Food Research International 43 (2010) 1321–1328

Contents lists available at ScienceDirect

Food Research International

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

Physicochemical changes in intermediate-moisture protein bars made with whey protein or calcium caseinate

Simon M. Loveday, Jason P. Hindmarsh, Lawrence K. Creamer, Harjinder Singh *

Riddet Institute, Massey University, Private Bag 11 222, Palmerston North, New Zealand

A R T I C L E I N F O

Article history:
Received 8 October 2009
Accepted 12 March 2010

Keywords:
High-protein snack bar
Whey protein isolate (WPI)
Calcium caseinate
Shelf life
Intermediate-moisture foods
Maillard reactions
Proton nuclear magnetic resonance (^{1}H-NMR)
Nuclear Overhauser effect spectroscopy (NOESY)

A B S T R A C T

This study examined model protein bars made with whey protein isolate (WPI) or calcium caseinate and stored at 20 °C for 50 days. WPI bars remained very soft and, throughout storage, confocal micrographs showed a continuous matrix containing soluble protein and increasing quantities of glucose crystals. In contrast, calcium caseinate bars had a firm texture within 1–5 days of manufacture (fracture stress 199 ± 16 Pa) and hardened progressively during storage (final fracture stress 301 ± 18 Pa). Electrophoresis showed no evidence of covalent protein aggregation, but there were substantial changes in microstructure over the first day of storage, resulting in segregation of a protein phase from a water–glucose–glycerol phase. Proton nuclear magnetic resonance (^{1}H-NMR) relaxometry and nuclear Overhauser effect spectroscopy (NOESY) experiments showed that water migration away from protein towards glucose and glycerol occurred 10–18 h after manufacture, lowering the molecular mobility of protein. Phase separation was probably driven by the high osmotic pressure generated by the glucose and glycerol. These results confirm that the hardening of protein bars is driven by migration of water from protein to glucose and glycerol, and microstructural phase separation of aggregated protein.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Intermediate-moisture foods have water activity (a_w) in the region 0.9–0.6 (Roos, 2001, p. 5), and high-protein snack bars (protein bars) fall within this region. The shelf life of intermediate-moisture foods is often limited by Maillard reactions (also known as nonenzymic browning reactions) between carbonyl groups on reducing carbohydrates and exterior amine groups on proteins. Maillard reactions can lead to unappealing color, texture, or flavor, and can seriously affect nutritional value.

As well as Maillard chemical reactions, physicochemical reactions can occur in multicomponent processed foods such as protein bars; these are often far from thermodynamic equilibrium (Mezzenga, 2007). Thermodynamic incompatibility of biopolymers (Tolstoguzov, 2003) and the existence of chemically heterogeneous micro-environments within foods (Kou, Ross, & Taub, 2002) can drive physicochemical reactions during storage. By judicious choice of ingredients and processing conditions, food manufacturers can deliberately create kinetically-limited conditions, such as glassy domains, in order to prolong the shelf life of foods.

The shelf life of protein bars is often limited by their tendency to become unacceptably hard during storage. In an earlier paper (Loveday, Hindmarsh, Creamer, & Singh, 2009), we discussed the occurrence of Maillard reactions and physicochemical reactions in protein bars during storage, as well as current approaches to mitigate hardening and extend shelf life.

In our earlier work with model protein bars containing milk protein concentrate (MPC), hardening did not correspond with changes in protein molecular weight or chemically available amine content (Loveday et al., 2009). Over the first 24 h after manufacture, the microstructure of MPC bars underwent a phase separation, concomitant with the transformation from a liquid batter to a solid bar. Subsequently, there were ongoing changes in the molecular mobility of glucose, glycerol, and water that suggested that the glucose was crystallizing.

This study investigated the effect of protein characteristics on bar hardening, by contrasting the physical and chemical changes in bars made with whey protein isolate (WPI) to those in bars made with calcium caseinate. WPI contains 90% whey protein, some of which is aggregated into dimers, trimers, etc. via disulfide bonding, but the aggregates are still small enough to be soluble. In contrast, calcium caseinate contains calcium-induced aggregates that are much larger and with lower solubility. As the methods

Abbreviations: a_w, Water activity; 2D NOESY, two-dimensional nuclear Overhauser effect spectroscopy; CPMG, Carr Purcell Meiboom Gill; MPC, milk protein concentrate; ^{1}H-NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; PHC, polyhydroxy compound; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; WPI, whey protein isolate.

* Corresponding author. Tel.: +64 6 350 4401; fax: +64 6 350 5655.
E-mail address: h.singh@massey.ac.nz (H. Singh).

0963-9969/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2010.03.013

and materials were very similar to those in the previous study with MPC, the findings are directly comparable.

2. Materials and methods

Materials, equipment, and protocols were identical to those described earlier (Loveday et al., 2009) with the exception of the proteins used and some proton nuclear magnetic resonance (^{1}H-NMR) protocols. Some experimental details are omitted here, but can be found in the earlier work.

2.1. Bar ingredients

Calcium caseinate (ALANATE™ 385, typical analysis 92.1% protein, 3.9% moisture, 1.5% fat, 0.1% lactose) and WPI (ALACEN™ 895, typical analysis 91.2% protein, 4.6% moisture, 0.3% fat, 0.7% lactose) were supplied by Fonterra Co-operative Group Limited, Auckland, New Zealand.

