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ABSTRACT

A wide range of compounds can be formed during thermal processing of food, some of which are relevant for aroma (e.g. furfural), while others are of great health-concern (e.g. furan). This paper presents the study of formulation as affecting the simultaneous generation of furan and furfural, along with other aroma quality markers, in sponge cake by means of headspace trap / GC-MS. Ingredients were screened according to their category (fat, salt, sugar, egg-based). Glucose-containing formulation resulted in the highest content of furan and furfural (12.5±0.5 ng g^{-1} and 9.2±0.2 µg g^{-1}, dry basis, respectively), while their lowest amount was found in the egg-white recipe (3.1±0.1 ng g^{-1} for furan and 0.287±0.078 µg g^{-1} for furfural, dry basis). The latter also related negatively to all studied compounds. This work will be useful for developing novel strategies to deliver safe foods with appealing organoleptic attributes.

KEYWORDS

formulation, furanic compounds, glucose, egg white, heat-processed foods, quantification, headspace trap, multivariate analysis

Highlights

- Food safety and aroma markers are studied simultaneously
- Assays are performed on a real food matrix (sponge cake)
- Glucose and egg are the most impacting ingredients on furan and furfural generation
- Fat removal, unsaturation or absence of antioxidants do not affect furan content
- Furfural is highly correlated to furan in all studied formulations

1 INTRODUCTION

Heat-treated foods, such as baked goods, are widely consumed among population, and their investigation is especially important from a food quality and safety point of view. During thermal processing food constituents may induce chemical changes through reaction pathways (such as caramelization, Maillard reaction and lipid oxidation), leading to the formation of a great number of compounds, some of which are of health concern, whereas others play a role in aroma and color. A current challenge is thus, the development of safe yet appealing food for which food reactivity must be taken into account.

In recent years, much attention has been paid to furan, a heat-generated compound of health-concern due to its possible carcinogenicity, occurrence and consumption by population (International Agency for Research on Cancer, 1995; Joint FAO/WHO Expert Committee on Food Additives, 2011). It is formed through reaction pathways that are common to other compounds having a role in sensory quality (Belitz, Grosch, & Schieberle, 2009; Crews & Castle, 2007; Kroh, 1994; Martins, Jongen, & van Boekel, 2001; Perez Locas & Yaylayan, 2004; Stadler, 2012), such as furfural, a naturally occurring odorant in many food items (Burdock, 2010; Stofberg & Grundschober, 1987). Getting insight into their simultaneous formation and their concentration in food is therefore important. Furan has been extensively quantified in a broad range of products, including baked goods (Crews & Castle, 2007; Joint FAO/WHO Expert Committee on Food Additives, 2011; Zoller, Sager, & Reinhard, 2007). Furfural has also been widely investigated in food, including qualitative, semi-quantitative and ultimately quantitative studies in bakery products (Ait Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013; C. Petisca, Henriques, Pérez-Palacios, Pinho, & Ferreira, 2014; Pico, Bernal, & Gómez, 2015; Poinot et al., 2008; Pozo-Bayón, Ruíz-Rodríguez, Pernin, & Cayot, 2007;

Rega, Guerard, Delarue, Maire, & Giampaoli, 2009). Up to date, however, furan and furfural simultaneous quantitative determination in food are scarce (Chaichi, Ghasemzadeh-Mohammadi, Hashemi, & Mohammadi, 2015; Pérez-Palacios, Petisca, Henriques, & Ferreira, 2013; Catarina Petisca, Pérez-Palacios, Pinho, & Ferreira, 2014b), and no studies are reported in baked goods.

Very recently, we optimized headspace trap (HS trap) extraction for furan and furfural simultaneous quantification in sponge cake by gas chromatography coupled to mass spectrometry (GC-MS), which proved to be sensitive, precise and competitive in comparison to other techniques (Cepeda-Vázquez, Blumenthal, Camel, & Rega, 2017). This method has been successfully applied to sponge cakes having different properties (fat content and humidity) and can thus be used for formulation studies in similar matrices. Sponge cake is indeed an interesting bakery product to study the relationship between quality and reactivity, since it is made-up with several ingredients (i.e. sugar, salt, egg, fat and flour), leading to a complex chemical composition, and contains a number of compounds that may also have a role in aroma quality (Maire et al., 2013; Pozo-Bayón et al., 2007; Rega et al., 2009). Moreover, studies in food are all-important because of the diverse nature of ingredients, their possible synergistic effect and impact on overall quality.

This paper presents the study of ingredients in sponge cake as influencing the generation of furan, furfural and other relevant aroma compounds (such as aldehydes and pyrazines) by means of HS trap / GC-MS.

2 MATERIALS AND METHODS

2.1 Chemicals and standards

Furan (purity: 99%), furfural (purity: 99%), 2-methylbutanal (purity: 95%), 2,5-dimethylpyrazine (purity: 99.9%), benzaldehyde (purity: 99%), hexanal (purity: 98%), 2-pentylfuran (purity: 97%), 1-hydroxy-2-propanone (purity: 90%) and d4-furan (purity: 98%) were supplied by Sigma-Aldrich. 3-Methylbutanal (purity: 98%) was obtained from Merck, 2,3,5-trimethylpyrazine was supplied by Acros (purity: 99%) and d4-furfural (purity: 99.7%) was obtained from CIL Cluzeau, CDN Isotopes (Pointe-Claire, Canada). Methanol (purity: 99.9%) was purchased from Carlo Erba (Val-de-Reuil, France). Ultrapure water (> 5 MΩ.cm) was produced using a Millipore Elix 3 System from Millipore SAS (Molsheim, France). Heptane and tetrahydrofuran were provided by Carlo Erba (Val de Reuil, France) and standard (±)-α-tocopherol by Sigma (Steinhein, Germany).

2.2 Ingredients

Flour was kindly provided by Grands Moulins de Paris (Ivry-sur-Seine, France). Caster sucrose was obtained from Tereos (Lille, France) and glucose (dextrose monohydrate) was kindly provided by Roquette Frères (Lestrem, France). Non hydrogenated palm oil was obtained from Palva Celys (Rezé, France) and sunflower oil by Lesieur (Asnières-sur-Seine, France). Pasteurized whole egg and egg white were supplied by Agrodoubs (Flagey, France) and Ovoteam (Massy, France). NaCl was supplied by Salinor (Clichy, France). Sucrose, glucose and salt were stored at room temperature, whole egg and sunflower oil were kept at 4°C and flour, egg white and palm oil were stored at -20°C. Flour and egg white were thawed at 4°C and palm oil in a water bath at 50°C prior to use.

