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Predicting lactulose concentration in heat-treated reconstituted skim milk powder using front-face fluorescence

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ABSTRACT

Industrial processing of milk and dairy foods requires heat treatment, which induces undesirable effects in milk such as protein denaturation, organoleptic and nutritional properties alteration, browning, etc. The hypothesis of the present study is that it is possible to use front-face measurement of fluorescent markers such as tryptophan, dityrosine, Maillard intermediate compounds and riboflavin as an inline, cheap, easy and fast way to evaluate lactulose formation in milk. Thus, the specific objective of this study was modeling the kinetics of lactulose formation during heat treatment of milk as well as evaluating the correlation of lactulose concentration with fluorescent markers in order to obtain prediction models of lactulose concentration in heat-treated milk based on front-face fluorescence.

Three replicates of the study were performed at three different temperatures (80, 90 and 100 °C) and six different times (0, 10, 15, 30, 45 and 60 min). Standard, low-heat, spray-dried skim milk powder, supplied by Chr. Hansen SL (Barcelona, Spain) was reconstituted at 12% and used in all trials to ensure consistency among the various experimental trials.

Lactulose concentration was significantly correlated ($P < 0.001$) with fluorescence intensity of different markers such as maximum intensity and maximum emission wavelength of tryptophan as well as the fluorescence intensity of riboflavin. Fluorescence changes associated to thermal treatment of milk were characterized and a series of fluorescent markers were identified as predictors allowing prediction of lactulose concentration with an R^2 of 0.91 at a temperature of 90 °C.

1. Introduction

Raw milk is subjected to strict sanitary control in addition to various treatment processes, all with the aim of killing pathogens that may be found in milk, prolong shelf-life and eliminate health risks. For the production of high quality milk, it is necessary to know the modifications suffered by milk components during heat treatment. Since quality is directly related to the intensity of heat treatment that is applied during milk processing, it is necessary to have indicators that can identify the treatment employed, i.e., treatment intensity, and could provide useful, inline control feedback.

Among "thermal markers", two groups can be distinguished according to the reactions and subsequent modifications undergone during heat treatment of milk. Type I processes are related to denaturation, degradation and/or inactivation of thermolabile compounds such as serum proteins, enzymes and vitamins. Type II markers include compounds which are not present in raw milk or appear in trace amounts and are formed upon heat treatment. For example, lactulose (4-*O*-ß-D-galactopyranosyl-D-fructofuranose) is mainly formed by an isomerization reaction of lactose by the Lobry de Bruyn-Alberda Van Ekenstein transformation. Since it is a suitable marker which provides the heat load to which the milk was subjected (Berg & Van Boekel, 1994), lactulosa has been adopted as analytical indicator to guarantee the quality of UHT milk by differentiating between UHT and in-bottle sterilized milk (threshold of 600 mg/L of lactulose) (EC, 1992; IDF, 1992, 1993). Some studies have focused on the characterization of industrial processed milk by determining the concentration of lactulose alone or in combination of other indices (Feinberg, Dupont, Efstathiou, Louâpre, & Guyonnet, 2006; Marconi et al., 2004; Morales, Romero, & Jiménez-Pérez, 2000; Pellegrino, De

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Noni, & Resmini, 1995; Pellegrino, Resmini, & Luf, 1995; Sakkas, Moutafi, Moschopoululou, & Moatsou, 2014). Despite the interest of lactulose as a marker of thermal damage, existing kinetic data in the literature regarding its formation in milk are certainly rare, probably due to the complexity of the chemical reactions involved and/or the limitations of the analytical methods available such as spectrophotometric (Adhikari, Sahai, & Mathur, 1991) and chromatographic methods (Olano & Calvo, 1989).

