

## Impact of Potassium Bromate and Potassium Iodate in a Pound Cake System

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This study investigates the impact of the oxidants potassium bromate and potassium iodate (8, 16, 32, 64, and 128  $\mu\text{mol/g}$  dry matter of egg white protein) on pound cake making. The impact of the oxidants on egg white characteristics was studied in a model system. Differential scanning calorimetry showed that the oxidants caused egg white to denature later. During heating in a rapid visco analyzer, the oxidants caused the free sulfhydryl (SH) group levels to decrease more intensively and over a smaller temperature range. The oxidants made the proteins more resistant to decreases in protein extractability in sodium dodecyl sulfate containing buffer during cake recipe mixing and less resistant to such decreases during cake baking. We assume that, during baking, the degree to which SH/disulfide exchange and SH oxidation can occur depends on the properties of the protein at the onset of the process. In our view, the prevention of extractability loss during mixing increased the availability of SH groups and caused more such loss during baking. During cooling, all cakes baked with added oxidants showed less collapse. On the basis of the presented data, we put forward that only those protein reactions that occur during baking contribute to the formation of a network that supports final cake structure and prevents collapse.

**KEYWORDS:** Pound cake; cake quality; egg protein; oxidants

### INTRODUCTION

Egg white proteins are frequently used in food recipes for their functional properties, such as foam formation and stabilization or protein aggregation during heating. Egg white indeed contains proteins that, in an aqueous continuous phase, contribute to incorporation and stabilization of air in the form of tiny bubbles (1).

Liquid egg heating results in protein denaturation and coagulation at a temperature range of 60–85 °C. Ovalbumin, the most abundant egg white protein, contains four free sulfhydryl (SH) groups, which are buried in the protein core (2). Denaturation exposes hydrophobic and SH groups. Subsequent aggregation involves noncovalent (hydrophobic and electrostatic) interactions or covalent (disulfide) reactions. This way, protein aggregates form a three-dimensional network (3). This process is complete when ovalbumin, the major protein, is denatured at 85 °C and becomes involved in the network.

Based on the production methods and formulations, cakes span a wide range of products (4). In many instances, in cake ingredient mixing, foaming properties are important for batter stability, while aggregation properties are important during baking. Mostly, the cake batter contains two gel-forming systems which, upon heating, combine to form the gel structure of the cell wall material (5). Cake setting in the oven appears to result from starch gelatinization and egg protein coagulation. Both processes tremendously increase the viscosity of the batter, result in a solid

appearance, and set the cake. Recently, Wilderjans et al. (6), using a model approach based on gluten-starch blends, observed that during baking proteins react to a degree that affects their extractability in sodium dodecyl sulfate (SDS) containing medium. The reaction products provide the cell walls with resistance to collapse. Therefore, the aggregation properties of egg white can play an important role in cake quality. The relationship between these properties and cake baking and quality is, however, not completely understood even if it can be studied by manipulating the structure and function of egg white proteins.

At present, oxidants are used in the bread making industry to improve dough handling properties and bread quality by affecting the degree of cross-linking of the gluten network (7, 8). Potassium iodate is a fast acting oxidant. Its effects upon dough properties are evident during or just after mixing. Potassium bromate is a slow acting oxidant. It exerts its major effect during the later stages of fermentation and during baking (9). In the presence of potassium iodate or potassium bromate, dough proteins become less reactive, which results in a higher residual protein extractability during bread baking (10, 11). Typical levels of potassium bromate addition in bread making are 10 ppm on flour basis. However, its use has greatly been reduced because of reports of a relationship between bromate intake and cancer (12). Its supplementation has been legally banned in many parts of the world. In the United States, potassium bromate can still be used at low levels (< 75 ppm) that must be indicated on the product label. However, industry has voluntarily decreased the use (12). Iodate, on the other hand, has GRAS status as food additive in the United States (9). In this study, both oxidants were used, not

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for commercial purposes, but as a tool to selectively modify protein properties.

While their effects on bread dough properties have been thoroughly investigated, the impact of such reagents on wheat and egg protein in cake making has, to the best of our knowledge, not been reported. In the particular case of egg white, earlier studies have mainly focused on the relationship between foaming properties of its proteins and their baking performance, especially in egg-foam cakes, i.e., a cake type containing little if any shortening and which depends on the air trapped in beaten eggs for almost all the leavening action (13).

Against this background, we set out to study the impact of the oxidants potassium bromate and potassium iodate on egg white characteristics during heating and on pound cake baking. First, egg white denaturation in the absence or presence of added oxidants was studied with differential scanning calorimetry (DSC). We further measured the level of free SH groups in the absence or presence of added oxidants during heating in a rapid visco analyzer (RVA).

In a second part, potassium iodate or potassium bromate was added during pound cake making, and the egg white characteristics were related to differences in cake baking and final cake quality. Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to investigate the status of the protein population during baking.

