Food Hydrocolloids 49 (2015) 208–215

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Storage induced conversion of ovalbumin into S-ovalbumin in eggs impacts the properties of pound cake and its batter

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ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form
11 March 2015
Accepted 17 March 2015
Available online 1 April 2015

Keywords:
S-ovalbumin
Pound cake
Protein network
Sulfhydryl content
Batter viscosity
Crumb springiness

ABSTRACT

Storage of shell eggs for 28 days at 6 or 23 °C converts ovalbumin (which represents over 50% of egg white protein) into its more thermostable intermediate and S-ovalbumin forms. Their increased thermostability causes them to expose their sulfhydryl groups only at higher temperatures than does ovalbumin itself. During pound cake baking, the loss in protein extractability under non-reducing conditions (further taken as a measure of protein network formation) occurred later when egg white from stored eggs rather than from fresh eggs was used. The incorporation of the more thermostable forms of ovalbumin (*i.e.* intermediate and S-ovalbumin) in the protein network occurred later than did that of ovalbumin. The moment at which ovalbumin denatures determines when the largest decrease in overall protein extractability occurs, which points to ovalbumin having a key role in protein network formation during cake baking. However, at the end of the process, the overall baking induced loss of protein extractability was similar when egg white from either fresh or from stored eggs was used. Rapid Visco Analyser measurements showed that this delay in protein network formation impacts cake batter viscosity. While cakes baked with fresh or stored egg white were of similar volume, cake crumb cohesiveness and springiness of cakes produced with fresh egg white were higher than those of cakes produced with stored egg white.

1. Introduction

Hen egg consists of shell (8–11%), egg white (56–61%) and yolk (27–32%). Fresh yolk contains 47–48% water, 16% protein, 32–35% lipid, and 1% carbohydrate. Fresh egg white contains about 89% water, 10% protein, 1% carbohydrate and almost no lipid (Powrie & Nakai, 1985). Egg white contains many different proteins (Mann & Mann, 2011), among which ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3%) and some globulins (about 8%). Ovalbumin is the only egg white protein which has free sulfhydryl (SH) groups (Powrie & Nakai, 1985). Native ovalbumin has four SH groups (Nisbet, Saundry, Moir, Fothergill, & Fothergill, 1981) which are buried within the protein core (Beveridge & Arntfield, 1979). It denatures at about 80 °C (Donovan, Mapes, Davis, & Garibaldi, 1975; Yamasaki, Takahashi, & Hirose, 2003). During heating, these SH groups become exposed and react with other SH groups to form disulfide (SS) bonds (Beveridge et al., 1979; Van der Plancken, Van Loey, & Hendrickx, 2005). SS bonds are crucial in the formation of protein networks and the texture of egg white gels (Van der Plancken et al., 2005).

While egg white has excellent gel forming and foaming properties, egg yolk has gel forming and emulsifying properties. These properties explain their use in many food products (Kiosseoglou & Paraskevopoulou, 2006).

European legislation allows for eggs to be stored for up to 28 days after being laid (European Commission, 2008). Multiple changes during storage have been described. These include weight

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http://dx.doi.org/10.1016/j.foodhyd.2015.03.014
0268-005X/

loss (Jones & Musgrove, 2005; Lucisano, Hidalgo, Comelli, & Rossi, 1996), moisture migration from egg white to egg yolk (Jones, 2007; Lucisano et al., 1996), increase in egg white pH (Lucisano et al., 1996), egg white liquefaction (Jones & Musgrove, 2005), weakening of the vitelline membrane (Kirunda & McKee, 2000) and conversion of ovalbumin into S-ovalbumin (Donovan & Mapes, 1976; Smith, 1964). Weight loss is mainly caused by loss of water from egg white through the shell to the surrounding air (Lucisano et al., 1996). Dissolved CO_2 migrates through the shell (Heath, 1977; Smith, 1931) to the environment. This increases the pH of the egg white from 7.6 at oviposition (Cotterill, Gardner, & Funk, 1958) to plateau values exceeding 9.0 after 4–5 days at different temperatures (Lapao, Gama, & Soares, 1999; Lucisano et al., 1996). High pH and storage temperature accelerate the conversion of ovalbumin into S-ovalbumin (Smith & Back, 1962, 1965). The denaturation temperature of the latter is 8 °C higher than that of native ovalbumin (Donovan et al., 1976; Smith, 1964). Yamasaki et al. (2003) found that three serine residues of ovalbumin (Ser-164, Ser-236 and Ser-320) isomerize into their D-amino acid forms during conversion of ovalbumin to S-ovalbumin. Isomerization of Ser-164 and Ser-320, and not that of Ser-236, increases the thermostability. Isomerization of both serines to form S-ovalbumin increases the denaturation temperature by 8 °C, while isomerization of only one of them yields intermediates with a denaturation temperature that is 4–5 °C higher than that of ovalbumin itself.

