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Impact of egg white and soy proteins on structure formation and crumb firming in gluten-free breads

Hanne G. Masure, Arno G.B. Wouters*, Ellen Fierens^a and Jan A. Delcour

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

^aCurrent affiliation: Flanders' FOOD, Wetenschapsstraat 14A, B-1040 Brussel, Belgium

*Corresponding author: Tel: +32-16-372035, Fax: +32-16-321997,

E-mail: arno.wouters@kuleuven.be

Abbreviations: ¹H-NMR, proton nuclear magnetic resonance; AP, amylopectin; CS-PS, cassava and potato starch ; CPMG, Carr-Purcell-Meiboom-Gill; DM, dry matter; DSC, differential scanning calorimetry; ERO, electrical resistance oven; EW, egg white; HEW, dry heated egg white powder; REW, regular egg white powder; FID, free induction decay; GF, gluten-free; MC, moisture content; PC, protein content; PID, proportional-integral-derivative; RF, rice flour; SPI, soy protein isolate; T₂, transverse relaxation time

Abstract

Regular or thermally treated egg white powder or soy protein isolate were included in the recipes of gluten-free (GF) breads based on rice flour (RF) or a mixture of potato and cassava starches (CS-PS) to improve bread texture and structure. Electrical resistance oven heating was combined with in-line measurements of batter height, viscosity and carbon dioxide release during fermentation and baking. Crumb firming during storage (6 days) of the different breads was studied by texture, moisture content, amylopectin (AP) crystal melting and proton nuclear magnetic resonance analyses. Drop shape tensiometry showed that both egg white types are highly surface active. Their use resulted in high gas cell stability and ultimately in high volume breads with fine crumb structure. Batters containing soy protein isolate, which had a lower surface-activity, had low stability. This resulted in low volume breads with inhomogeneous crumb structure. During the first 24 hours of storage, the crumb firmness increase was mainly caused by AP retrogradation and later on by crumb-to-crust moisture migration. Crumb firming was less pronounced in CS-PS than in RF breads. Egg white decreased the extent of crumb firming while soy protein increased it. Most notably, differences in crumb firming between samples were largely related to differences in initial bread specific volume and crumb structure rather than to differences in AP retrogradation or moisture migration. Thus, inclusion of ingredients which improve bread volume and crumb structure in GF bread recipes may also strongly improve the shelf-life of such breads.

Keywords: gluten-free bread; electrical resistance oven heating; shelf life; egg white protein; soy protein isolate

1. Introduction

Coeliac patients must refrain from consuming food containing wheat or related species and therefore rely on gluten-free (GF) alternatives. Unfortunately, the absence of gluten in GF breads results in low quality products. Low loaf volumes, dry and dense crumb and lack of taste, mouthfeel and acceptable color are common issues with GF breads. Furthermore, crumb firming happens faster in GF than in wheat bread.

Novel insights in crumb structure formation of GF breads based on rice flour (RF) or a combination of cassava and potato starch (CS-PS) and hen egg white (EW) powder (Masure, Wouters, Fierens, & Delcour, 2018) have resulted from combining electrical resistance oven (ERO) heating with in-line measurements of batter height, viscosity and carbon dioxide release during fermentation and baking. In an ERO, batter or dough functions as electrical resistance between two electrodes and the temperature increase is the result of Ohmic heating. This allows batter to rise uniformly without significant internal temperature gradients. Although there are some differences between ERO and conventional baking, bread volumes obtained either with ERO or with conventional baking correlate well. In combination with the above mentioned possibility to perform in-line measurements of several parameters, this implies that ERO baking is an excellent tool for probing structure formation in GF breads. Indeed, with this approach, the balance between the moment of gas cell opening and that of crumb structure setting can be studied. Controlling this balance allows impacting the volume and crumb structure of GF breads. One possible way to achieve this is by using ingredients that impact batter stability and thus the moment of gas release.

In the present research, several protein types were included in two GF bread recipes and crumb structure formation in such breads was investigated. Soy protein isolate (SPI) and hen EW are regularly used functional ingredients in GF bread making. They contain high levels of the essential amino acids lysine and methionine (Marco & Rosell, 2008a, 2008b; Wouters, Rombouts, Lagrain, & Delcour, 2016).

SPI increases water absorption and thereby impacts batter rheology. In most cases, the use of SPI in GF bread recipes leads to low volume and dense crumb structure (Crockett, Ie, & Vodovotz, 2011; Horstmann, Foschia, & Arendt, 2017; Srikanlaya, Therdthai, Ritthiruangdej, & Zhou, 2018; Ziobro, Juszczak, Witczak, & Korus, 2016; Ziobro, Witczak, Juszczak, & Korus, 2013). EW proteins have high foaming capacity and may stabilize gas cells during bread making. Indeed, as proteins diffuse to and adsorb at the air/water interface, surface tension decreases and adsorbed proteins with high surface activity form a coherent visco-elastic film around gas cells (Murray, 2007). This decrease in both surface tension and interfacial dilatational modulus can be derived from (oscillating) pendant drop measurements.

The inclusion of EW proteins in GF breads increase bread softness and volume (Han et al., 2019; Horstmann, Foschia, et al., 2017; Schoenlechner, Mandala, Kiskini, Kostaropoulos, & Berghofer, 2010; Ziobro et al., 2016). When EW proteins are first thermally treated in a dry state, they tend to form strong and stable gels (Kato, Ibrahim, Takagi, & Kobayashi, 1990; Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1990b). Moreover, such treatment increases their foaming capacity and foam stability (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1990a) and can thus increase batter stability. This makes preheated EW a promising ingredient in GF bread making.

Staling limits the shelf-life of bread. It involves crumb firming, crust softening and loss of aroma during storage (Gray & BeMiller, 2003). The mechanism whereby wheat bread crumb firms has been studied extensively, both on macroscopic (texture and moisture measurements) and molecular [differential scanning calorimetry (DSC) and proton nuclear magnetic resonance ($^1\text{H-NMR}$)] scales (Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2013; Choi, Kim, & Baik, 2010; Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014). When freshly baked wheat breads are cooled, rapid amylose crystallization determines the initial crumb texture. During storage of wheat bread, amylopectin (AP) retrogradation is the main factor contributing to crumb firming (D'Appolonia & Morad, 1981; Goesaert et al., 2005; Gray & BeMiller, 2003). Water redistribution during storage also plays a role in

the process. Indeed, it migrates from crumb to crust (Baik & Chinachoti, 2000; Piazza & Masi, 1995; Pisesookbunterngr & D'Appolonia, 1983), the amount of free water in the crumb decreases even further as water molecules are built in AP crystals formed during retrogradation, and water migrates from gluten to starch (Bosmans et al., 2013). GF bread stales faster than wheat bread (Moore, Schober, Dockery, & Arendt, 2004). The mechanism behind GF bread crumb firming is not well known. As reviewed earlier (Masure, Fierens, & Delcour, 2016), research on storage of GF bread has mainly focused on sensory and textural parameters. AP retrogradation measurements (Demirkesen, Campanella, Sumnu, Sahin, & Hamaker, 2014; Krupa, Rosell, Sadowska, & Soral-Smietana, 2010; Ronda & Roos, 2011; Sciarini, Ribotta, Leon, & Perez, 2012; Witczak, Korus, Ziobro, & Juszczak, 2010) have also been performed. Only few articles (Carini, Curti, Fattori, Paciulli, & Vittadini, 2017; Hager, Bosmans, & Delcour, 2014; Purhagen, Sjöo, & Eliasson, 2012) have used ^1H -NMR to study water dynamics in GF breads during storage. Much as in wheat bread, AP retrogradation and water inclusion in the starch network are the main contributors to crumb firming in GF rice and oat breads (Hager et al., 2014). Carini et al. (2017) observed a more pronounced decrease in springiness and cohesiveness during storage of four GF bread types than was the case for wheat bread. They ascribed this to more pronounced AP retrogradation during storage in the former. However, comprehensive research on the mechanism of GF bread crumb firming and the impact of different additives and ingredients thereupon is still lacking.

