Food Hydrocolloids 63 (2017) 226–232

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Protein network formation during pound cake baking: The role of egg yolk and its fractions

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ARTICLE INFO

Article history:
Received 24 April 2016
Received in revised form
12 July 2016
Accepted 27 July 2016
Available online 20 August 2016

Keywords:
Mixed protein network
Egg yolk granule
Egg yolk plasma
Disintegration
Batter liquor
Pound cake

ABSTRACT

Pound cake is made from equal portions of egg, wheat flour, sugar and margarine or butter. Its quality is codetermined by the protein network formed during baking. A key insight on egg yolk functionality is that during batter mixing the yolk granules disintegrate and dissolve most of their protein into the batter liquor. This was demonstrated by comparing the protein population of a reference batter liquor with that in batter liquor of which the added yolk was pretreated to disintegrate the granules. Using a ^{15}N-labeling approach, the protein network formation was monitored during cake baking in an electrical resistance oven. Protein was extracted in different media, *i.e.* diluted saline, aqueous ethanol, a buffer containing sodium dodecyl sulfate and one containing dithiothreitol, and analyzed by size-exclusion HPLC. Both egg yolk plasma and granule proteins are incorporated in the protein network by disulfide crosslinking during pound cake baking. Only phosvitin, which lacks both free sulfhydryl and intermolecular disulfide bonds, does not take part in the protein network via disulfide bridge formation.

1. Introduction

Hen eggs consist of about two thirds egg white and one third yolk. The latter is a suspension of granules in an aqueous phase, *i.e.* the plasma (Anton, 2013). Centrifugation of diluted egg yolk separates these components readily (Anton & Gandemer, 1997; Causeret, Matringe, & Lorient, 1991; Mc Bee & Cotterill, 1979). Overall, egg yolk dry matter (dm) content is 50–52%. It consists of approximately two thirds lipid and one third protein (Anton & Gandemer, 1997). These are distributed over low density lipoprotein (LDL; 70% of dm), high density lipoprotein (HDL; 16% of dm), livetin (10% of dm) and phosvitin (4% of dm) (Powrie & Nakai, 1986). Table 1 lists the major egg yolk proteins, their relative contribution to the population, molecular weights (MWs) and relative amounts of sulfhydryl (SH) groups and intramolecular disulfide (SS) bonds.

Plasma (77–81% of the egg yolk dm) contains 52–58% of the egg yolk protein and 89–93% of its lipid (Anton & Gandemer, 1997; Saari, Fennema, & Powrie, 1964). It consists of LDL and livetins. LDL dm is 85% lipid and 15% protein in the form of micelles (17–60 nm diameter) with a lipid core surrounded by phospholipid and protein, more particularly the vitellenins (Anton, 2007c; Mine & Anton, 2006). The density of LDL (0.98 kg/m^3) makes it relatively stable in aqueous media (Powrie & Nakai, 1986). Most identified vitellenins have intramolecular SS bonds and free SH groups of which the accessibility increases during heating (Nguyen & Burley, 1984). Livetin proteins consist of water soluble α-, β- and γ-types (Table 1) (Schade & Chacana, 2007).

Granules make up 19–23% of egg yolk dm (Anton & Gandemer, 1997; Burley & Cook, 1961) and 42–48% of the protein (Anton & Gandemer, 1997; Causeret et al., 1991). They contain HDL (70%), phosvitin (16%) and granular LDL (12%) (Burley & Cook, 1961). Five different vitellin protein types make up about 75–80% of the HDL mass (Table 1) (Anton, 2007b). In contrast to LDL, HDL does not form micelles. It resembles globular proteins (Anderson, Levitt, & Banaszak, 1998) and is soluble in media with ionic strength exceeding that of 0.3 M NaCl (Anton, 2007b). Phosvitin makes up 10–11% of the egg yolk protein and is one of the most

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http://dx.doi.org/10.1016/j.foodhyd.2016.07.036
0268-005X/

Table 1
Most abundant egg yolk proteins, their relative contribution to the yolk protein population, molecular weight [MW] and amount of sulfhydryl [SH] groups and intramolecular disulfide [SS] bonds.

Protein	Percentage of egg yolk protein (%)	MW (kDa)	SH[h]	SS[h]
Vitellin [HDL]	35[a]	35/50/80/100/110[b]	≥1[f]	≥1[f]
Livetin	30[a]	45/70/170[c]	≥1[g]	≥1[g]
Vitellenin [LDL]	23[a]	15/60/65/80/130[d]	≥1[g]	≥1[g]
Phosvitin	11[a]	35/40/45[e]	0[e]	0[e]

Low density lipoprotein [LDL]; High density lipoprotein [HDL].
[a] (Anton, 2007a).
[b] (Anton, 2007b).
[c] (Schade & Chacana, 2007).
[d] (Anton et al., 2003).
[e] (Anton et al., 2007).
[f] (Anderson et al., 1998).
[g] (Nguyen & Burley, 1984).
[h] Per molecule.