A batch of purified sunflower oil was also obtained by removing tocopherols (naturally occurring antioxidants). Purification was carried out in three successive vacuum filtrations

(150 mbar and twice 120 mbar) of 200 g of sunflower oil through 70 g of dry alumina (adapted from Yoshida, Kondo, & Kajimoto (1992)). This purified sunflower oil was put into amber glass bottles filled with nitrogen and stored at 4°C until use.

Absence of tocopherols in purified sunflower oil was confirmed by high performance liquid chromatography (HPLC), for which 1 g of oil was diluted in heptane for a final concentration of 0.1 g mL^{-1}. Sample was vortexed for 60 s at maximum speed and put into an ultrasound bath for 30 s for proper homogenization, followed by filtration through a 0.22 nm nylon filter membrane (Interchim, Montluçon, France). Analyses were carried out under isocratic conditions in an Alliance Waters 2695 system equipped with a photodiode array detector (298 nm) and a Lichrospher 100 DIOL column (5 µm, 250 x 4 mm, AIT France, Houilles, France). Mobile phase was a 96.15/3.85 (v/v) heptane/tetrahydrofuran mixture (1 mL min^{-1}) and standard tocopherol concentration was 100 mg L^{-1} (adapted from (AFNOR, 1996)).

2.3 Sponge cake preparation

2.3.1 Formulations for the screening of ingredients

Reference sponge cake (f1) was formulated according to Cepeda-Vázquez et al. (2017). Five other formulations (f2-f6) were initially prepared in order to perform the screening of ingredients in relation to their category (fat, salt, sugar, egg-based) and their ability to act as precursors of different reaction pathways, namely lipid oxidation, caramelization, Maillard reaction, Strecker's degradation and other chemical interactions (see Table 1).

Concerning the fat category, three types of formulas were designed based on polyunsaturated fatty acids (PUFAs) content (mainly linolenic and linoleic acids, well-known furan precursors) (Crews & Castle, 2007; Stadler, 2012), and thus their likeliness towards enhancing lipid oxidation. Palm oil (f1) is mainly composed of saturated and monounsaturated fat with around 9% of linoleic acid and a minor fraction of linolenic acid.

Sunflower oil (f3) is particularly rich in linoleic acid (> 65%) (U.S. Department of Agriculture (USDA), 2016). Since these two oils naturally contain tocopherols (i.e. endogenous antioxidants), an additional formulation (f7) was prepared with purified sunflower oil to further test the lipid oxidation pathway in the absence of antioxidants.

2.3.2 Batter preparation

Batter of reference sponge cake (500 g) was prepared according to Cepeda-Vázquez et al. (2017) by mixing and beating whole eggs, sucrose and salt using a kitchen appliance (Kenwood Chef, KM300, UK) for 10 min at maximum speed. Flour was gently added during 1.5 min at minimum speed and the whole was further beaten for 0.5 min. Palm oil was gently added during 15 s at minimum speed and the batter beaten for 1 min at the same speed. Additional sponge cakes were prepared by replacing or removing whole egg, sucrose, salt and palm oil from reference formulation according to Table 1. Formulation containing egg white was further battered for 120 s for proper incorporation.

2.3.3 Baking trials

For each formulation, aliquots of 20 g of batter were poured into 21 aluminum molds (8.0 cm x 4.5 cm x 3.5 cm) and baked at 170°C for 25 min in an instrumented oven (Bongard, Wolfisheim, France) specifically designed to ensure thermal homogeneity (Fehaili, Courel, Rega, & Giampaoli, 2010).

Immediately after baking, sponge cakes were put into hermetically sealed glass jars and frozen at -20°C. Twenty sponge cakes were used for composite sampling for chemical analyses, and one for color measurements.

Independent baking trials (n=3) were performed to check the reproducibility of sample preparation on the reference formula (f1) as detailed in section 2.8.

2.4 Composite sampling for chemical analysis

For each baking trial, sponge cakes were ground by means of a Grindomix GM200 knife mill, equipped with a stainless steel bowl and titan knifes (Retsch GmbH, Haan, Germany) using the following conditions: five cakes per grinding operation - 3,000 rpm for 10 s, 6,000 rpm for 20 s and 3,000 rpm for up to 50 s. All batches were then mixed together at 2,000 rpm for 5 s and the resulting composite sample was stored at -20°C until analysis. The same procedure was adopted for all formulas.

2.5 Physical and physicochemical properties

Sponge cake formulations (f1 to f6) were characterized by measuring the properties described below.

2.5.1 Dry matter determination

Moisture content was determined gravimetrically on 1 g of composite samples (n=3) after oven drying at 105°C (Memmert, Schwabach, Germany) to nearest milligram (AE 200, d: 0.1mg, Mettler, Switzerland) (adapted from (Fehaili et al., 2010)).

2.5.2 pH measurement

Aliquots of 1 g of ground samples were mixed with 25 mL of ultrapure water (n=3), stirred for 1 min and further stirred during pH measurement of the resulting mixture (PH3, Hach Lange, Barcelona, Spain) (adapted from (Ait Ameur et al., 2008)).

2.5.3 Color determination

Color measurements were performed on the upper face of a whole cake at 4 points using a 6834 spectro-guide sphere gloss colorimeter (BYK-Gardner, Germany) and expressed in the CIELab color space (adapted from (Poinot et al., 2008)).

2.6 Selection of quality markers

Furan and furfural were selected as main furanic quality markers for food safety and aroma, respectively. Other typical odorant compounds found in baked goods were selected for being representative of different chemical classes (furans, aldehydes, pyrazines, etc.) and reaction pathways (mainly caramelization, Maillard reaction, lipid oxidation and Strecker's degradation) (Table 2).

2.7 HS trap / GC-MS procedure

Furan and furfural were quantified by stable isotope dilution assays according to Cepeda-Vázquez et al. (2017), and concentrations were estimated. The remaining aroma compounds were studied using semi-quantitative analysis based on chromatographic peak areas and relative abundances were assessed.

Quantitative and semi-quantitative analyses (n=3) were performed simultaneously by means of a TurboMatrix Headspace Sampler HS 40 Trap (Perkin Elmer, Llantrisant, UK) equipped with an air monitoring trap containing a two-sorbent bed (Carbotrap and Carbosieve SIII, Perkin Elmer), and a Trace GC Ultra gas chromatography system coupled to an ISQ single quadrupole mass spectrometer (Thermo Scientific, Rodano, Italy).