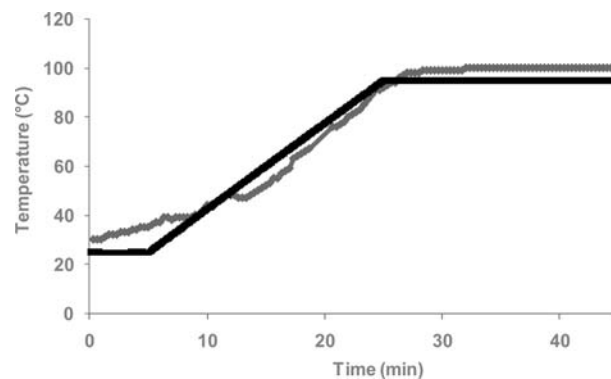
## MATERIALS AND METHODS

**Materials.** Commercial sugar (700  $\mu\text{m}$  average crystal size) and flour [14.0% moisture, 0.56% ash (dry base), 2.5% damaged starch (dry base)] were from Lotus Bakeries (Lembeke, Belgium). Moisture and ash contents were determined according to AACC Approved Methods 44-19 and 08-01 (14), respectively. Starch damage was determined according to the Megazyme (Bray, Ireland) procedure (AACC Method 76-31). Protein contents were determined using an adaptation of the AOAC Official Method (15), with an automated Dumas protein analysis system (EAS vario Max C/N, Elt, Gouda, The Netherlands), with 5.7 as the conversion factor for gluten and 6.25 as the conversion factor for egg protein. On an as is base, the flour and the egg contained 9.7% and 12.6% protein, respectively. The ratio between the quantities of egg and gluten proteins in the pound cake recipe was hence 1.3 to 1. The margarine (19.0% moisture) was from Puratos (Groot-Bijgaarden, Belgium). Sodium bicarbonate (BICAR) was from Solvay Chemicals International (Brussels, Belgium) and sodium pyrophosphate from Acacris Food Belgium (Londerzeel, Belgium). Salt and fresh eggs were purchased in a local supermarket. Chemicals, solvents, and reagents were of highest purity available and from Sigma-Aldrich (Steinheim, Germany), unless specified otherwise.

**Impact of Potassium Iodate or Potassium Bromate on Egg White Protein.** *Differential Scanning Calorimetry (DSC).* DSC was performed with a DSC Q1000 (TA Instruments, New Castle, DE). Potassium iodate (16  $\mu\text{mol/g}$  dry matter (dm) of egg white protein) or potassium bromate (16  $\mu\text{mol/g}$  dm of egg white protein) was dissolved in egg white immediately before analysis. As outlined above, potassium iodate reacts faster than potassium bromate (9).

Control egg white samples (ca. 8.0 mg) or such samples (ca. 8.0 mg) containing added potassium iodate or potassium bromate (16  $\mu\text{mol/g}$  dm of protein) were accurately weighed in triplicate into coated aluminum sample pans. The pans were sealed and equilibrated at 0 °C before heating from 0 to 140 °C at 4 °C/min (together with an empty reference pan). Calibration was with indium and tin. The thermograms were analyzed using TA Q Series Advantage Universal Analysis software (TA Instruments). Standard deviations of triplicate measurements of onset, peak, and conclusion temperatures did not exceed 5%.

**Controlled Heating.** A RVA (RVA-4D; Newport Scientific, Sydney, Australia) was used to study egg white aggregation properties during a heating step comparable to that experienced by the center point in the baking pans during cake baking. Temperatures during conventional oven cake baking were registered at the pan center by a thermocouple connected to a digital readout (Figure 1). The temperature profile included a holding



**Figure 1.** Temperature measured at the center point in the baking pan during baking for 45 min at 175 °C and corresponding heating profile used in the rapid visco analyzer.

