ISSN 0974 – 5211

Journal of Natural Products

Volume 3 (2010)

www.JournalofNaturalProducts.com

Research Paper

Anti-glycation Effect of Spices and Chilies Uses in Traditional Mexican Cuisine

Rosa Martha Pérez Gutiérrez[1*], Sandra Lugrado Diaz[1], Ivan Cordova Reyes[2], Adriana Maria Neira Gonzalez[1]

[1]Laboratorio de Investigación de Productos Naturales, [2]Laboratorio de Investigación de Catalisis, Escuela Superior de Ingeniería Química e Industrias extractivas IPN. Punto Fijo 16, Col. Torres Lindavista cp 07708, D.F. Mexico

*Corresponding author

(Received 22 October 2009; Revised 29 October -07 November 2009; Accepted 10 November 2009)

ABSTRACT

Non-enzymatic glycation and the accumulation of advanced glycation end products (AGEs) are associated with various diseases states, including complications of diabetes and aging. Hydroalcoholic extracts of several kinds of spices (16) and chilies (21) of culinary used in traditional Mexican cuisine were tested for *in vitro* inhibition of non-enzymatic glycation of bovine serum albumin. Of these, plants *Piper auritum, Origanum majorana, Crotolaria longirostrata, Bixa orellana*, *Satureja macrostema*, *Allium ascalonicum*, and *Curcuma longa* exhibited significant inhibitory activity against AGEs formation with IC_{50} values ranging from 23.1 to 188.8 µg/ml. The most active *P. auritum* displaying an IC_{50} value of 23.07 µg/ml, was more effective than aminoguanidine, (IC_{50} = 27.1 µg/ml) a know inhibitor of glycation. *O. majorana* with IC_{50} value of 38.16 µg/ml was the second most active extract followed by *C. longirostrata* IC_{50} being 77.24 µg/ml, *B. orellana* with an IC_{50} of 94.32 µg/ml, *S. macrostema* (IC_{50} = 106.1 µg/ml), *A. ascalonicum* (IC_{50} = 111.77 µg/ml), and *C. longa*, (IC_{50} of 188.77 µg/ml).

Keywords: Chilies; Spices; Glycation of protein.

INTRODUCTION

Nonenzymatic protein glycation by reducing sugars such as glucose, fructose or ribose is a complicated cascade of condensations, rearrangements, fragmentations, and oxidative modifications that lead to a training of compounds collectively called advanced glycation end products (AGEs). The reaction is initiated by the reversible formation of a Schiff base which undergoes a rearrangement to form a relatively stable Amadori product. The Amadori product will further undergo a series of reactions through dicarbonyl intermediates to form AGEs. It is also known that AGEs are formed by sequential glycation and oxidation reactions termed glycoxidation. The accumulation of the reaction products of protein glycation in living organisms leads to structural and functional modifications of tissue proteins (Takeuchi, et al., 2004).

Advanced glycated end-product formation and their patho-biochemistry particularly in relation to the diabetic microvascular complications of retinopathy, neuropathy and nephropathy as well as their role in the accelerated vasculopathy observed in diabetes has been amply demonstrated (Singh, et al., 2001). There is evidence that glycation leads to chemical modification of proteins, and other macromolecules and it contributes to the pathogenesis of diabetic complications (Mendez, 2003).

Hyperglycaemia has an important role in the pathogenesis of diabetic complications by increasing protein glycation and the gradual build-up of advanced glycation end products (AGEs) in body tissues. The formation of AGEs progressively increases with normal aging, even in the absence of disease. However, they are formed at accelerated rates in diabetes. AGEs are not only markers but also important causative factors for the pathogenesis of diabetes, cataracts, atherosclerosis, diabetic nephropathy, and neurodegenerative diseases, including Alzheimer's disease, Thus, the design and discovery of inhibitors of AGEs formation can offer a promising therapeutic approach for the prevention of diabetic or other pathogenic complications.

Aminoguanidine, a hydrazine-like small molecule, is the first AGEs inhibitor explored in clinical trials. However, the drug was not ultimately approved for commercial production because side effects were observed in phase III clinical trials in patients with diabetes, perhaps related to some extent to the sequestration of pyridoxal, resulting in vitamin B_6 deficiency (Brownlee, 1995). The present study was initiated with the aim of investigating the antiglycation properties of several natural spices and chilies of culinary uses in Mexican food.

MATERIALS AND METHODS

***Materials*:** Fatty acid-free Bovine serum albumin (BSA), sodium azide, phosphate buffer (PBS), and aminoguanidine (AG) were obtained from Sigma (USA). Fructose was purchased in Merck.