2.7.1 Standard solutions for furan and furfural quantification

Stock and standard solutions were prepared in a room at 18°C, while HS samples were prepared in a separate room to avoid any cross-contamination from the surrounding air. Stock solutions of d4-furan, d4-furfural, furan and furfural in methanol were prepared separately at a concentration of 2.5 g L^{-1}, put into hermetic amber glass bottles and stored at -20°C for up to one month. A standard solution of d4-furan at a concentration of 25 ng μL^{-1} was prepared daily by diluting the stock solution in ultrapure water. A mixed standard solution containing 0.5 and 15 ng μL^{-1} of d4-furan and d4-furfural, respectively, was prepared daily by diluting the standard solution of d4-furan and the stock solution of d4-furfural in ultrapure water and kept at 4°C until use. All glassware was previously baked out at 55°C for at least 24 h to avoid any contamination prior to analysis (Cepeda-Vázquez et al., 2017).

2.7.2 HS sample preparation

For vial preparation, sponge cake composite samples were kept in an ice bath to avoid volatile loss. Aliquots of ground sponge cake (0.588 g, dry basis) and ultrapure water (9.412 g) were weighed into 20 mL vials for a 16 water / sample amount ratio (dry basis) and 10 g of total amount (water + sample amount, dry basis) (Cepeda-Vázquez et al., 2017). All amounts were exactly measured in mass. A volume of 40 µL of the d4-furan + d4-furfural standard solution was added for a final set of 20 ng of d4-furan and 600 ng of d4-furfural per vial, respectively. Vials were capped with aluminum polytetrafluoroethylene coated silicone septa at each step of preparation and sealed immediately after labeled standards addition to avoid loss of volatiles. Vials were then vortexed for 5 s at maximum speed. All HS vials were previously baked out in a muffle furnace at 350°C for at least 1 h to avoid any HS glassware contamination before analysis (Cepeda-Vázquez et al., 2017).

2.7.3 HS trap conditions

Conditions were set as follows, according to the multivariate optimization of HS trap extraction for furan and furfural simultaneous quantitative analysis (Cepeda-Vázquez et al., 2017): thermostatting temperature (65°C) and time (15 min), number of pressurization cycles (4) and dry purge time (0.9 min). For all assays, shaker mode was on during vial thermostatting (automatically varying through a frequency range). Needle temperature was set to 75°C and vials were pressurized to 40 psi during 0.5 min, followed by a decay time of 2 min per pressurization cycle. After HS extraction, the trap was heated from 28 to 250°C at a 40°C s^{-1} rate and a pressure of 23 psi, in order to desorb and release the analytes into the transfer line for 0.2 min. Trap hold time was set to 15 min between GC runs and a sample blank (empty vial) was run every three injections to confirm complete desorption. Vial and water blanks were run daily to check for the absence of contaminants.

2.7.4 GC-MS analysis

Analysis conditions were adapted from Cepeda-Vázquez et al. (2017). A 100% polyethylene glycol column (VF-WAXms) was used for separation (60 m x 0.25 mm x 0.5 µm; Agilent, Netherlands). Helium was used as carrier gas at a constant flow rate of 1.2 mL min^{-1}. Injection was done in split mode (split ratio of 1:8). The GC oven was programmed as follows: initial temperature 40°C (held for 4 min), then raised at 10°C min^{-1} until 150°C and then raised at 20°C min^{-1} until reaching a final temperature of 240°C (held for 8 min). MS transfer line and ion source temperatures were set to 250°C and 200°C, respectively. The ionization mode was electron impact (EI), 70 eV. Data acquisition was done in nine segments, including a full scan mode from 25 to 150 m/z at 0.2 s per scan and the corresponding selected ion monitoring (SIM) mode with a dwell time of 0.1 s each, except

for furfural and d4-furfural 0.05 s each (Table 2). Chromatographic peak areas for each compound were calculated by extracting the quantifier ions from the SIM mode acquisition data using Quan Browser, Xcalibur 2.1.0 SP1, build 1160 (Thermo Fisher Scientific Inc., USA). Native molecules (i.e. generated in sponge cake during baking) were confirmed by means of the Wiley 8 and NIST 08 mass spectra libraries, calculation of normal alkane retention index (RI) and comparison to NIST Chemistry WebBook SRD 69 (2017) indices, and analysis of pure standards.

2.8 Sample preparation variability

In order to assess variability during batter preparation, baking and grinding, independent assays were run for reference formulation (three independent baking trials). Sponge cakes were sampled, frozen and ground as previously described to obtain a composite sample per baking trial. Each composite sample was then analyzed by HS trap / GC - MS in triplicate, both for furan and furfural quantitation, as well as aroma markers semi-quantitation as detailed in section 2.7.

2.9 Data treatment

One-way ANOVA, Tukey-Kramer's honest significant difference (HSD) and Dunnett's tests were performed on dry matter, pH, color measurements as well as furan and furfural quantitative results. Reference formulation (f1) was defined as the control (Dunnett's test). For the markers here considered, statistical differences among formulations were assessed by means of ANOVA and Tukey-Kramer's HSD test on raw chromatographic peak areas of quantifier ions, while principal component analysis (PCA) was carried out on the mean-centered areas. All analyses were performed using JMP 10.0.0 (SAS Institute Inc.).

3 RESULTS AND DISCUSSION

3.1 Variability in sample preparation

Low variability was observed for furan and furfural quantitation on single sponge cake preparation experiments (≤ 3.5% for both compounds, n=3), as previously reported for the method (Cepeda-Vázquez et al., 2017). Experiment reproducibility assessed through three independent trials, including batter preparation, baking, grinding and HS trap analysis, both for furan and furfural quantitation was also low: 6.4 and 6.8%, respectively. Higher variability was observed for semi-quantitative analyses: 2-methylbutanal and 3-methylbutanal (10%), hexanal (6%), 2-pentylfuran (11%), 1-hydroxy-2-propanone (11%), 2,5-dimethylpyrazine and 2,6-dimethylpyrazine (15%), 2,3,5-trimethylpyrazine (15%), benzaldehyde (8%). Yet, moderate variability was observed overall, showing good reproducibility throughout experiments.