**Table 1.** Batter Formulation for Standard Cake Baking

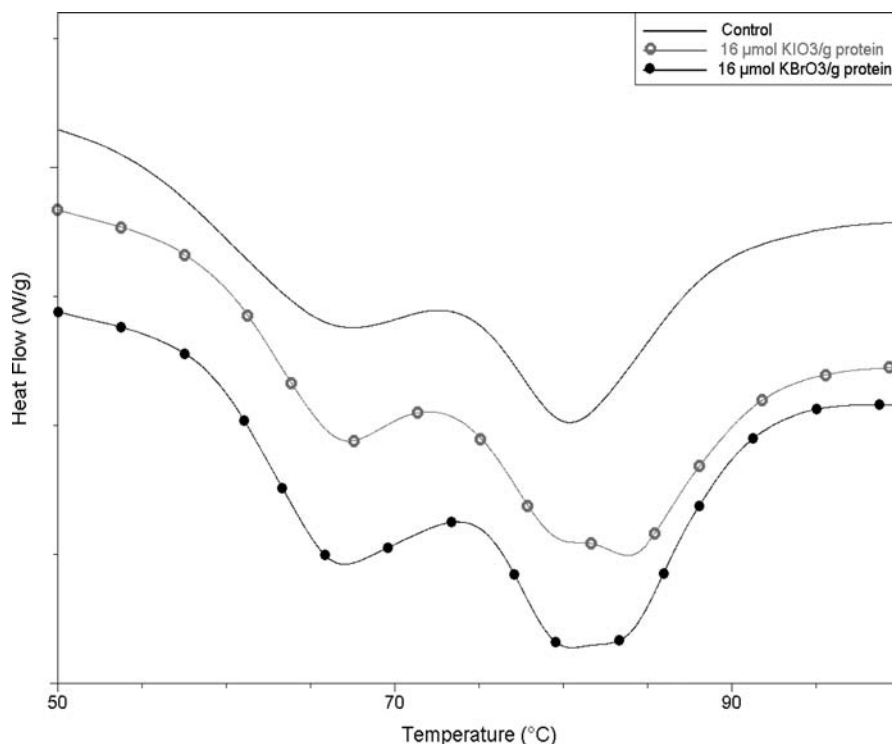
ingredient	g
flour (14% moisture base)	450
sugar	450
margarine	450
fresh egg white	351
fresh egg yolk	99
sodium bicarbonate	5.14
sodium pyrophosphate	3.86
salt	4.5

step of 5 min at room temperature, a linear temperature increase from room temperature to 95 °C in 20 min, and a holding step of 20 min at 95 °C (Figure 1). The temperature profile was applied to 20.0 g of control egg white or egg white containing potassium iodate (16  $\mu\text{mol/g}$  dm of protein) or potassium bromate (16  $\mu\text{mol/g}$  dm of protein). Samples were withdrawn at different stages during heating in the RVA (i.e., after 10, 20, 25, 30, and 45 min) and immediately frozen in liquid nitrogen. Afterward, samples were freeze-dried and milled.

**Free SH Determination.** Free SH groups were determined colorimetrically after reaction with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The freeze-dried control egg white samples and egg white samples containing potassium iodate or potassium bromate, both containing 1.0 mg of protein, were shaken in triplicate for 60 min in 1.0 mL of 0.05 M sodium phosphate buffer (pH 6.5) containing 2.0% (v/v) SDS, 3.0 M urea, and 1.0 mM tetrasodium ethylenediaminetetraacetate (EDTA). DTNB reagent [0.1% (w/v) in the same buffer, 100  $\mu\text{L}$ ] was mixed with 1.0 mL of sample. After 42 min, these samples were centrifuged (3 min, 11000g) before determining the absorbance at 412 nm. Absorbance values were converted to levels of free SH ( $\mu\text{mol/g}$  of protein) using a calibration curve with glutathione. Standard deviations for triplicate determinations were smaller than 5%.

**Protein Extractability.** The freeze-dried control egg white samples and egg white samples containing potassium iodate or potassium bromate, both containing 1.0 mg of protein, were extracted with 1.0 mL of 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% SDS (Acros Organics, Geel, Belgium). Size-exclusion high-performance liquid chromatography (SE-HPLC) was conducted as described by Lagrain et al. (16), using a LC-2010 system (Shimadzu Corp., Kyoto, Japan) with automatic injection. The extracts (60  $\mu\text{L}$ ) were loaded on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA). The elution solvent was acetonitrile/water (1:1 v/v) containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL/min, and the column temperature was maintained at 30 °C (17). Eluted protein was detected at 214 nm. Total SDS-extractable protein of the heated egg white samples was expressed as the percentage of the peak area of the chromatogram obtained following extraction of unheated egg white. All analyses were performed in triplicate. Standard deviations were smaller than 2%.

**Cake Batter Preparation and Baking Procedure.** Table 1 lists the cake batter recipe. The oxidants potassium iodate (8, 16, 32, 64, and 128  $\mu\text{mol/g}$  dm of egg white protein) or potassium bromate (8, 16, 32, 64,



**Figure 2.** Differential scanning calorimetry thermograms of control egg white and egg white samples containing potassium iodate or bromate (16  $\mu\text{mol/g}$  of protein).

and 128  $\mu\text{mol/g}$  dm of egg white protein) were dissolved in egg white immediately before mixing. The mixing and baking methods were those described by Wilderjans et al. (6). First, margarine and sugar were mixed for 3 min at speed level 6 in a Kitchen-Aid electric KPM5 mixer (St. Joseph, MI). Then, fresh egg white was poured into the mixer together with the egg yolk. After 30 s, the flour, salt, and baking powder were added. After another 4 min of mixing, 250 g of batter was placed into baking pans (length 180 mm, width 76 mm, and internal height 50 mm). For each recipe, batches of six cakes were baked in a rotary oven (National Manufacturing, Lincoln, NE) at 175  $^{\circ}\text{C}$  for 45 min. Time-lapse photography was conducted to measure the change in center height during baking with a camera (Canon Powershot S50; Canon, Machelen, Belgium). Photographs were taken every minute. A ruler behind the baking pan allowed monitoring of cake center height. The rate of oven rise was defined as the slope of the linear portion of the graph (i.e., between 15 min and the time at which the maximum height in the oven was reached) obtained by plotting cake center height versus time. Total collapse was calculated by subtracting the center height after cooling for 2 h from the maximum height noted in the oven.

