The role of exogenous lipids in starch and protein mediated sponge cake structure setting during baking

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Abbreviations used

AL, air-liquid; CPMG, Carr-Purcell-Meiboom-Gill; DSC, differential scanning calorimetry; DTT, dithiothreitol; ELs, exogenous lipids; FID, free induction decay; MAGs, monoacylglycerols; PGEs, polyglycerol esters of fatty acids; SCB(s), sponge cake batter(s); SC(s), sponge cake(s); SDS, sodium dodecyl sulfate; SDS EP, the percentage of proteins extractable in SDS containing medium; SE-HPLC, size-exclusion high performance liquid chromatography; TD ^{1}H NMR, time domain proton nuclear magnetic resonance spectroscopy; ΔH, enthalpy (J/g dry matter sample)

Abstract

While it is well established that using exogenous lipids (ELs) such as monoacylglycerols and polyglycerolesters of fatty acids improves gas cell incorporation and stability in sponge cake batter (SCB) and allows producing sponge cakes (SCs) with very high volume, fine grained crumb and soft texture, their impact on starch gelatinization and protein polymerization remained unknown. Here, differential scanning calorimetry and size-exclusion high performance liquid chromatography were performed on SC(B) samples prepared with or without ELs. Starch gelatinization and protein denaturation and polymerization started at temperatures exceeding 67 °C and mostly occurred up to a temperature of 96 °C. During further isothermal treatment at 96 °C the rigidity of the cake matrix (for which temperature-controlled time domain ^{1}H NMR T_2 relaxation times are a predictor) further increased mainly because of protein polymerization. While the temperature range of starch crystal melting was not affected by the use of ELs, protein polymerized more intensively in an 88 to 94 °C temperature range when SCB contained ELs. The more intense protein polymerization and the high water binding capacity of ELs presumably made the cake matrix more rigid at that point in time. The present results allow concluding that ELs not only impact air-liquid interface stability but also cake structure setting. Hence, both aspects most likely contribute to the superior quality of SCs containing ELs.

Keywords

sponge cake baking; α-tending exogenous lipids; polyglycerol esters of fatty acids and monoacylglycerols; cake structure setting; cake matrix rigidity; temperature-controlled time domain proton nuclear magnetic resonance; proton mobility; in situ analysis

1 Introduction

Sponge cakes (SCs) are classified as foam-type cakes (Godefroidt, Ooms, Pareyt, Brijs, & Delcour, 2019). As their name reveals, they have an airy and springy texture. SCs are mostly known as layer cakes and Swiss rolls. Apart from toppings and fillings, the principal ingredients for SCs are wheat flour, sugar, whole eggs and leavening agents (Lai & Lin, 2006; Shepherd & Yoell, 1976). Sponge cake (SC) recipes can also contain emulsifiers [*e.g.* monoacylglycerols (MAGs), diacylglycerols and polyglycerol esters of fatty acids (PGEs)]. They improve gas cell incorporation during mixing and gas cell stability after mixing and during early baking (Moonen & Bas, 2004; Norn, 2004; Pycarelle, Bosmans, Nys, Brijs, & Delcour, 2020; Richardson, Langton, Faldt, & Hermansson, 2002; Sahi & Alava, 2003; Shepherd & Yoell, 1976). Moreover, their excellent functionality allows single stage instead of multi-stage mixing to prepare sponge cake batter (SCB) which reduces production time and costs (Richardson et al., 2002; Rodríguez-García, Sahi, & Hernando, 2014b). In this work, these optionally used lipid-like components are further denoted as exogenous lipids (ELs) rather than as emulsifiers because they act at air-liquid (AL) rather than at oil-liquid interfaces.

ELs stabilize foams ***directly*** and ***indirectly***. The *direct* stabilization relies on adsorption of ELs at the AL interface and their α-tending behavior. The latter refers to their ability to form three-dimensional structures in the presence of water which are called α-gels, *i.e.* hexagonally packed lamellar crystalline mesophases which consist of lipid bilayers (Krog, 1997; Krog & Borup, 1973; Krog & Larsson, 1968; Richardson et al., 2002). ELs *indirectly* stabilize foams by binding high levels of water in between the lipid bilayers. This increases the viscosity of the continuous aqueous phase which in turn slows down drainage, coalescence and bubble rise (Hasenhuettl & Hartel, 2008; Moonen & Bas, 2004; Sahi & Alava, 2003; Shelke, Faubion, & Hoseney, 1990).

When included in the recipe ELs dominate the AL interface in the batter (Pycarelle et al., 2020). While endogenous lipids in such case most likely play a minor role at the AL interface (Pycarelle et al., 2020), in ELs-free recipes egg lipids such as lecithin have a very important role in SC making (Kamat, Lawrence, Hart, & Yoell, 1973). Whether ELs are necessary in SC making depends on the type of mixing method used to prepare the batter. When SCB is prepared in a single mixing step, ELs are definitely necessary for incorporating a

sufficient amount of gas cells and to obtain SCs with high volumes. When such batter is prepared in multiple mixing steps, ELs can be left out (Pycarelle et al., 2020). However, including ELs in the SC recipe generally leads to SCs of superior quality in terms of high volume, fine crumb and soft texture (Norn, 2004; Pycarelle et al., 2020; Richardson et al., 2002; Rodríguez-García et al., 2014b).

Cake baking is typically divided in an *early* and a *late baking* stage. During *early baking* temperature increases, moisture is lost, gas cells expand and batter viscosity decreases. AL interface stability is not only important during and after mixing but also during baking. During *late baking* the liquid foam structure is transformed into a solid sponge (Cauvain & Young, 2006; Godefroidt et al., 2019; Shepherd & Yoell, 1976). This is called cake structure setting and is the result of starch gelatinization and protein polymerization (Godefroidt et al., 2019; Wilderjans, Luyts, Brijs, & Delcour, 2013).

Recent research on SCs mainly focusses on the evaluation of SC quality when including a variety of ingredients in the recipe such as pea protein (Bustillos, Jonchere, Garnier, Reguerre, & Della Valle, 2020), olive stone powder (Jahanbakhshi & Ansari, 2020) or broccoli leaf powder (Krupa-Kozak et al., 2019) or when replacing (part of the) flour by rice starch (Wang, Zhao, Liu, & Xiong, 2020), jujube fruit flour (Hosseini, Bolourian, & Shahidi, 2019) or Eucheuma powder (Huang & Yang, 2019). From the above, it is clear that novel fundamental studies on SC structure setting during baking are lacking. Also, the impact of ELs on the key phenomena responsible for cake structure setting has to the best of our knowledge never been reported. Against this background, the present study aimed at unraveling the impact of ELs on the timing and extent of cake structure setting by combining ***online*** and ***offline*** techniques on samples prepared either with or without ELs.