3.2 Sample physical and physicochemical characterization

Low moisture content, neutral pH and mid-browning level (L*) were observed in general (Table 3). However, according to the ANOVA and Tukey-Kramer's test, significant differences were found among sponge cake formulations within each physical and physicochemical variable and were also grouped differently across them. Surprisingly, major differences were only found for glucose (f5) and egg white formulation (f6), exhibiting maximum and minimum values for pH, L* and b*. Indeed, glucose recipe showed the highest browning level (L*< 50) and the lowest pH (acid), as opposed to egg white formulation which exhibited the lowest browning level (L*> 50) and the highest pH (alkaline). Low pH in formulation with glucose might be due to the formation of acidic compounds and correlated to advanced browning, as previously reported in cookies (Ait Ameur et al., 2008).

Indeed, while a release of H^+ occurs during caramelization (Kroh, 1994), organic acids are also formed during the Maillard reaction (Martins et al., 2001), which may lead to pH fall. Egg white's pH (7.6 to 7.9), which rises with temperature (Belitz et al., 2009), could explain higher pH in formulation f6. Low dry matter content in this ingredient (12.1%) (Belitz et al., 2009) may also explain low dry matter in this formulation, and in turn result in a lower browning level, given its higher water content.

3.3 Furan and furfural content in sponge cake

Furan content in sponge cake remains in the range of 3.1-12.5 ng g^{-1}, dry basis (Figure 1) which can be considered as a low to mid-level when compared to different types of bread (≤ 30 ng g^{-1}), and similar or low when compared to other products, such as baby food containing fruit (≤ 16 ng g^{-1}), cereal-based snacks (≤ 143 ng g^{-1}) or coffee brew (≤ 199 ng g^{-1}) (Zoller et al., 2007). Furfural content in sponge cake (0.29-0.83 µg g^{-1}, dry basis), except in the case of glucose recipe (9.2 µg g^{-1}, dry basis), is similar to levels observed for cake/pastry (0.26-2.6 µg g^{-1}) (Petisca et al., 2014a).

3.4 Furan and aroma generation in sponge cake

A scheme based on current literature on the main reaction pathways (i.e. caramelization, Maillard reaction, Strecker's degradation and lipid oxidation) leading to the selected compounds (Table 2) and considering main precursors in sponge cake ingredients is here put forward (Figure 2). It will be discussed in the following sections to better understand how formulation impacts the generation of key quality markers in sponge cake.

3.4.1 Furan and furfural generation

Furfural can only be generated through caramelization or Maillard reaction (Belitz et al., 2009; Kroh, 1994; Martins et al., 2001; Srivastava et al., 2017; Whitfield, 1992; Yaylayan & Keyhani, 2000), while furan can be additionally formed by PUFAs oxidation, as well as amino acid thermal degradation (Crews & Castle, 2007; Perez Locas & Yaylayan, 2004; Srivastava et al., 2017; Stadler, 2012) (Figure 2). Yet, our results show that furfural generation in sponge cake seems to be favored over furan's, since furfural content (ppb to ppm) was found to be 65 to 730-fold that of furan (ppb), depending on the formulation under study. Both furan and furfural content were mainly impacted by two categories of ingredients, namely sugar and egg-based (Figure 1).

The glucose-containing recipe (f5) exhibited their highest concentration, suggesting that the extent of non-enzymatic browning reactions (caramelization, Maillard reaction) in this formulation is greater than in the reference cake (f1) containing sucrose. This result is not surprising since glucose (reducing sugar) can directly undergo enolization and further degradation (through caramelization or Maillard reaction) (Figure 2) (Kroh, 1994; Martins et al., 2001), while sucrose (non-reducing sugar) first has to undergo hydrolysis before releasing its reducing monomers (glucose and fructose) (Quintas, Guimarães, Baylina, Brandão, & Silva, 2007). Similar results were previously reported in sponge cake where sucrose replacement by reducing sugars led to higher amounts of furanic compounds (i.e. furfural and HMF (5-hydroxymethylfurfural)) (Zhang et al., 2012). Our results also show a higher molar yield regarding furan generation in the case of the glucose-containing formulation (f5) as compared to the sucrose recipe (f1) (18.0 and 13.0 ng g^{-1} mol^{-1}, dry basis, respectively). Opposite results were reported in model solutions (Perez Locas & Yaylayan, 2004), suggesting that sucrose undergoes hydrolysis less effectively in sponge cake than in simple models. Indeed, sucrose degradation into reducing sugars (fructose

and glucose) may be limited in sponge cake (Zhang et al., 2012). This can be easily explained: model systems (single or binary mixtures with a high water content, heated at elevated temperatures, i.e. 250°C) do not reproduce what really occurs during baking of sponge cake (complex chemical composition and structure, where gradual water evaporation and drying of the surface lead to crust formation, whose temperature tends to that of the oven, i.e. 170°C) (Fehaili et al., 2010). This underlines the importance of performing studies in complex matrices.

Interestingly, the increase in furfural content in our recipe (f5) with respect to our reference cake (f1) was much greater than that in furan (around 17-fold and 3-fold, respectively). This suggests that, although glucose is a precursor of both compounds, the pathway leading to furfural (i.e. 1,2-enolization) is favored over the one majorly contributing to furan (i.e. 2,3-enolization) (Belitz et al., 2009; Kroh, 1994; Martins et al., 2001; Perez Locas & Yaylayan, 2004). Indeed, in a recent kinetic study in cake models containing either glucose-only or glucose and leucine, furfural generation was also favored over furan's (Srivastava et al., 2017).

The egg white formulation (f6) showed the lowest furan and furfural concentrations. Indeed, whole egg is richer in free amino acids (U.S. Department of Agriculture (USDA), 2016), which might enhance the Maillard reaction pathway or might even form furan through amino acid thermal degradation (Perez Locas & Yaylayan, 2004) (Figure 2). Besides, while PUFAs are present in egg yolk, phospholipids also are and might exert a protective effect by forming antioxidant species when reacting with carbonyl compounds (Maire et al., 2013), hindering lipid oxidation as a result.

Interestingly, all formulations (f1-f3) designed to study furan formation via lipid oxidation had low and very similar furan contents, suggesting that furan formation through PUFA oxidation is not particularly favored. Likewise, in other products such as baby food model

systems containing starch, sugars and soybean oil, a higher furan content was only obtained when oxidizing oil in extreme conditions (14 days at 60°C) prior to use (Owczarek-Fendor et al., 2012). Also, naturally occurring tocopherols in our fat ingredients (sunflower and palm oil) could exert an antioxidant effect as reported by Maire et al. (2013), hence preventing further furan generation through PUFAs oxidation. However, this hypothesis is here discarded since formulations with raw (f3) or purified sunflower oil (f7) resulted in no differences in furan content (7.1 $\pm$ 0.3 and 7.1 $\pm$ 0.2 ng g^{-1}, dry basis, respectively, P: 0.337), even if (f7) did show a higher amount of other lipid oxidation markers (i.e. hexanal and 2-pentylfuran).