After cooling for 2 h to room temperature, cake weights (g) and rapeseed displacement volumes ( $\text{cm}^3$ ) were measured. The average volumes of six cakes baked on two different days did not differ by more than 5%.

**Protein Extractability during Mixing and Baking.** Cake and batter samples were freeze-dried and ground in a laboratory mill (IKA, Staufen, Germany). Samples (1.0 g) were shaken with 10.0 mL of hexane in a 30 mL test tube for 60 min. Hexane was removed and the extraction repeated. Finally, samples were dried under a stream of nitrogen.

Defatted batter and cake samples containing 1.0 mg of protein were extracted with 1.0 mL of 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% SDS (Acros Organics, Geel, Belgium). SE-HPLC was conducted as described above. Total SDS-extractable protein of the batter and cake samples was expressed as the percentage of the peak area of the chromatogram obtained following extraction of unheated batter with the above SDS-containing buffer in the presence of 1.0% dithiothreitol (Acros Organics) and 2.0 M urea. All analyses were performed in triplicate. Standard deviations were smaller than 2%.

**Statistical Analysis.** Analysis of variance (ANOVA) was conducted using the Statistical Analysis System software 8.1 (SAS Institute, Cary,

NC). All mean values were compared using *t* tests at  $\alpha = 0.05$  level of significance.