Online techniques, *i.e.* differential scanning calorimetry (DSC) and time domain proton nuclear magnetic resonance spectroscopy (TD ^{1}H NMR), were used to measure batter/cake properties *in situ* during baking and cooling. TD ^{1}H NMR was here used for the first time to study cake structure setting and relate changes in proton mobility of the continuous phase of the batter or matrix of the cake during baking to the timing and extent of starch gelatinization and protein polymerization. The applied temperature-time profile during DSC and TD ^{1}H NMR experiments was typical for conventional SC baking and cooling.

In *offline* experiments, SCB was heated during different time periods at 100 °C, frozen in liquid nitrogen and freeze-dried. After defatting, the samples were analyzed with (i) DSC to determine the extent of starch gelatinization and protein denaturation and with (ii) size-exclusion high performance liquid chromatography (SE-HPLC) to determine the extent to which proteins had become unextractable because of intermolecular cross-linking.

Earlier NMR studies dealing with cakes focused on proton mobility in (model) batter (Le Grand, Cambert, & Mariette, 2007; Luyts et al., 2013) or (model) cake (Hills, Benamira, Marigheto, & Wright, 2004; Le Grand et al., 2007; Luyts et al., 2013; Yildiz, Guner, Sumnu, Sahin, & Oztop, 2018) systems but never on changes during baking. Le Grand et al. (2007) and Luyts et al. (2013) determined T_2 relaxation times of both batter and cake. They observed five proton populations for cakes made from recipes including oil and margarine, respectively. Since SCs generally do not contain added fat such as oil or margarine (Lai & Lin, 2006), only the model system containing flour, sugar, eggs and water examined by Luyts et al. (2013) is here used as a point of reference. Hills et al. (2004) explored whether NMR two-dimensional T_1-T_2 correlation spectroscopy can be used as a quality control tool in food science. They examined egg, cellular tissue (fruit and vegetable) and hydrocolloids (dressings and cakes). In contrast to the results obtained by Le Grand et al. (2007) and Luyts et al. (2013), their results only showed three populations in cake based on T_2 relaxation times. More recently, TD ^{1}H NMR has been used to evaluate water retention and water-food matrix interactions in gluten-free cakes prepared with hydrocolloids (*e.g.* hydroxypropyl methylcellulose) (Yildiz et al., 2018).

The aim of this work was to study the impact of ELs on the timing and extent of structure setting during SC baking. This was done by performing online experiments in which cake structure setting was monitored *in situ* as well as offline experiments in which baking was interrupted and samples were taken at discrete time points. The novelty of this work thus not only resides in the insights gained, but also in the use of temperature-controlled TD ^{1}H NMR to simulate cake baking along with SE-HPLC and DSC to monitor key phenomena during baking.

2 Materials and methods

2.1. Chemicals and materials

White wheat flour [Halm commercial brand, 14.0% moisture content and 10.4% protein (N x 5.7) content], rice starch (10.0% moisture content) and the leavening agent [sodium acid pyrophosphate (number 15) and sodium bicarbonate] were from Paniflower (Merksem, Belgium), Beneo (Wijgmaal, Belgium) and Budenheim (Budenheim, Germany), respectively. The EL preparation (Puratos, Groot-Bijgaarden, Belgium) contained 35% lipids [*i.e.* a combination of PGEs, MAGs and diacylglycerols] and 65% rice starch as carrier. Eggs were purchased locally, stored at 3 °C and used before their "best before" date. Sodium dodecyl sulfate (SDS) and HPLC grade hexane were purchased at Sigma-Aldrich (Bornem, Belgium). Dithiothreitol (DTT) was from VWR International (Leuven, Belgium). Chemicals were of analytical grade.

2.2. Methods

2.2.1 Moisture and protein contents of raw materials

Moisture contents of flour and rice starch were determined according to AACC method 44-15.02 (AACCI, 1999). Flour protein content (N x 5.7) analysis was by using an automated Dumas protein analysis system (VarioMax Cube N, Elementar, Hanau, Germany) and based on method 990.03 of the Association of Official Analytical Chemists (AOAC, 1995). All analyses were in triplicate.

2.2.2 Sponge cake batter preparation

Sponge cake batters (SCBs) were prepared from recipes either containing ELs or not. Each batter sample was prepared in triplicate. The recipes are presented in Table 1 and the used multi-stage mixing method can be found in Pycarelle et al. (2020). Important to note is that egg white and egg yolk were separately whipped, each with part of the sugar. When the recipes contained ELs, these were mixed with egg yolk, part of the sugar, water and part of the flour. Since the ELs not only contained lipids but also rice starch as a carrier (see section 2.1), the latter was included in the recipe not containing ELs. As a result, differences between recipes either including ELs or not were only caused by adding the lipid fraction in the EL preparation. To prepare

batter not containing ELs, the rice starch was mixed together with egg yolk, part of the sugar, water and part of the flour (Pycarelle et al., 2020).

2.2.3 Batter and crumb temperature during online DSC and TD ^{1}H NMR measurements

The experimental temperature-time profile in DSC (see section 2.2.4) and TD ^{1}H NMR (see section 2.2.6) measurements was derived from that measured in the center of SC(B) during conventional baking and cooling (Pycarelle et al., 2020). SC(B) center temperature was monitored with a Datapaq (Cambridge, UK) setup consisting of a thermocouple (Multipaq 21 temperature logger, Datapaq), type T thermocouples and stainless steel casing to shield the data logger from high temperatures. Baking consisted of (i) an initial heating phase (from 25 to 98 °C at a heating rate of 7.3 °C/min) and (ii) an isothermal phase at 98 °C of 20 min. Cooling was from 98 to 25 °C at 1.9 °C/min.

2.2.4 Starch gelatinization and protein denaturation during sponge cake baking monitored using DSC

The onset of starch gelatinization and/or protein denaturation during SC baking was measured with a Q1000 DSC (TA Instruments, New Castle, DE, USA).

In a first instance, a method was optimized to monitor starch gelatinization and protein denaturation *in situ*. This was done by heating fresh SCB in a DSC device. For this, fresh SCB (5.0 – 10.0 mg) was accurately weighed in aluminum pans (Perkin-Elmer, Waltham, MA, USA). Pans were hermetically sealed and heated from 0 to 98 °C at 7.3 °C/min (together with an empty reference pan). This heating rate was chosen based on the one measured during conventional SC baking (see section 2.2.3). Calibration was with indium and tin. Onset temperatures associated with starch gelatinization and/or protein denaturation were determined with TA Instruments Universal Analysis software. For every batch of batter DSC analyses were performed at least in triplicate.