The objective of the present research was two-fold.

We first set out to study how modifying the balance between the moment of gas cell opening and that of crumb setting would impact the volume and crumb structure of these GF breads. This was done by including different protein types in two GF bread recipes, one based on RF and one based on CS-PS. Crumb structure formation was studied using the above mentioned set-up, thus combining ERO heating and in-line measurements of height, viscosity and carbon dioxide release.

We secondly used a comprehensive approach to study the crumb firming mechanism in GF RF or CS-PS based breads and the impact of EW and SPI thereupon. Hereto, conventional oven-baked breads were stored for six days. Their crumb texture and moisture content (MC) as well as AP retrogradation and $^1\text{H-NMR}$ proton mobility were studied.

We here report on the outcome of this work.

2. Materials and methods

2.1 Materials

Cassava starch [MC $12.7\% \pm 0.1\%$, protein content (PC, N x 6.25) $0.5\% \pm 0.2\%$] was from Cargill (Vilvoorde, Belgium), potato starch (MC $18.7\% \pm 0.1\%$, PC (N x 6.25) $0.2\% \pm 0.1\%$) from Caldic (Hemiksem, Belgium) and RF [MC $9.8\% \pm 0.1\%$, PC (N x 5.95) $4.8\% \pm 0.1\%$] was from Beneo-Remy (Wijgmaal, Belgium). Spray dried hen EW powder [REW, MC $7.5\% \pm 0.1\%$, PC (N x 6.25) $87.8\% \pm 1.5\%$] was from Pulviver (Bastogne, Belgium), dry heated EW powder [HEW, MC $7.3\% \pm 0.1\%$, PC (N x 6.25) $91.0\% \pm 1.3\%$] from Bouwhuis Enthoven (Raalte, The Netherlands) and SPI Wilpro D150 [MC $5.3\% \pm 0.1\%$, PC (N x 6.25) $84.3\% \pm 0.3\%$] from Wilmar (Singapore). MC was determined according to AACC method 44-15.02 (AACC, 1999). PC was determined using an adaptation of AOAC Official Method 990.03 (AOAC, 1995) to an automated Dumas protein analysis system (VarioMax Cube CN, Elementar, Hanau, Germany) and expressed on dry matter (DM) basis. Fresh yeast was from AB Mauri (Dordrecht, The Netherlands) and dry yeast from Puratos (Groot-Bijgaarden, Belgium). Sugar and salt were from a local supermarket and calcium propionate from Sigma-Aldrich (Saint Louis, MO, USA).

2.2 Bread making

2.2.1 Batter preparation

GF breads were prepared as in Masure et al. (2018) from the recipes listed in Table 1. Recipes were developed in preliminary trials based on specific volume and crumb softness and structure of the

different breads. A mixture of 67.5% (w/w) cassava starch, 22.5% (w/w) potato starch and 10.0% (w/w) REW is further referred to as 'flour' and was used for producing what is further referred to as control CS-PS+REW bread. Substitutions with HEW or SPI were done on a constant protein level basis. Control RF bread contained 100% RF, which was substituted by REW, HEW or SPI on flour DM basis. Total batter weights differed for different experiments (see below). For each bread type, all dry ingredients but yeast were premixed with a spoon. Dry yeast was first suspended in the total amount of tap water at 30 °C for 10 minutes while fresh yeast was used as such. Addition of yeast and water was followed by mixing all ingredients at speed 1 for 60 seconds in a Kitchen Aid (St. Joseph, MI, USA) KPM5 mixer equipped with a batter blade. The bowl contents were scraped down and a second mixing step was performed at speed 4 for 90 seconds. The amount of batter produced in one batch for different experiments ranged from 300 to 800 g.

2.2.2 Electrical resistance oven baking

ERO baking and the measurements of carbon dioxide release, height and batter viscosity were as in Masure et al. (2018). GF batters served as electrical resistance between two stainless steel electrode plates. A proportional-integral-derivative (PID) feedback controller (Jumo Automation, Eupen, Belgium) continuously adjusted the voltage to obtain the desired temperature - time profile. Batters were fermented in an ERO at 30 °C for 25 (RF based breads) or 45 (CS-PS based breads) minutes. During baking, a temperature-time profile similar to that in the center of 250 g batter baked in a conventional oven at 195 °C was applied. RF batters were kept at 30 °C for 7 minutes, heated to 95 °C in 30 minutes (2.2 °C per minute) and kept at 95 °C for 8 minutes. CS-PS batters were kept at 30 °C for 7 minutes, heated to 95 °C in 25 minutes (2.6 °C per minute) and kept at 95 °C for 13 minutes. The ERO dimensions and batter weights differed for different experiments (see below).

Carbon dioxide and height measurements

For carbon dioxide release and height measurements, 250 g batter was poured in an ERO (150 x 60 x 180 mm, l x w x h) equipped with a cover which linked it to a carbon dioxide data logger (CO2Meter,

Ormond Beach, FL, USA) (Figure 1a). During fermentation and baking, data loggers suitable for carbon dioxide concentration ranges of 0-10⁴ ppm and 0-10⁶ ppm recorded the carbon dioxide release. An internal pump constantly circulated air from the headspace above the batter to the data logger and back. The carbon dioxide concentration was recorded every 5 seconds. Prior to each analysis, the ambient carbon dioxide concentration was subtracted from further readings. Batter height was measured with a ruler every 5 (during fermentation) or 2 (during baking) minutes.

Batter viscosity measurements

A Marimex (Bottrop, Germany) ViscoScope viscometer probe was completely submerged in 600 g batter poured in an ERO (90 x 100 x 120 mm, l x w x h). (Figure 1b). The viscometer (based on small amplitude oscillating torsion) was connected to a PID controller that kept the small amplitude torsion oscillation frequency of the probe at 520 Hz. During baking, batter was allowed to overflow to ensure that the probe remained in contact with the batter at all times.