Salt removal from recipe (f4) did not seem to exert any effect on furan formation (P: 0.0779), while it did on furfural, since a lower amount was found with respect to (f1) (P: 0.0005). Previous studies on salt effect are scarce and results rather unclear. Undetectable furan levels were reported both in liquid models containing glucose or sucrose added with NaCl (Fan, Huang, & Sokorai, 2008). In the case of furfural, salt addition showed different effects depending on the complexity of the matrix: no effect on furfural generation in a glucose-containing solution, and different effects on furfural content in cookies (increasing in the presence of leavening agents and decreasing in their absence) (Kocadağlı & Gökmen, 2016). Indeed, the complexity of the food matrix is to be considered (e.g. use of leavening agents).

To conclude, furan generation in sponge cake would seem to proceed through common pathways to furfural (i.e. caramelization and Maillard reaction). Their generation is enhanced in the presence of glucose (reducing sugar) and hindered in the absence of egg yolk (probably due to differences in Maillard reaction progression, given the restriction on free amino acids).

3.4.2 Impact of formulation on selected quality markers

PCA was carried out on all considered markers in order to determine correlations among them and relate them to changes in formulation. According to this analysis, components 1, 2 and 3 account for 95.5% of variability (Figure 3). Three highly correlated groups of compounds are observed in the PC1 vs PC2 loading plot: 1) furan, furfural and hexanal, 2) Strecker aldehydes, 2-pentylfuran and 1-hydroxypropanone, and 3) pyrazines. However, in the PC1 vs PC3 loading plot, hexanal and 1-hydroxypropanone exhibit a particular behavior: hexanal is opposite to furan and furfural, and 1-hydroxypropanone to Strecker aldehydes, when considering PC3.

These results, along with possible reaction pathways (Figure 2), give rise to several remarks. First of all, furan strongly correlates to furfural and overall behaves differently from all the other volatile markers. 2-Pentylfuran and hexanal are both well-known lipid oxidation products (Belitz et al., 2009; Cerny, 2010; Perez Locas & Yaylayan, 2004) generated from linoleic and arachidonic acid via 2,4-decadienal (Grein, Huffer, Scheller, & Schreier, 1993; Whitfield, 1992); they strongly correlate when considering PC2, but not regarding PC1. This suggests that additional pathways could be active in the formation of 2-pentylfuran in sponge cake, either through cyclization of other alkenals (Adams, Bouckaert, Lancker, Meulenaer, & Kimpe, 2011; Hidalgo, Gallardo, & Zamora, 2005) or via a lipid oxidation and amino group interaction (Adams et al., 2011; Whitfield, 1992). It should also be noted that hexanal may be formed prior to sponge cake baking, that is during the beating of batter, as previously shown by Maire et al. (2013).

Pyrazines are produced through a well-known pathway involving aminoketone degradation (Cerny, 2010; Whitfield, 1992). This very specific origin is well represented on the PCA loading plots where they are closely correlated to one another (Figure 2). Benzaldehyde would be produced either by Strecker's degradation or lipid oxidation, as previously

reported by Whitfield (1992), explaining its high correlation both to other Strecker aldehydes and 2-pentylfuran.

1-Hydroxy-2-propanone is an especially interesting marker. It could be produced either through sugar degradation (Yaylayan & Keyhani, 2000) or Maillard reaction (Belitz et al., 2009; Martins et al., 2001) via 1-deoxy-2,3-hexodiulose, a common intermediate to furan, but also from aminoketones produced during Strecker's pathway (Whitfield, 1992). Moreover, as shown in simple models, 1-hydroxy-2-pronanone could yield pyrazines in the presence of NH_3 along with aliphatic aldehydes (e.g. hexanal) and these compounds may be formed during cake preparation (through Strecker degradation from cysteine and lipid oxidation, respectively (Whitfield, 1992)). All of the above would explain 1-hydroxy-2-propanone's particular location in the PCA loading plots (Figure 3), correlated to Strecker aldehydes, positively influencing PC1 (like pyrazines, unlike furan) and PC2 (like furan, unlike pyrazines).

The effect of sponge cake formulation is clearly visible in the PC1 vs PC2 plane (85%). Indeed, four clusters of recipes are observed in the score plot: glucose (f5, top left), egg white (f6, bottom left), with no added fat nor salt (f2, f4, center) and reference and sunflower oil (f1, f3, right center). This indicates that in the presence of glucose, the peak intensities of furan, furfural and hexanal are higher, and those of the remaining compounds are lower. However, given hexanal's particular behavior in the PC1 vs PC3 plane, it can only be concluded that the presence of glucose might induce a quicker progression of caramelization and Maillard reaction in sponge cake, yielding higher amounts of furan and furfural and limiting the formation of compounds related to amino acid degradation (mainly Strecker-related and pyrazines). In the absence of egg yolk (f6), the peak intensities of nearly all compounds are lower. In this case, the restriction on some precursors (e.g. amino

acids) might be responsible for limiting the progression of certain pathways, in particular those related to amino acid degradation. Indeed, our work confirms previous findings where a higher response of 2-methylbutanal, 3-methylbutanal, benzaldehyde and 2-pentylfuran were observed in a whole egg sponge cake recipe, compared to an egg white-only formulation (Maire et al., 2013).

All considered markers were significantly influenced by formulation as shown by the ANOVA procedure, confirming PCA results (see supplementary table). Overall, sponge cake reference formulation containing sucrose and whole egg has a greater variety in aroma compounds such as Strecker aldehydes and pyrazines; glucose recipe increases furan and furfural response, most likely due to this sugar's reducing nature; and egg white formulation causes a decrease in the peak intensity of aroma compounds, probably related to amino acid-related pathways.

Clearly, an appropriate selection of key markers, a suitable experimental design and the use of multivariate analysis tools make it possible to have better understanding of the link between formulation and reaction pathways in real food, as in this case with sponge cake.

4 CONCLUSIONS

Furan and furfural content, as well as the generation of key aroma markers from different reaction pathways taking place during baking, are clearly influenced by sponge cake formulation. Sugar and egg-based are undoubtedly the most interesting categories of ingredients involved in their formation in this product.