In a second instance, 200 – 300 mg SCB was heated in 2 mL Eppendorf tubes (with perforated lids to avoid pressure buildup) for 30, 60 and 90 s and for 2, 5, 10, 20 and 30 min in a water bath at 100 °C. This experimental approach ensured accurate and reproducible sample taking which is not possible when samples are withdrawn from SC(B) during conventional baking. In this type of experiment, SCB reached its maximum

temperature of 96 °C already after 2 min. Afterwards, samples were rapidly frozen in liquid nitrogen, freeze-dried, gently ground and defatted to avoid interference of lipid melting and extensive amylose-lipid complex formation in subsequent DSC analyses. Lipids were removed essentially as in Deleu et al. (2015). In the present study, 500 mg sample dry matter was suspended in 5.0 mL hexane and shaken for 60 min (150 rotations/min, room temperature). Following centrifugation (3,000 *g*, 10 min, 23 °C), the supernatant was removed and the lipid extraction repeated. The resulting pellet was dried under a fume hood overnight. For each recipe, the samples were prepared three times from individual SCBs. These samples are further denoted as heated + defatted samples. For DSC analysis, deionized water was added [1:3 (w/w) sample dry matter:water] to accurately weighed heated + defatted samples (2.0 – 5.0 mg), pans were hermetically sealed and heated from 0 to 130 °C at 4 °C/min (together with an empty reference pan) (Wilderjans, Kerckhofs, et al., 2010). Calibration and data processing were as outlined above.

2.2.5 Protein polymerization during sponge cake baking monitored using SE-HPLC

The extent of protein polymerization during SC baking was monitored by determining the percentage of proteins extractable in sodium dodecyl sulfate (SDS) containing medium (SDS EP) for the above heated + defatted samples (see section 2.2.4). For each sample, protein was extracted once with SDS containing medium resulting in what is further referred to as SDS extract. Total batter protein was extracted with SDS+DTT containing medium resulting in SDS+DTT extract. The SDS containing medium was 0.05 M sodium phosphate buffer containing 2.0% (w/v) SDS. The SDS+DTT containing medium additionally contained 2.0% (w/v) dithiothreitol (DTT). How proteins are extracted from batter/cake samples and how these extracts are then analyzed with SE-HPLC is thoroughly described elsewhere (Pycarelle et al., 2020). SDS EP values were calculated from the SE-HPLC data as follows.

$$\text{SDS EP (\%)} = \frac{\text{total area SDS extract of heated + defatted sample}}{\text{total area SDS+DTT extract of defatted SCB}} \times 100$$

The decrease in SDS EP values during baking reflects the extent of disulfide cross-linking between proteins (Lagrain, Thewissen, Brijs, & Delcour, 2007; Wilderjans, Lagrain, Brijs, & Delcour, 2010).

2.2.6 *Proton mobility during sponge cake baking and cooling monitored using temperature-controlled TD ^{1}H NMR*

The mobility of protons during SC baking and cooling was studied with temperature-controlled TD ^{1}H NMR such as recently developed for bread baking and cooling by Nivelle, Beghin, Bosmans, & Delcour (2019) who built on the work by Bosmans et al. (2012). In this novel approach, a temperature-time profile is applied to samples in the NMR device which simulates that during conventional baking and subsequent product cooling (see section 2.2.3) while measuring proton mobility at set temperatures. Measurements were with a Minispec mq 20 TD NMR spectrometer (Bruker, Rheinstetten, Germany) connected to a BVT3000 tempering unit (Bruker) that controls probe head temperatures with nitrogen gas and a heating coil inside the probe head. T_2 relaxation times were determined for less and more mobile protons by performing a single 90° pulse (free induction decay, FID) and a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, respectively. Instrument settings were as in Nivelle et al. (2019). Measurements were performed at 25, 50, 70, 80, 90 and 98 °C (the latter every five minutes) during baking and at 90, 70, 50 and 30 °C during subsequent cooling.

SCBs prepared with and without ELs (see section 2.2.2) were weighed (*ca.* 100 mg) into Bruker NMR glass inserts (external diameter 8 mm) with sealed bottom. The inserts were then transferred into larger Bruker NMR tubes (internal diameter 8.5 mm) and another insert was placed upside down on top of the one containing SCB to avoid excessive moisture loss. The NMR tube was then sealed. Sample height was initially *ca.* 4 – 6 mm and reached a maximum of *ca.* 10 mm during simulated SC baking and cooling. FID and CPMG measurements were performed on separate tubes. For every recipe, SCB was prepared in triplicate (see section 2.2.2). For every batter replicate one FID and one CPMG measurement were performed. Thus, in total three FID and three CPMG measurements were performed for each SC recipe.

Data processing was as in Nivelle et al. (2019). In short, T_2 relaxation curves were transformed to continuous distributions of T_2 relaxation times using the CONTIN algorithm of Provencher (Bruker software). The area and mean T_2 relaxation time of proton populations in these distributions respectively reflect the amount of protons in a given population and the mobility of the environment they are in. FID and CPMG measurements were corrected for temperature effects described by Curie's law using a correction factor [varying (with

temperature) between 0.996 and 1.284] based on the initial intensity of an FID measurement of olive oil subjected to the same temperature-time profile as SCB samples.

2.2.7 Statistical data-analysis

For variables with only two groups, significant differences were verified with a Student's t-test. For variables with more than two groups a one-way analysis of variance (ANOVA) was performed first after which a Tukey multiple comparison test was used to verify significant differences between mean values. The above statistical data-analyses were performed at a significance level (α) of 0.05 with JMP Pro 12 software (SAS Institute, Cary, NC, USA).

3 Results and discussion

3.1 Starch gelatinization and protein polymerization during sponge cake baking

3.1.1 Batter/cake not containing exogenous lipids

Figure 1 presents data of SCB samples heated for different time periods in a water bath at 100 °C. ΔHs and SDS EP values for samples heated for time periods exceeding 10 min are not shown because thereafter both remained constant.

ΔH readings showed an endothermic transition which started to decrease between 30 and 60 s of heating at 100 °C (*i.e. ca.* 67 and 88 °C) and was completed between 60 and 90 s of heating at 100 °C (*i.e. ca.* 88 and 94 °C) (Figure 1). In contrast, online DSC analyses (see section 2.2.4) of SCB indicated 87.4 ± 1.2 °C as the onset temperature of starch gelatinization and/or protein denaturation. Based on the above results and in line with literature data (Allan, Rajwa, & Mauer, 2018; Bean, Yamazaki, & Donelson, 1978; Donovan, 1977; Rodríguez-García, Sahi, & Hernando, 2014a; Wilderjans, Kerckhofs, et al., 2010; Wilderjans, Pareyt, Goesaert, Brijs, & Delcour, 2008), we conclude that the onset of starch gelatinization and/or protein denaturation during baking of SCs not containing ELs occurs between 85 and 90 °C. These phenomena are most likely completed during the isothermal phase of the heating process. Temperature ranges for starch gelatinization and/or

protein denaturation slightly differed between online and offline DSC analysis probably because of different heating rates during both treatments.

SDS EP values were slightly lower after 60 s (*i.e. ca.* 88 °C) than after 30 s (*i.e. ca.* 67 °C) of heating at 100 °C (Figure 1). They significantly decreased in a temperature range of 88 °C to 96 °C (*i.e.* between 1 and 2 min of heating at 100 °C, Figure 1) which is in line with earlier observations for pound cake baking (Deleu et al., 2015). During the isothermal phase of the heating process protein polymerization continues until constant SDS EP values are reached (Figure 1).