***Plant material*:** The plants were purchased in a market of the D.F. (Mexico). A voucher for each of the specimens was deposited in the herbarium of Universidad Autonoma Metropolitana-Xochimilco. The aerial parts of plants were air-dried, protected from direct sunlight, and then powdered. The powder was kept in a closed container at 8°C.

***Preparation of extracts*:** The powdered plant material (200 g) was macerated for 8 days with a mixture of ethanol-H_2O (1:1), at room temperature (25^{o}C). The extract was filtered through Whatman No. 42 filter paper, the filtrate was lyophilized to powdered form, freshly prepared, which was used in *in vitro* glycation studies.

***Advanced glycation end products (AGEs) in the BSA/fructose*:** BSA (10 mg/ml) was incubated with fructose (500 mM) in phosphate buffered-saline (PBS) (5 ml total volume, (pH 7.4) containing 0.02% sodium azide at 37°C. All of the reagents and extracts were sterilized by filtration through 0.2 µm membrane filters. From each crude extracts was prepared a different negative control, which contained BSA+extract+azide (without sugar), and incubated for 30 days as sample with fructose. To exclude the presence of high molecular weight substances in the crude extracts that may quench AGEs fluorescence, they were dialyzed and tested to see if these interfere with fluorescence measurements.

The protein, sugar and prospective inhibitor were introduced into the incubation mixture simultaneously. AG was used as a positive inhibitor, control reactions in the absence of the prospective inhibitor were also set up. Each solution was kept in the dark in a capped tube, and incubation was carried out in triplicate

tubes for 30 days. After the glycation period and prior to fluorescence analysis were separated from weight substances by centrifugation/filtration with 10kDa filter cartridges. A final concentration of AG (1mM) and ethanol-water (without addition of the inducer) were used as the positive and negative controls, respectively. Fluorescence standard was naphthalene with excitation and emission wavelength of 290 and 330 respectively to normalize all fluorescence measurements.

The formation of AGE was firstly assessed by the characteristic fluorescence (excitation wavelength of 370 nm and emission wave-length of 440 nm) (Kiho, et al., 2004). Percent inhibition was calculated as follows:

$$\text{Inhibition \%} = [1 - (A_o - A_b)/ (A_c - A_b)] \times 100$$

- Where A_o is the fluorescence of the incubated mixture with sample, and A_c and A_b are the fluorescence of the incubated mixture without sample as a positive control and the fluorescence of incubated mixture without sample as a blank control.

All experiments were performed in triplicate, and all extracts were tested at least at a minimum of eight dilutions of 10-1500 µg/ml for species and 1000-125,000 µg/ml for chilies.

***Free radical scavenging activity on DPPH*:** For 5 mg/ml of each extract in methanol, 0.1 mM of DPPH (1, 1-diphenyl-2-picrylhydrazyl) in methanol was added of a total volume of 5 ml. The mixture left standing at room temperature for 30 minutes, the absorbance was measured at 517 nm. Gallic acid (0.1 mg/ml) was used as the positive control (Lee, et al., 1998). The radical scavenging activity was calculated as a percentage of DPPH decolorization compared to control.

Statistical analysis: All analyses were run in triplicate and averaged. Results are expressed as mean ± S.D. Significance ($P< 0.05$) of mean differences was determined by Duncan's multiple range tests SAS, a software used worldwide for statistics, and recognized to be significantly differ if the probability was lower than 0.05 (Takeuchi, et al., 2004). Inhibition of glycation by extracts of herbs and spices is expressed as a percentage of the difference between control samples containing albumin plus fructose versus those with albumin without fructose.

RESULTS

The formation of total AGEs was assessed by monitoring the production of fluorescent products at excitation and emission maxima of 370 and 440 nm, respectively. The fluorescence intensity of this glycophore which is characteristic of AGEs, was highly increased through incubation of BSA with fructose. Tables 1 and 2 show the effect of fructose on the total AGEs formation during 30 days of BSA incubation at 37°C compared to the control values, the fluorescence intensity was significantly higher in samples with sugar. As it is evident in both Tables hydro-alcoholic extract at different concentrations has significantly quenched the fluorescence, has significantly suppressed the fluorescence intensity in a dose-dependent manner. In that respect, the extracts activities are comparable to the effect of 1 mM AG solution which is a known inhibitor of glycation process. The effectiveness are expressed in IC_{50} values (inhibits glycation 50%) on AGEs formation.

