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Short communication

Effect of heat treatment on lactoperoxidase activity in camel milk: A comparison with bovine lactoperoxidase

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ABSTRACT

The thermal inactivation of lactoperoxidase (LP) in camel and bovine milk was studied and compared in a temperature range of 67–73 °C. The analysis of inactivation rate constant (k) data for the process of thermal denaturation of LP in camel and bovine milk showed monophasic inactivation pattern. Based on the thermal death time model, decimal reduction time (D) and inactivation rate constant (k) values of LP in camel milk were more decreased and increased, respectively with increasing temperature in respect of the bovine LP. The corresponding thermal sensitivity values (z) calculated for camel and bovine LP were 6.42 °C and 4.7 °C, respectively. Thermodynamic analysis of LP showed lower values for activation energy and change in enthalpy of denaturation in camel than bovine milk. Overall the results obtained in this study suggest a lower heat stability of camel LP than in its bovine counterpart.

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1. Introduction

Lactoperoxidase (LP) (EC 1.11.1.7) is a glycoprotein that occurs naturally in colostrum, milk, and many other human and animal secretions (Kussendrager and van Hooijdonk, 2000; Conner et al., 2002). It contributes to the nonimmune host defense system, exerting bacteriostatic and bactericidal activity mainly on gram negative bacteria (Touch et al., 2004). For antimicrobial function, LP needs the presence of hydrogen peroxide and thiocyanate, which have been called together "LP system". Today this system is considered to be an important part of the natural host defense system in mammals (Boots and Floris, 2006). The antibacterial action of the LP system is due to the effect of reaction products of thiocyanate oxidation, $OSCN^-$ and HOSCN, which are able to oxidize free SH – groups of various proteins that are important for the viability of pathogens, thereby, inactivating crucial enzyme and protein systems (Sermon et al., 2005). The LP system could be used as an alternative method for the preservation of raw milk, which is produced under high ambient temperature and low hygienic conditions when a cooling process is not found (Haddadin et al., 1996). Furthermore, LP has been used for preservation of cosmetics, foodstuffs and protection of growing flowers, fruits, tubers, etc. (Le Nguyen et al., 2005; Touch et al., 2004).

LP-activity may be used as an indicator of a correct pasteurization process for bovine and camel milk. This enzyme can be activated in some cases after heat treatment, thus contributing to extend the shelf-life of pasteurized milk in locations with inefficient cold storage conditions (Barrett et al., 1999; Fox and Kelly, 2006).

The effect of heat treatment on milk LP activity has been examined in buffalo (Tayefi-Nasrabadi and Asadpour, 2008), bovine (Hernandez et al., 1990; Ludikhuyze et al., 2001; Marin et al., 2003) and caprine (Trujillo et al., 2007), but detailed quantitative kinetic thermal

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inactivation studies in camel milk are lacking. The aim of this study was to determine the differences in lactoperoxidase heat-resistance in camel and bovine milk based on kinetic and thermodynamic analysis in the range 67–73 °C.

2. Materials and methods

2.1. Chemicals

Pyrogallol, hydrogen peroxide (30% solution) and all the other chemicals used in this research were obtained from Merck (Darmstadt, Germany) and were of reagent grade.

2.2. Milk sampling

Fresh raw camel (*Camelus bactrianus*) and bovine (*Holstein*) milk were supplied from Khorkhor (Tabriz, East-Azerbaijan province, Iran) and was analysed for total protein (IDF, 1993), fat matter (ISO, 1976), ash content (AOAC, 1995) and dry matter (IDF, 1970). The milk was divided into small portions (50 mL) and stored at −20 °C until analysis.

2.3. Enzymatic activity assay

Milk LP activity was measured by following the H_2O_2-dependent oxidation of pyrogallol at 430 nm, using an extinction coefficient of 2470 M^{-1} cm^{-1} (Pruitt et al., 1990). 3 mL of TS buffer (0.1 M citrate–phosphate–borate buffer, pH 6.5), 0.15 mL pyrogallol (200 mM) and milk sample (0.05 mL) were added together in cuvette. The reaction was initiated by the addition of 0.03 mL hydrogen peroxide solution (61 mM) and immediately the measurement of absorbance started at 430 nm as a function of time for 2 min at 15 s intervals using an UNICO UV-2100 PC spectrophotometer (UNICO, China). Measurements were carried out against the reagent blank containing pyrogallol and enzyme solution only. Reaction velocity was computed from linear slopes of absorbance–time curve. One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of pyrogallol per min at room temperature (~22–25 °C).