The above results showed that formation of intermolecular protein disulfide bonds and starch gelatinization occur simultaneously in cake systems. Sucrose postpones both starch gelatinization and protein denaturation and in that way also protein polymerization (Donovan, 1977; Wilderjans et al., 2013). Together the above phenomena are responsible for the formation of the cake matrix and the final cake texture (Wilderjans, Luyts, Goesaert, Brijs, & Delcour, 2010).

3.1.2 Batter/cake containing exogenous lipids

SCB containing ELs displayed lower endothermic ***ΔH readings*** than SCB not containing ELs (Figure 1). Possibly, although most of the ELs had been removed by hexane defatting, the exothermic transition associated with amylose-lipid inclusion complex formation reduced the size of the overall resultant endothermic transition associated with starch gelatinization and/or protein denaturation. Indeed, type I amylose-lipid inclusion complexes are formed during starch gelatinization (Delcour & Hoseney, 2010) in an exothermic process (Biliaderis, Page, Slade, & Sirett, 1985). That this was the case follows from the observation that DSC showed an endothermic transition with a peak temperature of *ca.* 96 °C (results not shown) which corresponds to dissociation of such complexes (Biliaderis et al., 1985; Goderis, Putseys, Gommes, Bosmans, & Delcour, 2014; Karkalas, Ma, Morrison, & Pethrick, 1995). Irrespective of whether or not ELs were used, the temperature range over which starch gelatinization and/or protein denaturation occurred was similar for both samples (Figure 1). In addition, the onset temperature of starch gelatinization and/or protein denaturation in SCB containing ELs (88.3 ± 0.6 °C) determined by online DSC analyses did not significantly differ from that observed in SCB not containing ELs.

When SCB samples either containing ELs or not were heated for 60 or 90 s at 100 °C (*i.e.* when SCB samples reached *ca.* 88 and 94 °C, respectively), the ***SDS EP values*** of the former were significantly lower than those of the latter (Figure 1). This indicates that use of ELs led to an increased protein polymerization at those points in time. It is tempting to speculate that this contributed to the higher volume of cakes containing ELs (Pycarelle et al., 2020) since protein networks give strength to the cake structure and limit collapse (Wilderjans et al., 2008). It is here hypothesized that ELs bind to hydrophobic patches in the protein structure and thereby induce conformational changes which make thiol groups available for intermolecular disulfide bond formation. Earlier, it has been shown that β-lactoglobulin has a high affinity binding site for Tween-20 (Wilde & Clark, 1993) and lysophosphatidylcholines (Sarker, Wilde, & Clark, 1995). Moreover, partial denaturation of globular proteins into a molten globule state also increases the degree to which proteins mutually interact since it exposes more hydrophobic domains (Dickinson & Matsumura, 1994; Mine, 1995).

3.2 Proton mobility during sponge cake baking and cooling

3.2.1 Assignment of proton populations

In this study, proton populations A, B, C and D were observed in SCB and proton populations A, B, D and E in SC (Table 2 and Figure 2). Protons that are part of these populations were assigned in this study based on earlier work by Luyts et al. (2013). They performed TD ^{1}H NMR measurements on model systems with flour, sucrose, egg and water. The authors distinguished non-exchanging CH protons and exchanging protons of hydroxyl, thiol, and amino groups by exchanging the latter with deuterium which is not measured during ^{1}H NMR analyses. Slight differences between proton populations in SC(B) and those observed by Luyts et al. (2013) can originate from different ingredient ratios or the use of different probe heads in the NMR device.

Proton population A contains non-exchanging CH protons of gluten and crystalline and amorphous starch not in contact with water and non-exchanging CH protons of egg proteins. Proton populations B and C contain non-exchanging CH protons of gluten and amorphous starch in little contact with water. Population C also contains (i) non-exchanging CH protons of sucrose and (ii) exchanging protons of intra-granular water, starch and gluten. Population D contains mobile protons in the aqueous phase of the batter or in the gel network

294 of the cake. In batter, this population represents (i) exchanging protons of extra-granular (bulk) water,
295 sucrose, starch, gluten and egg proteins and (ii) non-exchanging CH protons of flour and egg lipids. Although
296 the T_2 relaxation time of population D is a measure of the moisture content of the system, this population
297 cannot be regarded as free water as the latter has a T_2 relaxation time of 2.0 – 3.0 s (Bosmans et al., 2012;
298 Luyts et al., 2013; Schmidt, 2007). A very mobile population E [often ascribed to non-exchanging CH protons
299 of lipids (Bosmans et al., 2012; Luyts et al., 2013; Todt, Guthausen, Burk, Schmalbein, & Kamlowski, 2006)] is
300 present in cake. In SC recipes not containing ELs, lipids originate from flour and egg. For the sake of clarity,
301 non-exchanging CH protons are further denoted as CH protons.

302 The molecular dynamics of starch and protein during SC baking (and cooling) are largely explained by proton
303 populations A, D and E which make up the majority of the protons (Figure 2).

304 *3.2.2 Batter/cake not containing exogenous lipids*

305 During simulated SC baking and cooling, proton populations A and B mostly merged into one population
306 denoted as population AB (Figure 3) during SC baking and cooling. As a result of ***initial heating*** from 25 to 98
307 °C, the area of proton population A(B) significantly decreased (Table 3) indicating increasing contact between
308 CH protons in population A and water. As a result, protons shifted from the more rigid population A to
309 populations D and E which contained protons that were more mobile. Indeed, the areas of proton population
310 D increased during the initial heating phase even if part of this population D shifted to higher T_2 relaxation
311 times during baking and formed an additional proton population E (Figure 2, Figure 4 and Table 3). In a flour-
312 sucrose-water model system, a highly mobile population was assigned to exchanging protons of sucrose
313 (Luyts et al., 2013). Therefore, this population E likely contains exchanging protons of sucrose and water
314 which are less in contact with protein and starch than those in population D. In addition, the highly mobile
315 CH protons of lipids are possibly also part of this population. In SC, a small proton population E, attributed to
316 CH protons of egg and flour lipids, was observed (Table 2 and Figure 2).

317 Taking into account the above DSC results (see section 3.1.1), it is suggested that at temperatures below 85
318 to 90 °C protons shifted from population A to populations D and E due to hydration of protein, hydration and
319 swelling of starch and possibly some amylose leaching. This shift is similar to the one observed during baking

of bread at temperatures lower than 60 to 65 °C (*i.e*. starch gelatinization temperature in the absence of high sucrose concentrations) (Nivelle et al., 2019). Next to CH protons from starch, population A also contains protons from gluten and egg protein. Hence, besides swelling and hydration of starch, the mobility of flour and egg protein may also increase with temperature and contribute to protons shifting from this population to population D and E. At temperatures exceeding 90 °C, further starch swelling and protein denaturation also likely contributed to the increased mobility of protons of population A until the isothermal phase of the heating process started (Figure 3).