2.2.3 Conventional baking

RF (250 g) or CS-PS (200 g) based batters were poured in baking tins (143 x 79 x 119 mm, l x w x h). Batter weights were determined for each bread type in preliminary trials to bring about a sufficiently large bread volume for texture measurements on the one hand and to avoid that the batter would overflow during fermentation and baking on the other. From each batch of batter, three breads were produced. Baking tins were then placed in a fermentation cabinet (National Manufacturing, Lincoln, NE, USA) at 30 °C and 90% relative humidity for 25 (RF based breads) or 45 (CS-PS based breads) minutes. The loaves were baked for 45 minutes at 195 °C (RF breads) or 175 °C (CS-PS breads) in a Condilux deck oven (Hein, Strasse, Luxemburg). Bread loaves were immediately removed from the tins after baking and left to cool at room temperature (23 °C) for two hours. In what follows, such cooled breads are referred to as fresh breads. They were weighed and their volume was measured with a Volscan Profiler (Stable Microsystems, Godalming, Surrey, UK).

2.3 Storage and sampling of bread

Twelve RF or CS-PS based breads (see section 2.2.3) were baked in one session from four batches of batter. Fresh breads were divided into four groups of three breads from three different batches of batter. One group of breads was analyzed fresh, while the other breads were hermetically packed in plastic foil and stored at 23 °C until further analyses, which took place on days 1, 3 and 6 after baking. On each day of analysis, three center slices with a thickness of 25 mm were removed from three different bread loaves. From these slices, cylindrical samples with a diameter of 30 mm were obtained with a sharp borer and used for texture analysis (see section 2.5). After measurement, two (compressed) cylindrical samples were frozen with liquid nitrogen, freeze-dried, milled in a laboratory mill, sieved through a 250 µm sieve and used for DSC analyses (see section 2.6). The remaining crumb was used for MC (analysis as in 2.1) and ¹H-NMR (see section 2.7) measurements.

2.4 Image analysis of bread crumb

Fresh bread slices (1.0 cm thickness) cut with an electric slicer (Affettatrice Slicer 30N, Galesecca, Italy) were analyzed using a flatbed scanner (Scanjet 3800, Hewlett-Packard, Beijing, China) as in Masure et al. (2018). An image with an area of 35 x 35 mm at the center of each slice was recorded. Images were in 256 grey scale with a resolution of 300 dots/inch and stored in .bmp format (413 x 413 pixels).

2.5 Texture analysis

Texture analysis on fresh and stored (days 1, 3 and 6) RF and CS-PS based bread crumb samples was with an Instron (Norwood, MA, USA) 3342 texture analyzer. A cylindrical probe (diameter 75 mm) compressed the cylindrical crumb samples (see section 2.3) at 100 mm/minute to 70% of their initial height. The probe was then raised from the sample at the same rate. During this process, the load, i.e. the resistance to compression expressed as a force (N), was recorded continuously. From the obtained curve, crumb firmness was calculated as the maximal force during sample compression (Trinh & Glasgow, 2012).

2.6 Differential scanning calorimetry

DSC measurements were as in Masure et al. (2018) with a DSC Q1000 device (TA Instruments, New Castle, DE, USA) on freeze-dried RF and CS-PS based bread crumb samples (2.5 – 4.0 mg) accurately weighed into aluminum sample pans (Perkin Elmer, Waltham, MA, USA). Deionized water was added to obtain a water: sample DM ratio of 3:1 and pans were hermetically sealed. Samples were equilibrated at 0 °C before heating to 120 °C at 4 °C/minute together with an empty reference pan. The system was calibrated with indium. The enthalpies (J/g sample, DM basis) corresponding to melting of AP crystals (formed as a result of retrogradation) were calculated from the thermograms using TA Instruments Universal Analysis software.

2.7 Proton nuclear magnetic resonance

Proton distribution measurements were conducted on crumb samples from fresh and stored RF and CS-PS based breads. Crumb samples were removed from breads stored as in section 2.3 on days 1, 3 and 6. Three crumb samples (approximately 0.30 g, accurately weighed) were then transferred into three Bruker (Ettlingen, Germany) NMR tubes (10 mm external diameter) and tightly compressed to a height of 8 mm to avoid the presence of large air bubbles. The tubes were then sealed to prevent moisture loss. All measurements were as in Bosmans et al. (2012) with a Bruker Minispec mq 20 low-field pulsed NMR spectrometer (operating resonance frequency 20 MHz for ^1H , magnetic field strength 0.47 T). Each tube was analyzed three times and the sample weight in each tube was taken into account. An external water bath kept the probe head at 25 ± 1 °C. Transverse relaxation curves were acquired with a single-pulse [signal after a 90° pulse, free induction decay (FID)] and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences. The CONTIN algorithm of Provencher (software provided by Bruker) was used to transform the transverse relaxation curves with an inverse Laplace transformation to continuous distributions of transverse relaxation time (T_2) values. The less mobile protons were detected in the FID experiments (FID proton populations), the more mobile ones with the CPMG pulse sequence (CPMG proton populations). The area under the curve of a population with

a given T_2 value is proportional to the number of protons in that population. The inhomogeneity of the static magnetic field affected the output for the most mobile FID population (with a T_2 of around 0.5 ms). This population was not further taken considered.

Bosmans et al. (2012) detected six proton populations in wheat bread crumb. In GF bread crumb, similar proton populations were identified (Hager et al., 2014). Population assignment was done as in these references. Capital letters were assigned to the different proton populations in order of increasing mobility (Figure 2). The most rigid (and thus the least mobile) population A contains CH protons of amylose crystals and amorphous starch and of proteins not in contact with water. Populations B and C contain more mobile CH protons from amorphous starch and of proteins in little contact with water. Population D consists of CH protons of proteins and exchanging protons of confined water, starch and protein. In CS-PS+REW bread crumb, an additional population (D_{bis}), containing protons similar to those of population D was observed in some but not all cases. Population E contains mobile exchanging protons of water, starch and proteins in the gel network formed after gelatinization. In RF based bread crumb, an additional small population with high mobility (population F) was visible. It contains lipid protons originating from RF.

2.8 (Oscillating) pendant drop measurements

Solutions of REW, HEW and SPI (0.50% w_{protein}/v) were prepared by suspending 5.0 mg of protein material in 1.0 ml water, shaking for 60 minutes and centrifuging for 10 minutes at 10,000 g. They were then analyzed using a Theta optical tensiometer (Biolin Scientific Attension, Stockholm, Sweden) using OneAttension software as in Wouters et al. (2017). The decrease of surface tension was measured continuously over a 10 minute time interval by using a pendant drop with a fixed volume of 8 μL . This was done to assess protein adsorption and rearrangement at the air–water interface. Surface tension values were determined based on drop shape analysis of images taken (1 frame per 7 seconds) during the analysis. Subsequently, the same drop was sinusoidally oscillated (50 cycles) at a frequency of 1 Hz with an amplitude set at 1.00, which corresponded to a volume of ± 1

μL. During oscillation, images were recorded (7 frames per second). The calculation of the surface dilatational elastic modulus E of the interfacial film was based on drop shape analysis data during oscillation. Measurements were conducted at 22 ± 2 °C. After each measurement, the device was thoroughly cleaned and the surface tension of pure water was checked to be 72.0 ± 0.5 mN/m.

2.9 Statistical analysis

All measurements were at least in triplicate. Error bars or values in figures and tables represent the standard deviation from the means. Data from specific loaf volume, texture analysis, DSC, $^1\text{H-NMR}$ and pendant drop analysis was analyzed using statistical software JMP Pro 13 (SAS Institute, Cary, NC, USA) with a Tukey multiple comparison test at a significance level $\alpha = 0.05$.

3. Results and discussion

Two control GF bread recipes (CS-PS+REW and RF bread) were developed in preliminary trials. Ingredient ratios, water contents, fermentation times and baking temperatures were chosen based on specific volume and crumb softness and structure of the different breads. In earlier work on these control recipes by our group, we related batter properties during fermentation and baking in an ERO to specific volume and crumb structure of conventionally baked breads (Masure et al., 2018). As mentioned in section 1, this allowed concluding that the volume and crumb structure of GF bread is determined by the balance between the moment of gas cell opening and that of crumb structure setting. Controlling this balance allows impacting the volume and crumb structure of GF breads. One way to achieve this is by using ingredients that impact batter stability and thus the moment of gas release.

To further explore this, we here used proteins with different properties [REW, HEW, and SPI]. First, baking trials in a conventional oven with different proportions of REW, HEW or SPI were executed to determine the concentration of HEW or SPI to be used in further experiments. Based on measurements of specific volume, crumb texture and structure, a concentration was selected which resulted in optimal (high) volume, desirable (soft) crumb texture and a fine and homogeneous crumb (see Table 1). For CS-PS based breads, all (CS-PS-HEW bread) or part (CS-PS-SPI bread) of the REW was substituted by an amount of sample such that the end product would contain a constant level of protein. For RF based breads, substitutions were done on flour DM basis.

3.1 Loaf specific volume and crumb structure of conventionally baked bread

Specific loaf volumes and crumb scans of conventionally baked breads are shown in Figure 3. The specific loaf volume of CS-PS+HEW bread was significantly ($P < 0.05$) higher than that of the control CS-PS+REW bread, and that of CS-PS+SPI bread significantly ($P < 0.05$) lower. CS-PS+REW and CS-PS+HEW bread had a fine crumb with small gas cells. The crumb of CS-PS+SPI bread was less homogeneous and had larger gas cells.

RF+REW and RF+HEW bread specific volumes were significantly ($P < 0.05$) higher than that of control RF bread. In contrast, the specific volume of RF+SPI bread did not differ significantly ($P > 0.05$) from that of control RF bread. Crumb structure (Figure 3) of control RF bread was inhomogeneous, with large gas cells surrounded by smaller ones. Crumb of RF+REW or RF+HEW breads was more homogeneous with smaller gas cells. Crumb of RF+SPI bread was inhomogeneous and had very large gas cells.

3.2 Evolution of batter properties during ERO fermentation and baking

Figures 3 and 4 show batter height, batter viscosity and CO_2 concentration in the headspace above batters during fermentation and baking in an ERO of RF and CS-PS based batters/breads. Earlier, we discussed the evolution of these properties for control RF and CS-PS+REW batters/breads as well as for RF+REW batter/bread Masure et al. (2018). In short, the crumb of CS-PS+REW bread had already (partly) set (measured by a pronounced increase in batter viscosity) at the moment of gas cell opening (as assessed by a sudden release of carbon dioxide from the batter), which resulted in almost no collapse and thus a high bread volume. In control RF bread making, crumb setting only started when a large part of the carbon dioxide had already been lost, resulting in collapse and thus low volume. In RF+REW bread, the presence of EW proteins led to delayed carbon dioxide release, which resulted in less pronounced batter collapse, higher volume and a finer crumb structure than noted for control RF bread. The presence of proteins able to stabilize air-water interfaces such as those of EW thus seems of major importance in controlling the balance between crumb setting and carbon dioxide release. To further support these findings and elaborate on the structure formation in GF bread making, two other protein types were investigated, *i.e.* HEW and SPI.

3.2.1 Cassava and potato starch based breads

ERO parameters for CS-PS+REW (control), CS-PS+HEW and CS-PS+SPI batters and breads are shown in Figure 4. During fermentation in the ERO, the viscosity of CS-PS+HEW batter was similar to that of CS-PS+REW batter, while that of CS-PS+SPI batter was higher. Masure et al. (2018) showed that

water uptake during mixing influences initial batter viscosity. SPI has high water binding capacity (Marco & Rosell, 2008a), which probably resulted in the higher viscosity of the CS-PS+SPI batter than that of CS-PS+REW batter, both initially and during fermentation. Heights of CS-PS+REW and CS-PS+HEW batters were similar at the start of fermentation, while that of CS-PS+SPI batter was lower. This indicates that either less air was incorporated in the CS-PS+SPI batter or that it was less stable and more air was lost when manipulating it than was the case for the control CS-PS+REW batter. Batter heights increased linearly during fermentation and at a similar rate, thus resulting in a lower fermented batter height for CS-PS+SPI batter than for CS-PS+REW and CS-PS+HEW batters.

During ERO heating (baking) of CS-PS+REW batter, a pronounced viscosity increase indicated the start of crumb structure setting at about 59 °C, while gas cell opening occurred at about 68 °C. In CS-PS+HEW batter, this pronounced viscosity increase was observed at approximately the same temperature as was the case in CS-PS+REW batter, but carbon dioxide release in the former started at about 75 °C rather than at about 68 °C in the latter. This allowed for an even longer oven spring in the CS-PS+HEW batter. Nevertheless, the end heights of CS-PS+REW and CS-PS+HEW breads were similar.

In CS-PS+SPI batter, viscosity started increasing steeply at a similar temperature as in CS-PS+REW batter. Carbon dioxide release in CS-PS+SPI batter also started at a similar temperature as that in CS-PS+REW batter, but the end height was lower. The higher viscosity of the batter probably prevented it from expanding to the same maximal height as CS-PS+REW batter. The moment of gas cell opening was not influenced to a large degree by the SPI present. However, the rate of carbon dioxide release and the total amount of carbon dioxide released (area under the curve) were higher for CS-PS+SPI than for control CS-PS+REW batter, which suggested a less efficient gas cell stabilization. In any case, the concentration of REW in CS-PS+SPI batter was still sufficiently high so that it could stabilize gas cells until the moment of crumb structure setting.

3.2.2 Rice flour based breads

Figure 5 shows ERO parameters for control RF, RF+REW, RF+HEW and RF+SPI batters and breads. The viscosity of control fermenting RF batter was higher than those of fermenting RF+REW and RF+HEW batters. Furthermore, RF+SPI batter viscosity was higher than that of control RF batter. For all RF batter samples, viscosity decreased during fermentation as the density decreased with the increase in batter height. An initial viscosity increase, which was most pronounced in control RF and RF+SPI batters, may have resulted from gas cell coalescence and disproportionation during the first minutes of fermentation. Indeed, as the size of gas cells increases, the biaxial stress around them also increases (van Vliet, 2008) which may result in a higher batter viscosity. At the start of fermentation, batter heights and thus densities of all RF batters were similar. Batter height increased linearly, resulting in similar batter heights after fermentation for control RF, RF+REW and RF+SPI batters, while that of RF+HEW batter was slightly lower. During ERO heating, gas cell opening in control RF batter started at about 56 °C, which was just before the viscosity of control RF batter started to increase strongly (at about 58 °C) resulting in batter collapse. Indeed, the batter was not able to retain its gas until crumb structure started setting. In contrast, in RF+REW batter, gas cell opening (at about 64 °C) started after batter viscosity already started increasing [at about 50 °C, which was ascribed to swelling of starch granules as a result of competition for water between starch and EW protein (Masure et al., 2018)]. As a result, batter collapse was less pronounced and the end height was higher. In line with results obtained for RF+REW, gas cell opening and carbon dioxide release in RF+HEW batter started at a higher temperature (about 67 °C) than in control RF batter. Viscosity increase in RF+HEW batter started at a higher temperature (about 63 °C) than in control RF batter, but still before gas cell opening occurred. Thus, batter collapse was much less pronounced than in control RF and a high end bread height was reached. In RF+SPI batter, a steep viscosity increase was observed at lower temperature (about 50 °C) than in control RF batter. However, carbon dioxide release already began at about 44 °C, and thus before crumb setting. This resulted in batter collapse

and a low end height. Although gas cell stability in RF+SPI batter was even lower than in control RF batter, both breads had similar heights.

3.3 Surface-active properties of REW, HEW and SPI

The presence of REW and HEW made CS-PS and RF based batters more stable. Batters containing SPI were less stable. To further investigate the relationship of these observations to the surface-active properties of the proteins used, pendant drop analyses of 0.50% (w_{protein}/v) solutions of REW, HEW and SPI were performed. Surface tension is a measure for diffusion to and adsorption at the interface of surface-active constituents. The surface dilatational modulus E is a measure of the viscoelasticity of adsorbed films. A high E value of an adsorbed protein film is generally related to delayed gas cell coalescence and disproportionation and thus high foam stability (Janssen, Wouters, Pauly, & Delcour, 2018). The evolution of surface tension in time and the E values of the different protein solutions are shown in Figure 6. The decrease in surface tension in time was more pronounced for REW and HEW solutions than for the SPI solution. The lower surface tension values 10 minutes after drop formation of the former indicate higher surface activity of REW and HEW than of SPI. E values of the solutions of REW and HEW were similar, while that of the SPI solution was substantially lower ($P < 0.05$). It should be noted here that while pendant drop analyses were performed at room temperature, gas cell opening in GF batters occurred at higher temperatures and thus under conditions of different surface activity of the tested proteins. Unfortunately, due to rapid evaporation of water from the pendant drop, it is not possible to perform such measurements at elevated temperatures in the set-up used. Thus, direct comparison of the observations in pendant drop analyses with those during bread baking is not entirely possible. Nevertheless, there is merit in comparing the different proteins used in terms of their inherent surface activity, be it at temperatures lower than those during bread baking. Indeed, Janssen et al. (2018) found that the inherent surface activity of dough aqueous phase constituents at room temperature is to some extent related to their ability to stabilize gas cells during bread fermentation and baking. The results obtained here are, while still keeping in mind the difference in temperatures at which the measurements were performed, in line with what was

observed when heating RF batter in the ERO, where gas cell opening occurred earlier in batters containing SPI than in those containing REW or HEW. In CS-PS batter, the moment of gas cell opening was not influenced by SPI, as this recipe still contained a high level of REW. Nevertheless, CS-PS+SPI breads had much lower volumes than the control CS-PS based breads. Overall, inclusion of proteins with high surface activity and a good ability to efficiently stabilize air-water interfaces under the conditions of pendant drop analyses and thus at room temperature in GF bread recipes improved the stability of gas cells in batter and eventually led to higher bread volume and more homogeneous crumb structure.

3.4 Changes during storage of conventionally baked bread.

3.4.1 Cassava and potato starch based breads

Table 2 shows firmness readings of CS-PS+REW, CS-PS+HEW and CS-PS+SPI bread crumb over the course of 6 days of storage. On day 0, the readings of the different CS-PS based bread crumbs were influenced by rapid shrinking of the samples after cutting and hence were not fully reliable. However, all CS-PS based breads showed an increase in firmness which was most pronounced in the first 24 hours of storage. Replacing REW by HEW in the CS-PS based bread led to lower ($P < 0.05$) crumb firmness after 6 days of storage. In contrast, CS-PS+SPI bread had higher ($P < 0.05$) crumb firmness after 6 days of storage than the control CS-PS+REW bread.

Table 2 also shows the melting enthalpy of AP crystals of CS-PS bread crumb samples over the course of 6 days of storage. Both in wheat and GF breads, AP retrogradation during storage contributes to crumb firming (D'Appolonia & Morad, 1981; Horstmann, Lynch, & Arendt, 2017). Indeed, a strong increase in AP melting enthalpy was observed during the first 24 hours of storage which was in line with the substantial increase in firmness in the same time frame. However, after day 3, the melting enthalpy of retrograded AP did not increase further, while firmness continued to increase significantly ($P < 0.05$) between days 3 and 6. Thus, factors other than AP retrogradation contribute to crumb firming during storage. Melting enthalpies of retrograded AP of CS-PS+HEW and CS-PS+SPI bread crumbs were similar ($P > 0.05$) to that of CS-PS+REW crumb. The MC of all CS-PS based bread

crumbs decreased ($P < 0.05$) during storage (Table 2). Both in wheat and GF breads, such decrease of the crumb MC content is due to crumb-to-crust moisture migration (Baik & Chinachoti, 2000; Osella, Sanchez, Carrara, de la Torre, & Buera, 2005; Piazza & Masi, 1995; Pisesookbuntern & D'Appolonia, 1983). The evolution of crumb MC throughout storage was not influenced by including HEW or SPI in the CS-PS based bread recipe.