Eggs are essential in cake making (Bennion & Bamford, 1997). Depending on the type of cake, they have foaming, emulsifying and/or gel forming functionalities (Bennion et al., 1997; Kiosseoglou et al., 2006). In egg foam cake (*e.g.* angel food and sponge cake) making, air is incorporated into batter by producing an egg (white) foam (Wilderjans, Luyts, Brijs, & Delcour, 2013). For different types of such cakes, stored eggs result in a lower cake volume and poorer texture than fresh eggs (Jones, 2007; Jones et al., 2005; Meehan, Sugihara, & Kline, 1962; Pyke & Johnson, 1941). In multistage pound and layer batter mixing, air bubbles are beaten into the fat phase (Wilderjans et al., 2013). Because of this, the foaming properties of egg white are less important in such systems. Still, the gel forming properties of egg white and egg yolk are very important for the crumb structure of these cakes. As described by Wilderjans et al. (2010), when a covalent protein network has been formed (*e.g.* with ovalbumin) during cake baking, the proteins are no longer extractable with a sodium dodecyl sulfate (SDS) containing medium, and the loss of protein extractability in said medium can be considered to be a measure for protein network formation.

The different impact of fresh or stored egg white on pound cake has not yet been investigated in detail. In this study, eggs were stored at 6 °C or at 23 °C and the changes in egg white pH and denaturation temperature during storage were monitored. Levels of exposed SH groups in fresh and stored egg white, and therefore also in ovalbumin or in its more thermostable forms were measured at different temperatures using Ellman's reagent. Egg white contains high levels of SH groups. Such groups are crucial in the formation of protein networks. The temperature at which SH groups become exposed may therefore influence protein network formation during cake baking. This was investigated by measuring protein extractability in SDS containing medium for cake batter samples containing fresh or stored egg white. The viscosity of the batters with fresh or stored egg white was evaluated during heating in a Rapid Visco Analyser (RVA) and allowed relating the changes at batter level to the properties of the egg white samples. Cake quality (volume and texture) was analyzed for cakes containing fresh or stored egg white. We here report on the outcome of this work.

2. Experimental

2.1. Materials

Fresh eggs were collected from Isa Brown hens (52–60 weeks old) within 4 h after being laid. Flour (Halmbloem) [14.0% moisture, 10.2% protein (as is basis)] was from Paniflower (Merksem, Belgium). Margarine (19.3% moisture) was from Puratos (Groot-Bijgaarden, Belgium) and sodium bicarbonate and sodium acid pyrophosphate from Budenheim (Budenheim, Germany). AACC International Approved Method 44-15.02 (AACCI, 1999) was used to determine flour moisture content. An adaptation of the AOAC Official Method (AOAC, 1995) was used to determine flour protein ($N \times 5.7$) content with an automated Dumas protein analysis system (EAS VarioMax C/N, Elt, Gouda, The Netherlands). Sugar was purchased in a local supermarket. All reagents, solvents and chemicals were of analytical grade and obtained from Sigma–Aldrich (Bornem, Belgium) unless indicated otherwise.

2.2. Egg storage

Fresh eggs (collected as outlined above) were stored at 6 °C or at 23 °C during 28 days. Samples were coded as in Table 1.

2.3. Egg properties

pH of FW, 6°W and 23°W was measured with a pH meter (HI 9025, Hanna Instruments, Woonsocket, RI, USA) at 23 °C. The standard deviation of at least 5 measurements was less than 0.2.

Differential Scanning Calorimetry (DSC) measurements were performed with a Q1000 DSC (TA Instruments, New Castle, DE, USA) calibrated with indium. Freeze-dried egg white samples (FW, 6°W and 23°W) (2.5–4.0 mg) were brought into aluminum pans (Perkin–Elmer, Waltham, MA, USA) and deionized water was added to obtain a dry matter content of 1:3 (w/w). The pans were hermetically sealed and placed in the DSC together with a sealed empty pan as reference. The heating profile started with an equilibration step at 0 °C before heating from 0 to 120 °C at 4 °C/min. The thermograms were analyzed using TA Q Series Advantage Universal Analysis software (TA Instruments). Peak temperatures (Tp) (°C) and enthalpies (J/g dry matter sample) were calculated from the thermograms. The standard deviations of triplicate measurements were below 0.6 J/g for enthalpy and 1 °C for Tp.

Free SH groups of freeze-dried egg whites (FW, 6°W, 23°W) were determined colorimetrically after reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent). An aliquot (1.0 mL) of Tris–HCl buffer [50 mM Tris-(hydroxymethyl)-aminomethane, 0.1 M HCl and 1 mM tetrasodium ethylenediamine tetraacetate, pH 8.0] was added to egg white samples containing 1.0 mg protein and shaken for 60 min at respectively 23 °C, 65 °C, 75 °C or 85 °C. DTNB reagent [100 mL, 0.1% (w/v) in the same Tris–HCl buffer] was added to the suspension. Samples were shaken for another 10 min and centrifuged (10,000 *g*) for 3 min. Exactly 30 min after adding the DTNB reagent, the absorbance at 412 nm was measured. The values were corrected for background absorbance of the sample and DTNB and converted to concentrations of exposed SH groups (μmol/g protein) by using a calibration curve with reduced glutathione (Veraverbeke, Larroque, Bekes, & Delcour, 2000). Measurements were executed in triplicate and standard deviations did not exceed 0.5 μmol/g protein.

2.4. Pound cake making

Pound cake batters were prepared with egg blends (450.0 g), flour (450.0 g), sugar (450.0 g), margarine (450.0 g) and leavening