That starch gelatinization, protein denaturation and polymerization started at temperatures between 85 and 90 °C (see section 3.1.1) was further evidenced by a pronounced increase of the area of proton population D in a similar temperature range (Figure 4A). Both starch gelatinization and protein denaturation result in an increased viscosity/rigidity of the cake matrix which was observed as a significantly lower T_2 relaxation time of proton population D at the end of baking than that observed in SCB (Figure 4A and Table 3). That there is a relation between T_2 relaxation times and viscosity has also been postulated for biscuit (Assifaoui, Champion, Chiotelli, & Verel, 2006) and bread (Nivelle et al., 2019) doughs. Although an overall decreased mobility was noted, the T_2 relaxation time of proton population D in SCB not containing ELs slightly increased again between 90 and 98 °C probably because of starch granule rupture and amylose leaching. In contrast to T_2 relaxation times of proton population D, those of proton population E increased during initial heating (Figure 4B) because of the implications of Arrhenius' law which relates molecular mobility with temperature (Lucas, Wagner, Quellec, & Davenel, 2006; Nivelle et al., 2019; Rondeau-Mouro et al., 2015). This means that during initial heating of SCB from 25 to 98 °C, the mobility of protons contained in population E was determined by the Arrhenius effect, while that of protons in population D was determined by the viscosity increase associated with cake structure setting. The latter thus overruled the Arrhenius effect.

During the ***isothermal phase of the heating process*** (*i*.e. from 10 to 30 min at 98 °C) the areas of populations A, D and E remained constant, while T_2 relaxation times of the latter two (significantly) decreased (Figure 3, Figure 4 and Table 3) indicating further stiffening of the cake matrix most likely because of protein polymerization (Goetz & Koehler, 2005; Indrawati, Stroshine, & Narsimhan, 2007). Indeed, when SCB was

heated in a water bath at 100 °C, SDS EP values decreased during the isothermal stage (see section 3.1.1, Figure 1). Possibly, starch gelatinization associated phenomena (*e.g.* amylose and/or amylopectin leaching) and water evaporation also further stiffened the network as stated for bread baking by Nivelle et al. (2019).

As a result of ***cooling*** the area of proton population A(B) increased because of amylose crystallization and an increased portion of protons in little contact with water (Nivelle et al., 2019). Protons thus shifted from population D and E to population A(B) as the cake was cooled to 30 °C. At the same time, the mobility of proton populations D and E decreased because of the formation of a mixed gel network containing starch and protein (Wilderjans et al., 2013) and because of the implications of Arrhenius' law (Nivelle et al., 2019). In the end, a large proton population D and a small proton population E were left (Table 3). The latter then only contained protons from flour and egg lipids.

3.2.3 Batter/cake containing exogenous lipids

Proton populations in SCB containing ELs were assigned as before (Table 2). In which population(s) protons of ELs appeared was unclear because irrespective of whether or not ELs were used, the areas of proton populations A(B) and D and their T_2 relaxation times were similar (Figure 3, Figure 4 and Table 3). When ELs dispersed in deionized water (similar concentration as in SCB) were subjected to the same temperature-time profile in the NMR device as SCB, a main CPMG proton population was found at T_2 relaxation times of 100 ms or higher. Protons of ELs were thus probably part of populations D and E. To further unravel interactions between these protons and other batter constituents during SC baking, analysis of model systems would be a valuable next step.

Irrespective of whether or not ELs were used, the areas and T_2 relaxation times of proton populations A(B), D and E were similar as a result of ***initial heating*** from 25 to 98 °C (Figure 3 and Figure 4). In contrast, at the end of initial heating, proton population D in SCB containing ELs had a significantly lower mobility than that observed in SCB not containing ELs (Figure 4A and Table 3). This indicates that at the end of this phase, the molecular environment of protons contained in proton population D was more rigid in the former which matched with more pronounced protein polymerization (see section 3.1.2).

As mentioned above, during the ***isothermal phase of the heating process*** T_2 relaxation times of proton population D decreased indicating further stiffening of the cake matrix (Figure 4A). In the presence of ELs, the matrix was more rigid during the isothermal phase of the heating process as T_2 relaxation times of population D were lower than those measured in the absence of ELs (Figure 4A and Table 3). This may be related to the high water binding capacity of ELs (Krog, 1997; Krog & Borup, 1973; Krog & Larsson, 1968; Richardson et al., 2002; Sahi & Alava, 2003) or to the formation of a different protein network in the presence of ELs even if SDS EP values did not differ between both samples at the end of baking (Figure 1). At the end of the isothermal phase of the heating process, T_2 relaxation times of proton population E did not differ between both samples, whereas its area was higher when ELs were used (Figure 4B and Table 3). As this population contains very mobile protons that are less in contact with starch and protein than protons from population D, it is plausible that these additional protons are water and sucrose protons that reside in the water layer between lipid bilayers of the α-gel structure.

At the end of ***cooling***, proton population A in SC containing ELs contained more protons than that in SC not containing ELs (Figure 3 and Table 3) which is probably because of amylose-lipid complex formation (see section 3.1.2). As the areas and T_2 relaxation times of proton population D were similar for both SC samples, the mobility of the protons confined in the gel network of the cooled cake is comparable (Figure 4A and Table 3).

3.3 An overarching view on the impact of exogenous lipids during the sponge cake making process

Air incorporation during ***mixing*** and air retention after mixing highly determine SC quality. To obtain an adequate batter density, the AL interface surrounding the gas cells needs to be stabilized (Godefroidt et al., 2019; Sahi & Alava, 2003).

When no ELs are used the molecular population at the AL interface consists of both protein and lipid (Pycarelle et al., 2020). It has been suggested that the AL interface in SCB is mainly stabilized by proteins, especially egg white ovalbumin and wheat flour α- and γ-gliadins, and that lipids disturb the formation of a

viscoelastic protein layer at that interface (Pycarelle et al., 2020; Pycarelle et al., 2019). *When ELs are part of the SC recipe*, they enhance air incorporation during mixing (Pycarelle et al., 2020; Richardson et al., 2002; Sahi & Alava, 2003) and increase batter stability by dominating the AL interface (Pycarelle et al., 2020). As a result, it is possible to prepare SCB in a single mixing step and reduce production time and costs (Rodríguez-García et al., 2014b). The presence of other surface-active molecules at the AL interface and/or their interaction with ELs can however not be ruled out (Pycarelle et al., 2020).

During ***early baking***, the area of the AL interface increases due to gas expansion and the production of carbon dioxide and water vapor (Godefroidt et al., 2019). Evidently, for air to be retained in the batter until the cake structure sets, there is a need for (i) additional surface-active lipids and/or proteins to adsorb from the bulk at the AL interface and/or (ii) the formation of a strong viscoelastic layer at the AL interface.

In *SCB not containing ELs,* both lipid and protein most likely stabilize the AL interface during early baking. However, AL interface stability is in this case not optimal and as a result coalescence and disproportionation are likely. In *SCB containing ELs*, the latter dominate the AL interface by forming three-dimensional multi-lamellar structures (*i.e.* α-gels) which stabilize gas cells. Additionally, water is captured within these structures which increases batter viscosity and in turn probably also batter stability (Krog, 1997; Krog & Borup, 1973; Krog & Larsson, 1968; Richardson et al., 2002; Sahi & Alava, 2003).