Furan content is mainly affected by the presence of glucose (increase) and the absence of egg yolk (decrease). Reaction pathways active in its formation in sponge cake are most likely caramelization, Maillard reaction or even amino acid degradation, but not lipid

oxidation. Furfural is highly correlated to furan, but its higher content in all recipes suggests that its generation is favored over furan's.

Key aroma compounds (Strecker aldehydes, pyrazines and 2-pentylfuran) are positively related to formulation containing whole egg, probably due to a higher contribution of free amino acids in egg yolk.

This work represents a step forward in delivering baked goods having optimal food safety and aroma properties. It contributes to the understanding of the two-way relationship among key quality markers and formulation. Our findings will be useful for developing furan mitigation strategies based on formulation and also for understanding how changes in recipes (e.g. sucrose replacement, low fat, fat free or salt free recipes) might affect the generation of key compounds in these bakery products.

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Table 1 Sponge cake formulation according to ingredient categories

Formulation description		Testing for	Ingredients (g)									
			Flour	Fat			Salt	Sugar		Egg-based		Total
			Type 55	*PO*	*SO*	*pSO*	*NaCl*	*S*	*G*	*E*	*EW*	
f1	reference (palm oil, salt, sucrose, whole egg)	effect of fat type and amount on lipid oxidation; effect of salt and sugar type on browning reactions; effect of egg-based ingredients on aroma generation	125	20	0	0	5	125	0	225	0	500
f2	no added fat	effect of fat amount on lipid oxidation	125	0	0	0	5	125	0	225	0	480
f3	sunflower oil (linoleic)	PUFAs reactivity towards lipid oxidation in the presence of tocopherols (antioxidants)	125	0	20	0	5	125	0	225	0	500
f4	no salt	salt effect on browning reactions	125	20	0	0	0	125	0	225	0	495
f5	glucose	sugar type effect on browning reactions	125	20	0	0	5	0	125	225	0	500
f6	egg white	effect of egg-based ingredients on aroma generation	125	20	0	0	5	125	0	0	225	500
f7	purified sunflower oil (linoleic, without tocopherols)	PUFAs reactivity towards lipid oxidation in the absence of tocopherols (antioxidants)	125	0	0	20	5	125	0	225	0	500

PO: palm oil; SO: sunflower oil; pSO: purified sunflower oil; S: sucrose; G: glucose; E: whole egg; EW: egg white; PUFAs: polyunsaturated fatty acids

Table 2 Selected volatile markers in sponge cake, associated pathways, sensory attributes and identification method

Compound	Class	Reaction pathway	Flavor/odor	CAS No.	$RI_{ref.}$*	$RI_{exp.}$	Selected ions (m/z)**	Identification
furan	furan	MR, C, LO, AA degradation [a, b, c, d]	-	110-00-9	813 [p]	811	39, **68**	MS, RI, STD
2-methylbutanal	aldehyde	S [e, f, g]	almond, cocoa, fermented, hazelnut, malty [n, o]	96-17-3	930 [p]	928	41, 44, **57**	MS, RI, STD
3-methylbutanal	aldehyde	S [e, f, g]	malty, roasty cucumber-like [o]	590-86-3	934 [p]	933	41, 44, **57**	MS, RI, STD
hexanal	aldehyde	LO [e, f, g]	apple, fat, fresh, green, oil, grassy, tallow [n, o]	66-25-1	1103 [p]	1099	44, **56**	MS, RI, STD
2-pentylfuran	furan	LO, LO & AA interaction [b, e, g, h, i, j]	butter, floral, fruit, green bean, mushroom, raw nuts [n, o]	3777-69-3	1240 [p]	1248	**81**, 138	MS, RI, STD
1-hydroxy-2-propanone	hydroxyketone	MR, C, S [e, g, k, l]	pungent, sweet caramellic, ethereal [o]	116-09-6	1319 [p]	1345	**43**, 74	MS, RI, STD
2,5-dimethylpyrazine	pyrazine	S interaction [g]	cocoa, roast beef, roasted nut, crust-like, popcorn [n, o]	123-32-0	1357 [p]	1357	42, **108**	MS, RI, STD
2,6-dimethylpyrazine	pyrazine	S interaction [g]	cocoa, coffee, green, roast beef, roasted nut, roasted [n, o]	108-50-9	1362 [p]	1362	42, **108**	MS, RI
2,3,5-trimethylpyrazine	pyrazine	S interaction [f, g]	cocoa, earthy, must, potato, roast [n, o]	14667-55-1	1429 [p]	1439	42, **122**	MS, RI, STD
furfural	furan	MR, C [e, g, k, l, m]	almond, baked potatoes, bread, burnt, spice, soil, roasted, sweet, toasted [n, o]	98-01-1	1503 [p]	1507	39, 95, **96**	MS, RI, STD
benzaldehyde	aldehyde	S, LO [g]	bitter almond, burnt sugar, cherry, malt, roasted pepper, caramel [n, o]	100-52-7	1533 [p]	1587	77, **106**	MS, RI, STD
Internal standards								
d4-furan				6142-90-1	-	-	42, **72**	-
d4-furfural				1219803-80-1	-	-	42, 98, **100**	-

MR: Maillard reaction; C: caramelization; LO: lipid oxidation; AA: amino acid; S: Strecker's degradation; RI: normal alkane retention index; MS: mass spectra; STD: pure standard
a Crews & Castle, 2007; b Perez Locas & Yaylayan, 2004; c Srivastava et al., 2017; d Stadler, 2012; e Belitz, Grosch, & Schieberle, 2009; f Cerny, 2010; g Whitfield, 1992; h Adams, Bouckaert, Lancker, Meulenaer, & Kimpe, 2011; i Grein, Huffer, Scheller, & Schreier, 1993; j Hidalgo, Gallardo, & Zamora, 2005; k Martins, Jongen, & van Boekel, 2001; l Yaylayan & Keyhani, 2000; m Kroh, 1994; n Flavor & Extract Manufacturers Association (FEMA), 2017; o Pico, Bernal, & Gómez, 2015; p National Institute of Standards and Technology (NIST), 2017
*Retention indices on a ZB-WAX-like column; **Quantifier ions are in bold

