Food Chemistry 196 (2016) 815–820

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Application of a dye-binding method for the determination of available lysine in skim milk powders

Kataneh Aalaei [a,*], Marilyn Rayner [a], Eden Tareke [b], Ingegerd Sjöholm [a]

[a] Department of Food Technology, Engineering and Nutrition, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b] Food for Health Science Centre, Lund University, Medicon Village, SE-223 81 Lund, Sweden

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 29 September 2015
Accepted 1 October 2015
Available online 13 November 2015

Keywords:
Available lysine
Skim milk powders
Storage stability
Drying
Dye binding methods

ABSTRACT

A dye-binding method using Acid Orange 12 was investigated regarding its suitability for the quantification of available lysine, as a means of monitoring the Maillard reaction in skim milk powders. The method was evaluated by analyzing a wide range of milk powders produced by three different drying methods and stored under various conditions. A pilot-scale freeze-dryer, spray-dryer and drum-dryer were used to produce skim milk powders and the samples were stored at two temperatures (20 °C and 30 °C) and two relative humidities (33% and 52%) under strictly controlled conditions. Moreover to validate the method, two protein isolates; bovine serum albumin and casein were investigated for their available lysine content. The results demonstrate the suitability of this method for measuring the available lysine in skim milk powders with good precision and high reproducibility. The relative standard deviations obtained from the 125 freeze-dried powders were 1.8%, and those from the 100 drum-dried samples were all 1.9%. The highest variation was found for the spray-dried powders, which showed relative standard deviations between 0.9% and 6.7%.

1. Introduction

One common group of reactions in foods is called Maillard or non-enzymatic browning reactions. Reducing sugars together with amino acids in proteins are involved in the initial step in this complex series of reactions. Lactose and lysine residues in caseins and whey proteins are the main reactants in milk, and lysine, which is an essential amino acid for the body, loses its bioavailability during the initial step. This chemical reaction has been the subject of many studies in recent years due to its impact on nutritional aspects, as well as potential health risks under certain conditions (O'Brien, 2009). Therefore, the determination of reactive lysine is not only important from a nutritional point of view, but can also be used as a marker to monitor the Maillard reaction after processing and during the storage of food products.

A large number of methods can be found in the literature for the determination of available lysine in foods. Considering the pros and cons of each method and also depending on the sample, perhaps dye binding methods are among the most convenient and reliable methods in this regard. Some of the advantages mentioned in the literature include: that they are simple and fast, there is no need for acid hydrolysis prior protein extraction, they show good precision and reproducibility, there is no interference from carbohydrates, and they are appropriate for routine daily analysis and show high correlation with animal studies (Anderson, Sneed, Skurray, & Carpenter, 1984; Barlow et al., 1984; Hurrell, Lerman, & Carpenter, 1979; Moore, DeVries, Lipp, Griffiths, & Abernethy, 2010; Perl, Szakács, Kővágó, & Petróczy, 1985; Sherbon, 1978).

Initially, anionic dyes were mostly used to estimate the protein content of samples, and not specifically for the determination of lysine. The method was first applied to quantify the protein content in food matrices in 1956 (Udy, 1956). Numerous modifications have been reported since then, and dye-based methods became very common, especially during the 1980s. The most commonly used dyes are Acid Orange 12, Orange G and Amido Black 10B, all belong to a group of dyes called Azo dyes possessing sulfonic groups (Ashworth, 1966; Goh & Clandinin, 1978; Moore et al., 2010; Sherbon, 1978).

The reaction between the dye and basic amino acids in proteins starts when the negatively charged dye is added to the protein, which already has some positively charged constituents. Lysine with its ε-NH_2 group, histidine with its imidazole group, and arginine with a guanidine group, combine stoichiometrically with the sulfonic groups of the dye, rapidly forming a protein–dye complex. If the basicity of the lysine group is neutralized, in this case by

* Corresponding author.
E-mail address: Kataneh.aalaei@food.lth.se (K. Aalaei).

http://dx.doi.org/10.1016/j.foodchem.2015.10.004
0308-8146/

propionic anhydride, the dye will only react with arginine and histidine. An estimate of the amount of lysine in the sample can then be obtained from the difference between the two steps. The dye–protein complex is separated by centrifugation, and the concentration of the dye in the supernatant, can be used indirectly to calculate the lysine quantity in the test material by a mass difference. Therefore, for the final part of the analysis the concentration of the remaining dye is quantified spectrophotometrically.

The protein content as well as the available lysine content of a wide range of food products have been successfully determined using this method, by various researchers, giving results in agreement with those obtained using other methods, including 1-fluoro-2,4-dinitrobenzene and O-phthalaldehyde (Barlow et al., 1984; Carpenter et al., 1989; Goh & Clandinin, 1978; Hendriks, Moughan, Boer, & van der Poel, 1994; Khan, 1978; Perl et al., 1985; Walker, 1979). In particular, this method has been applied to a number of dairy products, which is the focus of the present work, including milk and milk powder, ice-cream, and cheese (Ashworth, 1966; Ashworth & Chaudry, 1962; El & Kavas, 1997; Hurrell, Finot, & Ford, 1983; Molnár-Perl et al., 1986).