When temperature further rises during ***late baking***, cake structure sets as a result of the simultaneous occurrence of two gel-forming phenomena: starch gelatinization and protein polymerization (Cauvain & Young, 2006; Godefroidt et al., 2019). The presence of sucrose postpones both phenomena (Beleia, Miller, & Hoseney, 1996; Donovan, 1977; Donovan, Mapes, Davis, & Garibaldi, 1975; Godefroidt et al., 2019; Semenova, Antipova, & Belyakova, 2002) with onset temperatures ranging between 85 and 90 °C irrespective of whether or not ELs were included in the SC recipe.

Whether ELs are used or not setting continues during the isothermal phase of the heating process most likely because of further protein polymerization. Possibly, starch gelatinization associated phenomena (*e.g.* amylose leaching) and water evaporation also contribute to further stiffening of the cake matrix during this

phase. *When ELs are used* more extensive protein polymerization occurs in an 88 to 94 °C temperature interval and results in an increased stiffening of the cake matrix. This increased rigidity is maintained during the isothermal phase of the heating process even when the extent of protein polymerization is similar to that in SCs not containing ELs at that time during baking. The water binding capacity of ELs and/or the formation of an altered protein network may be the cause of the above.

During ***cooling***, moisture is lost, the cake shrinks to some extent and the matrix further stiffens due to amylose crystallization and protein gel formation (Cauvain & Young, 2006; Gough, Whitehouse, Greenwood, & Miller, 1978; Wilderjans et al., 2013). *Irrespective of whether or not the SC recipe contains ELs*, the gel network in cooled SC has the same rigidity. However, in *SCs containing ELs* the latter likely formed inclusion complexes with amylose during cooling (Krog & Jensen, 1970; Wang & Marangoni, 2016).

It is clear that including ELs in the SC recipe alters batter properties and some phenomena during baking (and cooling). As a result, SCs with high volumes, soft texture and fine crumb are produced (Norn, 2004; Pycarelle et al., 2020; Sahi & Alava, 2003).

4 Conclusions

Proton mobility prior to starch gelatinization and protein denaturation and polymerization during baking changes due to hydration of protein and hydration and swelling of starch granules. Starch gelatinization and protein denaturation and polymerization during baking of SCB **not containing ELs** start in an 85 to 90 °C temperature interval. At temperatures exceeding 90 °C, starch gelatinizes, proteins polymerize, and cake structure sets. As protein polymerization continues during the isothermal phase of baking, the cake matrix further stiffens. This was deduced from decreasing T_2 relaxation times of the proton population representing the gel network of the cake.

Both offline and online measurements indicated similar onset temperatures for starch gelatinization and protein denaturation and polymerization during baking of SCB either **containing ELs** or not. However, when ELs were used protein polymerization was more pronounced at temperatures in an 88 to 94 °C range and

coincided with formation of a more rigid gel network. The latter, such as evidenced by TD ^{1}H NMR measurements, was maintained during the isothermal phase of baking where further stiffening occurred. To the best of our knowledge, this study is the first to demonstrate that ELs impact cake structure setting. Their impact is attributed to their high water binding capacity and/or to their impact on the formation of the protein network.

The work has high scientific value as it revealed that ELs not only impact AL interface stability but also cake structure setting. Both aspects may thus contribute to the very high quality of SCs containing ELs and have to be considered if one aims at preparing ELs-free SCs. The latter is relevant in the context of the search for clean label food products.

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Figure captions

Figure 1: Enthalpy (ΔH) of the endothermic transition during differential scanning calorimetry (DSC) analyses and the percentage of protein extractable in sodium dodecyl sulfate containing medium (SDS EP) of heated + defatted sponge cake batter (SCB) samples prepared with (ELs +) or without (ELs -) use of exogenous lipids (ELs). Samples were heated for up to 10 min at 100 °C. For heating times exceeding 10 min, parameters remained constant. Vertical bars indicate standard deviations. Values with an asterisk indicate significant differences ($\alpha < 0.05$) between ELs + and ELs - samples.

Figure 2: Free induction decay (FID, left) and Carr-Purcell-Meiboom-Gill (CPMG, right) proton distributions of sponge cake batter (SCB) and of sponge cake (SC) prepared without use of exogenous lipids (ELs) at the end of baking and after cooling. Populations are as assigned in Table 2. Amplitudes are given in arbitrary units (au).

Figure 3: Areas of free induction decay (FID) proton population (pop) A(B) as a function of time and temperature during simulated baking and cooling of sponge cake (SC) prepared with (ELs +) and without (ELS -) use of exogenous lipids (ELs). Populations are as assigned in Table 2. Amplitudes are given in arbitrary units (au). Vertical bars indicate standard deviations.

Figure 4: Areas and T_2 relaxation times of Carr-Purcell-Meiboom-Gill (CPMG) (A) proton population (pop) D and (B) pop E as a function of time and temperature during simulated baking and cooling of sponge cake (SC) prepared with (ELs +) and without (ELs -) use of exogenous lipids (ELs). Populations are as assigned in Table 2. Amplitudes are given in arbitrary units (au). Vertical bars indicate standard deviations.