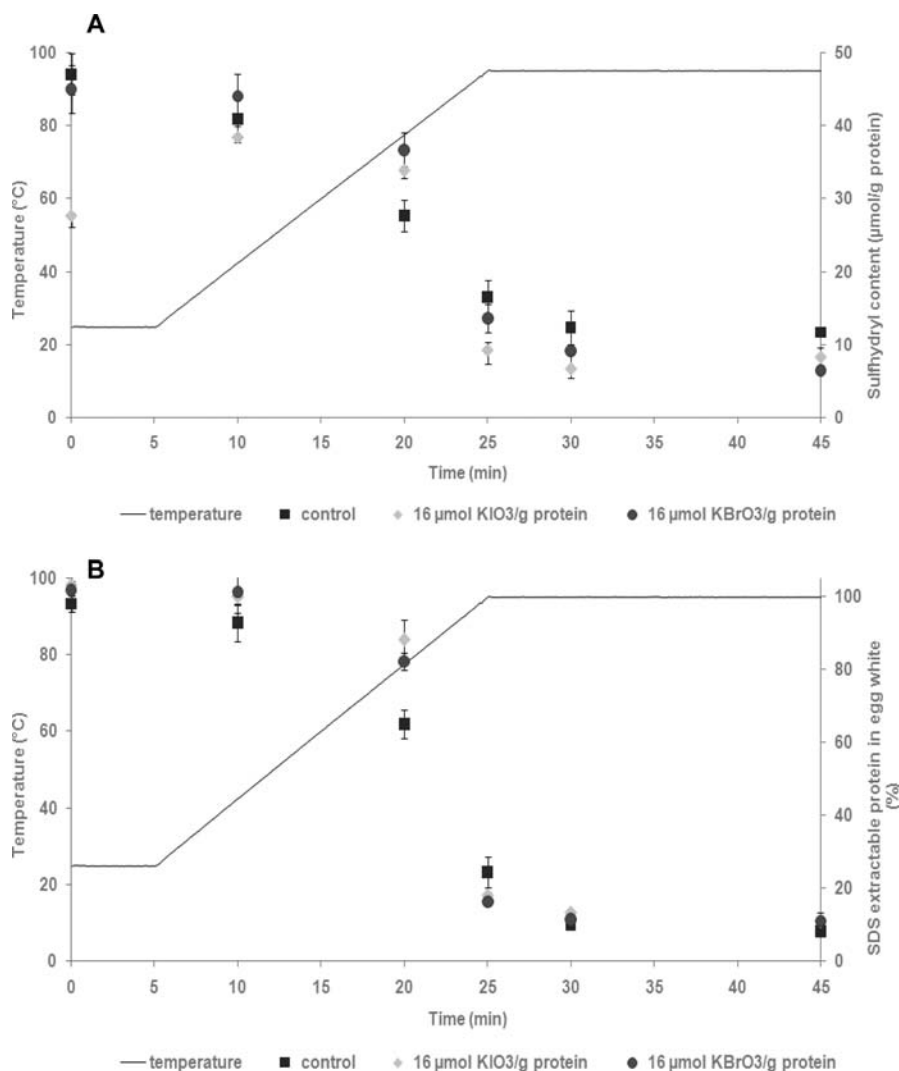
## RESULTS AND DISCUSSION

**Impact of Potassium Iodate or Potassium Bromate on Egg White Protein. Denaturation Temperature.** Figure 2 shows the DSC temperature range for denaturation of egg white. The two prominent endotherms with peaks at 65 and 80  $^{\circ}\text{C}$  have been ascribed to denaturation of ovotransferrin and ovalbumin (18). Ovotransferrin denatured between 60 and 73  $^{\circ}\text{C}$  and ovalbumin between 75 and 90  $^{\circ}\text{C}$  (Figure 2).

The addition of potassium iodate or bromate mainly impacted the ovalbumin denaturation peak (Figure 2). The peak broadened and moved to higher temperatures, which can point to an increased heat stability of the egg white proteins. Formation of disulfide bonds can indeed stabilize proteins and increase their denaturation temperature (19). The increase in protein stability has been related to changes in conformational entropy (20). In earlier scanning electron microscopy studies on bread, it was observed that potassium iodate or bromate addition renders dough proteins less reactive (21). Further, heat resistance upon addition of potassium iodate or bromate resulted in a higher protein extractability during bread baking (10, 22).

**Free SH Content and Protein Extractability.** Panels A and B of Figure 3 show the decrease in SH content and in protein extractability, respectively, of egg white during a temperature profile in the RVA corresponding to that in the cake center during baking.

The initial SH content of egg white was 47  $\mu\text{mol/g}$  of protein, in line with observations by Beveridge et al. (23) and Van der Plancken et al. (24). Upon addition of potassium iodate, it immediately decreased to 25  $\mu\text{mol/g}$  of protein, while addition of potassium bromate did not have an immediate effect (Figure 3A). Iodate is a fast acting oxidant while bromate is slow acting (9), which can be due to the lower oxidation potential of



**Figure 3.** (A) Free sulfhydryl (SH,  $\mu\text{mol/g}$  of protein) content of control egg white and egg white containing potassium iodate or bromate ( $16 \mu\text{mol/g}$  of protein) during a heating step in the rapid visco analyzer comparable to that experienced by the center point in the baking pans during cake baking. (B) Extractabilities (%) of egg white in 2.0% SDS buffer of control egg white and egg white containing potassium iodate or bromate ( $16 \mu\text{mol/g}$  of protein) during a heating step in the rapid visco analyzer comparable to that experienced by the center point in the baking pans during cake baking.

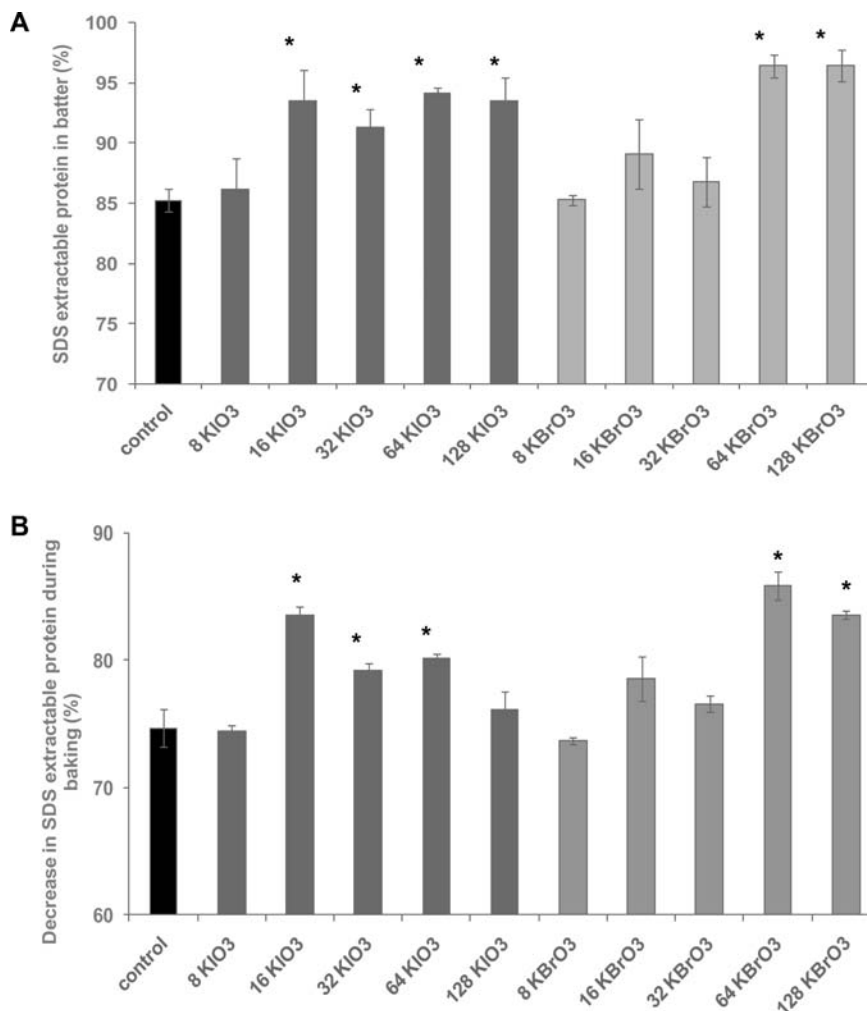
potassium bromate than that of iodate (23). Potassium bromate reacts faster at lower pH (25), which can explain the low reactivity of bromate in egg white (pH 7.6).