phosphorylated proteins known in nature with over 40% of its amino acids being phosphorylated serines. It has a MW between 35 and 45 kDa (Table 1) and is soluble in water (Anton, Castellani, & Guérin-Dubiard, 2007). In egg yolk (pH 6.0–6.5; ionic strength 0.17 M NaCl), granules appear as complexes (0.3–2 μm diameter) of HDL and phosvitin linked by phosphocalcic ionic bonds between negatively charged phosphoserines of HDL and phosvitin on the one hand and calcium ions on the other (Anton, 2007a; Causeret et al., 1991). Ways to break the phosphocalcic bridges and disintegrate the granules include increasing the ionic strength (Anton & Gandemer, 1997; Causeret et al., 1991, 1992) and adjusting the pH (Causeret et al., 1991; Le Denmat, Anton, & Beaumal, 2000). The ionic strength can be increased by adding monovalent (e.g. sodium) or multivalent (e.g. calcium and iron) cations (Anton & Gandemer, 1997; Causeret et al., 1991, 1992). Increasing the pH also causes disintegration (Anton, 2013; Causeret et al., 1991; Le Denmat et al., 2000) and increases the solubility of the granule proteins. HDL, like LDL, is stable in aqueous media (Anton & Gandemer, 1997; Anton, 2007b). However, the proteins from disintegrated granules hardly occur in their original molecular form but appear as micelles (100–200 nm diameter) of HDL and phosvitin, much as is the case for casein. These micelles can spread at oil-water interfaces (Anton, 2013).

Heating egg yolk from 65 to 70 °C onwards produces a three-dimensional protein network (Powrie & Nakai, 1985) which mainly consists of the plasma proteins livetin and vitellenin. When heating the separated plasma fraction, the proteins denature in the order γ-livetin, α-livetin, vitellenin and β-livetin (Le Denmat, Anton, & Gandemer, 1999). This way livetin initiates gel formation, which is then enforced by vitellenin (Kiosseoglou & Paraskevopoulou, 2006). Granule proteins denature at higher temperatures, but their network formation is hindered because of their granular organization. Indeed, due to the high level of ionic bonds and the resultant compact structures, thermal aggregation between proteins in different granules is low and no gel is formed. However, when granules are first disintegrated, thermally induced granule protein gels can be formed as a result of the aggregation of LDL and some HDL components (Anton, Le Denmat, & Gandemer, 2000). Whatever be the case, be it in egg yolk or its separate fractions, both hydrophobic and SS bonds play an important role in the formed gel (Kiosseoglou & Paraskevopoulou, 2005).

Pound cake is made from equal portions of egg, wheat flour, sugar and margarine or butter. Egg yolk accounts for about one quarter of the protein with the other protein originating from egg white and wheat flour. The protein network formed during baking is crucial for product volume and texture (Deleu et al., 2015; Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008, Wilderjans, Lagrain, Brijs, & Delcour, 2010, Wilderjans, Luyts, Goesaert, Brijs, & Delcour, 2010).

The contribution of egg white to pound cake structure and its role during network formation has recently been studied using electrical resistance oven based baking (Deleu et al., 2015). In an electrical resistance oven, in contrast to what is the case in a conventional oven, there is hardly any temperature gradient in the baking batter/cake. This allows withdrawing homogeneous samples during the process. In this paper, the corresponding contribution of egg yolk protein was evaluated. The incorporation of egg yolk protein was monitored during cake baking by using ^{15}N-labeled egg protein much as done for egg white in Deleu, Wilderjans, Van Haesendonck, Brijs, and Delcour (2016a). First, whether or not egg yolk granules are disintegrated in pound cake batter preparation was examined. Protein in a reference batter liquor (aqueous phase of the batter) was compared with that in batter liquor of which the added egg yolk was pretreated to disintegrate the granules. To study the specific role of plasma and granules in protein network formation, batters were prepared substituting egg yolk by only one of these fractions. The outcome of this work is here reported.

2. Experimental

2.1. Materials

^{15}N-labeled eggs were obtained by feeding Isa Brown hens with ^{15}N-leucine-supplemented feed as in Deleu, Wilderjans, Van Haesendonck, Brijs and Delcour (2016b). Sugar and unlabeled cooled eggs were bought at a local supermarket and further stored at 6 °C before use. This way, the conversion from ovalbumin into S-ovalbumin (Deleu et al., 2015) was limited. Wheat flour (Halm flour) [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). Solvents and chemicals (analytical grade) were from Sigma-Aldrich (Bornem, Belgium).

2.2. Egg yolk fractionation

Egg yolk was fractionated into plasma and granules based on Mc Bee and Cotterill (1979). Yolk was diluted with an equal weight of water, shaken (60 min) and centrifuged (10,000 g; 45 min; 6 °C). The supernatant was decanted and centrifuged for a second time (10,000 g; 15 min; 6 °C). It was then freeze-dried to obtain freeze-dried yolk plasma powder. The pellet from the first centrifugation