Figure 1

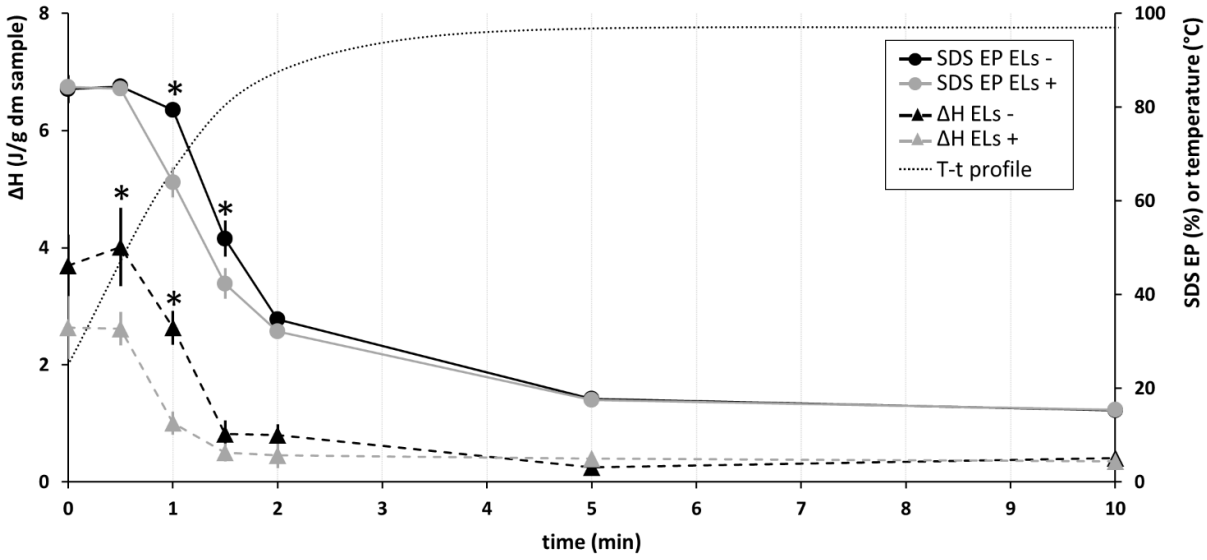
ΔH (J/g dm sample)
SDS EP (%) or temperature (°C)
time (min)
SDS EP ELs -
SDS EP ELs +
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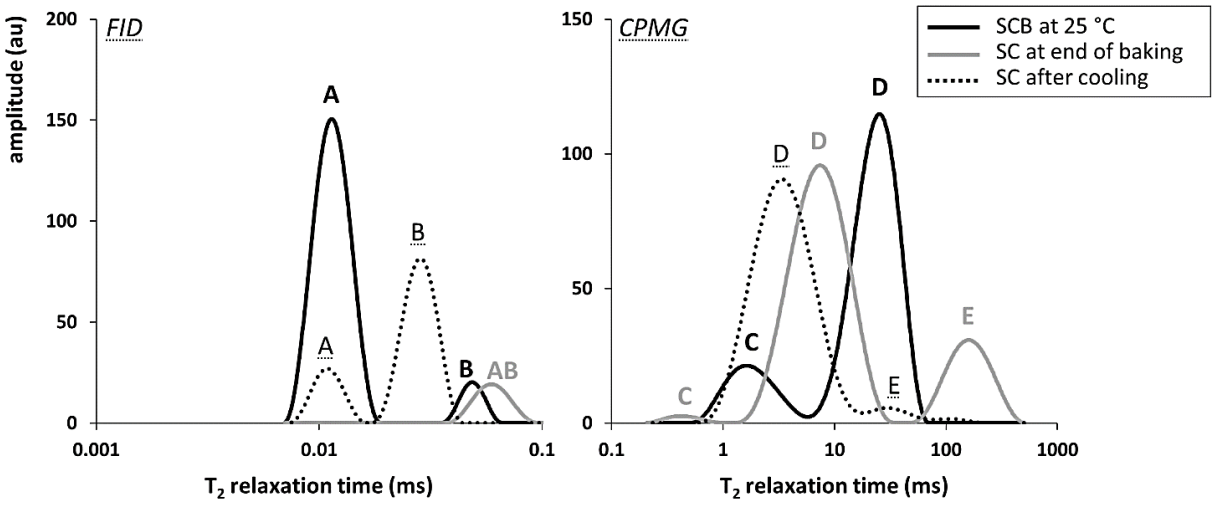
Figure 2


FID
CPMG
amplitude (au)
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A
B
AB
C
D
E
T2 relaxation time (ms)
T2 relaxation time (ms)
SCB at 25 °C
SC at end of baking
SC after cooling

Figure 3

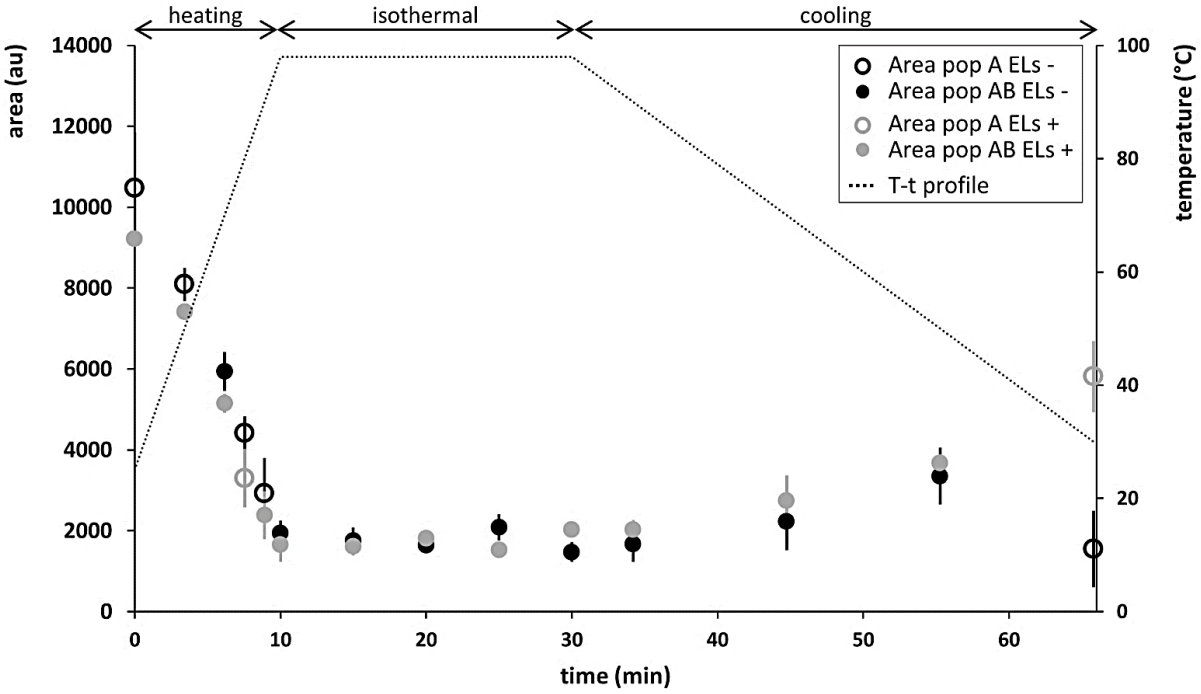
heating
isothermal
cooling
area (au)
temperature (°C)
time (min)
Area pop A ELs -
Area pop AB ELs -
Area pop A ELs +
Area pop AB ELs +
T-t profile
0
2000
4000
6000
8000
10000
12000
14000
0
20
40
60
80
100
0
10
20
30
40
50
60