In the context of this project we are interested in the loss of available lysine in skim milk powders after processing by three different methods (freeze drying, drum drying and spray drying) and during storage under different conditions (controlled relative humidity and temperature) as an indication of the initiation and development of Maillard reactions. Therefore, the aim of this study is to apply a dye-binding method in the determination of available lysine in the various skim milk powders. Moreover there is also a need to validate this method using known dairy ingredients of practical interest, in this case BSA and casein. Furthermore the robustness, precision and variability of the method was evaluated with respect to inter- and intraday variations, as well as small changes in sample matrix, dye to sample ratios, and material preparation. Additionally, we set out to clarify any ambiguity in this method by describing the method in a more detailed manner, since most of the published literature refers to the original paper from 1979 (Hurrell et al., 1979), which does not provide sufficiently detailed information to carry out the method in a straightforward way.

2. Materials and methods

2.1. Chemicals and instruments

Sodium acetate anhydrous reagent grade (CAS: 127-09-3) was purchased from Scharlau (Sentmenat, Spain). Acid Orange 12 (Crocein Orange G) (CAS: 1934-20-9 and MW = 350.32) was supplied by Tokyo Chemical Industry (Tokyo, Japan). Potassium dihydrogen phosphate for analysis (CAS: 7778-77-0) and magnesium nitrate hexahydrate for analysis (CAS: 13446-18-9) were obtained from Merck (Darmstadt, Germany). Propionic anhydride 99% (CAS: 123-62-6) was acquired from ACROS organics (Geel, Belgium). Magnesium chloride hexahydrate (CAS: 7791-18-6) was purchased from VWR international (Leuven, Belgium). Oxalic acid dehydrate >99.5% (CAS: 6153-56-6) and casein sodium salt from bovine milk (CAS: 9005-46-3) were supplied by Sigma–Aldrich (Steinheim, Germany), while albumin from bovine serum 96–99% (CAS: 9048-46-8) was obtained from Sigma–Aldrich (St. Louis, USA).

The instruments used included a 3005 orbital lab shaker type from (Gesellschaft for Labortechnik), an Aqualab Series 3 water activity meter (Decagon Devices), a wireless Hygroclip with temperature and air humidity sensors (Mätman 3) from Eltex of Sweden, an Optima LE-80K ultracentrifuge (Beckman Coulter) and a Varian Cary 50 Bio UV–Vis spectrophotometer.

2.2. Drying experiments

2.2.1. Freeze-drying

Freeze-drying was executed using a pilot-scale freeze-dryer (Labconco, Missouri, USA). Skim milk (0.1% fat) was placed into aluminum trays (1 cm thickness). The samples were then put into the freezer at −20 °C for 24 h before freeze-drying. The freeze-drying temperature was −20 °C in the beginning and reached 20 °C with a 1 °C/h increase. The condenser had a temperature of −50 °C and the vacuum pressure was 0.02 mbar. The freeze-drying duration was 7 days and the samples were immediately ground, vacuum-packed and placed into the freezer at −20 °C until further analysis.

2.2.2. Drum-drying

The process was carried out using a Goudsche Machinefabriek drum-dryer (Waddinxveen, Netherlands). The surface temperature of the drum was 115 °C on average and the drying was completed in 40 s. The obtained flakes were then ground, vacuum packed and placed into the freezer at −20 °C until further analysis.

2.2.3. Spray-drying

A lab-scale Büchi mini spray-dryer B-290 (Flawil, Switzerland) was utilized for spray-drying. The inlet temperature of 150 °C and outlet temperature of 85 °C was applied. The flow rate of the feed was 0.6 L/h and the pre-heated air had a flow rate of 540 L/h. The powder was immediately collected, vacuum-packed and put into the freezer at −20 °C until further analysis.

2.3. Water activity and water content measurements

Since water plays a critical role in the Maillard reaction, and also due to the importance of the water content in the final calculations, the water activity and water content of the powders were quantified before analyzing the available lysine. The water activity was measured in duplicate using Aqua-lab water activity meter, and water content, or the dry matter, of the sample was determined using the standard method of the International Dairy Federation (Rückold, Grobecker, & Isengard, 2000). Briefly, samples were dried in an oven at 102 °C for 2 h. Following this, the samples were kept in a desiccator for one hour to reach constant weight before analysis.

2.4. Storage of the samples

Storage of the samples was carried out in desiccators at two temperatures (20 °C and 30 °C) and two relative humidities (33% and 52%). In order to achieve 33% relative humidity inside the desiccator, 200 g magnesium chloride was mixed with 25 ml distilled water and stirred until a homogeneous solution was obtained, while 52% relative humidity was obtained by dissolving 200 g magnesium nitrate in 30 ml distilled water (Motarjemi, 1988). The solutions were placed in the desiccators and allowed to equilibrate for one week before adding the samples. This is an important step often neglected in other studies. The temperature and relative humidity in the desiccators were monitored once prior to putting the samples in the desiccators, and regularly during storage, with a wireless Hygroclip.

Several glass Petri dishes containing 7 g milk powder each were placed in the desiccator, which was then placed in an incubator to reach the desired storage temperature. The temperature of the incubators was controlled regularly with the thermometer. At each pre-determined sampling point one plate was taken out and analyzed.