To study the contribution of covalent disulfide bonds to egg white protein aggregation, SDS-containing buffers have been used before (26). Before processing, all egg white proteins were extractable in such a buffer (Figure 3B). This was expected, as egg white proteins are globular in structure and small in size with molecular weights below 80000 (26).

During heating egg white in the RVA system, the SH content decreased. The decrease was significant between 43 and 78 °C and between 78 and 95 °C (Figure 3A). This is due to denaturation of ovotransferrin and ovalbumin, respectively (Figure 2). The denaturation resulted in exposure of SH groups and decreased the SH content due to formation of disulfide bonds, as also shown by Van der Plancken et al. (24). The total decrease in free SH content of egg white during the RVA heating step experienced by the cake center during baking was  $35 \mu\text{mol/g}$  of protein (Figure 3A). Exposure of reactive SH groups and subsequent formation of disulfide bonds drastically decreased protein extractability, as shown in Figure 3B. Panels A and B of Figure 3 show similar behavior, with significant decreases between 43 and 78 °C and between 78 and 95 °C.

Potassium iodate, because of its rapid action, immediately decreased the initial free SH concentration of egg white proteins and, hence, impacted their structure by formation of disulfide bridges. It is logical to assume that potassium iodate induces formation of intramolecular disulfide bridges, and that way, the SH groups were enclosed. During heating, free SH content first increased, because SH groups were exposed that, for steric reasons, did not react immediately (Figure 3A). The largest decrease in SH content ( $25 \mu\text{mol/g}$  of protein) was then measured upon further heating between 78 and 95 °C, and this decrease was larger than for the control sample ( $11 \mu\text{mol/g}$  of protein). This can be related to the increased heat stability of the egg white proteins, as shown by the large impact of potassium iodate on ovalbumin denaturation (Figure 2). Protein extractability also decreased substantially when the temperature increased from 78 to 95 °C (Figure 3B). Compared to the control egg white sample, a larger and later decrease in protein extractability was measured (Figure 3B). Combined with Figure 3A, this can be related to the later exposure of SH groups and subsequent later disulfide cross-linking.

Heating egg white in the presence of potassium bromate to a temperature of 43 °C did not change the free SH content, in line with its lower reactivity than that of potassium iodate. It seems



**Figure 4.** (A) Extractabilities (%) of protein in 2.0% SDS buffer of control batter and batter samples containing potassium iodate (8, 16, 32, 64, and 128  $\mu\text{mol/g}$  of protein) or potassium bromate (8, 16, 32, 64, and 128  $\mu\text{mol/g}$  of protein). (B) The decrease in SDS extractable protein during baking, defined as the difference between extractabilities (%) of protein in 2.0% SDS buffer of the cake samples and of the batter samples. Sample codes as in **Figure 3A**. Asterisks indicate values differing significantly ( $P < 0.05$ ) from the values for control samples.

plausible that the consumption of SH groups by bromate was compensated for by additional exposure when heating up to 78 °C (**Figure 3A**). Between 78 and 95 °C, a sudden large decrease in SH content occurred (**Figure 3A**). This decrease (23  $\mu\text{mol/g}$  of protein) was larger and later than for the control sample (11  $\mu\text{mol/g}$  of protein), which again can be due to the increased heat stability and later denaturation of the egg white proteins imparted by potassium bromate (**Figure 2**). Again, protein extractability (**Figure 3B**), just as free SH content (**Figure 3A**), decreased larger and later than the control egg white.

These observations indicate that denaturation and coagulation of egg white occurred later in the presence of potassium iodate or bromate, due to increased heat stability of the proteins (**Figure 2**). Furthermore, the larger decrease in SH content between 78 and 95 °C points to more protein reactions taking place in a smaller temperature range (**Figure 3**).

**Cake Recipe Mixing.** During mixing, protein extractability decreased, probably due to the shear action, which denatured some egg proteins and exposed SH groups that subsequently formed intermolecular disulfide bonds. Johnson and Zabik (27) also suggested that the mechanical whipping action can alter egg protein conformation, such that subsequent SH–disulfide interchange reactions become involved.

