A new approach to measure and evaluate inhibitory activity against protein glycation during heating

Glycation is the addition of a sugar moiety into a protein molecule occurring during Maillard reaction. It takes place both endogenously in the body and exogenously in food products. So-called advanced glycation end products (AGEs), lead to several consequences in the body. AGEs may contribute to the decline in tissue and organ function with age and are related to chronic and degenerative diseases; such as diabetes, renal failure, atherosclerosis, Alzheimer's and Parkinson's diseases. Exogenous glycation occurs during food processing and severe heat treatment causes advance glycation in food products. Dietary intake of exogenous AGEs may cause their accumulation in blood stream and in tissue proteins, then undergo further reactions in the body. Due to these consequences of advanced glycation, prevention of protein glycation is an emerging issue.

There are many studies dealing with anti-glycation activity of different compounds. However the activity of these compounds are not really measurable and comparable. There is not any methodological approach to express the degree of inhibition, which takes mechanism of glycation reaction into account. Therefore, we aimed to investigate the inhibition of glycation during heating by using a kinetic approach.

Fig. 1. Inhibition pattern of protein glycation in the presence of anti-glycation agent (I). OVA; ovalbumin, G; glucose, FL; fructoselysine.

Model systems composed of ovalbumin, glucose, and anti-glycation agents (tannic acid or calcium ion) at different molar ratios were heated at 90°C for different times in dry state or in solution. Heated samples were analyzed for furosine, acid derivative of N-fructoselysine (FL), to monitor the progression of early glycation stage.

The complex mechanism of Maillard reaction (MR) is mostly tried to be investigated in terms of specified compounds and products, such as lysine amino acid and its glycation products. At the initial stage of MR, glucose reacts with lysine residues of the protein to give the condensation product N-fructoselysine (FL). What happens when an inhibitor agent is incorporated into the reaction medium? In order to investigate in-depth the inhibition effect in the presence of anti-glycation agents, we proposed that glycation reaction could be analyzed similarly with enzyme-substrate-inhibitor kinetics; because glycation is a site-specific reaction like an enzymatic reaction and inhibitors affect glycation rate through non-covalent interactions.

Figure 1 shows the general inhibition pattern of anti-glycation agents, resembling enzyme inhibition model. An anti-glycation agent or inhibitor (I) binds to protein leading to a complete or partial inhibition of the formation of N-fructoselysine during glycation. For example, if the agent binds to ovalbumin (OVA), then ovalbumin-agent complex (OVAI) is formed and glucose (G) would bind to this complex resulting in less product formation (FL). The agent may bind to ovalbumin-glucose complex (OVAG), resulting again less product formation. Or sometimes both situations may take place at the same time, depending on the inhibition type.

The Michaelis-Menten equation was adapted for glycation reaction (Eq. (1)). K_G is the glycation constant and it indicates the affinity of glucose towards protein; low value means high affinity and high value means low affinity. The K_G and V_{max} values can be estimated from the double reciprocal form of the Michaelis-Menten equation as given in Eq. (2). Determination of K_G, K'_G, K_I, and K'_I may help to understand which mechanism takes place during glycation reaction in the presence of an anti-glycation agent, and can be realized by solving related equations given through Eq. (3-5).

$$V_0 = \frac{V_{max}\,[G]}{K_G + [G]} \qquad \text{Eq. (1)}$$

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_G}{V_{max}[G]} \qquad \text{Eq. (2)}$$

$$V_{max} = V_{max}/(1 + [I]/K'_I) \qquad \text{Eq. (3)}$$

$$K_G = (K_G + K_G \times [I]/K_I)/(1 + [I]/K'_I) \qquad \text{Eq. (4)}$$

$$V_{max} = V_{max}/(1 + [I]/K_I) \qquad \text{Eq. (5)}$$

Comparing to control, it was found that presence of calcium ions and tannic acid decreased FL formation significantly (p