Some autofluorescent molecules might be useful as "thermal markers" since they show a change in their fluorescence due to thermal denaturation or formation. For example, riboflavin (*Rb*); loss in milk during pasteurization and exposure to light is 10–20% (McDowell, 2000). But much larger losses (50–70%) can occur if bottled milk is left standing in bright sunlight for more than 2 h (McDowell, 2000). Another useful fluorescent marker is tryptophan (*Trp*) since it occurs in most proteins and biologically active peptides and its fluorescence is highly sensitive to environment, making it an ideal marker for reporting protein conformation changes and interactions with other molecules (Chen & Barkley, 1998; Pan, Muiño, Barkley, & Callis, 2011). Dityrosine (*Dt*) is a marker that has been proposed to evaluate oxidative stress in proteins (Heineckes, Li, Daehnke, & Goldstein, 1993) and, since it forms in vivo, it may also serve as a useful intrinsic fluorescent probe of protein environments and internal motions (Harms, Pauls, Hedstrom, & Johnson, 1997; Malencik & Anderson, 2003). Fluorescent products have been proposed as early indicators of the Maillard reaction since, in stages prior to the formation of brown pigments, fluorescent compounds are formed (Bastos, Monaro, Siguemoto, & Séfora, 2012). In particular, the progress of the Maillard reaction in milk and milk-resembling systems during heating might be followed by monitoring free fluorescent intermediary compounds (Morales, Romero, & Jiménez-Pérez, 1996), hereafter referred as Maillard compounds (*MC*). In an early stage, fluorescence experiments done by the classical right-angle fluorescence spectroscopy were carried out by diluting milk down to an absorbance of 0.1. At higher absorbance, the screening effect (or inner filter effect) provokes distortion of the excitation spectra and a decrease of fluorescence intensity (Genot, Tonetti, Montenay-Garestier & Drapron, 1992). To avoid these problems, a few studies have focused on the use of front-face fluorescence spectroscopy (*FFFS*) in the field of milk analysis (Dufour & Riaublanc, 1997; Hougaard, Lawaetz, & Ipsen, 2013; Karoui, Martin, & Dufour, 2005; Kulmyrzaev & Dufour, 2002; Kulmyrzaev, Levieux, & Dufour, 2005; Liu & Metzger, 2007; Schamberger & Labuza, 2006), one of which focuses on lactulose as thermal heat index (Kulmyrzaev & Dufour, 2002).

Therefore, the specific objective of the present study was to obtain prediction models through kinetic modeling of lactulose formation during heat treatment of milk and the evaluation of its correlation with fluorescent markers determined by *FFFS*.

2. Materials and methods

2.1. Experimental design

Kinetic study for the evaluation of lactulose and autofluorescent markers (*Dt*, *Trp*, *MC* and *Rb*) during heat treatment was performed using a factorial design with two factors: temperature and time, with three (80, 90 and 100 °C) and six (0, 10, 15, 30, 45 and 60 min) levels, respectively. For each treatment, determinations of lactulose and all fluorescent markers evaluated were run in parallel in order to correlate the concentration of lactulose with fluorescent markers and obtain the corresponding predictive models. The whole experiment was carried out in three independent occasions.

2.2. Sample preparation and heat treatment

For all trials, reconstituted standard skimmed milk was obtained from atomized dried powder with high-quality functional and microbiological grade (extra-grade, low-heat, spray-dried skim milk powder, pH = 6.5, 800 cfu g^{-1}) supplied by Chr. Hansen SL (Barcelona, Spain). Milk was reconstituted to 12% (w/w) with distilled water at ~40 °C by stirring for 15 min at 150 rpm (Agimatic-E, JP. Selecta, Barcelona, Spain) and leaving it undisturbed at room temperature for 30 min.

To study the kinetic formation of lactulose, heat treatments were applied to samples of 15 mL of milk in tubes of 30 mL capacity sealed and immersed in a thermostatic bath (mod. OvanTherm TC00E C, Lovango SL; control accuracy ±0.1 °C) at the target temperature. At the different treatment time intervals, samples were removed from the bath and placed on iced-water to cool down to room temperature.

2.3. Determination of lactulose concentration

Lactulose quantification was achieved by chemical essay following the enzymatic method ISO 11285 (2004). Enzymatic determinations of lactulose with a Glucose/Fructose kit (Boehringer Mannheim/R-Biopharm Enzymatic BioAnalysis/Food Analysis, Germany) were performed in triplicate.

2.4. Modeling the kinetic data

For the modeling of kinetic data, kinetic equations zero-, first-, and second-order were evaluated to select the most appropriate kinetic model in each case. The effect of temperature on the reaction rate was modeled with the Arrhenius equation (Peleg, Normand, & Corradini, 2012). To estimate the rate constant of lactulose formation (k) and its activation energy (E_a), once identified the order, the following equations were used (Claeys, Loey, & Hendrickx, 2003):

a) zero-order rate equation:

$$[L] = [L]_0 + kt \quad [1]$$

b) Arrhenius equation applied to two conditions of temperature T_1 and T_2:

$$k_1 = Ae^{-\frac{Ea}{RT_1}} \quad [2]$$

$$k_2 = Ae^{-\frac{Ea}{RT_2}}. \quad [3]$$

Dividing equations (2) and (3) yields:

$$\frac{k_1}{k_2} = e^{\left(-\frac{Ea}{RT_1} + \frac{Ea}{RT_2}\right)} \quad [4]$$

then, solving for $k_1$yields:

$$k_1 = k_2 e^{-\frac{Ea}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)} \quad [5]$$

assuming T_1 and T_2 as working and reference temperatures, respectively, and substituting Eqn. (5) into Eqn. (1), it yields: