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**Electrical resistance oven baking as a tool to study crumb structure
formation in gluten-free bread**

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Abstract

Gradientless baking by means of ohmic heating was used for the first time in gluten-free (GF) bread making. Combination thereof with in-line measurements of batter height, viscosity and carbon dioxide (CO₂) release proved to be powerful for studying structure formation in GF breads. GF breads studied here were based on (i) a mixture of potato and cassava starches and egg white powder (C/P-S+EW), (ii) rice flour (RF) or (iii) a mixture of RF and egg white powder (RF+EW). The work revealed that bread volume and crumb structure rely heavily on the balance between the moment of CO₂ release from batter during baking and that of crumb setting. At the moment of CO₂ release, C/P-S+EW bread crumb had already (partly) set, while this was not the case for RF bread crumb, resulting in a collapse and thus low volume of the latter. When a part of RF was replaced by egg white powder, the moment of CO₂ release was postponed and the batter collapse was less pronounced, leading to a higher volume and a finer crumb. The presence of egg white proteins in C/P-S+EW or RF+EW batters improved gas cell stabilization. Thus, increasing batter stability or altering the moment of crumb setting results in GF breads with higher volume and a finer crumb structure.

Keywords: gluten-free bread; ohmic heating; batter viscosity; carbon dioxide release; batter stability

1. Introduction

In many parts of the world including Western Europe, wheat bread is an important part of the daily diet. Wheat glutenin and gliadin, its main storage proteins, make up the gluten fraction and give wheat dough its visco-elastic properties. Glutenins are polymeric proteins which provide dough with elasticity and strength, while monomeric gliadins act as plasticizers and give rise to dough viscosity (Veraverbeke & Delcour, 2002). During wheat dough mixing, gluten and starch are hydrated, a continuous gluten network with embedded starch granules is formed (Hug-Iten, Handschin, Conde-Petit, & Escher, 1999), and air is incorporated in the dough (Cauvain, 2015). The gluten network retains gas cells even as they expand during fermentation and the early stages of baking. In the later steps of baking, gas cells open which results in an open bread crumb spongy structure. However, the need for gluten-free (GF) alternatives is growing. Celiac patients suffer from an auto-immune disease requiring them to follow a strict GF diet as no adequate alternative is currently available. Furthermore, people suffering from non-celiac gluten sensitivity and wheat allergy exclude gluten from their diet (Mulder et al., 2013).

The absence of such gluten network in GF batter or dough is responsible for the low quality of GF breads which generally have low specific loaf volumes and dry and crumbly crumb structures which firm faster than those of wheat bread. Furthermore, GF breads in many instances lack taste, mouthfeel and an acceptable color (Arendt, Morrissey, Moore, & dal Bello, 2008; Gallagher, Gormley, & Arendt, 2004; Houben, Hochstotter, & Becker, 2012). In addition, they generally have lower fiber and protein and higher fat contents than wheat bread (Matos & Rosell, 2015; Saturni, Ferretti, & Bacchetti, 2010).

GF bread recipes are usually based either on GF flours, starches or a combination of both. In attempts to make breads with an appearance and texture comparable to that of wheat bread, these GF flours or starches are often combined with ingredients or additives such as hydrocolloids, proteins or enzymes (Capriles & Arêas, 2014; Masure, Fierens, & Delcour, 2016). While GF batters/doughs and

bread has been extensively studied (Masure et al., 2016; Matos & Rosell, 2015), it has been difficult to relate the properties of the former to those of the latter. Matos and Rosell (2015) have compiled data from seven studies on batter or dough characteristics and bread quality. They found it difficult to draw conclusions on the ideal properties of GF systems. Indeed, many parameters could not be correlated although certain Mixolab® batter or dough parameters strongly correlated with crumb texture profile analysis parameters (Matos & Rosell, 2015). We hypothesize that insights in the phenomena taking place during GF bread making will lead to better understanding of how different ingredients can be used to improve the quality of GF breads.

A useful research approach is one using an electrical resistance oven (ERO). In an ERO, batter or dough functions as electrical resistance between two electrodes and baking is performed by Ohmic heating. Doing so allows batter to heat uniformly without significant internal temperature gradients (Luyts et al., 2013). This makes it possible to study temperature-dependent phenomena such as starch gelatinization and crumb structure setting. ERO heating has been used to study different parameters in wheat bread making (Derde, Gomand, Courtin, & Delcour, 2014; Gally et al., 2017; He & Hosney, 1991b). A major advantage of ERO technology is that it can be combined with in-line measurements during the bread making process. *In situ* monitoring of the viscosity and volume of batter can be used to assess structure formation phenomena and batter expansion (Shelke, Faubion, & Hosney, 1990). Measurement of carbon dioxide (CO₂) release in a closed ERO system further provides information on the permeability of the batter during fermentation and the initial stages of baking as well as on the moment of gas cell rupture later in the process (He & Hosney, 1991a). He and Hosney (1991b) with such approach showed that gas retention during fermentation and early baking of doughs based on different cereals, rather than their total gassing power, is crucial for the resultant loaf volume. It is evident that measuring such parameters continuously over the course of fermentation and baking can also provide valuable insights in the 'black box' process of GF bread making. To the best of our knowledge, ERO technology has not been used before in research on GF bread making.

Against this background, the objective of the present research was to study the sequence of events leading to structure formation in GF breads. Hereto, two GF bread models were studied. Most GF bread recipes are either based on a GF flour, or on starch with additional proteins or hydrocolloids. One model bread was based on rice flour (RF). RF is much used in GF bread making due to its bland taste, light color and low allergenicity (Capriles & Arêas, 2014). The other model bread was based on a mixture of cassava and potato starches and egg white (EW) powder as protein source (C/P-S+EW). Finally, to study the role of EW proteins in GF bread structure formation, a RF based bread containing EW proteins (RF+EW) was also studied. The number of ingredients in these recipes was purposely kept limited, in order to be able to gain insights in the structure formation mechanisms taking place in GF bread making. To achieve this, ERO heating was combined with in-line measurements of height, viscosity and CO₂ release (fermentation and baking).

2. Materials and methods

2.1 Materials

C/P-S+EW bread contained cassava starch [moisture content (MC) $12.7\% \pm 0.1\%$, protein content (PC) $0.5\% \pm 0.2\%$] from Cargill (Vilvoorde, Belgium), potato starch (MC $18.7\% \pm 0.1\%$, PC $0.2\% \pm 0.1\%$) from Caldic (Hemiksem, Belgium), freeze dried hen EW powder (MC $7.5\% \pm 0.1\%$, PC $87.8\% \pm 1.5\%$) from Lodewijckx (Veerle-Laakdal, Belgium), and fresh yeast from AB Mauri (Dordrecht, The Netherlands). RF bread contained RF [MC $9.8\% \pm 0.1\%$, PC $4.8\% \pm 0.1\%$] from Beneo-Remy (Wijgmaal, Belgium) and dry yeast from Puratos (Groot-Bijgaarden, Belgium). 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. Nitrogen to protein conversion factors were 5.95 for RF and 6.25 for all other analyzed materials. 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

For C/P-S+EW bread production, a mixture of 67.5% (w/w) cassava starch, 22.5% (w/w) potato starch and 10.0% (w/w) EW powder is further referred to as 'flour'. RF breads contained only RF while in RF+EW breads 6.0% (w/w) of RF was replaced by EW powder. Other ingredients of GF breads were tap water, fresh or dry yeast, sugar, salt and calcium propionate. The GF bread formulations are summarized in Table 1. The choice for fresh and dry yeast in C/P-S+EW and RF based formulations, respectively, was based on preliminary experiments. Fresh yeast resulted in C/P-S+EW breads with higher volumes. In RF bread, dry yeast was used to obtain a more reproducible bread quality. Indeed, the RF recipe was sensitive to variations in fresh yeast quality. 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 tap water at 30 °C for 10 minutes while fresh yeast

was used as is. Addition of yeast and water to the dry ingredient blend 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 in the same device with the same batter blade was performed at speed 4 for 90 seconds.

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2.2.2 Electrical resistance oven baking

ERO baking was performed essentially as in Luyts et al. (2013) for pound cake with ERO dimensions depending on the experiment (see below). As already mentioned, GF batters served as electrical resistance between the 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 the ERO at 30 °C for 45 (C/P-S+EW bread) or 25 minutes (RF and RF+EW bread). During baking, a temperature-time profile similar to that in the center of a 250 g batter baked in a conventional oven at 195 °C was applied. The temperature-time profiles applied in the ERO baking step were as follows. C/P-S+EW batter was kept at 30 °C for 7 minutes, heated to 95 °C in 25 minutes and kept at 95 °C for 13 minutes. RF and RF+EW batters were kept at 30 °C for 7 minutes, heated to 95 °C in 30 minutes and kept at 95 °C for 8 minutes.

Carbon dioxide and height measurement

For CO₂ release and height measurements, batters (250 g) were poured in an ERO (150 x 60 x 180 mm, l x w x h) which was then closed with a cover. A CO₂ data logger (CO2Meter, Ormond Beach, FL, USA) was connected to the ERO chamber (Figure 1a). Data loggers suited for CO₂ concentration ranges of 0-10⁴ ppm and 0-10⁶ ppm were used to record CO₂ release during fermentation and baking, respectively. Air was constantly circulated from the headspace above the batter to the data logger and back. The CO₂ concentration was recorded every 5 seconds. Prior to every analysis, the ambient CO₂ concentration was measured and subtracted from further readings. Batter height was measured with a ruler every 5 minutes (during fermentation) or 2 minutes (during baking).

Batter viscosity measurement

For viscosity measurements, batters (600 g) were poured in an ERO (90 x 100 x 120 mm, l x w x h). A Marimex (Bottrop, Germany) ViscoScope small amplitude oscillating torsion viscometer probe was completely submerged in the batter (Figure 1b). The viscometer was connected to a PID controller.

The dynamic viscosity was calculated from the power needed to keep the oscillation frequency of the probe at its resonance frequency of 520 Hz (Marimex, 2018). During baking, the batter was allowed to overflow to ensure that the probe remained in contact with the batter at all times.

2.2.3 Conventional oven baking

C/P-S+EW (200 g), RF or RF+EW (250 g) batters in baking tins (143 x 79 x 119 mm, l x w x h) were placed in a fermentation cabinet (National Manufacturing, Lincoln, NE, USA) at 30 °C and 90% relative humidity for 45 (C/P-S+EW bread) or 25 minutes (RF or RF+EW bread). The loaves were baked for 45 minutes at 175 °C (C/P-S+EW bread) or 195 °C (RF or RF+EW bread) 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).

2.4 Image analysis of bread crumb

Bread slices (1.0 cm thickness) were cut with an electric slicer (Affettatrice Slicer 30N, Galesecca, Italy) and analyzed using a flatbed scanner (Scanjet 3800, Hewlett-Packard, Beijing, China). 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 Water retention capacity of batter

C/P-S+EW, RF and RF+EW batters were prepared as in section 2.2.1, without addition of yeast. A batter weight corresponding to 1.0 g of DM in a test tube covered with a cap was placed in water baths at 30, 45, 55, 65, 75 or 85 °C for 30 min. After centrifugation (30 min, 4,000 g, 20 °C) and removal of supernatant, the sediment was weighed and the ratio of water retained in batter after heating over initial water in the batter was calculated. The analysis was done at least in triplicate.

2.6 Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a DSC Q2000 device (TA Instruments, New Castle, USA). Freeze-dried C/P-S+EW, RF and RF+EW batter samples (2.5 – 4.0 mg) were accurately weighed into aluminum sample pans (Perkin Elmer, Waltham, MA, USA). Deionized water was added to obtain a MC as in fresh batter and the pans were hermetically sealed. Samples were equilibrated at 0 °C before heating to 120 °C at 4 °C/minute. The system was calibrated with indium. The onset temperatures of starch gelatinization were calculated from the thermograms using TA Instruments Universal Analysis software.

2.7 Statistical analysis

Statistical analyses were conducted using JMP Pro 13 (SAS Institute, Cary, NC, USA). Data were analyzed with one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test (significance level $p < 0.05$) as post-hoc analysis to detect significant differences.

3. Results and discussion

Two distinct model GF bread recipes (C/P-S+EW and RF bread) were developed independently from each other 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. C/P-S+EW bread (produced as described in sections 2.2.1 – 2.2.3), which contained EW protein, had a relatively high volume and a soft and homogeneous crumb structure. In contrast, RF bread (produced as described in sections 2.2.1 – 2.2.3), which contained a low level of rice proteins of low functionality, had a low volume and a rather hard and inhomogeneous crumb (see section 3.1). To evaluate whether EW proteins are responsible for the better quality of C/P-S+EW than of RF bread, a third recipe containing RF and EW powder (RF+EW bread; baking procedure and parameters identical to RF batter) was also considered. Replacing 6.0% (w/w) of RF by EW protein resulted in a higher specific volume and softer crumb than noted for RF bread, without making the crumb excessively sticky (which occurred at even higher EW protein concentrations).

3.1 Loaf specific volume and crumb structure of fresh conventionally baked bread

Specific volumes of conventionally baked C/P-S+EW, RF and RF+EW breads were $3.2 \pm 0.1 \text{ cm}^3/\text{g}$; $2.3 \pm 0.1 \text{ cm}^3/\text{g}$ and $2.9 \pm 0.1 \text{ cm}^3/\text{g}$, respectively. Figure 2 shows images of the crumbs of conventionally baked C/P-S+EW, RF and RF+EW breads. The structure of the crumb of these GF breads depends for a large part on gas cell stability during baking. Both wheat dough and GF batter can be considered as foam-like systems with gas cells dispersed in a continuous phase. Several processes cause instability of dough or batter and can lead to inhomogeneous crumb structures (Mills, Wilde, Salt, & Skeggs, 2003). Disproportionation, in which larger gas cells grow at the expense of shrinking smaller gas cells, and coalescence, which is the merging of two neighboring gas cells due to rupture of the thin dough or batter film surrounding the gas cells, are the main destabilization mechanisms (Mills et al., 2003). C/P-S+EW bread crumb was homogeneous with a large number of small pores (Figure 1). This was in agreement with findings of Pasqualone et al. (2010) for GF bread containing cassava flour and EW. RF

bread crumb structure was more inhomogeneous and consisted of some very large pores surrounded by smaller ones. These large pores probably originated from the instability of the batter which caused gas cell coalescence. RF+EW bread crumb was more homogeneous than RF bread crumb and had smaller pores, although still not similar to those in C/P-S+EW bread crumb. Surface-active components such as EW proteins can stabilize air-liquid interfaces in the batter and slow down destabilization (Mine, 1995). In GF bread making, the use of EW powder can increase loaf volume (Crockett, Ie, & Vodovotz, 2011; Pasqualone et al., 2010) and decrease (Ziobro, Witczak, Juszczak, & Korus, 2013) or increase (Ziobro, Juszczak, Witczak, & Korus, 2016) crumb firmness depending on which part of the original formulation was replaced by EW powder. In the present case, gas cells in C/P-S+EW and RF+EW batter were probably stabilized by EW proteins, leading to breads with higher specific volume and a more desirable initial crumb structure than RF breads.

To study the phenomena occurring during fermentation and baking leading to these differences in the breads, we studied the evolution of batter height, batter viscosity and the CO₂ concentration in the headspace above the batter during fermentation and heating in an ERO.

3.2 Evolution of batter properties during ERO fermentation and baking

Figure 3 shows viscosity and height of and CO₂ release from C/P-S+EW, RF and RF+EW batters during fermentation in an ERO. At the start of fermentation, RF and RF+EW batter heights were lower than that of C/P-S+EW batter (Figure 3). Thus, mixing caused more air to be incorporated in C/P-S+EW than in the other batters. The amount of gas cells incorporated during mixing is a first important parameter determining the crumb structure of GF bread (Elgeti, Jekle, & Becker, 2015). Indeed, yeast does not form new gas cells but only expands existing ones and GF batter cannot be sheeted to redistribute the gas cells as can be done for wheat flour dough. The excellent foaming of EW proteins (Kiosseoglou & Paraskevopoulou, 2014; Mine, 1995) likely explains the higher incorporation of air in C/P-S+EW than in RF batter as well as the better gas cell stabilization in the former. However, the batter heights of RF and RF+EW batters were still similar, despite the presence of EW proteins in the

latter. RF+EW batter contained a lower EW and a higher water content than C/P-S+EW batter. Thus, the EW in the former may have been diluted too much to impact air retention during mixing. During fermentation, batter heights increased linearly over time. A small, linear increase in CO₂ concentration in the headspace above all batters was also noticed, indicating that some (but not much) CO₂ was not retained by the batters. At the start of fermentation, C/P-S+EW batter had a slightly higher initial viscosity than RF and RF+EW batters, which was probably due to the higher DM content of the former. Still, the differences in batter viscosity at the start of fermentation were minor. The viscosity of C/P-S+EW batter decreased during fermentation, probably due to the decrease in density. In RF batter, an initial viscosity increase in the early stages of fermentation was followed by a more or less equal decrease. Throughout fermentation, the viscosity of RF batter was higher than that of C/P-S+EW batter, which could be related to the higher density of the former. The viscosity of RF+EW batter was lower than that of RF batter (both initially and throughout fermentation), probably because RF+EW batter contained a lower level of starch than RF batter, and thus absorbed less water during mixing (see also section 3.3).

Figure 4 shows viscosity, height of and CO₂ release from C/P-S+EW, RF and RF+EW batters/breads during ERO baking. The rate of batter expansion increased as a result of the increase of temperature. This phenomenon also occurs in conventional bread making and is referred to as oven spring. It has several causes. First, yeast activity increases with temperature and temporarily produces more CO₂ before becoming inactive at 55 °C. Second, the solubility of CO₂ in the aqueous phase decreases as temperature increases. It therefore increasingly migrates to the preexisting gas cells. Third, due to the temperature increase, the CO₂ in the gas phase expands. Finally, evaporation of water and ethanol contributes to the expansion of gas cells (Delcour & Hosney, 2010).

Oven spring ended when the batter could no longer cope with gas cell expansion. At that moment, the (sudden) rupture of gas cells caused a large increase in CO₂ concentration in the headspace above the batters/breads (Figure 4). The batter viscosities slowly decreased in the early stages of

baking as the batter heights increased. Next, there was a sharp increase in viscosity as a result of crumb structure setting. The viscosity readings were considered to be relevant only up to the point where the batter had transformed into a solid, the readings thereafter are represented by lighter grey lines in Figure 4. In all cases, the height of the batters increased to a maximum, after which they either remained more or less at this peak value (C/P-S+EW bread), decreased slightly (RF+EW bread) or decreased substantially because of collapse (RF bread). Thus, similar phenomena were observed in all batters, but the extent to which and the temperatures at which they took place differed and determined the bread properties.

In **C/P-S+EW batter**, a sudden large viscosity increase occurred at about 59 °C. In this system, the viscosity increase occurred simultaneously with the onset of starch gelatinization at about 60 °C, as determined with DSC (Figure 5). Therefore, the viscosity increase during baking of the C/P-S+EW system was probably mainly determined by structure setting. The CO₂ concentration in the headspace above the batter started to increase substantially at about 68 °C (Figure 4). Thus, the crumb structure had already been set to a large extent at the moment of significant CO₂ release and, thus, gas cell opening. The height of the product then continued to increase at a lower rate until a maximum was reached at about 81 °C. Only a limited decrease in height after reaching this maximum height was noted. At that time, no more or less CO₂ was released from the crumb than the amount of CO₂ leaking from the ERO baking chamber. Some minor loss of CO₂ was inevitable during circulation and analysis. As will be further discussed, EW proteins in C/P-S+EW batter probably stabilize gas cells during baking and thereby probably delay CO₂ release until the crumb structure is already partly set. This probably prevents major structural collapse of the C/P-S+EW system.

In **RF batter**, a first increase in the rate of CO₂ release was observed at 56 °C, presumably when the gas cells started opening. The viscosity started to increase substantially at about 60 °C and thus after a considerable amount of CO₂ had already been released. However, as observed with DSC, starch gelatinization only began at 77 °C (Figure 5). Thus, the viscosity increase in RF batter was not caused

by structure setting, as was the case for C/P-S+EW bread. Probably, the viscosity increase could be explained as resulting from starch granule swelling (see section 3.3). In any case, the crumb structure had not been set at the moment of substantial CO₂ release, resulting in a pronounced collapse of the system at about 62 °C and low product height at the end of baking (Figure 4). The collapse evidently led to a sudden increase in density and to a further acceleration of CO₂ release, which could be observed as a second increase in the steepness of the CO₂ concentration versus time curve of RF batter at 63 °C.

In **RF+EW batter**, viscosity already increased substantially at about 50 °C. This may have been due to a competition for water between rice starch and EW protein during starch granule swelling. Water uptake during starch granule swelling was further investigated in section 3.3. In this system, the CO₂ concentration in the headspace started to increase substantially at about 64 °C. The height increased up until about 65 °C, after which a limited collapse of the batter was noticed. As measured with DSC, starch gelatinization began at about 78 °C (Figure 5). The end height of the RF+EW bread was substantially higher than that of RF bread (Figure 4). Gas cells in RF+EW batter were properly stabilized, probably due to the presence of EW proteins, until the crumb structure was (at least partly) set, thereby preventing major batter collapse.

The above suggests that the extent to which the crumb structure is already set at the moment of CO₂ release is crucial in defining the volume of the final loaf. In order to produce bread of high volume, gas cells need to be sufficiently stable during baking to delay CO₂ release. The presence of proteins which can stabilize air-water interfaces, such as those of EW, seems of major importance in this regard.

To further investigate the initial differences in batter viscosity, the water retention capacity at 30 °C of batter was determined. Since the moment of viscosity increase was similar to the start of starch gelatinization in C/P-S+EW batter, but not in RF or RF+EW batters, we hypothesized that in the latter two batters starch swelling prior to gelatinization, and a concomitant uptake of water, might have

been the reason for the viscosity increase. To further investigate this, the water retention capacity of batter was also determined after heating at different temperatures.

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3.3 Water retention capacity of batter

Table 2 shows water retention capacities of C/P-S+EW, RF and RF+EW batters after heating at different temperatures. At 30 °C, while C/P-S+EW and RF batters had similar water retention capacities, the latter retained significantly ($P < 0.05$) more water than RF+EW batter, probably due to the higher water holding capacity of rice starch than of EW. The water retention capacity after heating at 30 °C could be related to the viscosity of the batters at the start of fermentation (section 3.2). C/P-S+EW and RF batters had similar water retention capacities, but since the former had the lower water content, the amount of free water remaining in the batter was also lower, which may have resulted in the slightly higher viscosity at the start of fermentation (Figure 3). The larger amount of free water remaining in the RF+EW than in the RF batter probably resulted in the lower initial viscosity of the former (Figure 3). After heating at 45 °C, water retention capacity of C/P-S+EW batter was significantly ($P < 0.05$) higher than after heating at 30 °C. This indicates that some starch swelling had already occurred between 30 and 45 °C. At higher temperatures, water retention continued to increase significantly, and after heating of C/P-S+EW batter at temperatures of 65 °C or higher, all water was retained in the sample (Table 2). This was consistent with starch gelatinization in C/P-S+EW batter, which started at about 60 °C as measured with DSC (Figure 5). For RF batter, water retention at 55 °C did not differ from that at 30 °C (Table 2). However, that at 65 °C did even if the onset for starch gelatinization in this batter occurred at a much higher temperature (Figure 5). Thus, some swelling of starch granules had occurred at 65 °C. Therefore, we hypothesize that the start of viscosity increase in RF batter at about 60 °C was caused by water uptake as a result of starch granule swelling. At 75 °C, further swelling of rice starch granules occurred and caused retention of all available water. Indeed, starch gelatinization began at about 77 °C as measured with DSC (Figure 5). In RF+EW batter, water retention increased slightly ($P < 0.05$) with temperature up to 55 °C. A further, more substantial increase in water retention was observed for RF+EW batter when increasing the temperature from 55 °C to 65 °C. Similar to what occurred in RF batter, further swelling of rice starch granules occurred between 65 and 75 °C, and starch gelatinization in the

batter started at about 78 °C (as measured with DSC, Figure 5). As analyzed with the method this resulted in retention of all available water in RF+EW batter after incubation at 75 or 85 °C. However, the reason for the relatively pronounced viscosity increase at about 50 °C in RF+EW batter (see section 3.2) remains unclear. Between 45 and 55 °C there was a significant ($P < 0.05$) but rather limited increase in water retention in RF+EW batter and not in RF batter which probably cannot fully explain the viscosity increase at about 50 °C in RF+EW batter.

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4. Conclusions

This research showed that the volume and crumb structure of GF breads to a large extent depend on the balance between the moment of CO₂ release from the GF batter and that of crumb structure setting.

In order to produce a GF bread with high volume and fine crumb, it is crucial that collapse during baking is avoided. In the case of C/P-S+EW breads, such collapse was very limited while for RF breads, it was substantial. The presence and absence of EW proteins, which have the ability to stabilize batter gas cells, in C/P-S+EW and RF breads, respectively, plays a major role in this regard. Indeed, replacing 6.0% of RF by EW powder to a large degree prevented collapse and resulted in end products with better volume and texture. Important in this regard was the observation that, when the batters lost their ability to retain the CO₂ in the expanding gas cells, the C/P-S+EW bread crumb had already (partly) set, while RF bread crumb had not. Thus, controlling the balance between the moment of CO₂ release from the batter and that of crumb setting is of major importance in GF bread making. In principle, this can be achieved in two ways. First, the moment of CO₂ release can be postponed. To this end, the batter structure and stability can be modified by using specific ingredients that promote network formation (such as enzymes or proteins), stabilize gas cells (such as surface-active proteins) or increase batter viscosity (such as gums or hydrocolloids) (Zannini, Jones, Renzetti, & Arendt, 2012). Second, the moment of crumb setting can be altered, for example by using starches with different gelatinization temperatures. Modification of these properties can yield breads with excellent loaf volume and crumb structure. The approach with ERO baking used here yields invaluable insights in the crumb structure formation phenomena taking place in GF bread making. In future research, the approach developed here will be used to study the impact of the inclusion of several of the aforementioned ingredients in GF bread recipes on their structure formation mechanisms.

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Tables

Table 1. Formulations of gluten-free breads based on cassava-and potato starch and egg white powder (C/P-S+EW), on rice flour (RF) or on rice flour and egg white powder (RF+EW). Mixtures of starch or flour and protein are regarded as 'flour' and 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' basis (fwb).

		C/P-S+EW	RF	RF+EW
'Flour'	<i>Cassava starch (% w/w)</i>	62.5		
	<i>Potato starch (% w/w)</i>	22.5		
	<i>Rice flour (% w/w)</i>		100.0	94.0
	<i>Egg white powder (% w/w)</i>	10.0		6.0
Other ingredients	<i>Tap water (% fwb)</i>	90.0	115.0	115.0
	<i>Fresh yeast (% fwb)</i>	6.2		
	<i>Dry yeast (% fwb)</i>		3.0	3.0
	<i>Sugar (% fwb)</i>	6.0	6.0	6.0
	<i>Salt (% fwb)</i>	1.5	1.5	1.5
	<i>Calcium propionate (% fwb)</i>	0.1	0.1	0.1

Table 2. Percentage of water retained by cassava and potato starch (C/P-S+EW), rice flour (RF) and rice flour with 6% egg white (RF+EW) based batters after heating for 30 min at different temperatures.

<i>Temperature</i>	C/P-S+EW batter	RF batter	RF+EW batter
30 °C	78.9 (0.7 A,d)	79.0 (0.6 A,c)	70.1 (0.3 B,e)
45 °C	82.4 (0.8 A,c)	79.6 (0.6 B,c)	71.5 (0.6 C,d)
55 °C	89.2 (2.6 A,b)	79.4 (0.4 B,c)	72.4 (0.7 C,c)
65 °C	100.0 (0.1 A,a)	85.2 (0.8 C,b)	87.8 (0.8 B,b)
75 °C	99.9 (0.1 A,a)	100.0 (0.1 A,a)	100.0 (0.1 A,a)
85 °C	99.9 (0.1 A,a)	100.0 (0.1 A,a)	100.0 (0.1 A,a)

Standard deviations are given between brackets. Different capital letters indicate a significant difference between batters heated at the same temperature. Different lowercase letters indicate a significant difference between samples of the same batter type heated at different temperatures.

List of figure captions

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. PID, proportional-integral-derivative.

Figure 2. Crumb images of cassava and potato starch (C/P-S+EW), rice flour (RF) and rice flour with 6% egg white (RF+EW) based breads, measured 1 day after baking.

Figure 3. Height (— —), viscosity (—) and carbon dioxide (CO_2) concentration in headspace (.....) of cassava and potato starch (C/P-S+EW), rice flour (RF) and rice flour with 6% egg white (RF+EW) based batters during fermentation at 30 °C in an electrical resistance oven (ERO).

Figure 4. Height (— —), viscosity (—) and carbon dioxide (CO_2) concentration in headspace (.....) of cassava and potato starch (C/P-S+EW), rice flour (RF) and rice flour with 6% egg white (RF+EW) based batters/breads during the baking phase (temperature profile: —) in an electrical resistance oven (ERO).

Figure 5. Differential scanning calorimetry thermogram of cassava and potato starch (C/P-S+EW), rice flour (RF) and rice flour with 6% egg white (RF+EW) based batters. Temperature values in the graphs indicate the starch gelatinization onset temperatures (T_0).

Figures

Figure 1

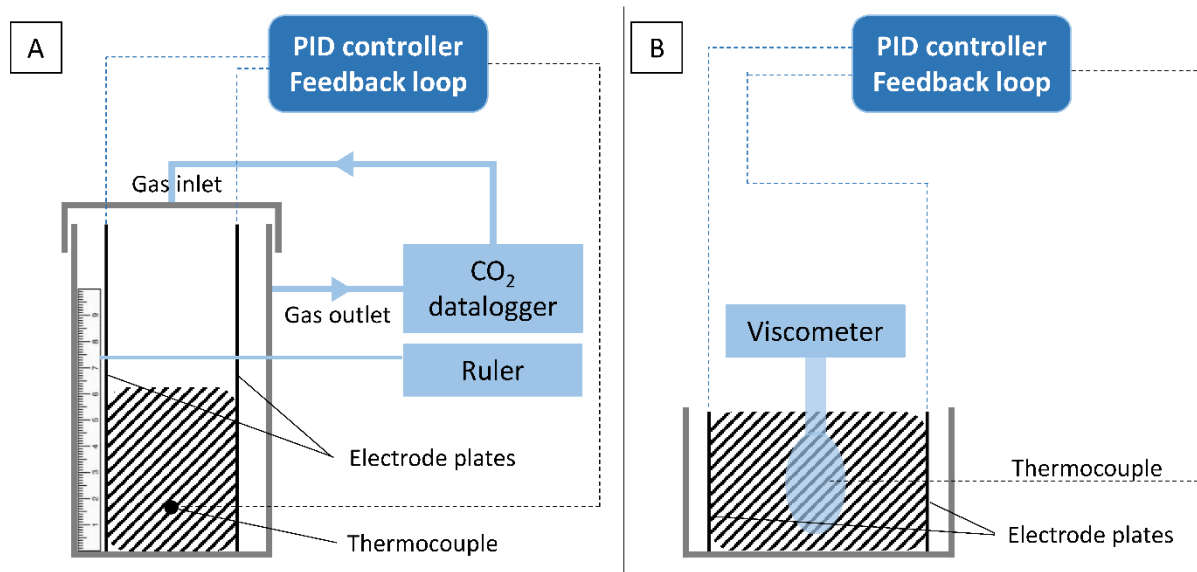
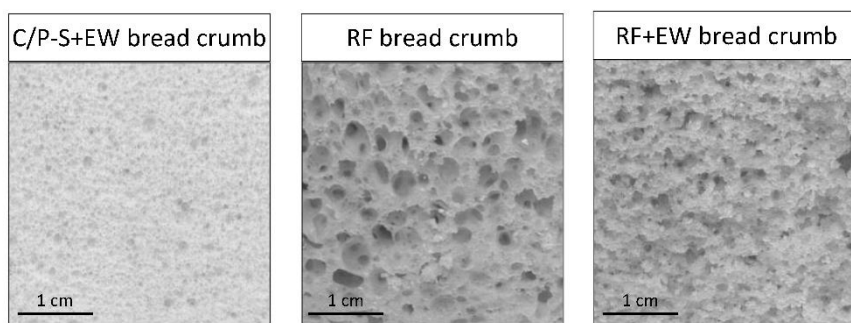


Figure 2



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Figure 3

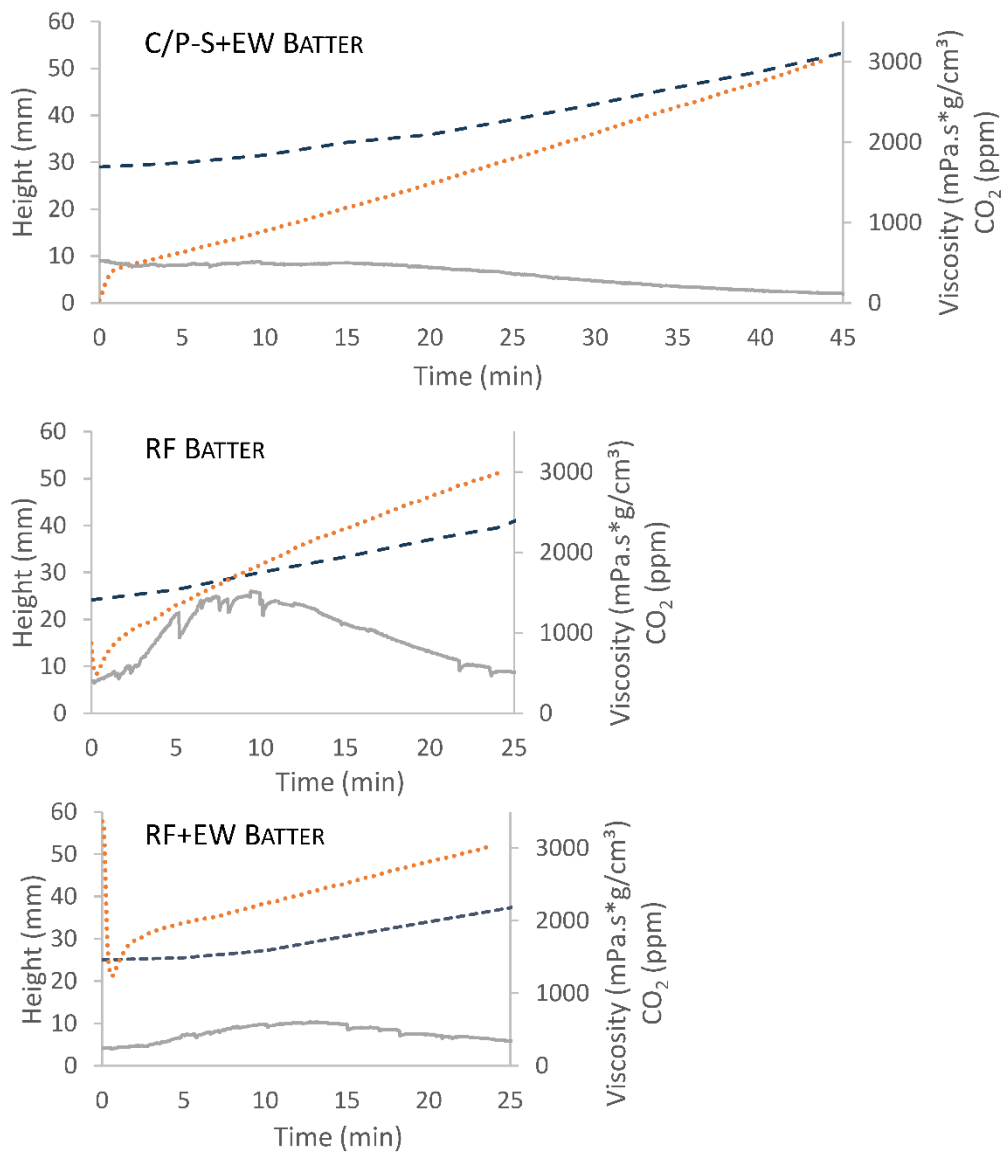


Figure 4

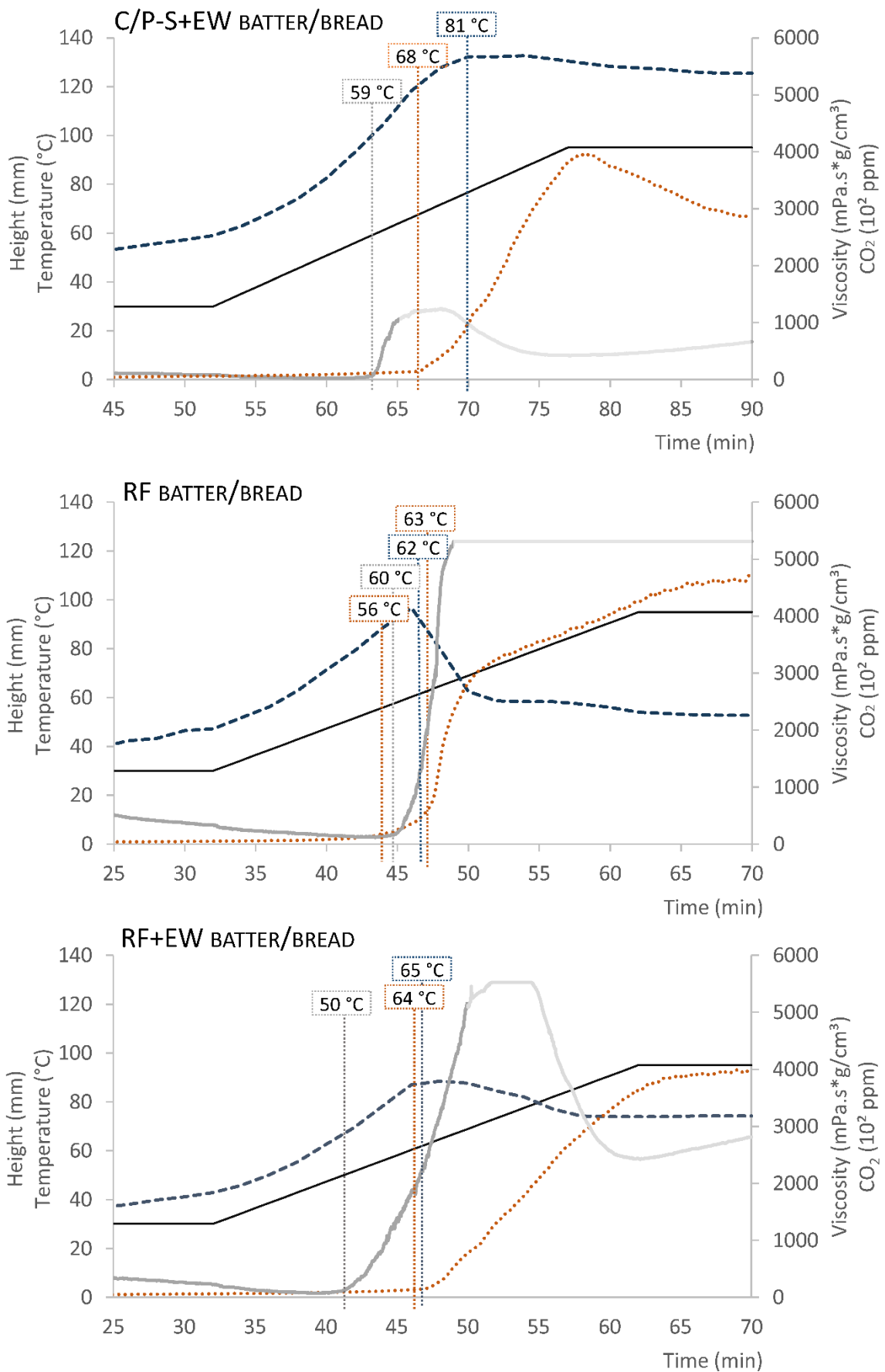
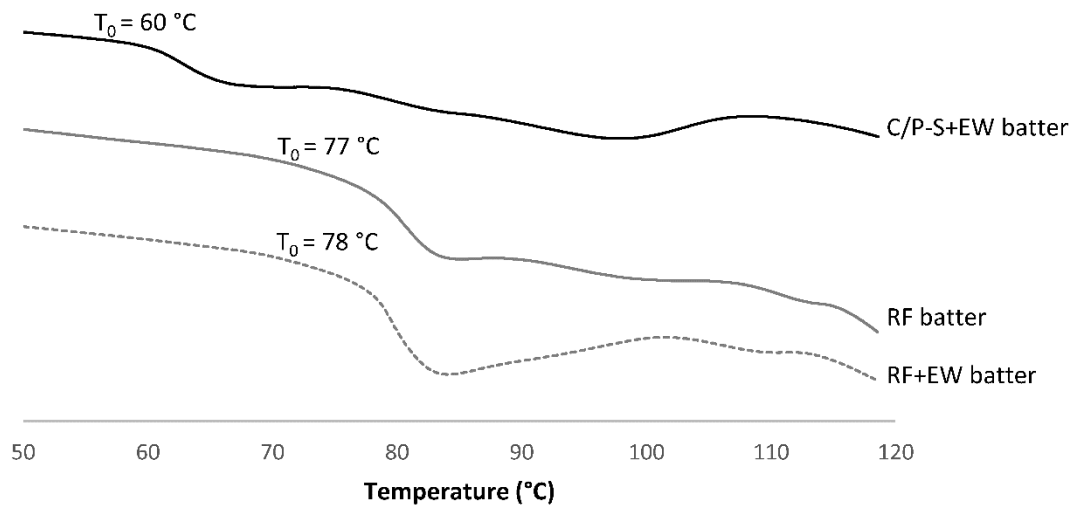
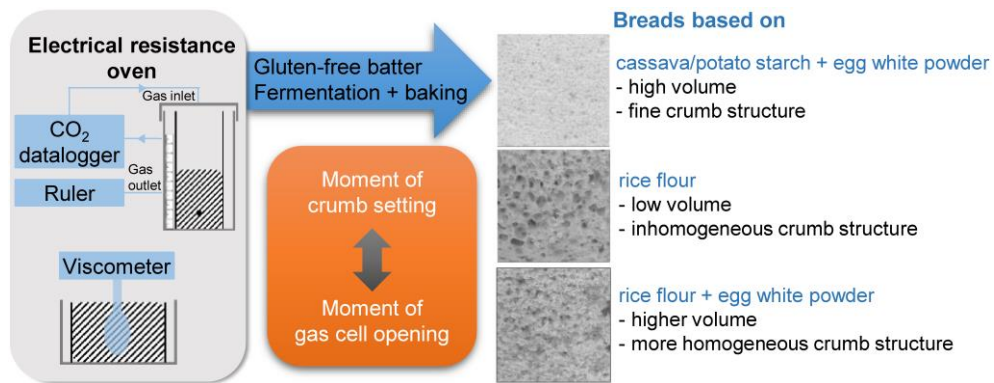


Figure 5



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Highlights

- Gluten-free bread was made for the first time in an electrical resistance oven
- This approach yielded insights in structure formation mechanisms
- The balance between the time of CO₂ release and crumb setting defines bread quality
- Egg white protein stabilizes gas cells, leading to high bread volume and fine crumb

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