2.2. Product manufacture

Bar material was made in 1 kg batches, consisting of 40% w/w glucose, 20% w/w protein powder, 15% w/w glycerol, 15% w/w water, and 10% w/w cocoa butter. The ingredients were mixed at room temperature (approximately 20 °C) with a Kenwood mixer (Kenwood Corporation, Long Beach, CA) fitted with a flat K-bar type blade. The mixing protocol and the preparation of subsamples were as previously described. Calcium caseinate bars had a_w of 0.65 immediately after manufacture, and a_w for fresh WPI bars was 0.68.

2.3. Texture analysis

A lubricated uniaxial compression test was adapted from methods described previously (Watkinson & Jackson, 1999; Watkinson et al., 1997, 2001). A cylindrical core of protein bar material was placed upright on a lubricated Teflon plate and then compressed vertically with a parallel lubricated Teflon plate attached to a TA.HD texture analyzer, equipped with a 500 N load cell (Stable Micro Systems, Godalming, UK) and driven by Texture Expert Exceed software (version 2.64, Stable Micro Systems). Samples were compressed to 80% Cauchy strain at a crosshead speed of 0.83 mm/s.

Force–distance–time data were converted into stress and Hencky strain, and the fracture stress was approximated by the local maximum in stress.

2.4. Confocal laser scanning microscopy

During manufacture of the protein bars, a subsample was withdrawn after the final mixing and a few drops of dye were added. The dye was a mixture of Nile Blue (lipid stain) and Fast Green FCF (protein stain) dissolved at 0.2% w/v in a commercial antifading mountant medium, Citifluor (Citifluor Ltd, Leicester, UK). A drop of the mixture was placed on a glass cavity slide and a coverslip was applied. The slides were stored at 20 °C. For one slide of WPI bar material, the coverslip was taped down and the slide was stored upside down.

The slides were examined with a Leica model TCS SP5 DM6000B confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany), initially approximately 30 min after the completion of mixing and then again the next day, approximately 24 h later, and after 4 and 18 days.

The images were processed with Adobe Photoshop CS version 8.0 (Adobe Systems Incorporated, San Jose, CA) and ImageJ 1.38× (National Institutes of Health, USA; http://rsb.info.nih.gov/ij/). Some images were processed with the built-in fast Fourier transform filter plug-in.

2.5. Proton Nuclear magnetic resonance (^{1}H-NMR)

All ^{1}H-NMR experiments were undertaken in a Bruker (Rheinstetten, Germany) AMX 200 MHz horizontal wide-bore magnet. The proton transverse (or spin–spin) relaxation was measured using the Carr Purcell Meiboom Gill (CPMG) spin–echo pulse sequence (Callaghan, 1991). The major components of the bar material – water, lipid, and polyhydroxy compounds (PHCs) – could be analyzed separately via their representative peaks in the proton NMR spectrum. The major PHCs, glucose and glycerol, could not be separated, as both appear at the –OH position in the proton spectrum. An example spectrum of protein bar material is shown in Loveday et al. (2009).

The transverse relaxation signal of each component was well-fitted in all cases by a bi-exponential model (Schuck et al., 2002):

$$S = A\exp\left(\frac{t}{T_{2,\mathrm{FAST}}}\right) + B\exp\left(\frac{t}{T_{2,\mathrm{SLOW}}}\right) \tag{1}$$

$T_{2,\mathrm{FAST}}$ and $T_{2,\mathrm{SLOW}}$ are the transverse relaxation rate constants of fast- and slow-relaxing protons of each compound, respectively. The values A and B are the proportions of each component. The T_2 of a compound is related to its molecular mobility and molecular interactions (Lin et al., 2006). As the molecular mobility decreases and/or the level of molecular interactions increases, T_2 will decrease.

The standard Bruker phase-sensitive nuclear Overhauser effect (NOE) pulse sequence (NOESYPH) was used to acquire a two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY) data set with a mixing time (τ_m) of 200 ms. In 2D NOESY, the NOEs appear as cross-peaks, indicating transfer of spin polarization from one spin population to another (cross-relaxation). NOEs can occur between adjacent nuclei within a molecule and/or between nuclei in different molecules. An NOE occurs when two nuclei are close in space (within 5 Å) (Otting & Liepinsh, 1995). The intensity of the NOE peak is proportional to the spin exchange rate and is inversely proportional to the distance between the nuclei.

The forward (k_1) and backward (k_{-1}) magnetization exchange rates between water and PHCs can be calculated using the method described by Zolnai, Juranic, Vikic-Topic, and Macura (2000). The magnetization exchange rate is generally proportional to the chemical exchange rate (in this case, the proton exchange rate between water and PHCs). This method requires two 2D NOESY experiments to be acquired with mixing times τ_m of 0 and 200 ms. The standard Bruker phase-sensitive NOE pulse sequence NOESEYPH was used.

2.6. Protein extraction and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE)

After storage for 1–50 days at 20 °C, samples of bar material were frozen in liquid nitrogen, ground to a fine powder, lyophilized, and stored at −20 °C. Protein extraction and SDS–PAGE followed the procedure of Loveday et al. (2009).

2.7. Chemically available amine

The protein bar extracts prepared for SDS–PAGE were also assayed for chemically available amine content. In a 1.5 mL acrylic cuvette (path length 10 mm), 50 μL of extract was added to 950 μL of reagent (Goodno, Swaisgood, & Catignani, 1981). The reader is referred to Loveday et al. (2009) for details of reagent

Download English Version:

https://daneshyari.com/en/article/4562598

Download Persian Version:

https://daneshyari.com/article/4562598

Daneshyari.com