In this study were tested 16 spices and 21 chilies for their inhibitory activity on protein glycation (Table-1; 2). The fluoresecence of AGEs was shown to be remarkably inhibited by several extracts comparing with the positive (AG). The most active extract was that of hoja Santa (*Piper auritum*) with IC_{50} value of 23.07 µg/ml and lower than that of AG (IC_{50} = 27.1 µg/ml). *Origanum majorana* with IC_{50} value

of 38.16 µg/ml was the second most active extract followed by chepil (*Crotolaria longirostrata*) with IC_{50} value of 77.24 µg/ml, *Bixa orellana* (IC_{50} = 94.32 µg/ml), *Satureja macrostema* (IC_{50} = 106.1 µg/ml), in the literature found few data on the antioxidant activity and other bioactive constituents of *Satureja macrostema. Allium ascalonicum* (IC_{50} = 111.77 µg/ml), and *Curcuma longa*, IC_{50} being 188.77 µg/ml. The IC_{50} values of these plants were below 200 µg/ml.

Among the tested chilies, the chipotle (*Capsicum anuum*, IC_{50} value of 1,604 µg/ml*)* and morita chico (*Capsicum anuum*, IC_{50} value of 6,854 µg/ml) were the most actives. Whereas other chilies extracts showed weak activity with IC_{50} values of 12,000 at 87.000 µg/ml. In addition, the morron chilies (green, red and yellow) showed $_{no}$ activity at the concentration tested of 350,000 µg/ml.

The free radical scavenging abilities of peppers (red, yellow and green) were high for the green (86.10 %) and red (88.77 %) pepper but not significantly different from the yellow peppers (76.98 %). Also, *Capsicum chinensis* (chile de agua, 70.53%), *C. anuum* (chilaca, 77.81 %), *C. pubensis* (habanero, 85.64%) *C. pubensis* (manzano, 70.63%) *C. anuum* (morita chico, 72.10%) showed high free radical scavenging properties.

Among the tested spices, *Curcuma longa* (86.54%), *Crotolaria longirostrata* (75.87%), *Myristica fragans* (77.26%), *Azadirachta indica* (69.89%), *Bixa orellana* (74.95%), and *Satureja macrostema* (87.21%) showed high effect on free radical scavenging.

DISCUSSION

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves and blood vessels. The underlying mechanism responsible for its complications, as well as for diabetes itself, remains unclear, though possible events such as activation of protein kinase C, the polyol patway, non-enzimatic glycation and oxidative stress have been suggested (Giugliano, et al., 1996; King, 1996).

Advanced glycation end-products are well-known contributors to the pathophysiology of aging and diabetic chronic complications (Lunceford and Gugliucci, 2005). Increased glycation during hyperglycaemia can cause intra or inter molecular cross linking of proteins as they accumulate advanced glycation endproducts. Numerous studies have shown that build up of crosslinked advanced glycation endproducts on long-lived proteins may underlie the development of complications affecting diabetes and ageing (Ahmed, 2005). Furthermore, the levels of serum advanced glycation endproducts reflect the severity of these complications whereas therapeutic interventions aimed at reducing advanced glycation endproducts can inhibit or delay their progression (Monnier, 2003).

Several plants showed antiglycation activities in BSA-fructose model as they all had high contents of phenolic compounds. On the basis of a literature search, many purified phenolic compounds (including flavones, flavanones, flavonols, isoflavones, proanthocyanidins, and other phenolics) and phenolic-rich plant extracts have been found to have strong inhibitory activity in this bioassay (Materska and Perucka, 2005).

A good correlation exists between their free radical scavenging capacity and AGE inhibitory activity *in vitro* (Matsuda, et al., 2003; Yokozawa, and Nakagawa, 2004). This suggests that they exert their inhibitory activity by interrupting the autoxidative pathways. In fact, there is growing evidence that production of ROS is increased in diabetes patients and that oxidative stress is associated with diabetic

complications. In contrast, numerous clinical trials have failed to provide conclusive evidence for the efficacy of natural antioxidant therapy in diabetic patients (Rahbar, and Figarola, 2003). These findings strongly suggest that free radical scavenging may be effective in suppressing AGE formation only under certain *in vitro* conditions and that inhibiting autoxidation alone is unlikely an effective way of preventing or treating diabetic complications when more complex physiological environments are involved.

The BSA-fructose model adopted in this study provides a useful tool for assessing the effects of hydro-alcoholic extracts of spices and chilies on the nonenzymatic glycation process. Table 1 and 2 displays the inhibitory effects of these plants on AGE formation in this model.