Batters prepared with potassium iodate addition (in concentrations exceeding 8  $\mu\text{mol/g}$  of protein) showed higher protein

extractabilities than the control batter (**Figure 4A**). Due to the lower reactivity of potassium bromate, higher concentrations (64  $\mu\text{mol/g}$  of protein and 128  $\mu\text{mol/g}$  of protein) were needed to result in significantly higher protein extractabilities of the batter than of the control batter (**Figure 4A**). Potassium iodate or bromate can oxidize free SH groups to intramolecular disulfide bridges and, as indicated above, increase protein stability. As those proteins were more resistant to denaturation, SH–disulfide interchange reactions during mixing were inhibited.

**Cake Baking. Protein Extractability.** As shown earlier (6, 28), the largest decrease in protein extractability occurred during cake baking. Above 70 °C, egg proteins denature and become involved in disulfide cross-linking. Different reactions lead to a three-dimensional protein network (2).

As outlined above, in our recipe, the ratio between egg and gluten proteins is 1.3. As egg protein contains seven times more free SH groups than gluten, nine times more SH groups originate from egg than from gluten in pound cake batter. Therefore, we assume that oxidants exert a greater effect in cake batter on egg than on gluten proteins when oxidizing free SH groups.

The decrease in SDS-extractable protein levels during baking was defined as the difference between the level of the batter samples and that of the cake samples. **Figure 4** thus indicates that whereas, from control cake batter, 85% of all proteins were extractable in the SDS-containing medium and, after baking,

**Table 2.** Time-Lapse Photography Characteristics and Density of Cakes Prepared with Different Levels of Potassium Iodate or Bromate (8, 16, 32, 64, and 128  $\mu\text{mol/g}$  of Protein)<sup>a</sup>

cake type	rate of oven rise (cm/min)	max height (cm)	collapse (%)	density (g/cm <sup>3</sup> )
control	0.320 $\pm$ 0.017b	8.88 $\pm$ 0.08b	18.44 $\pm$ 1.79a	0.317 $\pm$ 0.006a
8 KIO <sub>3</sub>	0.346 $\pm$ 0.016ab	9.15 $\pm$ 0.07ab	15.30 $\pm$ 1.43ab	0.299 $\pm$ 0.004ab
16 KIO <sub>3</sub>	0.355 $\pm$ 0.010a	9.20 $\pm$ 0.04a	11.65 $\pm$ 0.17c	0.297 $\pm$ 0.003b
32 KIO <sub>3</sub>	0.342 $\pm$ 0.021ab	9.00 $\pm$ 0.04ab	15.38 $\pm$ 1.27ab	0.301 $\pm$ 0.006ab
64 KIO <sub>3</sub>	0.295 $\pm$ 0.021b	8.95 $\pm$ 0.03ab	15.95 $\pm$ 2.17ab	0.306 $\pm$ 0.002ab
128 KIO <sub>3</sub>	0.307 $\pm$ 0.002b	8.88 $\pm$ 0.04b	16.65 $\pm$ 1.27a	0.314 $\pm$ 0.008a
8 KBrO <sub>3</sub>	0.346 $\pm$ 0.006ab	8.95 $\pm$ 0.07ab	14.41 $\pm$ 1.69ab	0.305 $\pm$ 0.007ab
16 KBrO <sub>3</sub>	0.370 $\pm$ 0.018a	9.40 $\pm$ 0.01a	16.09 $\pm$ 0.17a	0.301 $\pm$ 0.004ab
32 KBrO <sub>3</sub>	0.351 $\pm$ 0.016ab	9.25 $\pm$ 0.01ab	14.67 $\pm$ 0.77ab	0.291 $\pm$ 0.003b
64 KBrO <sub>3</sub>	0.340 $\pm$ 0.001ab	9.05 $\pm$ 0.07ab	12.23 $\pm$ 0.64b	0.307 $\pm$ 0.006ab
128 KBrO <sub>3</sub>	0.346 $\pm$ 0.029ab	9.03 $\pm$ 0.11ab	13.57 $\pm$ 1.02ab	0.304 $\pm$ 0.005ab

<sup>a</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

only 10% of all proteins were extractable, 75% of all proteins had become unextractable in the same medium during baking (Figure 4B). For cakes prepared with potassium iodate addition, more proteins lost extractability during baking. Combined with Figure 3, these results can be related to the later and larger decrease in free SH content of egg white during heating in the presence of potassium iodate. Upon addition of potassium iodate, protein denaturation occurred later, and more protein extractability loss occurred over the same temperature range.

It was further obvious that more protein extractability loss during mixing resulted in less such loss during baking (Figure 4). It is logical to assume that, during baking, the degree to which SH–disulfide exchange and SH oxidation can occur depends on the properties of the protein at the onset of the process. For the control cake, some protein cross-linking had already taken place during mixing. Upon addition of potassium iodate, egg white proteins were oxidized immediately and became more resistant to denaturation, which prevented extractability loss during mixing and resulted in more available SH groups and more such loss during baking (Figure 4).