2.4. Heat incubation study

Thermal stability of milk LP was studied by incubating aliquots of milk at various temperatures (67, 69, 71 and 73 °C) up to 60 min in a thermostatic water bath and measuring their activity at room temperature after brief cooling in ice. The incubation was carried out in sealed vials to prevent change of volume of the sample and, hence, the enzyme concentration due to evaporation. Assays at the different temperatures were done at least in 3 separate experiments and the mean values of data were used to obtain the different kinetic and thermodynamic parameters.

2.5. Kinetic data analysis

Inactivation kinetics of milk LP toward thermal processes was subjected to reaction kinetic analysis. According to Eq. (1), loss of enzyme activity rate ($-dA/dt$) is proportional to the inactivation rate constant (k) and enzyme activity at each treatment time (A).

$$-\frac{dA}{dt} = kA^n \tag{1}$$

The experimental raw data are plotted according to the equation $\ln A/A_0 = kt$ derived from Eq. (1), where A is the response value after heating treatment, A_0 is the initial enzyme activity at time t_0, and t is the exposure time (min). We also calculated D-values (decimal reduction time) and z-value (temperature necessary to reduce D-value by 1 logarithmic cycle) according to Eq. (2).

$$\text{Log}\left(\frac{A}{A_0}\right) = -\frac{1}{D} \times t \tag{2}$$

For estimation of z-value, the linear regression of log D-values versus corresponding temperatures was performed using the SigmaPlot for windows version 10.0 (Systat software, Germany). In a denaturation process, the rate constant (k) and the temperature of treatment are related according to the Arrhenius equation:

$$\ln\ k = \ln A - \frac{E_a}{RT} \tag{3}$$

where A is the Arrhenius constant, E_a the apparent activation energy, R the universal gas constant, and T the absolute temperature. Activation energy can be calculated from the slope of the line.

From activation energy (E_a), different thermodynamic parameters such as variations in enthalpy (ΔH°), Gibbs free energy (ΔG°) and entropy (ΔS°) can be estimated according to the following expressions:

$$\Delta H^\circ = E_a - RT \tag{4}$$

$$\Delta G^\circ = -RT\ \ln\left(\frac{kh}{k_B T}\right) \tag{5}$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \tag{6}$$

where h and k_B are the Planck's and the Boltzmann's constants, respectively.

3. Results and discussion

Composition of camel and bovine milk used in this study was: total protein (3.4%, 3.1%), fat matter (4.2%, 3.5%), ash content (1.33%, 0.71%) and dry matter (12.14%, 11.82%), respectively.

Effects of heat treatment on the enzymatic activity of camel and bovine milk LP at different temperatures are

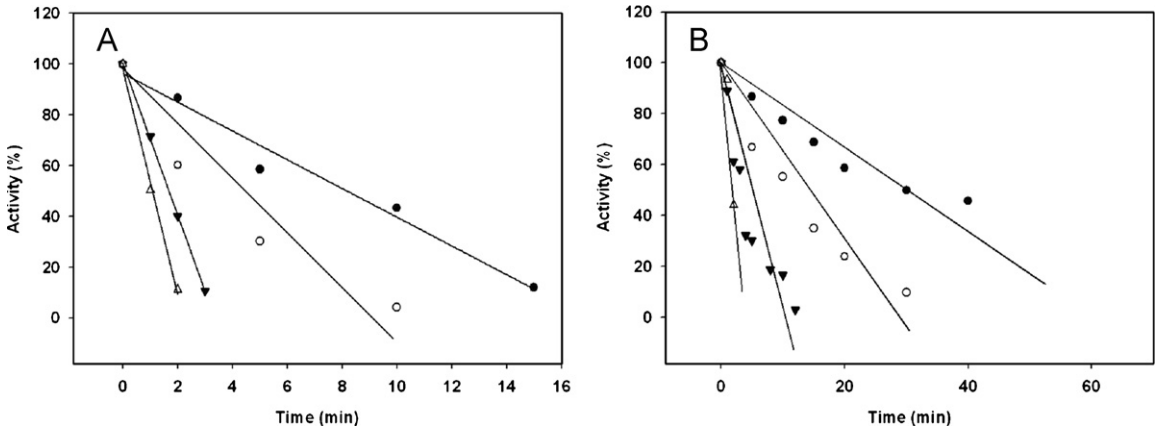

Fig. 1. Effect of heat treatment on camel (A) and bovine (B) milk lactoperoxidase activity as a function of treatment time at different temperatures: 67 °C (●), 69 °C (○), 71 °C (▼), 73 °C (△). The activity expressed as the percentage of initial activity.

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