It was a surprise to find that leaves of *Origanum majorana, Allium ascalonicum* and *P. auritum* showed high antiglycation activities in BSA-fructose model being capable of reduce appreciably the formation of fluorescent since it not contains a high content of phenols, was shown *P. auritum* to be more promising antiglycation candidates than the other thirty six plants studied.

Vitamin C, phenolics, flavonoids, carotenoids, capsaicinoids, capsaicin, dihydrocapsaicin isolated from the pepper fruit *C. annuum*, C. *baccatum, C. chinensis, C. pubensis, C. anuum grossum* and peppers (red, yellow and green), showed high antioxidative effect. However, these chilies reduced slightly the glycation.

Instead, *Crotolaria longirostrata, Bixa orellana*, *Satureja macrostema* and *Curcuma longa* have high antioxidant and high antiglycation activities.

In several studies showed that antiglycation activities of plants indeed, as having been repeatedly reported, were relevantly and directly related to its polyphenolic content, yet it seemed to us that several plants also possessed a rather specific and somewhat different degree of free radical scavenging ability, thus it was speculated that the reaction mechanism of plants might have occurred in the initiation rather than the propagation phase, a mechanism being quite different from the conventional free radical scavenging (Prior, et al., 1998). Antioxidant activity was evaluated quantitatively in the current study, our results indicated in some cases the antioxidant and antiglycation activities are high (Moyer, et al., 2002), although this does not happen in other cases.

CONCLUSION

It is concluded, therefore that these plants have antioxidant activity as well as AGEs inhibitory effects on phosphate-buffered fructose and BSA reaction. As a result, these plants could be offered as leading compounds for further study as a new products drug for diabetic complications. Our research work provides additional evidence to support the health benefits of spices and chilies for diabetic patients.

REFERENCES

Ahrned, N., (2005): Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Res Clin Pract.,* 67: 3-21.

Brownlee, M., (1995): Advanced protein glycosylation in diabetes and anging. *Annu Rev Med*., 146: 223-234

Giugliano, D., Ceriello, A., Paolisso, G., (1996): Oxidative stress and diabetic vascular complications. *Diabetes Care*., 19: 257-267.

Kiho, T., Usui, S., Hirano, K., Aizawa, K., Inakuma, T., (2004): Tomato paste fraction inhibiting the formation of advanced glycation end-products. *Biosci Biotechnol Biochem.,* 68: 200-205.

King, G. L., (1996): The role of hyperglycemia and hyperinsulinaemia in causing vascular dysfunction in diabetes. *Ann. Med*., 1 28: 427-432

Lee, S.K., Mbwambo, Z.H., Cheng, H.S., Luyengi, L., Games, E.J., Mehta, R.G., Kinghorn, A.D., Pezzuto. J.M., (1998): Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screen*, 1: 35-46.

Lunceford, N. and Gugliucci, A., (2005): Ilex paraguariensis extracts inhibit AGE formation more efficiently than green tea. *Fitoterapia*, 76: 419-427.

Materska, M., Perucka, I., (2005): Antioxidant activity of the main phenolic Compounds isolated from hot pepper fruit (*Capsicum annuum* L.) *J. Agric. Food Chem*., 53: 1750-1756.

Matsuda, H., Wang, T., (2003) Managi, H.; Yoshikawa, M. Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg Med Chem.*, 11: 5317-5323.

Mendez, J.D., (2003): Advanced glycosylation end products and chronic complications of diabetes mellitus. *Gac Med Mex*., 139: 49-55.

Monnier, V.M., 2003. Intervention against the Maillard reaction *in vivo*. *Arch* Biochem Biophys., 419:1-15.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., Wrolstad, R.E., (2002): Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes. *J Agric Food Chem.*, 50: 519-525.

Navarro, M.C., Montilla, M.P., Cabo, M.M., Galisteo, M., Cáceres, A., Morales, C., Berger, I., (2003): Antibacterial, antiprotozoal and antioxidant activity of five plants used in Izabal for infectious diseases. *Phytother Res.*, 17: 325-329.

Prior, R.L., Cao, G., Martin, A., (1998): Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of vaccinium species. *J Agric Food Chem., 46*: 2686-2693.

Rahbar, S., Figarola, I. L., (2003): Novel inhibitors of advanced glycation endproducts. *Arch. Biochem. Biophys*. 419: 63-79.

Singh, R., Barden, A., Mori, T., Beilin, L., (2001): Advanced glycation end-products: a review. *Diabetologia*., 44: 129-46.