Figure 4

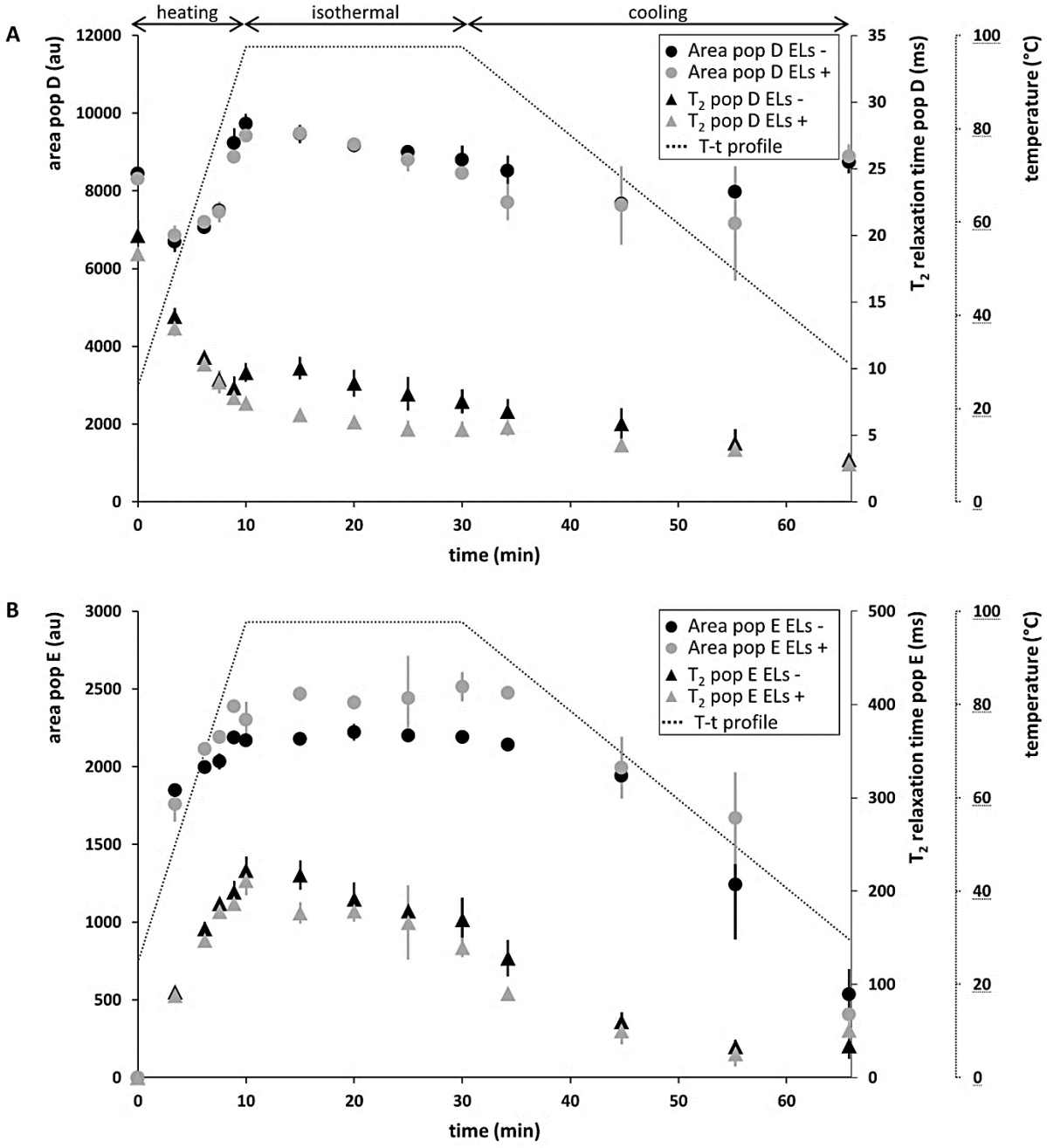
A
heating
isothermal
cooling
area pop D (au)
T_2 relaxation time pop D (ms)
temperature (°C)
Area pop D ELs -
Area pop D ELs +
T_2 pop D ELs -
T_2 pop D ELs +
T-t profile
time (min)
B
area pop E (au)
T_2 relaxation time pop E (ms)
temperature (°C)
Area pop E ELs -
Area pop E ELs +
T_2 pop E ELs -
T_2 pop E ELs +
T-t profile
time (min)

Table 1: Recipes of sponge cakes prepared without (ELs -) and with use of exogenous lipids (ELs) (ELs +).

Ingredient (g)	ELs -	ELs +
Flour (MC*: 14.9%)	284.0	284.0
Sugar	247.0	247.0
Egg white	176.0	176.0
Egg yolk	64.0	64.0
Sodium bicarbonate	4.8	4.8
Sodium acid pyrophosphate	6.6	6.6
Rice starch (MC*: 10.0%)	30.8	0.0
ELs	0.0	47.0
Deionized water	90.6	100.0

*MC: moisture content

Table 2: Proton populations in sponge cake batter (SCB) and sponge cake (SC) not containing exogenous lipids (ELs).

Proton population	SCB	SC
A	CH protons of gluten and crystalline and amorphous starch and CH protons of egg proteins	
B	CH protons of gluten and amorphous starch	CH protons of gluten and amorphous starch
C	CH protons of gluten and amorphous starch CH protons of sucrose exchanging protons of intra-granular water, starch and gluten	not detected
D	exchanging protons of extra-granular water, sucrose, starch, gluten and egg proteins CH protons of flour and egg lipids	exchanging protons of extra-granular water, sucrose, starch, gluten and egg proteins
E	not detected	CH protons of flour and egg lipids

Table 3: T_2 relaxation times and/or areas of free induction decay (FID) proton population A(B) and Carr-Purcell-Meiboom-Gill (CPMG) proton populations D and E during simulated baking of sponge cake batter (SCB) and cooling of sponge cake (SC) prepared with (ELs +) and without (ELs -) use of exogenous lipids (ELs). SCB heated for 10 min was subjected to heating from 25 to 98 °C. SCB heated for 30 min was also subjected to this heating phase and additionally to a 20 min isothermal phase at 98 °C. Populations are as assigned in Table 2. Areas are given in arbitrary units (au). Standard deviations are indicated between brackets.

		SCB at 25 °C	SCB heated for 10 min	SCB heated for 30 min	SC at the end of cooling at 30 °C
Area A(B) (au)	ELs -	10466 (1384)^{A,a}	1934 (310)^{B,a}	1473 (246)^{B,b}	1543 (948)^{B,b}
	ELs +	9224 (94)^{A,a}	1659 (426)^{C,a}	2028 (152)^{C,a}	5813 (882)^{B,a}
Area D (au)	ELs -	8439 (109)^{B,a}	9766 (249)^{A,a}	9121 (383)^{AB,a}	8865 (292)^{B,a}
	ELs +	8311 (16)^{C,a}	9450 (136)^{A,a}	8770 (169)^{BC,a}	9015 (325)^{AB,a}
T_{2D} (ms)	ELs -	20.0 (1.2)^{A,a}	9.7 (0.7)^{B,a}	7.5 (0.9)^{B,a}	3.2 (0.4)^{C,a}
	ELs +	18.6 (0.5)^{A,a}	7.4 (0.2)^{B,b}	5.4 (0.6)^{C,b}	2.8 (0.3)^{D,a}
Area E (au)	ELs -	n.d.	2178 (19)^{A,a}	2271 (18)^{A,b}	543 (163)^{B,a}
	ELs +	n.d.	2311 (116)^{B,a}	2606 (97)^{A,a}	411 (35)^{C,a}
T_{2E} (ms)	ELs -	n.d.	222 (15)^{A,a}	169 (24)^{B,a}	34 (14)^{C,a}
	ELs +	n.d.	211 (16)^{A,a}	140 (11)^{B,a}	51 (10)^{C,a}

n.d.: not detected. Results in the same row with different upper case letters and results in the same column with different lower case letters are significantly different ($\alpha < 0.05$).