatment of non insulin dependent diabetes mellitus (NIDDM) patients (Beisswenger *et al.*, 1999), which is derived from a medicinal plant *Galega officinalis* and historically used for treatment of diabetes (Oubre *et al.*, 1970). There are many anti-diabetic plants, which

might provide useful sources for the development of drugs, in the treatment of diabetes mellitus. The literature on medicinal plants with hypoglycemic activity is vast. As many of these plants were used for many centuries and some times as regular constituents of the diet, it is assumed that they do not have many side effects (Shnkar *et al.*, 1980).

Synthetic inhibitors and inhibitors from plant extracts have their own importance and now are studied extensively. There are reports of some natural substances isolated from plants with AGE-inhibitory effects. One such compound is curcumin isolated from *Curcuma longa* (Turmeric), commonly known as Haldi. Ginger (*Zingiber officinale* Rosc.) is another spice useful for diabetic therapy (Broadhurst, 2000). When type 2 diabetic rats are fed ginger, they show hypoglycemic activity, thus improving their diabetic condition (Kar *et al.*, 2003). Now it is the need of time to develop or isolate new compounds either from plants or synthetically to control diabetes and other age accelerating diseases. As plants have fewer side effects so these should be preferred to study. In this study onion (*Allium cepa* L.) extract was used to study its affect on glycation and Maillard products. The major object of this study was to investigate the effect of onion as inhibitor of AGE or Maillard reaction under *in vitro* conditions and measure its activity against AGE production or inhibition.

MATERIALS AND METHODS

Preparation of onion (*Allium cepa* L.) extract

Dried and ground onion plant (5 g) was extracted with 30 mL of 50% ethanol at 37 °C for 10 days and then filtered and stored at 4°C.

Sample recovery

Sample was recovered by evaporating the ethanol using Rotary Evaporator. Samples after drying were dissolved in 25 mL of phosphate buffer saline and stored at 4°C for further use.

In vitro glycation inhibition with onion (*Allium cepa* L.) extract

Conditions and concentrations selection for onion (*Allium cepa* L.) extract

To measure glycation inhibition with onion extract, different concentrations of protein (BSA) and glucose (two of each) and three concentrations or volumes of onion extract were used. These are given in table 1.

Table 1. Concentration of different components used to study glycation inhibition under *in vitro* conditions with onion (*Allium cepa* L.) extract.

S.No.	Components of reaction	Concentration
1	B = Buffer (PBS)	0.075M
2	P ₁ = Protein (BSA)	10 mg/mL
3	P ₂ = Protein (BSA)	5 mg/mL
4	G ₁ = Glucose	250 mM
5	G ₂ = Glucose	5.5 mM
6	F ₁ = Filtrate	250 µL
7	F ₂ = Filtrate	125 µL
8	F ₃ = Filtrate	50 µL

In vitro glycation of BSA with onion (*Allium cepa* L.) extract

Glucose, BSA with or without inhibitor (plant extracts in PBS pH 7.4) were prepared and their mixture was incubated at 37°C and 50°C for 5 weeks. During this, samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation. The samples kept at 4°C until analysis.

Trichloroacetic acid (TCA) method for Maillard reaction inhibitory activity of onion (*Allium cepa* L.) extract

This method is also known as TCA treatment method described by Matsuura *et al.* (2002) was followed with some modification.

RESULTS AND DISCUSSIONS

Onion plant was selected for experiments as it is in routine use for daily food cooking and salads in Pakistan. Onion plants were dried in hot air oven at 37°C. After drying, ground to powder form and kept in

50% ethanol for extraction. Samples were recovered by Rotary Evaporator. Heat and liquid nitrogen method were also tried but Rotary evaporator was cheaper, less time consuming and simple to perform than other two. Samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation.