Takeuchi, M., Kikuchi, S., Sasaki, N., Suzuki, T., Watai, T., Iwaki, M., Bucala, R., Yamagishi, S., (2004): Involvement of advanced glycation end-products (AGEs) in Alzheimer's disease. *Curr Alzheimer Res*., 1: 39-46.

Yokozawa, T., Nakagawa, T., (2004): Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. *Food Chem Toxicol.*, 42: 975-981.

Table-1: Effect of hydro-alcoholic extracts of spices on glycation induced by BSA-fructose reaction *in vitro*.

Common name	Botanical name	Family	AGEs (IC_{50}) µg/ml	Activity on DPPH (%)
Albahacar	*Ocimum basilicum*	Lamiaceae	219.4	42.08
Cilantro	*Coriandrum sativum*	Umbelliferaceae	419.3	54.14
Curcuma	*Curcuma longa*	Zingiberaceae	188.7	86.54
Epazote morado	*Chenopodium ambrosioides*	Chenopodiaceae	411.0	19.80
Epazote verde	*Chenopodium ambrosioides*	Chenopodiaceae	280.0	26.52
Hoja de aguacate	*Persea americana*	Lauraceae	1,330	73.39
Hoja Santa	*Piper auritum*	Piperaceae	20.07	12.34
Mejorana	*Origanum majorana*	Lamiaceae	38.16	46.04
Margosa	*Azadirachta indica*	Meliaceae	359.0	69.89
Nuez moscada	*Myristica fragans*	Myristicaceae	213.54	77.26
Perejil	*Petroselinum crispum*	Apiaceae	400.6	2.03
Salvia	*Salvia officinalis*	Lamiaceae	620	57.92
Shalott	*Allium ascalonicum*	Liliaceae	111.77	4.14
Chepil	*Crotolaria longirostrata*	Fabaceae	77.24	75.87
Poleo	*Satureja macrostema*	Lamiaceae	106.1	87.21
Achiote	*Bixa orellana*	Bixaceae	94.32	74.95
Aminoguanidine	-	-	28.2	89.6

- Assay was conducted in 5 ml of volumes.
- Concentration of the plant extract residue required to inhibit 50% (IC_{50}).

Table-2: Effect of hydro-alcoholic extracts of chilies on glycation induced by BSA-fructose reaction *in vitro*.

Common name	Botanical name	Family	AGEs (IC_{50}) µg/ml	Activity on DPPH (%)
De agua (a)	*Capsicum anuum*	Solanaceae	12,720	70.53
De arbol (b)	*Capsicum anuum*	Solanaceae	39,480	54.33
ancho (b)	*Capsicum anuum*	Solanaceae	41,220	55.89
Cascabel (b)	*Capsicum anuum*	Solanaceae	86,970	66.11
Catarina (b)	*Capsicum anuum*	Solanaceae	78,540	16.48
Chipotle (b)	*Capsicum anuum*	Solanaceae	1,604	52.39
Jalapeño (a)	*Capsicum anuum*	Solanaceae	71,438	28.36
Pasilla (b)	*Capsicum anuum*	Solanaceae	35,835	42.36
Chilaca (a)	*Capsicum anuum*	Solanaceae	22,980	77.81
Guajillo (b)	*Capsicum anuum*	Solanaceae	15,270	47.24
Guero (a)	*Capsicum baccatum*	Solanaceae	111,600	60.22
Habanero (a)	*Capsicum chinensis*	Solanaceae	71,438	85.64
Manzano (a)	*Capsicum pubensis*	Solanaceae	(c)	70.63
Morita chico (b)	*Capsicum anuum*	Solanaceae	6,854	69.80
Morita grande (b)	*Capsicum anuum*	Solanaceae	49,689	72.10
Mulato (b)	*Capsicum anuum*	Solanaceae	35,247	52.58
Poblano (a)	*Capsicum anuum*	Solanaceae	115,460	52.85
Morron amarillo (a)	*Capsicum anuum grossum*	Solanaceae	(c)	76.98
Morron rojo (a)	*Capsicum anuum grossum*	Solanaceae	(c)	88.77
Morron verde (a)	*Capsicum anuum grossum*	Solanaceae	(c)	86.10
Piquin (b)	*Capsicum anuum*	Solanaceae	12,449	42.08
*Aminoguanidine	-	-	28.2	89.6

- Assay was conducted in 5 ml volumes.
- Concentration of the plant extract residue required to inhibit 50% (IC_{50}). Fresh (a), dry (b) and (c) no activity at concentration of 350,000 µg/ml.
- Aminoguanidine, a hydrazine-like small molecule, is the first AGEs inhibitor explored in clinical trials.