^1H -NMR measurements yielded further insights in molecular changes during storage. Figure 2 shows ^1H -NMR profiles of CS-PS+REW and control RF bread crumbs, both fresh and after 6 days of storage. In GF bread crumb, proton populations similar to those in wheat bread [(Bosmans et al., 2012; Hager et al., 2014), section 2.7] were recognized, and similar changes could be observed during storage. In all CS-PS and RF based bread crumbs, the area of population A increased as a result of storage (Figure 2 and Supplementary table 1). Both for wheat and GF breads, this has been attributed to AP retrogradation, as water is enclosed in starch crystals and becomes less available (Bosmans et al., 2013; Carini et al., 2017; Curti, Bubici, Carini, Baroni, & Vittadini, 2011; Hager et al., 2014). Much as noted for wheat bread, the areas of populations C and D decreased during storage (Figure 2). Their protons were probably incorporated in the crystalline structure during retrogradation and as a result appeared in population A. During storage of CS-PS based bread, both T_2 relaxation time ($T_{2,E}$) and the area of population E of the crumb samples decreased over time. In the case of wheat bread, the decrease in $T_{2,E}$ has been related to the rigidity of the gel network, while the decrease in area of population E has been related to crumb-to-crust moisture migration (Bosmans et al., 2013), which is very likely also the case here. In CS-PS based breads, the most remarkable differences between recipes were noticed in the T_2 of population E (Supplementary table 1). In CS-PS+SPI bread crumb, both the $T_{2,E}$ of the initial sample and that of the sample after 6 days of storage were significantly ($P < 0.05$) lower than those of CS-PS+REW. Thus, in CS-PS+SPI, the gel network was more rigid than in the control, which relates to the higher crumb firmness during storage of the former. After 6 days storage, $T_{2,E}$ of CS-PS+HEW crumb was similar to that of CS-PS+REW crumb. The lower firmness of

CS-PS+HEW than of CS-PS+REW crumb was therefore probably not related to the rigidity of the gel network, but more likely a result of the higher volume (lower density) of the former.

Thus, firming of all CS-PS based breads during the first day of storage was probably related to both AP retrogradation and moisture migration, while moisture migration caused a further increase in firmness during later storage. Nevertheless, the differences in crumb firming between CS-PS+REW, CS-PS+HEW and CS-PS+SPI samples, the latter of which had a respectively lower and higher firmness at the end of storage than CS-PS+REW bread, were caused by the initial crumb structure and rigidity of the gel network, since there were no significant differences in AP retrogradation or crumb-to-crust moisture migration.

3.4.2 Rice flour based breads

Table 3 shows firmness of control RF, RF+REW, RF+HEW and RF+SPI over the course of 6 days of storage. The firmness of RF based bread crumbs increased even more drastically during storage than was the case for CS-PS based bread crumbs, resulting in significantly higher ($P < 0.05$) firmness of control RF than of CS-PS+REW bread crumb on days 1, 3 and 6. On day 0, the firmness readings of control CS-PS+REW and control RF bread crumb were not different ($P > 0.05$). However, the CS-PS+REW samples were perceived to be softer than the control RF samples. As was the case for CS-PS breads, the increase in firmness was most pronounced during the first 24 hours of storage, after which it increased more gradually. After 6 days of storage, crumb firmness readings of RF+REW and RF+HEW breads were significantly ($P < 0.05$) lower than that of control RF bread while firmness of RF+SPI bread was significantly ($P < 0.05$) higher than that of control (Table 3). As in CS-PS+REW bread crumb, the enthalpy of retrograded AP of control RF bread crumb (Table 3) increased mainly during the first 24 hours of storage, and did not increase further after day 1. Furthermore, there were no significant ($P < 0.05$) differences between the melting enthalpies of retrograded AP in RF or CS-PS+REW bread crumb during storage, despite large differences in firmness after storage. AP retrogradation enthalpies after 6 days of storage were similar ($P > 0.05$) for all RF based breads

(Table 3). Also similar to what was observed for CS-PS+REW bread, crumb MC of control RF bread decreased ($P < 0.05$) during storage with the greatest decrease occurring between days 1 and 3. The relative crumb-to-crust moisture migration was less pronounced in control RF (decrease of crumb MC from 49.5% to 46.1%) than in control CS-PS+REW (decrease of crumb MC from 45.7% to 40.5% moisture) bread crumb. The inclusion of REW, HEW or SPI in RF based breads did not influence crumb-to-crust moisture migration, since crumb MC after 6 days of storage was similar for all RF based breads. Proton populations in ^1H -NMR measurements of fresh crumb samples of control RF and CS-PS+REW breads were rather similar. Similar to what happened in CS-PS breads, both the area and T_2 relaxation time of population E decreased during storage. Population E had a higher T_2 and its peak was more narrow for CS-PS+REW than for control RF bread crumb (Figure 2). The higher $T_{2,E}$ of the former indicates that the CS-PS+REW bread crumb gel network was less rigid than that of control RF bread crumb, which could be related to the lower firmness of the former than the latter. Together with the lower density (higher specific volume) of control CS-PS+REW bread than control RF bread, this resulted in the large difference in firmness of both bread types. Furthermore, over the course of 6 days of storage, the relative decrease in area of population E of CS-PS+REW bread crumb was more pronounced than that of control RF bread crumb (Supplementary table 1, Supplementary table 2). This was in line with the more pronounced crumb-to-crust moisture migration in CS-PS+REW than in control RF bread. When comparing the RF based breads, RF+REW and RF+HEW bread crumbs had higher $T_{2,E}$ than control RF bread before and after storage, which indicated a less rigid gel network in the former samples than in the latter. This concurred with the lower firmness in both recipes containing EW than in the control RF bread. As in CS-PS bread, the lower density (larger specific volume) of RF+REW and RF+HEW bread than of control RF bread probably further decreased crumb firmness. RF+SPI bread crumb had a lower $T_{2,E}$, and thus a more rigid network, than control RF bread crumb on days 0, 1 and 3 which concurred to its higher firmness than control RF bread. However, after 6 days storage $T_{2,E}$ of the former was similar as in control RF, while the firmness was still higher ($P < 0.05$).

In conclusion, AP retrogradation and water redistribution in the crumb play an important role in GF bread crumb firming. In the early stages of storage, AP retrogradation and water redistribution both contribute to the increase in firmness. There were no differences between the enthalpies of retrograded AP of control RF and CS-PS+REW bread crumbs after 6 days of storage. Towards the end of storage, water redistribution primarily caused the further increase in firmness, although there were no notable differences between control RF and CS-PS+REW bread in terms of the changes in proton distributions which could explain their difference in crumb firmness throughout storage. The latter was probably related to differences in the initial rigidity of the gel network, as could be seen by differences in the T_2 relaxation time of population E for both bread types. All this may have originated from the difference in initial density of the two bread types, which is caused by the major collapse of control RF breads at the end of baking. Furthermore, the differences in firmness of breads with different protein types, also seemed to mainly depend on the differences in initial rigidity of the gel network.

4. Conclusions

This research showed that high loaf volume and fine and homogeneous crumb structure can be achieved by including highly surface-active proteins such as REW and HEW in the bread recipe. Even though direct comparison between the observations at room temperature in pendant drop analyses and those during bread baking at elevated temperatures is not entirely possible, the inherent surface activity of the proteins used seems to be related to their ability to stabilize gas cells during bread fermentation and baking. By adsorbing at the air/water interface, these proteins stabilized the batter and delayed gas cell opening until the crumb structure started setting. Differences between REW and HEW were limited in terms of surface activity and bread making properties. On the contrary, batters containing SPI had high viscosity and were characterized by poor gas cell stabilization which resulted in low volumes and inhomogeneous crumb structures of CS-PS+SPI and RF+SPI breads. The initial loaf volume and crumb structure also largely determined differences in the extent of crumb firming during storage, as AP retrogradation and crumb-to-crust moisture migration behavior was rather similar in all recipes. Thus, controlling batter stability and crumb structure setting to modify the volume and crumb structure of GF bread, also has a major impact on crumb firming during storage.