Due to lower reactivity of potassium bromate, only higher concentrations (64 and 128  $\mu\text{mol/g}$  of protein) resulted in a greater loss of protein extractability during baking (Figure 4B). This was expected as such concentrations were also needed to make egg white proteins more resistant to protein extractability loss during mixing (Figure 4A).

**Oven Rise.** Table 2 shows the effect of increasing levels of oxidants on maximum center height during baking and rate of oven rise. With increasing concentrations of the oxidants, maximum center height and rate of oven rise increased until optimum levels were attained. Previous studies suggested that differences in bread oven rise during baking may be attributed to differences in the time during which oven rise occurs and/or the rate of oven rise (11). In Table 2, the rate of oven rise and the maximum center height showed a similar trend. As mentioned above, setting of cakes in the oven appears to result from starch gelatinization and egg protein coagulation. As egg white proteins become more heat stable after oxidation, the delay of setting and thus higher oven rise can be due to the later egg protein denaturation.

Optimum levels for oven rise were 16  $\mu\text{mol/g}$  of protein for both potassium iodate or potassium bromate. Increases in the level of the oxidants above the optimum resulted in decreases in maximum height (Table 2). As for bread (11), higher dosages resulted in a loss of oven rise and corresponding decrease in quality. For bread, the differences in expansion time with addition of oxidants have been attributed to a combination of changes in the rheological dough properties and the denaturation temperature of the protein matrix (11).

**Cake Quality.** During cooling, cakes show some collapse (Table 2). In earlier work, our group already found a correlation between protein extractability and collapse (6, 28). This suggests that protein provides the cell walls with structural material and a higher resistance to collapse. More protein extractability loss during baking may well contribute to a stronger protein network. In the present case, all cakes baked with added oxidants showed less collapse (Table 2). This could be related to the larger decrease in protein extractability during baking for cakes containing oxidants than for the control cake (Figure 4). As indicated above, addition of potassium iodate (in concentrations exceeding 8  $\mu\text{mol/g}$  of protein) or potassium bromate (higher concentrations) reduced protein extractability loss during mixing and caused more protein to react during baking (Figure 4). The optimum levels for inhibition of collapse were 16  $\mu\text{mol/g}$  of protein for potassium iodate and 64  $\mu\text{mol/g}$  of protein for potassium bromate. Such results were expected, as these concentrations also resulted in the greatest loss in protein extractability during baking (Figure 4B). On the basis of our data, we speculate that only the protein reactions during baking contribute to the formation of a network that supports final cake structure. Those reactions produce the cell wall material and prevent it from collapsing (6, 28).

The positive effect of oxidants on cake volume can, partly, be due to prevention of cake collapse (Table 2). The higher volumes of cakes baked with added oxidants can be explained by the higher oven rise which itself results from the later denaturation of egg protein (Table 2). The optimum cake volume was obtained at lower concentrations of potassium iodate than of potassium bromate, i.e., 16 vs 32  $\mu\text{mol/g}$  of protein (Table 2), in line with observations for bread (11). Furthermore, cake quality was more sensitive to overtreatment with potassium iodate than to overtreatment with potassium bromate (Table 2).

Taken together, the present results indicate that protein properties play an important role in cake baking and final cake quality. Egg white characteristics during heating and their influence on cake baking were studied by dissolving potassium iodate or potassium bromate in egg white. In DSC, the oxidants broadened the egg white denaturation peak, which indicates an increased heat stability of the proteins. Denaturation occurred later in the presence of potassium iodate or potassium bromate, and more proteins reacted in a smaller temperature range during heating in the RVA.

During mixing, upon addition and immediate action of potassium iodate, proteins became more resistant to denaturation, and protein reactions resulting in loss of extractability were inhibited. During baking, egg white proteins denatured later, and the prevention of reactions during mixing resulted in more available SH groups and more protein reactions. For potassium

bromate, due to its lower reactivity, higher concentrations were needed to obtain similar results. With increasing concentrations of the oxidants, maximum center height and rate of oven rise increased until optimum levels were attained. During cooling, all cakes baked with oxidant addition showed less collapse. More protein reaction during baking can contribute to the formation of a network that supports final cake structure and prevents collapse.

The positive effect of oxidants on cake volume can be due to the combination of less collapse, on one hand, and higher oven rise due to the increased heat stability of the proteins, on the other.

#### ABBREVIATIONS USED

ANOVA, analysis of variance; dm, dry matter; DSC, differential scanning calorimetry; RVA, rapid visco analyzer; SDS, sodium dodecyl sulfate; SE-HPLC, size-exclusion high-performance liquid chromatography; SH, sulfhydryl.

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