Glucose concentration 250 mM and 5.5 mM was selected as it was cleared that 250 mM is a hyperglycaemia condition (Rahbar and Figarola, 2003) and 5.5 mM is a normal glucose level (human body). Bovine serum albumin (BSA) was used as protein for glycation and its concentration was i.e. 10 mg/mL and 5 mg/mL. Onion extracts (250 µL, 125 µL and 50 µL) in phosphate buffer were prepared. Each extract along with glucose and BSA were incubated at 37°C and 50°C for five weeks to monitored glycation and Maillard reaction inhibitory activity. TCA treatment method described by Matsuura *et al.* (2002) was adopted with modifications. The absorbance change based on Schiff base formation was measured by spectrophotometer at 350 nm. This gave the apparent inhibitory activity. Real inhibitory activity was estimated by subtracting the quenching effect from the apparent inhibitory activity. The results obtained by this method are explained below.

Maillard reaction inhibitory activity by onion (*Allium cepa* L.) extract

The results obtained from onion (*Allium cepa* L.) extract after TCA treatment at 37°C and 50 °C. At temperature 37°C (Figure 1), G₁ P₁ produced more Maillard products (0.099) after 5th week. While G₂P₂ produced minimum products 0.029 after 1st week at same temperature. G₁P₂ and G₂P₁ produce moderate level of products. Onion extract followed the pattern from F₁ to F₃ as F₁ (250 µL) generated maximum response while F₃ (50 µL) generated least or no response at 37°C.

BSA (10mg/mL, 5mg/mL), glucose (250mM, 5.5mM) with or without Onion (*Allium cepa* L.) extract (250 µL, 125 µL and 50µL) in phosphate buffer(0.075M), pH 7.4 and mixture was incubated at 37°C for five weeks. Samples were drawn for glycation inhibition activity after 1st, 3rd and 5th week of incubation. Absorbance was recorded at 350nm.

At 50°C (Figure 2) G₁ P₁ produced more Maillard products (0.109) after 5th week. While G₂P₂ produced minimum products 0.037 at same temperature after 1st week of incubation. Here it was also seen that G₂P₂ generated maximum products at 3rd week (0.048) in spite of 5th week at 50°C. F₁ (250 µL) generated maximum response while F₃ (50 µL) generated least response at 50°C and minimum inhibitory activity was observed. At both temperatures F₂ (150 µL) produced

Effect of onion extraction on maillard reaction

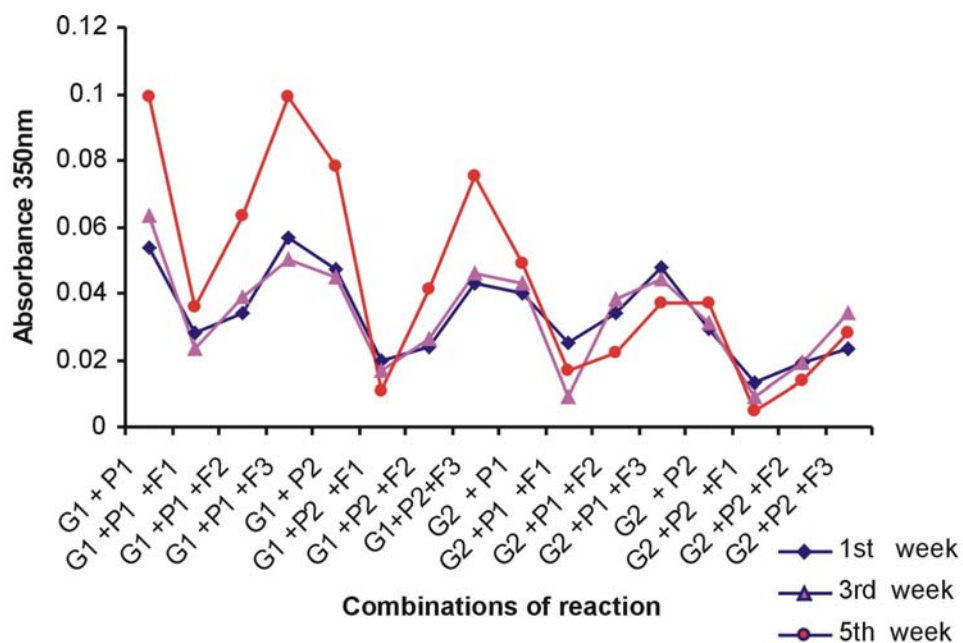


Figure 1. Maillard Reaction inhibitory activity by Onion (*Allium cepa* L.) with TCA Treatment Method under *in vitro* conditions measured at 37°C temperature