Table 3 Sponge cake physical and physicochemical characterization

Formulation	dry matter (%)	pH	L*	a*	b*
f1	92.8 [a]	7.0 [d]	53.2 [c]	12.8 [a]	15.5 [c]
f2	90.0 [bc]	7.1 [d]	56.3 [c]	13.7 [a]	18.6 [bc]
f3	89.5 [c]	7.2 [c]	55.5 [c]	14.7 [a]	19.0 [bc]
f4	93.6 [a]	7.7 [b]	62.6 [b]	12.9 [a]	21.7 [b]
f5	91.3 [b]	5.5 [e]	43.9 [d]	6.2 [b]	4.6 [d]
f6	87.3 [d]	8.0 [a]	70.1 [a]	12.9 [a]	26.5 [a]

Values are the means from 3 replicates for dry matter and pH, and 4 replicates for color measurements. Values followed by different letters within columns are significantly different (P: <0.05)

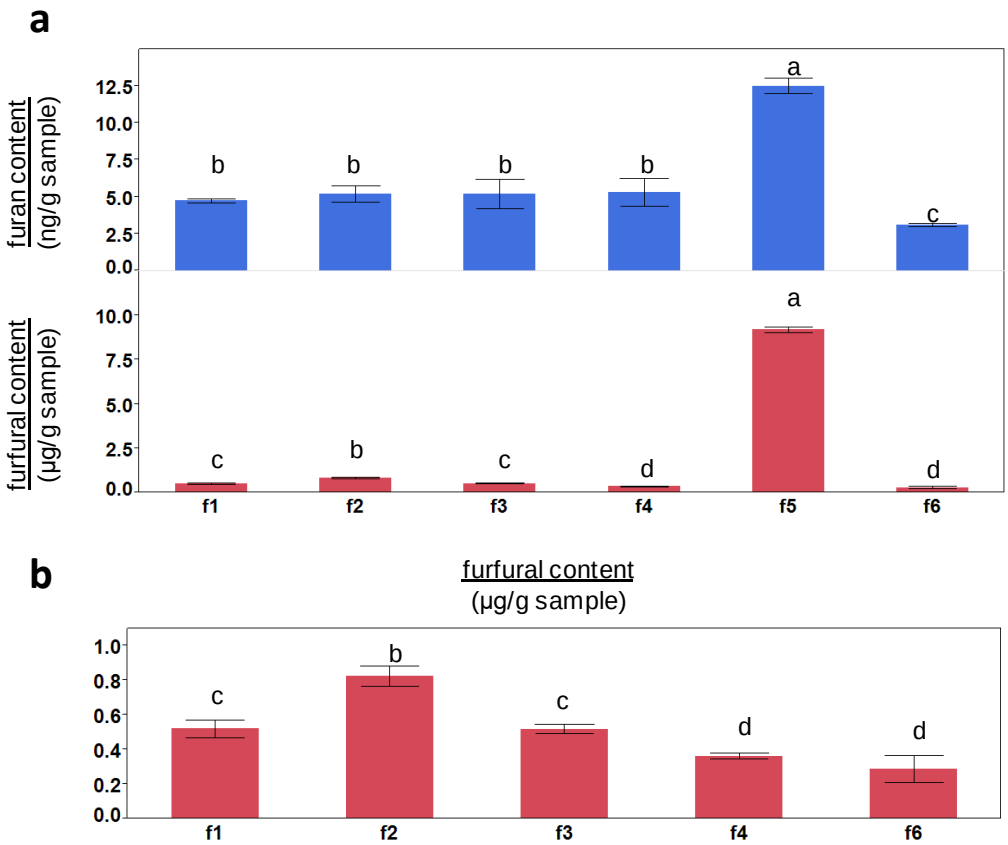

Figure 1 Furan (ng/g) and furfural (µg/g) content in sponge cake according to formulation. (a) furan and furfural content in formulations 1 to 6; (b) formulations having the lowest furfural content; f1: reference; f2: no added fat; f3: sunflower oil; f4: no salt; f5: glucose; f6: egg white; values are the means and 95% confidence intervals (n=3); mean values with different letters are significantly different (P: <0.05)

