Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Research International 38 (2005) 1045–1050
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

Effect of Maillard reaction conditions on browning and antiradical activity of sugar–tuna stomach hydrolysate model system

M.T. Sumaya-Martinez, S. Thomas, B. Linard, A. Binet, F. Guerard *

ANTiOX Laboratory, Université de Bretagne Occidentale, Pôle Universitaire PJ Helias, F-29000 Quimper, France

Received 6 June 2004; accepted 30 March 2005

Abstract

The antiradical activity of Maillard reaction products (MRPs) made from sugar–tuna stomach hydrolysate model system was tested. The antiradical activity of the MRPs derived from ribose was 11-fold higher than that of MRPs derived from glucose due to the acyclic form of the ribose. The activity reached the plateau at a 30 mg/mL ribose concentration. The ribose caramelization contributed to the antiradical activity and browning reactions at 95 °C and 115 °C. The increase in DPPH$^{\bullet}$ radical scavenging of MRPs is attributed not only to the temperature but also to the buffer type and buffer concentration. Phosphate buffer showed the most efficient compared to citrate or Tris–HCl buffers. A positive correlation ($R^2 = 0.98$) was observed between the antiradical activity, the browning and the phosphate concentration. The MRPs obtained under these mild experimental conditions exhibited no toxicity towards Vero cells and 3T3 cells, despite their high antiradical activity.

Keywords: Maillard reaction; Protein hydrolysate; Ribose; Antiradical activity; DPPH$^{\bullet}$; Browning; Phosphate buffer; Temperature

1. Introduction

The Maillard reaction (MR), which links the carbonyl group of reducing carbohydrates and the amino group of free amino acids as well as of lysyl residues in proteins, may have either beneficial or detrimental effects (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001). This reaction is classified as non-enzymatic browning reactions and has been associated with the formation of compounds with strong radical scavenging activity. The Maillard reaction takes place in three major stages:

- At an early stage of the reaction, the free amino groups of proteins such as the ε-NH_2 groups of lysine, react with carbonyl groups of sugar to form a reversible Schiff base, which rearranges to stable, covalently bonded Amadori products (Jing & Kitts, 2002; Rizzi, 1994). The radical scavenging activity derived from the uncolored reaction products is smaller than the brightly colored pigments (Murakami et al., 2002).
- At intermediate stages, highly-UV-absorbing and colorless compounds are continually formed. In the advanced phase of the reaction, Amadori products undergo further transformation to fluorescent, colored substances and cross-linked polymers (Ames, 1990; Morales, Romero, & Jimenez-Perez, 1996; Van Boekel, 1998; Van Boekel, 2001).
- Formation of melanoidins and heterocycles compounds in the advanced stage of the Maillard reaction could explain the ability of glycated hydrolysate to react with radical compounds (Friedman, 1996).

Thus, the Maillard reaction is a complex reaction, since it is influenced by many factors such as temperature, pH, time, water activity, type and concentration of buffer, reactant source and sugar involved (Ames, 1990; Wijewickreme, Krejpcio, & Kitts, 1999). Changing

* Corresponding author. Fax: +33 2 98 10 00 62.
E-mail address: guerard@univ-brest.fr (F. Guerard).

0963-9969/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2005.03.015

any of these factors will alter reaction rate, reaction pathways and reaction end-products. Little information is available on the chemical structure of the hundreds of unknown products which are formed by a series of consecutive and parallel reactions including oxidations, reductions, and aldol condensations among others (Eriksson, 1981). In addition, since the majority of studies was carried out on model systems, little is known about sugar–protein hydrolysate systems. Chevalier, Chobert, Genot, and Haertle (2001) reported that the radical scavenging activity of glycated β-lactoglobulin and its tryptic and peptic hydrolysates depended on the sugar used for the modification. Guerard and Sumaya-Martinez (2003) showed that the antiradical scavenging effect was improved by 75% when protein hydrolysates from casein or fish were incubated in the presence of glucose. Although certain protein hydrolysates had been reported to be antioxidative per se, the effect was considerably improved when reacting the hydrolysates with glucose (Lingnert & Eriksson, 1981).

Finally, the MR is a cascade of consecutive and parallel reaction steps and has been shown to produce some desirable components such as antioxidative molecules, other undesirable compounds such as mutagenic, DNA-damaging, and cytotoxic substances. Brands, Alink, Boekel, and Jongen (2000) demonstrated that ketose sugars (fructose and tagatose) exhibited a remarkably higher mutagenicity compared with their aldose isomers (glucose and galactose), which was due to a difference in reaction mechanism. The cytotoxic effects of MRP have been studied in different model systems and are associated with the use of high temperatures (Jing & Kitts, 2002).The degree of browning is often used analytically to assess the extent to which the MR has taken place. Simple positive or complex correlation between color and antioxidant properties have been found depending on the composition and technological history of the product (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001).

The present investigation was undertaken to study the effect of experimental conditions (sugar type and concentration, buffer type and temperature incubation) on the development of Maillard reaction, the degree of browning and the formation of the antiradical compounds. The objective was to maximize the antiradical activity without the production of the cytotoxic effect related to MRPs. The contribution of the caramelization of the reducing sugar in the development of antiradical activity was also evaluated.

2. Materials and methods

2.1. Materials

D-Glucose, D-ribose and 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), citrate buffer salts, TRIS buffer salts and phosphate buffer salts were purchased from Sigma/Aldrich (St Louis, MO, USA). All reagents were of analytical grade.

2.2. Preparation of the tuna stomach hydrolysate

The tuna (*Thunnus Albacares*) stomachs were taken from frozen fish and heat inactivation of endogenous stomach enzymes (100 °C, 20 min) was carried out prior pH adjustment and addition of Alcalase®2,4L (Novo Nordisk). Hydrolysis experiments were performed in a batch reactor using the pH-stat method in controlled conditions (pH, temperature and stirring speed) as follows: The grinded stomachs (25 kg) were mixed with an equal amount of water and temperature of the mixture was adjusted to 50 °C. The enzyme concentration was fixed to 4% (w/w protein) and pH was kept constant (pH 8) by addition of a 5 N NaOH solution. The reaction was stopped after 15 min by heating treatment at 85 °C. The hydrolysis degree was calculated from the amount of base consumed according to Alder-Nissen (1982) and was stated to be 18% after 4 h hydrolysis. The DPPH scavenging activity of native hydrolysate was 72 ± 5 μmol equiv TROLOX/L.

2.3. Preparation of MRPs from sugar–tuna stomach hydrolysate model system

Glucose, fructose and tuna stomach hydrolysate (5 mg/mL of protein according to Lowry assay) were used to prepare different model aqueous solutions: A mixture of sugar and tuna stomach hydrolysate was heated for 17 h in pyrex tubes (15-mL) at different temperatures ranging from 35 to 115 °C. The assay conditions were: sugar concentration (ranging from 5 to 90 mg/mL), buffer type (Tris–HCl, citrate and phosphate), buffer concentration (ranging from 0 to 0.5 M) and pH (ranging from 4 to 12).

The DPPH· scavenging activity was evaluated by triplicate after appropriate dilution of heated solutions.

2.4. Measurement of browning

Browning of the samples was evaluated by reading the absorbance at 420 nm on a Hitachi U-2000 UV–vis spectrophotometer using a 1 cm path length cell after appropriate dilution.

2.5. Measurement of free radical scavenging activity

The antiradical activity of MRPs was evaluated according to the procedure reported by Morales and Jimenez-Perez (2001), which was sightly modified. An aliquot of sample (200 μL) was added to 1 mL of a daily-prepared solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH·) in ethanol (74 mg/L). The mixture was shaken