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Tables

Table 1. Recipes of gluten-free (GF) breads based on a mixture of cassava-and potato starch (CS-PS) or on rice flour (RF) with regular egg white powder (REW), high gel egg white powder (HEW) or soy protein isolate (SPI). Mixtures of starches and protein are referred to as 'flour' as are mixtures of RF and protein. Percentages of the different ingredients represent their weight fraction in the 'flour'. Other ingredients in the formulation are expressed as percentages on dry matter 'flour' weight basis (fwb).

		CS-PS+REW (control)	CS-PS+HEW	CS-PS+SPI	
'Flour 1'	Cassava starch (% w/w)	62.5	62.5	62.5	
	Potato starch (% w/w)	22.5	22.5	22.5	
	Regular egg white powder (% w/w)	10.0	0	7.7	
	High gel egg white powder (% w/w)	0	10.0	0	
	Soy protein isolate (% w/w)	0	0	2.3	
Other ingredients	Tap water (% fwb)	90.0	90.0	90.0	
	Fresh yeast (% fwb)	6.2	6.2	6.2	
	Sugar (% fwb)	6.0	6.0	6.0	
	Salt (% fwb)	1.5	1.5	1.5	
	Calcium propionate (% fwb)	0.1	0.1	0.1	
RF (control)		RF+REW	RF+HEW	RF+SPI	
'Flour 2'	Rice flour (% w/w)	100.0	94.0	94.0	96.0
	Regular egg white powder (% w/w)	0	6.0	0	0
	High gel egg white powder (% w/w)	0	0	6.0	0
	Soy protein isolate (% w/w)	0	0	0	4.0
Other ingredients	Tap water (% fwb)	115.0	115.0	115.0	115.0
	Dry yeast (% fwb)	3.0	3.0	3.0	3.0
	Sugar (% fwb)	6.0	6.0	6.0	6.0
	Salt (% fwb)	1.5	1.5	1.5	1.5
	Calcium propionate (% fwb)	0.1	0.1	0.1	0.1

Table 2. Moisture content, amylopectin (AP) melting enthalpy and crumb firmness of breads based on cassava and potato starch (CS-PS) and regular or high gel egg white powder (REW or HEW) or a combination of REW and soy protein isolate (SPI). Standard deviations from the mean are given between brackets.

		Firmness (N)	Moisture content (%)	Enthalpy (J/g)
CS-PS+REW (control)	Day 0	3.16 (0.79 d,A)	45.70 (0.48 a,A)	1.86 (0.35 c,A)
	Day 1	12.69 (0.60 c,B)	45.03 (0.10 a,B)	3.60 (0.30 b,AB)
	Day 3	18.20 (2.00 b,B)	41.52 (0.68 b,B)	5.27 (0.58 a,A)
	Day 6	20.30 (0.64 a,B)	40.45 (0.74 b,A)	4.87 (0.46 a,A)
CS-PS+HEW	Day 0	1.78 (0.32 d,B)	45.87 (0.28 a,A)	0.62 (0.24 c,B)
	Day 1	6.54 (0.57 c,C)	45.72 (0.12 a,A)	3.13 (0.36 b,B)
	Day 3	9.86 (0.59 b,C)	42.21 (0.67 b,B)	4.41 (0.47 a,A)
	Day 6	13.26 (0.99 a,C)	40.28 (0.91 c,A)	4.34 (0.58 a,A)
CS-PS+SPI	Day 0	2.58 (0.62 d,A)	45.17 (0.22 a,A)	1.26 (0.50 b,AB)
	Day 1	14.00 (0.97 c,A)	45.33 (0.25 a,AB)	3.96 (0.52 a,A)
	Day 3	24.31 (2.67 b,A)	44.37 (0.18 a, A)	5.15 (0.62 a,A)
	Day 6	31.31 (3.48 a,A)	41.98 (0.69 b,A)	5.37 (0.91 a,A)

Values in one column of the same bread type with a different lowercase letter are significantly different ($P < 0.05$). Values in one column on the same day between bread types with a different capital letter are significantly different ($P < 0.05$).

Table 3. Moisture content, amylopectin (AP) melting enthalpy and crumb firmness of breads based on rice flour (RF) without or with regular egg white powder (REW), dry heated egg white powder (HEW) or soy protein isolate (SPI). Standard deviations are given between brackets.

		Firmness (N)	Moisture content (%)	Enthalpy (J/g)
RF (control)	Day 0	3.80 (0.85 d,B)	49.55 (0.29 a,B)	0.27 (0.09 b,B)
	Day 1	31.75 (3.57 c,B)	49.38 (0.17 a,AB)	4.73 (0.33 a,A)
	Day 3	41.25 (3.87 b,A)	46.94 (0.82 b,B)	4.38 (0.55 a,A)
	Day 6	47.11 (5.32 a,B)	46.06 (0.40 b,A)	5.07 (0.26 a,AB)
RF+REW	Day 0	2.73 (0.46 d,B)	50.35 (0.12 a,A)	0.30 (0.11 b,B)
	Day 1	8.62 (1.29 c,D)	50.31 (0.07 a,A)	4.14 (0.48 a,A)
	Day 3	12.55 (2.19 b,B)	48.66 (0.12 ab,A)	3.63 (0.71 a,A)
	Day 6	16.44 (3.06 a,C)	47.47 (1.24 b,A)	4.00 (0.13 a,B)
RF+HEW	Day 0	2.88 (0.98 c,B)	50.70 (0.07 a,A)	0.38 (0.05 b,B)
	Day 1	13.80 (2.00 b,C)	49.78 (0.67 a,A)	2.82 (0.64 a,B)
	Day 3	17.91 (4.06 ab,B)	48.54 (0.46 ab,A)	4.55 (1.28 a,A)
	Day 6	20.23 (5.36 a,C)	47.25 (1.10 b,A)	4.37 (0.77 a,B)
RF+SPI	Day 0	5.91 (1.70 c,A)	48.79 (0.50 a,B)	2.15 (0.16 b,A)
	Day 1	40.74 (3.78 b,A)	48.61 (0.21 a,B)	5.14 (0.60 a,A)
	Day 3	46.22 (7.92 b,A)	47.94 (0.56 a,AB)	5.44 (0.54 a,A)
	Day 6	57.25 (9.30 a,A)	45.79 (0.83 b,A)	5.96 (0.65 a,A)

Values in one column of the same bread type with a different lowercase letter are significantly different ($P < 0.05$). Values in one column on the same day between bread types with a different capital letter are significantly different ($P < 0.05$).

Supplementary table 1. Transverse (T_2) relaxation times and areas of populations A and E of proton nuclear magnetic resonance spectra (all with standard deviations) of bread crumb samples based on cassava and potato starch and regular egg white powder (CS-PS+REW), dry heated egg white powder (CS-PS+HEW) or a combination of REW and soy protein isolate (CS-PS+SPI).