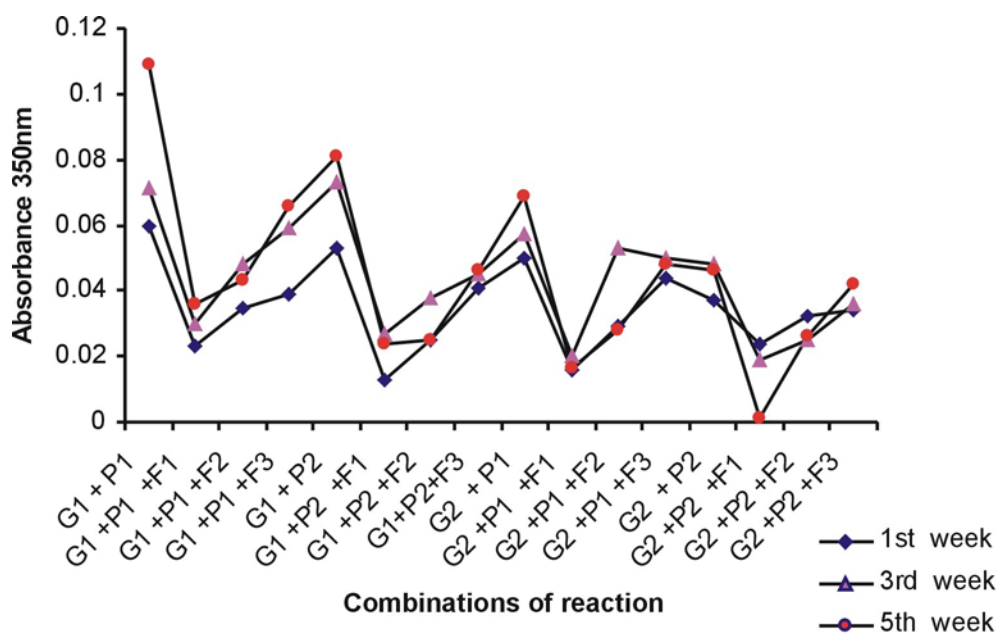


Figure 2. Maillard Reaction inhibitory activity by Onion (*Allium cepa* L.) with TCA Treatment Method under *in vitro* conditions measured at 50°C temperature

moderate response against Maillard products inhibition. It was also observed from both figures that high temperature facilitate the production of Maillard products. Moreover, high temperature did not effect the

activity of onion extract i.e. their trend towards inhibition is same.

Onion and garlic have significant blood sugar lowering action. Our results are supported by Demerdash *et al.*

(2005). They carried out the study to investigate the effects of onion (*Allium cepa*) and garlic (*Allium sativum*) juices on biochemical parameters, enzyme activities and lipid peroxidation in alloxan-induced diabetic rats. Their results showed that garlic and onion juices exerted antioxidant and antihyperglycemic effects. Our findings were contrary to Jelodar *et al.* (2005) as they carried out the study to clarify the effect of fenugreek, garlic and onion in the treatment of diabetes, on blood glucose and their possible effect on pancreatic tissues. The results of their study indicated that only garlic was able to reduce blood glucose significantly compared with the control group. However, most studies confirm our findings and showed onion decreased the hyperglycemic peak in rabbits (Romas *et al.*, 1995). In addition, onion amino acid α -methyl cysteine sulfoxide contributed to antidiabetic effects in affected rats, controlling blood glucose in addition to other diabetic effects comparable to insulin (Shella, 1995).

CONCLUSIONS

Our studies concluded that onion has ability to inhibit Maillard products that ultimately lead to AGEs production. It was also cleared that 10mg/ mL concentration of BSA was more active towards glycation of glucose. However, F₃ (50 μ L) concentration of onion extract was almost unable to produce any effect at 37 °C. So it is suggested that more concentration of onion extract should be used to stop or decrease glycation in human body level in case of diabetes mellitus and persistent hyperglycaemia.

Abbreviations: AGEs, Advanced Glycation End-Products; NIDDM, Non insulin dependent diabetes mellitus; BSA, Bovine serum albumin; PBS, Phosphate buffer saline; TCA, Tri-chloro acetic acid.

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