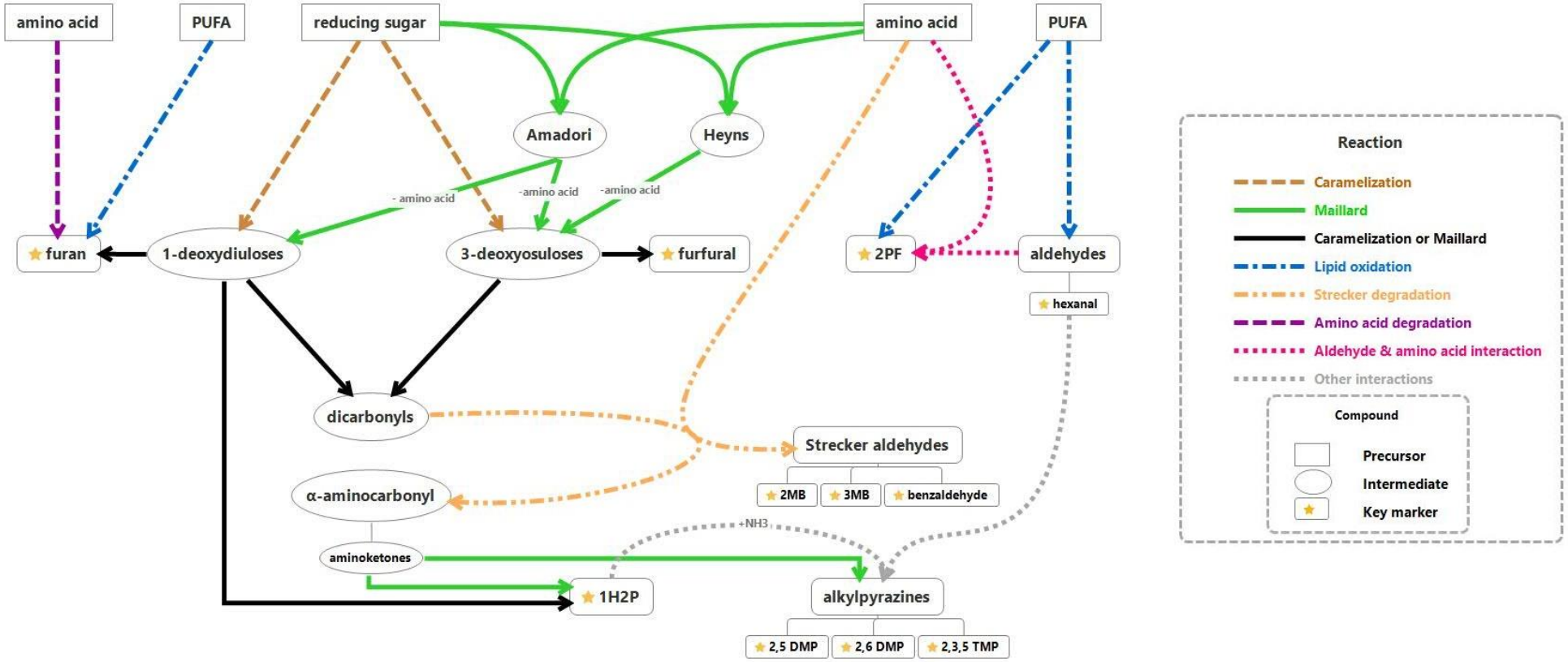

Figure 2 Reaction pathways leading to key quality markers in sponge cake. PUFA: polyunsaturated fatty acids; 2PF: 2-pentylfuran; 2MB: 2-methylbutanal; 3MB: 3-methylbutanal; 1H2P: 1-hydroxy-2-propanone; 2,5 DMP: 2,5-dimethylpyrazine; 2,6 DMP: 2,6-dimethylpyrazine; 2,3,5 TMP: 2,3,5-trimethylpyrazine (Crews & Castle, 2007; Perez Locas & Yaylayan, 2004; Srivastava et al., 2017; Stadler, 2012; Belitz, Grosch, & Schieberle, 2009; Cerny, 2010; Whitfield, 1992; Adams, Bouckaert, Lancker, Meulenaer, & Kimpe, 2011; Grein, Huffer, Scheller, & Schreier, 1993; Hidalgo, Gallardo, & Zamora, 2005; Martins, Jongen, & van Boekel, 2001; Yaylayan & Keyhani, 2000; Kroh, 1994)

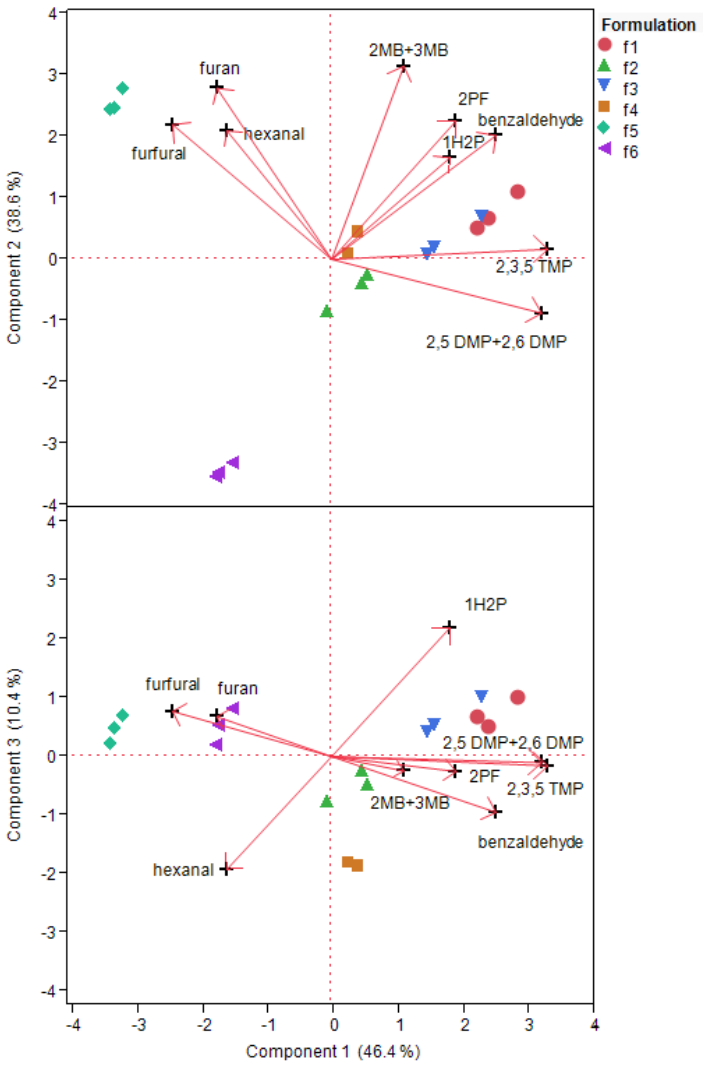

Figure 3 Principal component analysis on selected volatile compounds in sponge cake formulations. f1: reference; f2: no added fat; f3: sunflower oil; f4: no salt; f5: glucose; f6: egg white; 2MB: 2-methylbutanal; 3MB: 3-methylbutanal; 2PF: 2-pentylfuran; 1H2P: 1-hydroxy-2-propanone; 2,5 DMP: 2,5-dimethylpyrazine; 2,6 DMP: 2,6-dimethylpyrazine; 2,3,5 TMP: 2,3,5-trimethylpyrazine

Supplementary Table Peak areas of the selected markers in sponge cake

Formulation	furan	2MB+3MB	hexanal	2PF	1H2P	2,5 DMP+2,6 DMP	2,3,5 TMP	furfural	benzaldehyde
1	1019293 b	117334872 a	1036261 d	6123387 b	793669 a	2165852 a	401325 a	983668 bc	1737559 a
2	897436 cd	80511591 c	3833174 c	2383420 d	621995 bcd	1791143 b	280398 bc	1332845 b	1364217 c
3	969202 bc	91631525 b	1073709 d	7135143 a	746201 ab	1924555 b	308601 b	927058 bc	1516750 bc
4	869030 d	95836968 b	4831596 b	6144870 b	476103 d	1458376 c	245609 c	598459 c	1632867 ab
5	2291855 a	110936634 a	5566375 a	4429624 c	641014 abc	35616 e	21244 e	16721826 a	1029226 d
6	506805 e	9527479 d	426097 e	737079 e	488099 cd	1146420 d	98061 d	468726 c	384190 e

2MB: 2-methylbutanal; 3MB: 3-methylbutanal; 2PF: 2-pentylfuran; 1H2P: 1-hydroxy-2-propanone; 2,5 DMP: 2,5-dimethylpyrazine; 2,6 DMP: 2,6-dimethylpyrazine; 2,3,5 TMP: 2,3,5-trimethylpyrazine

Values are the means of raw chromatographic peak areas from 3 replicates. Values followed by different letters within columns are significantly different ($P<0.05$)