		T_2A (ms)	Area A (au)	T_2E (ms)	Area E (au)
CS-PS+REW (control)	Day 0	0.014 (0.001 a,A)	4,548 (38 d,B)	18.82 (0.70 a,A)	8,615 (113 a,A)
	Day 1	0.013 (0.001 a,A)	7,083 (85 c,A)	17.33 (0.67 b,A)	8,390 (90 b,AB)
	Day 3	0.013 (0.001 a,A)	8,046 (189 b,A)	15.39 (0.61 c,A)	8,102 (105 c,A)
	Day 6	0.014 (0.001 a,A)	9,116 (500 a,A)	13.11 (1.43 d,A)	7,527 (293 d,B)
CS-PS+HEW	Day 0	0.014 (0.001 a,A)	4,277 (83 d,C)	19.44 (0.53 a,A)	8,652 (155 a,A)
	Day 1	0.014 (0.001 b,A)	6,633 (107 c,B)	17.80 (0.37 b,A)	8,487 (109 a,A)
	Day 3	0.014 (0.001 b,A)	7,761 (304 b,B)	15.00 (1.14 c,A)	8,152 (174 b,A)
	Day 6	0.013 (0.001 b,A)	8,508 (135 a,B)	13.41 (0.50 d,A)	7,838 (80 c,A)
CS-PS+SPI	Day 0	0.011 (0.001b,B)	4,739 (181 d,A)	16.93 (0.71 a,B)	8,390 (203 a,B)
	Day 1	0.014 (0.001a,A)	6,166 (102 c,C)	16.08 (0.37 b,B)	8,289 (91 a,B)
	Day 3	0.014 (0.001a,A)	7,377 (165 b,C)	13.46 (0.70 c,B)	7,776 (157 b,B)
	Day 6	0.014 (0.001a,A)	7,870 (157 a,C)	11.75 (0.49 d,B)	7,300 (78 c,C)

Values in one column of the same bread type with a different lowercase letter are significantly different ($P < 0.05$). Values in one column on the same day between bread types with a different capital letter are significantly different ($P < 0.05$).

Supplementary table 2. Transverse (T_2) relaxation times and areas of populations A and E of proton nuclear magnetic resonance spectra (all with standard deviations) of rice flour (RF) based bread crumb samples with regular egg white powder (RF+REW), dry heated egg white powder (RF+HEW) or soy protein isolate (RF+SPI).

		T_2A (ms)	Area A (au)	T_2E (ms)	Area E (au)
RF (control)	Day 0	0.013 (0.001 a,B)	5,826 (223 d,AB)	11.73 (0.2 a,B)	9,606 (115 a,B)
	Day 1	0.012 (0.001 a,B)	8,223 (134 c,A)	10.88 (0.41 b,B)	9,555 (90 a,C)
	Day 3	0.013 (0.001 a,A)	9,178 (129 b,A)	9.59 (0.15 c,B)	9,220 (104 b,B)
	Day 6	0.013 (0.001 a,A)	10,082 (135 a,A)	7.91 (0.37 d,C)	8,608 (97 c,C)
RF+REW	Day 0	0.013 (0.001 a,B)	5,757 (284 d,AB)	12.80 (0.20 a,A)	9,710 (177 a,B)
	Day 1	0.013 (0.001 a,B)	7,899 (80 c,B)	12.23 (0.14 b,A)	9,720 (97 a,B)
	Day 3	0.013 (0.001 a,A)	8,934 (225 b,B)	11.45 (0.27 c,A)	9,486 (104 b,A)
	Day 6	0.013 (0.001 a,A)	9,922 (165 a,A)	9.01 (0.42 d,B)	8,897 (146 c,B)
RF+HEW	Day 0	0.013 (0.001 a,A)	5,945 (224 d,A)	12.66 (0.18 a,A)	10,021 (126 a,A)
	Day 1	0.013 (0.001 ab,A)	7,837 (63 c,B)	12.11 (0.20 a,A)	9,831 (65 b,A)
	Day 3	0.013 (0.001 b,A)	8,795 (188 b,BC)	11.11 (0.50 b,A)	9,486 (114 c,A)
	Day 6	0.013 (0.001 b,A)	9,500 (263 a,B)	9.88 (0.66 c,A)	9,076 (186 d,A)
RF+SPI	Day 0	0.013 (0.001 a,AB)	5,519 (318 d,B)	10.73 (0.41 a,C)	9,534 (89 a,B)
	Day 1	0.013 (0.001 a,A)	8,212 (160 c,A)	9.69 (0.30 b,C)	9,398 (84 a,D)
	Day 3	0.013 (0.001 a,A)	8,690 (123 b,C)	9.14 (0.38 c,C)	9,027 (191 b,C)
	Day 6	0.013 (0.001 a,A)	9,479 (133 a,B)	8.31 (0.29 d,C)	9,005 (78 b,AB)

Values in one column of the same bread type with a different lowercase letter are significantly different ($P < 0.05$). Values in one column on the same day between bread types with a different capital letter are significantly different ($P < 0.05$).

Figures

Figure 1. Schematic representation of an electrical resistance oven (ERO) combined with measurement of (a) carbon dioxide (CO_2) concentration in the headspace and batter height or (b) batter viscosity. Proportional-integral-derivative (PID).

706 Figure 2. Proton nuclear magnetic resonance proton distributions of control rice flour (RF) and
 707 cassava and potato starch and regular egg white powder (CS-PS+REW) based bread crumb stored
 708 with crust. Fresh samples (—) and samples stored during 6 days (.....) were measured. FID, Free
 709 induction decay; CPMG, Carr-Purcell-Meiboom-Gill; au, arbitrary units.

Figure 3. Crumb structure scans and specific volumes of conventionally baked rice flour (RF) or cassava and potato starch (CS-PS) based bread crumb without or with regular egg white powder (REW), dry heated egg white powder (HEW) or soy protein isolate (SPI). It was not possible to produce CS-PS breads without adding egg white protein. Values of the same bread type with a different capital or lowercase letter are significantly different ($P < 0.05$).

Figure 4. Batter viscosity, height and carbon dioxide (CO₂) concentration in the headspace of batters based on cassava and potato starch and regular egg white powder (CS-PS+REW), dry heated egg white powder (CS-PS+HEW) or soy protein isolate (CS-PS+SPI) during fermentation and baking in an electrical resistance oven (ERO).

Figure 5. Batter viscosity, height and carbon dioxide (CO₂) concentration in the headspace of batters based on rice flour (RF) without or with regular egg white powder (RF+REW), dry heated egg white powder (RF+HEW) or soy protein isolate (RF+SPI) during fermentation and baking in an electrical resistance oven (ERO).

729 Figure 6. Surface tension as a function of time and surface dilatational moduli (E) of the interface of a
 730 pendant drop of 0.5% (w/v) solutions of regular (REW) or dry heated egg white powder (HEW) and
 731 soy protein isolate (SPI).

Highlights

- Balance between moment of CO₂ release and of crumb setting defines bread quality
- Surface active proteins stabilize gas cells and positively influence bread quality
- Crumb firmness increases during storage due to retrogradation and moisture migration
- Initial high volume and fine crumb result in high shelf life